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Ramadas Nayak
Rakshatha Nayak

Exam Preparatory Manual for Undergraduates

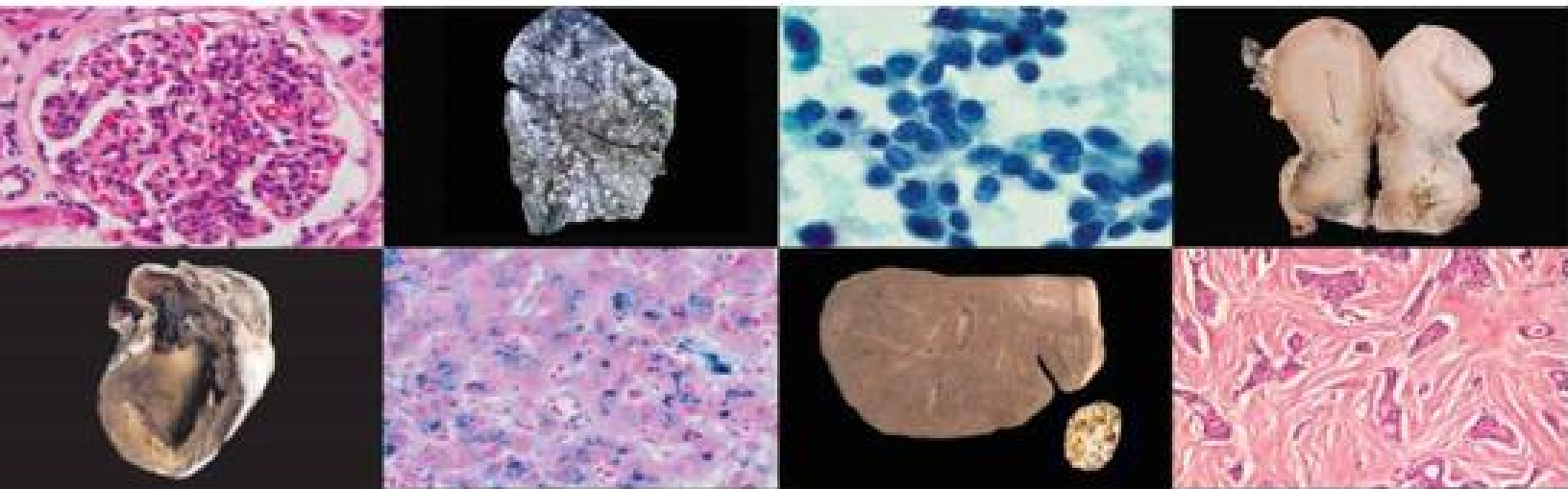
Pathology

HIGHLIGHTS

- **Incorporated competency-based undergraduate curriculum for the Indian medical graduate as per Medical Council of India (MCI).** Recent updates in pathology is covered
- Easy to understand and memorize entire subject including hematology and clinical pathology in short time
- Assured success with high scoring in theory, viva voce and competitive examination
- Helps both undergraduates and postgraduates in medical, dental and allied health sciences
- Contains everything which an undergraduate student should know before the pathology examination in a comprehensive compact text, boxes, flowcharts and figures making it a ready reckoner before the examination
- Key points are highlighted. Exclusive coverage of high yielding points in text boxes which is important for answering MCQs.

4th Edition

Foreword
K Ramnarayan



Exam Preparatory Manual for Undergraduates

PATHOLOGY



Exam Preparatory Manual for Undergraduates

PATHOLOGY

Fourth Edition

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Foreword
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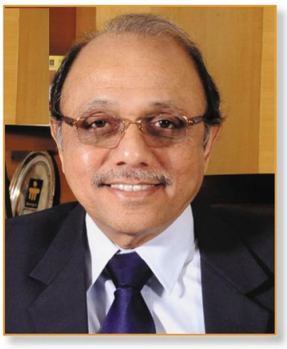
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Dedicated to

Students who inspired us,
Patients who provided the knowledge,
Our teachers, parents and family members,
who encouraged and supported us.

Foreword



“Any intelligent fool can make things bigger and more complex,” said Albert Einstein. To make things understandable and appealing is the persisting and daunting task of a passionate teacher. It is in this context that Dr Ramadas Nayak’s book assumes a considerable significance. In this book, he has provided conceptual clarity that it is both astounding and amazing.

The veritable qualities of a review book include simplicity, structure, sequence, and standardization. To this, must be added another ‘s’, i.e., sympathy—sympathy for the reader who is grappling with the essentials. Dr Nayak’s endeavor to have all these qualities in the book is a testimony of his expertise and experience as an effective and exemplary teacher.

I am delighted to write this Foreword to *Exam Preparatory Manual for Undergraduates—Pathology*, which, I am certain, will be an invaluable resource for students and teachers in pathology.

K Ramnarayan
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Preface to the Fourth Edition

We are proud to bring the fourth edition of this *Exam Preparatory Manual for Undergraduates—Pathology* which has become popular within a few years of publishing the first edition. We had to bring this edition little prematurely to incorporate "Competency-based undergraduate curriculum for the Indian medical graduate" by Medical Council of India (MCI) in 2018. This book is organized to incorporate modification as suggested by this new "Competency-based Medical Education (CBME)". As per CBME following have been incorporated:

- Interpretation of liver function and viral hepatitis along with serology panel. Features to distinguish obstructive from nonobstructive jaundice based on clinical features and liver function tests. Cases have been provided with history and liver function tests for interpretation.
- Abnormal urinary findings and few abnormal renal function tests in various disease states are provided with clinical history. Common urinary abnormalities in almost all renal diseases have been included.
- Abnormal findings in body fluids in various disease states.
- Clinical history with cerebrospinal fluid (CSF) findings has been included so that learner can identify the etiology of meningitis based on CSF parameters.
- Core competency is marked as (CC) and non-core competency as (NC).
- DOAP (Demonstration-Observation-Assistance-Performance): A practical session that allows the student to observe, demonstrate, assist the performer, perform in a simulated environment, perform under supervision or perform independently has been mentioned in relevant topics.

As the saying goes "Change is inevitable", this book also has undergone changes as per the need of the student. Pathology is one of the rapidly-expanding, ever-changing and interesting field in medicine. This book is an endeavor to present the vast knowledge of pathology in a lucid manner for undergraduate and postgraduate medical students as well as those

undergoing training in paramedical courses. The main aim of this book is to provide a sound knowledge of pathology and understand disease mechanisms which lays the foundation for basic science. It gives insight into etiology, pathogenesis, pathology and the disease course. Every attempt has been made to present information in a simplified text augmented with the use of colored illustrations, tables, text boxes and flowcharts. There was a tremendous increase in the understanding of molecular pathology and same is highlighted in all the relevant chapters. Thus, this edition is completely revised, updated, better illustrated and a complete manual for scoring high marks in all pathology examinations. Two chapters have been reorganized to include pediatric diseases and environmental diseases. Two new chapters namely basic diagnostic cytology and blood banking and transfusion as well as many flowcharts are added. Each chapter has been updated incorporating all the core and non-core competencies as per CBME. Few figures and illustrations have been replaced with better quality photomicrographs or illustrations. As far as possible, we have used clear and simple language that can be understood by students who studied their primary school in any of the regional language.

Organization

This book consists of 27 chapters and is organized into three sections namely General Pathology, Hematology and Clinical Pathology, and Systemic Pathology.

Section 1—General Pathology: It provides an overview of the basic pathologic mechanisms underlying diseases including—introduction to pathology; cell injury and adaptation; inflammation and healing; infections and infestations; hemodynamic disorders, thromboembolism, and shock; immunopathology, AIDS, and amyloidosis; neoplasia; genetic and pediatric diseases; environmental and nutritional diseases, and basic diagnostic cytology.

Section 2—Hematology and Clinical Pathology: It consists of—introduction to hematology and disorders of red blood cell; disorders of white blood cell, lymph node, and spleen; disorders of hemostasis; clinical pathology, and blood banking and transfusion essential for the undergraduate students. Students are requested to go through the second edition of the book titled

Essentials in Hematology and Clinical Pathology authored by Dr Ramadas Nayak and Dr Sharada Rai, M/s Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, for detailed knowledge of hematology and clinical pathology.

Section 3—Systemic Pathology: It deals with systemic pathology with chapters devoted to diseases of various organ systems including—cardiovascular disorders; respiratory disorders; disorders of oral cavities and gastrointestinal tract; hepatobiliary and pancreatic disorders; kidney and urinary tract disorders; male genital tract disorders; female genital tract disorders; breast disorders; endocrine disorders; bones, joints, and soft tissue tumors; skin disorders, and disorders of central nervous system and eye.

We found that undergraduate students find it difficult to understand, remember and answer the questions during examinations, in a satisfying way. There are many pathology textbooks, but undergraduates face difficulty to refresh their knowledge during examinations. This book fills the niche, to provide basic information to an undergraduate in a nutshell. The text provides all the basic information the student will ever need to know. Keywords are shown in bold words so that the student can rapidly go through the book on the previous day or just before the examination. Most students are fundamentally "visually oriented". As the saying "a picture is worth a thousand words", it encouraged us to provide many illustrations.

This book can serve as a source of rapid review of pathology for even postgraduates in pathology. Numerous illustrations, gross photographs, photomicrographs, tables, text boxes, flowcharts, and X-rays have been incorporated for easy understanding of the subject. Appendices provide various important bodies and its associated conditions, important cells in various lesions, pathognomonic structures in diseases, and peripheral blood smear.

How to use this book

Before you start reading

We recommend that this book to be used by all students for understanding the basic knowledge and refresh their knowledge during examinations.

- **CC (core curriculum) and NC (non-core curriculum)** is highlighted in a box with lines.

- **Bold words:** The readers are requested to give more emphasis on word in bold letters which represents the keyword to be remembered.
- **Text boxes:** Text boxes have been designed to provide information useful during viva-voce. Most important points to be remembered are presented as text box with yellow background and blue background for not so important points.
- **Questions:** Commonly expected pathology questions during undergraduate examination is also provided at relevant places. In this edition, **most frequently asked questions are highlighted in red colored font** and **not so frequently asked in blue colored font**.
- **Illustration and figures:** One of the important notes provided in all pathology theory examination is "Draw diagrams wherever necessary." Hence, illustrations, figures and flowcharts are provided for those questions where it is mandatory to answer with diagrams.
- **Definition:** One of the frequent viva as well as theory questions is "definition" of the disease. It is provided in a yellow box.
- **Pathogenesis:** As far as possible pathogenesis have been provided with flowchart as well as text for better understanding of difficult concepts.
- **Classification:** Only classifications usually asked in examinations are provided.
- **Morphology:** Another frequent question in theory is "Morphology" which include gross and microscopic findings. Hence, it is also provided in a box to draw your attention during reading.
- **Clinical cases:** Common clinical-oriented questions (asked in few universities) have been incorporated.
- **Interpretation laboratory results**: In "Competency-based undergraduate curriculum for the Indian medical graduate" a skill attribute has been introduced. It test how as student is able to interpret/demonstrate a complex procedure requiring thought, knowledge and behavior. Hence, in this addition following has been provided:
  - Abnormal urinary findings in disease states and identify and describe common urinary abnormalities in a clinical specimen.
  - Interpret the abnormalities in renal function tests and liver function tests.
  - Cerebrospinal fluid analysis.



Ramadas Nayak



Rakshatha Nayak

Preface to the First Edition

Pathology is a rapidly-expanding and ever-changing field and lays the foundation for understanding diseases. This book is an endeavor to present the vast knowledge of pathology in a lucid manner for the second year medical and dental students, and those undergoing training in paramedical courses. My aim is to provide a sound knowledge of pathology and hence give insight into etiology, pathogenesis, pathology and the disease course. Every attempt has been made to present information in a simplified text augmented with the use of colored illustrations.

Organization

This book consists of 23 chapters and is organized into two sections namely general pathology and systemic pathology.

Section 1—General pathology: It provides an overview of the basic pathologic mechanisms underlying diseases including cellular adaptations, inflammation, tissue repair, chronic inflammation, hemodynamic disorders, immunological disorders, neoplasia, and genetics.

Section 2—Systemic pathology: It deals with chapters devoted to diseases and disorders of various organ systems including vascular, cardiac, respiratory, gastrointestinal, liver and biliary tract, pancreas, kidney, male and female genital tract, bones, endocrines, skin, and central nervous system. For hematology section, readers are requested to refer to *Rapid Review of Hematology* authored by Dr Ramadas Nayak and Dr Sharada Rai and *Essentials in Hematology and Clinical Pathology* by Dr Ramadas Nayak, Dr Sharada Rai and Dr Astha Gupta.

After many years (more than 34 years) of teaching undergraduates, I found that undergraduate students find it difficult to understand, remember and answer the questions during examinations, in a satisfying way. There are many pathology textbooks, but undergraduates face difficulty to refresh their knowledge during examinations. This encouraged me to write a book to fill

the niche, to provide basic information to an undergraduate in a nutshell. The text provides all the basic information the student will ever need to know. Keywords are shown in bold words so that the student can rapidly go through the book on the previous day or just before the examination. Most students are fundamentally "visually oriented." As the saying "a picture is worth a thousand words", it encouraged me to provide many illustrations.

How to use this book

I recommend that this book to be used by all students for understanding the basic knowledge and refresh their knowledge during examinations. The readers are requested to give more emphasis on word in bold letters that represents the keywords to be remembered. One of the aims of the students after getting undergraduate degree is to fetch a good ranking in the postgraduate entrance examination. Most graduates cannot answer multiple choice questions (MCQs) in entrance examination by just reading the usual textbooks in pathology. In this book, text boxes have been designed at the sides of main text that provide information useful in answering these MCQs. These boxes also provide commonly expected pathology questions during undergraduate examination. This book can serve as a source of review of general and systemic pathology for even postgraduates in pathology.

About 350 illustrations, 82 gross photographs, 162 photomicrographs, 152 tables, 3 X-rays, and a clinical photograph have been incorporated for easy understanding of the subject. Appendices provide various important bodies and its associated conditions, important cells in various lesions and pathognomonic structures in diseases.

Ramadas Nayak

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- We would like to express our gratitude to all our friends, colleagues, undergraduate and postgraduate students (Department of Pathology, Yenepoya Medical College, Mangaluru) who helped us in the different stages of preparing the manuscript; to all those who provided support, talked things over, read, offered comments and assisted in the editing, proofreading and design.
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Images Contribution

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- Dr Janaki M, MD, DGO, DFWCD, Professor and Head, Department of Pathology, Santhiram Medical College, Nandyal, Kurnool, Andhra Pradesh, India.

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CHAPTER

1

Introduction to Pathology

INTRODUCTION

Definition: Pathology is the **scientific study (logos) of disease (pathos)**. It mainly focuses on the study of the structural, biochemical and functional changes in cells, tissues and organs in disease.

Learning Pathology

Study of pathology can be divided into general pathology and systemic pathology.

- **General pathology:** It deals with the study of mechanism, basic reactions of cells and tissues to abnormal stimuli and to inherited defects.
- **Systemic pathology:** This deals with the changes in specific diseases/responses of specialized organs and tissues.

Scientific Study of Disease

CC: Enumerate common definitions and terms used in pathology.

Disease process is studied under following aspects. The common definitions and terms used in pathology are also mentioned below.

Etiology

The etiology of a disease is its **cause** and modifying factors which are responsible for the initiation and progression of disease process. It describes why a disease arises or what sets the disease process in motion. The

causative factors of a disease can be divided into two major categories: Genetic and acquired (e.g., infectious, chemical, hypoxia, nutritional, physical).

- **Causative agents:** Some diseases are closely linked with etiologic factors and these etiological factors are said to be the causative agents for the disease. For example, microbial pathogens are considered as the causative agents for infectious diseases: *Mycobacterium tuberculosis* causes pulmonary tuberculosis, human immunodeficiency virus causes HIV disease, *Entamoeba histolytica* causes amebic dysentery. These diseases cannot develop without pathogens in the body; however, this does not mean that the all individual exposed to infection will have the same consequences, because many host factors affect the clinical course.
- **Etiological factors:** Even when there is a strong link between disease and etiologic agent, only a proportions of the individuals exposed to the etiological factor may develop the disease. For example, if an individual consume large quantities of alcohol he/she may develop liver cirrhosis. In this case, alcohol consumption is considered to be the cause, yet only proportions of individuals who drink alcohol heavily will develop cirrhosis. The concept of one etiologic agent as the cause of one disease is not applicable to the majority of diseases. The exact causes of most disorders remain incompletely understood.
- **Multifactorial:** Most common diseases are multifactorial due to combination of causes (several different etiologic factors that contribute to their development), i.e., inherited genetic susceptibility and various environmental triggers (e.g., coronary heart disease, hypertension, diabetes, and cancer). For example, coronary heart disease is a result of the interaction of genetic predisposition, elevated blood pressure, exposure to cigarette smoke, diet, and many other lifestyle factors acting together. None of these individual factors can be said to cause the disease.
- **Idiopathic:** When the **cause of the disease is unknown**, a condition is said to be **idiopathic.**
- **Iatrogenic:** If the **cause of the disease is** the result of an **unintended or unwanted medical intervention**, the resulting condition is said to be **iatrogenic.**
- **Risk factor:** When the link between a causative/etiologic factor and development of a disease is less than certain but the probability is

increased when the factor is present, it is called a risk factor. The identification of risk factors is important for the prevention of the disease.

Pathogenesis

Pathogenesis refers to the development or evolution of a disease from the initial stimulus to the ultimate expression of the manifestations of the disease. Pathogenesis deals with **mechanisms of development, progression of disease** and the sequence of physiologic events that follow the exposure of cells or tissues (i.e., from the beginning of any disease process) to an injurious agent (initial contact with an etiologic agent/causative factor). It is a dynamic interplay of changes in cell, tissue, organ, and systemic function. It produces cellular, biochemical and molecular abnormalities and ultimate expression of a disease. With the present advances in technology, it is possible to identify the changes occurring at cellular and molecular level that give rise to the specific functional and structural abnormalities of any particular disease. Thus, it describes how a disease process develops or evolves. This knowledge is helpful for designing new therapeutic approaches. In summary, pathogenesis is a description of how etiologic factors alter physiologic function and lead to the development of clinical manifestations in a particular disorder or disease.

Pathophysiology: In pathology, the study and diagnosis of disease is performed through examination of organs, tissues, cells, and bodily fluids. Physiology is the study of the mechanical, physical, and biochemical functions of living organisms. Together, as pathophysiology, the term refers to the study of abnormalities in physiologic functioning (i.e., physiological changes) of an individual or an organ due to a disease.

- **Latent period:** Few causative agents produce signs and symptoms of the disease immediately after exposure. Usually, etiological agents take **some time to manifest the disease** (e.g., carcinogenesis) and this time period is called as the latent period. It varies depending on the disease.
- **Incubation period:** In disorders caused by infectious (due to bacteria, viruses, etc.) agents, the **period between exposure and the development of disease** is called the incubation period. It usually ranges from days to weeks. Most of the infectious agents have characteristic incubation period.

MORPHOLOGIC CHANGES

All diseases start with structural changes (pathologic changes) in cells. **Rudolf Virchow (**known as the **father of modern pathology**) proposed that injury to the cell is the basis of all disease. Morphologic changes refer to the **gross and microscopic structural changes** in cells or tissues affected by disease. **Lesion** is the term used for the characteristic changes in tissues and cells produced by disease in an individual. The term "**pathology**" (pathological feature) is **sometimes used synonym with morphology.**

Gross

Many diseases have characteristic gross pathology and a fairly confident diagnosis can be given before light microscopy. For example, serous cystadenoma of ovary usually consists of one cystic cavity containing serous fluid; cirrhosis of liver is characterized by total replacement of liver by regenerating nodules.

Microscopy

Light microscopy: Abnormalities in tissue architecture and morphological changes in cells can be studied by light microscopy.

- **Histopathology:** Sections are routinely cut from tissues and processed by paraffin-embedding. The sections are cut from the tissue by a special instrument called microtome and examined under light microscope. In certain situations (e.g., histochemistry, rapid diagnosis) sections are cut from tissue that has been hardened rapidly by freezing (frozen section). The sections are stained routinely by hematoxylin and eosin.
  - **Pathognomonic abnormalities:** If the **structural changes are characteristic of a single disease or diagnostic of an etiologic process** it is called as **pathognomonic.** Pathognomonic features are those features which are restricted to a single disease, or disease category. The diagnosis should not be made without them. For example, **Aschoff bodies** are pathognomonic of **rheumatic heart disease** and **Reed-Sternberg cells** are pathognomonic of **Hodgkin lymphoma** (*refer* Appendix 2).
- **Cytology:** The cells from cysts, body cavities, or scraped from body surfaces or aspirated by fine needle from solid lesions can also be studied under light microscope. This study of cells is known as cytology and is used widely especially in diagnosis and screening of cancer.
- **Histochemistry (special stains):** Histochemistry (*refer* **Table 2.4**) is the study of the chemistry of tissues, where tissue/cells are treated with specific reagent so that the features of individual cells/structure can be visualized, e.g., **Prussian blue reaction for hemosiderin (***refer* **Fig. 2.12).**
- **Immunohistochemistry and immunofluorescence:** Both utilize antibodies (immunoglobulins with antigen specificity) to visualize substances in tissue sections or cell preparations. Former uses **monoclonal antibodies** linked chemically to enzymes and latter uses **fluorescent dyes**.

Electron microscopy: Electron microscopy (EM) is useful to study the changes at ultrastructural level, and for the demonstration of viruses in tissue samples in certain

diseases. The most common diagnostic use of EM is for the interpretation of biopsy specimen from kidney.

Functional Derangements and Clinical Manifestations

Functional Derangements

The effects of genetic, biochemical and structural changes in cells and tissues are **functional abnormalities**. For example, excessive secretion of a cell product (e.g., nasal mucus in the common cold); insufficient secretion of a cell product (e.g., insulin lack in diabetes mellitus).

Clinical Manifestations

The functional derangements produce clinical manifestations of disease, namely symptoms and signs.

Symptoms: The subjective feelings of an abnormality in the body are called as symptoms. It is any change in the body or its functions as perceived by the patient. The symptoms are subjective experience of disease and can only be described to an observer by the affected patient.

Signs: Manifestations of disease that are observed by physician/observer are termed signs of disease and is an objective evidence of a disease. For example, the feeling of nausea is a symptom, whereas vomiting is objectively observed and is a sign. Signs are apparent to observers, whereas symptoms may be obvious only to the patient.

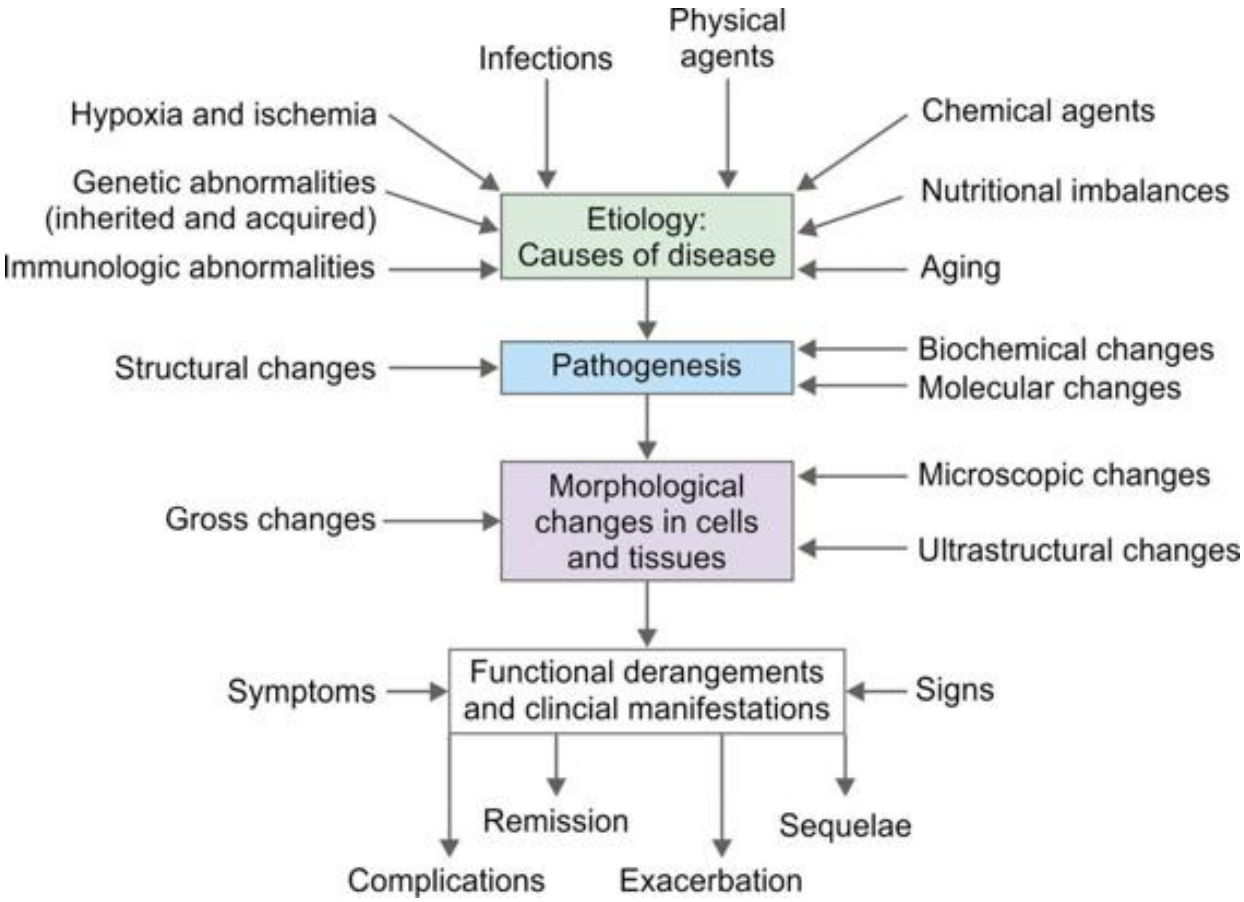


Fig. 1.1: Steps involved in study of disease.

Laboratory tests and investigations: Some signs and symptoms (e.g., fever, headache) are nonspecific and do not indicate a specific cause. In such situation further clinical examination and, often, laboratory tests (biochemical analysis, hematological, microbiological, cytological examination, etc.) and investigations (e.g., X-ray, diagnostic imaging) may be required to know the possible causes of the signs and symptoms.

Diagnosis: It is the art or act of identifying (determine the cause) a disease (pathological condition) from its signs and symptoms. Many diseases/disorders are characterized by a particular constellation of signs and symptoms, the knowledge of which is essential for accurate detection and diagnosis.

Syndromes: Diseases characterized by **multiple abnormalities** (symptom complex) are called syndromes. A **syndrome** is a group (collection) of medical signs, symptoms, laboratory findings, and physiological disturbances occurring together that are often associated with a particular

disease or disorder. For example, carpal tunnel syndrome; irritable bowel syndrome; Klinefelter syndrome, Down syndrome.

Prognosis

The prognosis forecasts (predicts) the known or likely **course (outcome) of the disease** and, therefore, the fate of the patient.

Complications

It is a **negative pathologic process** or event occurring **during the disease** which is not an essential part of the disease. It usually aggravates the illness. For example, perforation and hemorrhage are complications which may develop in typhoid ulcer of intestine.

Sequelae

It is a pathologic condition **following as a consequence of a disease**. For example, intestinal obstruction following healed tuberculosis of intestine, mitral stenosis following healed rheumatic heart disease.

Remission and Relapse/Exacerbation

Some diseases may pass through several cycles/alteration of remission and relapse/exacerbation. For example, inflammatory bowel disease (Crohn's disease and ulcerative colitis), some autoimmune diseases. An exacerbation is characterized by a relatively sudden increase in the severity of a disease or any of its signs and symptoms. A remission is characterized by a decline in severity of the signs and symptoms of a disease. If a remission is permanent, the patient is said to be cured.

Various steps involved in study of disease are presented in **Figure 1.1.**

ROLE OF A PATHOLOGIST IN DIAGNOSIS AND MANAGEMENT OF DISEASE

CC: Describe the role of a pathologist in diagnosis and management of disease.

Pathology is a branch of medicine involved in understanding the cause of disease, the processes involved in testing the disease and the reporting of diagnostic tests.

TABLE 1.1: Major pathology disciplines and roles.

Discipline/subspecialty	***Role***
Anatomic pathology	
• Histopathology	Study of disease in human tissue samples (e.g., in cancer management through the staging and grading of tumors)
• Cytopathology	Study of disease in individual cells
• Forensic pathology	Determination of cause and manner of death for legal purposes
• Autopsy	Determination of cause and manner of death
Hematology	Study of the blood and bone marrow
Clinical pathology also called laboratory medicine	It is largely concerned with analysis of blood and other fluids and involves, for instance, clinical biochemistry, microbiology, and hematology
• **Chemical pathology** (clinical biochemistry)	Study of the biochemical tests to diagnose and treat patients. Also to understand the biochemical basis/mechanisms of the body related to disease. Body fluids such as blood or urine are used for screening, diagnosis, prognosis and management through chemical and biochemical tests
• Medical microbiology	Study of infection
Molecular and genetics pathology	Study of the molecular and genetic basis of diseases and heritable conditions. Variety of tests of molecules within organs, tissues or bodily fluids are performed
Immunopathology	Study of the immunologic basis of disease
Transfusion medicine	Study of the collection, preparation, storage, and clinical use of blood products

Subdivisions of Pathology

The study of pathology can be divided into different branches (disciplines) and subspecialties **(Table 1.1).**

Anatomic Pathology/Histopathology

In anatomic pathology, microscopic examination is an essential tool for the study. It includes histopathology, cytopathology and autopsy. Histopathology is the classic and most useful method of study. The term histopathology is used synonymously with anatomic pathology or morbid anatomy. Modern anatomic pathology is divided into subspecialities such as renal pathology, dermatopathology, gastrointestinal pathology, cardiac pathology, pulmonary pathology, neuropathology, gynecologic pathology, breast pathology, oral pathology, etc.

Surgical Pathology

It deals primarily with the study of structure changes in tissues/organs removed from the living patients by biopsy or surgical resection. Surgical pathology includes both gross or naked eye examination referred to as gross or macroscopic changes, as well as examination of processed tissue under a compound light microscope. Intraoperative examination is done by use of frozen tissue sections and is employed for rapid diagnosis.

Microscopic examination: It requires tissues be cut with a microtome into thin sections that can be stained with routine stains such as hematoxyline and eosin (H & E). Two types of sectioning methods are most commonly used: *frozen sections* and *paraffin-embedded* or *permanent sections*.

- **Frozen sections:** Frozen sections are cut by using cryostat (microtome in a refrigerated chamber) or freezing microtome from tissue that has been hardened rapidly by freezing (hence termed frozen section). They can be prepared rapidly (within minutes) during the course of surgery while the patient is still under anesthesia (for details *refer* page 230).
- **Permanent sections**: In most instances, pathologists rely on the study of better-preserved tissue by conventional permanent tissue sections with paraffin embedding technique stained with hematoxylin and eosin. These are prepared from tissues that have been fixed, dehydrated and embedded in paraffin wax as a supporting medium prior to sectioning. Though it needs more time for preparation (about 24 hours), permanent sections have several advantages over frozen tissue sections. These include thinner (typically 5 μm) and better-quality sections without any freezing

artefacts. They permit greater certainty of interpretation. Some tissues (e.g., those containing fat or bone) cut poorly as frozen sections but may be satisfactorily studied in permanent sections. H & E may be further supported by numerous special stains (histochemistry) for specific tissue components (e.g., mucus, glycogen, collagen, bacteria, and fungi) in certain situations.

- **Ancillary staining and analytical methods:** Histochemistry (special stains), immunohistochemistry, and electron microscopy are ancillary technique in surgical pathology that may be helpful or necessary supplements for diagnosis.
  - **Special stains** may aid in the diagnosis of one or another type of tumor (i.e., differential diagnosis) and classification. Examples include special stains for mucins, amyloid, lipids and glycogen.

Surgical pathology helps to diagnose a disease and determine a treatment plan and has a definitive role in tumor diagnosis. With very few exceptions, definitive therapy for cancer should not be undertaken in the absence of a tissue diagnosis.

Role of Pathologists

Diagnosis

Diagnostic role is a **key aspect of pathology laboratories**. Pathologist examines the changes in the tissue samples/biopsies of the body (removed at surgery or autopsy), in the cells obtained from needle-aspirated specimens, in blood and other body fluids/secretions (including urine, cerebrospinal fluid, sputum, pleural/ascitic/pericardial fluid and synovial fluid). Some of these changes in test show the causes (e.g., lepromatous leprosy with positive lepra bacilli in the tissue sections, malarial parasites in the peripheral blood smear, *Candida albicans* in vaginal smear).

Tumor Grading, Staging, and Prognosis of Disease

Pathologists helps in clinical care by assessing disease severity and prognosis. For example, determining the tumor staging [e.g., the well-known **TNM system** (*refer* pages 237-8)] and grading of a cancer to estimate tumor prognosis by histopathology; this information determines the precise type and severity (stage) of the cancer. Staging attempts to measure the extent of

spread of a malignant tumor within a patient on the basis of tumor size, lymph node involvement, and the presence of other metastases. **Tumor grading is a tumor's degree of differentiation** and growth rate, often graded as I to III where III represents the least differentiated, fastest dividing tumors and presumed to have the worst prognosis.

Management of the Disease

Some of the test are fundamental in deciding and managing treatment plans for patients. Examples include, use of antiestrogenic agents in estrogen receptor-positive breast cancers. A newer approach has been to target the HER2/*neu* antigen (*refer* **Fig. 23.14C**) which is overexpressed in certain breast and ovarian cancers. The HER2/*neu* antigen is targeted by a therapeutic agent, trastuzumab (Herceptin, Genentech) consisting of a humanized monoclonal antibody that binds to the HER2/*neu*. A complete and accurate pathology report is crucial for precise diagnosis and to decide the best treatment plan for patient. In the present era, importance is given to **identify markers that help to predict the response to new and different therapeutic modalities**.

Monitor the Response to Treatment

Pathology reports may be helpful in monitoring the clinical response to treatment. For example, analyzing blood levels of markers of renal function (renal function tests) in patients with renal failure, minimal residual disease in lymphomas after treatment.

To Comment of Excision Margin in Surgical Specimens

An important concern in surgical pathology is the **adequacy of tumor excision**. Depending on the tissue or type of excision or resected specimen, this decision can be made on either frozen or permanent sections. It is necessary to know the **distances between the edges of the tumor and the excision (resection) margins**. If the tumor forms a discrete mass and the margins of the specimen are well/clearly recognizable/defined, then

determination of excision margins is usually straightforward. Examples of tumors whose excision is likely to have clearly recognizable margins include tumors arising in the gastrointestinal tract, lung, and skin. Excision margins should be identified and painted with ink before any dissection, thus permitting accurate measurement of these distances microscopically. All lymph nodes associated with a cancer specimen need to be dissected out, described along with their location, and processed for histology.

Other roles: Pathology plays a number of other key roles. One is **quality assurance** within the healthcare system. Pathologists routinely supervise the laboratory to ensure pathology results are appropriate and of the highest standard.

Cytopathology

Cytopathology consists of study of exfoliated cells [i.e., cells shed off from the lesions (exfoliative cytology)] scraped, or brushed cells or fine-needle aspiration cytology (FNAC) of superficial and deep-seated lesions for diagnosis. These can be a rapid, efficient, and low-risk technique for establishing an accurate diagnosis. Role of a cytopathologist in diagnosis and management of disease are discussed in Chapter 10.

Forensic Pathology and Autopsy

This includes the study of organs and tissues removed at postmortem (autopsy) for medicolegal work and for determining the underlying sequence and cause of death. Forensic pathology is integral to legal system. By this, the course of events that occurred in the patient during life and the probable cause of death can be ascertained. Post-mortem anatomical diagnosis helps the clinician to enhance their knowledge about the disease and their judgement. Forensic autopsy is done for medicolegal purposes. In post-mortem examination 'the dead teach the living' and has an important role in patient care, around the world.

Hematology

Hematology deals with the study of diseases of blood. It includes laboratory hematology and clinical hematology. The hematological diseases include

disorders of the red and white blood cells, platelets and the coagulation system. It may be non-neoplastic, certain inflammatory diseases, benign or malignant disorders. Red blood cell (RBC) disorders include disorders of hemoglobin (anemia such as iron deficiency, sickle cell disease) and white blood cell (WBC) disorders include quantitative disorders of white blood cells and malignant disorders such leukemia, lymphoma or myeloma. The disorders of platelets (e.g., thrombocytopenia) and disorders of blood coagulation (e.g., hemophilia) are also part of hematology. Hematologists receive blood samples for checking any abnormalities. They examine the blood film for any abnormality (e.g., anemia, leukemia) and also perform a bone marrow aspiration and biopsy. Common hematologic test is a complete blood count (CBC), which includes white blood cell count, red blood cell count, platelet count, hematocrit/red blood cell volume (HCT), hemoglobin concentration (HB), differential white blood count and red blood cell indices. Other examples of hematologic tests include prothrombin time (PT), and partial thromboplastin time (PTT).

Clinical Pathology (Laboratory Medicine)

Clinical pathology includes analysis of various fluids including blood and body fluids such as urine, semen, cerebrospinal fluid (CSF) and other body fluids. Such analysis may be qualitative, semiquantitative or quantitative. Wide range of laboratory test results assist clinicians in their diagnosis and treatment of disease. These results help hospital departments including intensive care, operating theaters, special care baby units and oncology.

Clinical Biochemistry

This includes quantitative determination of various biochemical constituents in serum and plasma, and in other body fluids. There is some overlapping between clinical pathology and clinical biochemistry.

Microbiology

This includes the study of disease-causing microbes. Depending upon the type of microorganism studied, it is further developed into bacteriology,

parasitology, mycology, virology, etc.

Immunology

In this, abnormalities in the immune system of the body comprising of immunology and immunopathology are studied.

Medical Genetics

It deals with the relationship between heredity and disease. It is useful in blood grouping, inborn errors of metabolism, chromosomal aberrations in congenital malformations and neoplasms, etc.

Molecular Pathology

Most of the diseases can be diagnosed by the morphological changes in tissues. But, with the present advances in diagnostic pathology, we realize that cellular disturbances develop due to alterations in molecules (genes, proteins, and others) that influence the survival and behavior of cells. Thus, in the present modern pathology it is necessary to understand the cellular and molecular abnormalities that give rise to diseases. The detection and diagnosis of abnormalities at the molecular level (i.e., DNA of the cell) is performed by molecular pathology. Molecular pathology has revealed the biochemical basis of many diseases, mainly congenital disorders and cancer. These techniques can detect changes in a single nucleotide of DNA and include in situ hybridization, polymerase chain reaction (PCR), etc. In situ hybridization can detect the presence of specific genes or their messenger RNA in tissue sections or cell preparations. Minute quantities of nucleic acids can be amplified by the use of the polymerase chain reaction. DNA microarrays can be used to determine patterns of gene expression (mRNA). These methods are used for research purposes as well as a part of diagnostic pathology reports. Molecular pathology, for example, using nucleic acid probes with or without amplification by the polymerase chain reaction is used to detect expression of specific tumor genes or gene mutations.

Transfusion Medicine

This branch ensures adequate stocks of safe blood when needed for blood transfusions. The blood bank/center confirms that the donated blood is the right match for the patient's blood group. This branch deals with blood and transplant service providing vital support for blood transfusion, organ and stem cell transplantation.

HISTORY AND EVOLUTION OF PATHOLOGY

NC: Describe the history and evolution of pathology.

Medicine is as old as humankind. In the beginning of mankind, there was a desire and need to know about the causes, mechanisms and nature of diseases. This knowledge was gained over the centuries from supernatural beliefs to the present knowledge of modern pathology. Pathology has evolved over the years as a distinct discipline or branch apart from others such as anatomy, medicine, surgery, etc. For an undergraduate beginner in pathology, it is necessary to know brief review of history of pathology and personalities with their outstanding contribution.

Prehistoric Times to Medieval Period

During prehistoric time; religion, magic and medical treatment were quite linked to each other. It was believed that the disease was the outcome of 'curse from God' or it had supernatural origin from 'evil eye of spirits.' The disease was treated by priests through prayers and sacrifices, and magicians by magic power. Some ancient superstitions still exist in some parts of the world.

Religious and magical belief: The link between medicine and religion was firmly believed throughout the world. This has resulted in belief in different societies that there are gods and goddesses of healing. For example, *Dhanvantri* was considered as the deity of medicine in India, and orthodox Indians' belief in *Mata Sheetala Devi* as the pox goddess. Same way Greeks had *Aesculapius* and Apollo as the principal gods of healing.

Philosophical and rational approach: This approach was by the methods of observations and this followed the earlier religious and magical beliefs.

This philosophical concept to all-natural phenomena was introduced by Greek philosophers namely *Socrates, Plato* and *Aristotle.*

Hippocrates: The real practice of medicine began with the great Greek clinical genius *Hippocrates* (460–370 BC). He is regarded as 'the father of medicine' and he dissociated medicine from religion and magic. He revolutionized the practice of medicine and established the basic foundations of the role of the physician. He studied patient's symptoms and described methods of diagnosis. He recorded his observations on cases and his writings called Hippocratic Corpus remained for nearly two thousand years as the mainstay of learning of medicine. However, Hippocates too propagated the prevailing concept on mechanism of disease based on disequilibrium of our basic humors (water, air, fire, and earth). Later this concept was abandoned. Hippocrates set standards for patient care and the physician's attitudes and philosophies that persist today. Many aspects of the Hippocratic approach to medicine are still with us today. Hippocrates rational and ethical attitudes in practice and teaching of medicine are being respected by the medical profession and *'Hippocratic oath'* is taken by all medical students at the time of entry into practice of medicine.

After Hippocrates, Greek medicine reached Rome (now Italy). After 146 BC, Rome controlled Greek world and dominated in ancient Europe. Many terms in medicine have their origin from Latin language which was the official language of countries included in ancient Roman empire (Spanish, Portuguese, Italian, French and Greek languages have their origin from Latin). Hippocratic teaching was propagated in Rome by Roman physicians. Prominent among them are *Cornelius Celsus* (53 BC to AD 7) and *Claudius Galen* (AD 130–200).

- Celsus first described four cardinal signs of inflammation namely rubor (redness), tumor (swelling), calor (heat), and dolor (pain).
- Galen postulated humoral theory, later called Galenic theory. According to this theory, the illness was considered as due to imbalance between *four humors* (or body fluids): blood, lymph, black bile (believed at that time to be from the spleen), and biliary secretion from the liver.

In India, in the centuries before and after Hippocrates, the hypothesis of disequilibrium of four elements of the body (Dhatus) similar to Hippocratic doctrine was mentioned in ancient Indian medicine books during AD 200. Susruta (Sushruta Samhita) and Chakara (Charaka Samhita) produced

encyclopedic founding works of Ayurvedic medicine. Charaka listed 700 plant-derived medicines/remedies and surgical sciences was documented by Sushruta.

Backwardness during Medieval period: During Middle Ages (or medieval period that lasted from the 5th to the 15th century) there was marked backwardness in medicine. During this period, there were widespread and devastating epidemics. This resulted in reversal of the process of rational thinking back to supernatural concepts and divine punishment for 'sins.' The dominant belief was that life was due to influence of vital substance under the control of soul *(theory of vitalism)*. During this period, the dissection of human body was strictly forbidden because it was considered to hurt the 'soul.'

Human Anatomy and Era of Gross Pathology

Renaissance period: The backwardness during Medieval period was followed by the Renaissance period (i.e., revival of learning). This began from Italy in late 15th century and spread to whole of Europe. During this period, there was quest (an attempt to achieve something) for advances in art and science. There was freedom of thought and emphasis on philosophical and rational attitudes again.

Human Anatomy

- The development of human anatomy took place at the beginning of this period with the art works and **drawings of human muscles and embryos** by famous Italian painter **Leonardo da Vinci** (1452–1519).
- **Vesalius (1514–1564) dissected human body** of freshly executed criminals. His pupils, Gabriel Fallopius (1523–1562) described human oviducts (Fallopian tubes) and Fabricius discovered lymphoid tissue around the intestine of birds (bursa of Fabricius).
- The special human anatomic dissection in the form of **postmortem** came into practice in various parts of ancient Europe.

Microscope

- **Antony van Leeuwenhoek** (1632–1723), a cloth merchant by profession in Holland, during his spare time **invented** the first ever **microscope** by grinding the lenses. He also introduced histological staining using saffron to examine muscle fibers.
- **Marcello Malpighi** (1624–1694) observed the **presence of capillaries** by using microscope. He described the malpighian layer of the skin, and lymphoid tissue in the spleen (malpighian corpuscles). **Malpighi is known as 'the father of histology.'**

Morbid Anatomy

Morbid anatomy (pathologic anatomy) is a branch of medical science concerned with the study of the structure of diseased organs and tissues.

- **Giovanni B Morgagni** (1682–1771): The beginning of study of morbid anatomy was by Italian anatomist, pathologist, *Giovanni B Morgagni* (1682–1771). He was an excellent teacher in anatomy, a prolific writer and a practicing clinician. Morgagni dismissed the ancient humoral theory of disease and published his life-time experiences based on 700 postmortems and their corresponding clinical findings. Thus, he laid the foundations of clinicopathologic methodology in the study of disease. He introduced the concept of clinicopathologic correlation (CPC), establishing a coherent sequence of cause, lesions, symptoms, and outcome of disease.
- **Sir Percival Pott** (1714–1788) was a famous surgeon in England. He **described tuberculosis of the spine** (Pott's disease). He was also **first to identify occupational cancer** (cancer of scrotal skin) in the chimney sweeps in 1775 and discovered chimney soot as the first chemical carcinogenic agent.
- During the latter part of 18th Century, **John Hunter** (1728–1793) who was a student of Sir Percival Pott became the greatest surgeon-anatomist of all times. His elder brother **William Hunter** (1718–1788) was a reputed anatomist-obstetrician. These brothers **developed the first museum of comparative anatomy and pathology** in the world by collecting surgical specimens and arranged them into separate organ systems. This museum knows as the Hunterian Museum, now housed in Royal College of Surgeons of London.
- **Edward Jenner** (1749–1823) who demonstrated **inoculation in smallpox**.

- **Matthew Baillie** (1760–1823) is a prominent English pathologist published first-ever systematic textbook of morbid anatomy in 1793.
- **Era of gross pathology**:
  - **Richard Bright** (1789–1858) described non-suppurative nephritis which was later termed as glomerulonephritis or **Bright's disease**.
  - **Thomas Addison** (1793–1860) gave an account of chronic adrenocortical insufficiency and was termed as **Addison's disease**.
  - **Thomas Hodgkin** (1798–1866) observed the complex of chronic enlargement of lymph nodes, often with enlargement of the liver and spleen, which was later called **Hodgkin's disease** (presently known as Hodgkin lymphoma).
  - **Xavier Bichat** (1771–1802) in France described that organs were composed of tissue and divided the study of morbid anatomy into General Pathology and Systemic Pathology.
  - **RTH Laennec** (1781–1826), a French physician had numerous discoveries. These include **description of several lung diseases** (tubercles, caseous lesions, miliary lesions, pleural effusion, and bronchiectasis), **chronic sclerotic liver disease** (later called Laennec's cirrhosis) and **invented stethoscope**.
  - **Carl F von Rokitansky** (1804–1878) is a self-taught German pathologist who **performed nearly 30,000 autopsies** himself. He described acute yellow atrophy of the liver, wrote monograph on diseases of arteries and congenital heart defects. Unlike most other surgeons during that time, Rokitansky did not do clinical practice of surgery but instead introduced the concept that pathologists should confine themselves to making diagnosis which became the accepted role of pathologist later.

Era of Technology Development and Cellular Pathology

Correlation of clinical features with autopsy findings: Up to middle of the 19th century, the major method of study of disease was by correlating clinical manifestations of disease with gross pathological findings at autopsy.

Advances in Pathology

In the 19th century, sophistication in surgical techniques led to advancement in pathology. The anatomist-surgeons of earlier centuries got replaced largely with surgeon-pathologists. **In later half of the 19th century, pathology became a diagnostic discipline** with the evolution of cellular pathology. This was closely linked to technology advancements in machinery for cutting thin tissue sections, improvement in microscope, and development of dyes for staining.

Identification of Microorganisms

- French chemist **Louis Pasteur** (1822–1895) **discovered the disease-causing microorganisms** and this replaced the prevailing theory of spontaneous generation of disease and firmly established **germ theory of disease.**
- In 1873, **GHA Hansen** (1841–1912) in Germany identified **Hansen's (lepra) bacillus** as the first microbe that caused leprosy (Hansen's disease).

Immunity and Phagocytosis

- The **basis of immunization** was initiated by **Edward Jenner** while studying infectious diseases.
- **Metchnikoff** (1845–1916), a Russian zoologist, **discovered phenomenon of phagocytosis** by human defense cells against invading microbes.

Staining

Developments in chemical industry lead to switching over from earlier dyes of plant and animal origin to **synthetic dyes**. In 1856, **Perkin** prepared first synthetic dye namely aniline violet. This led to emergence of a viable dye industry for histological and bacteriological purposes. Various pioneers who contributed the use for dye are as follows:

- **Paul Ehrlich** (1854–1915), German physician, conferred Nobel prize in 1908 for his work in immunology. He described **Ehrlich's test for urobilinogen** using **Ehrlich's aldehyde reagent** and staining techniques

of cells and bacteria. These developments led to the foundations of clinical pathology.

- **Christian Gram** (1853–1938), Danish physician, used **crystal violet for staining bacteria**.
- **DL Romanowsky** (1861–1921), Russian physician, developed **stain for peripheral blood film** using eosin and methylene blue derivatives.
- **Robert Koch** (1843–1910), German bacteriologist **described Koch's postulate and Koch's phenomena**. He also developed techniques of fixation and staining for identification of bacteria. He **discovered tubercle bacilli** in 1882 and cholera vibrio organism in 1883.
- **May-Grünwald** in 1902 **and Giemsa** in 1914 developed **stains for blood and bone marrow**. They applied them for classification of cells in blood and bone marrow.
- **Sir William Leishman** (1865–1926) in 1914 described **Leishman's stain for blood smear/films** and observed Leishman-Donovan bodies (LD bodies) in leishmaniasis.
- **Robert Feulgen** (1884–1955) described **Feulgen reaction for DNA staining**. This laid the foundations of cytochemistry and histochemistry.

Technical Advances

Advances in techniques and machinery led to development and upgrading of microtomes for obtaining thin sections of organs/tissues and staining by dyes for enhancing detailed study of sections. The presence of cells in thin sections of non-living object cork was first demonstrated by Robert Hooke in 1667. In the 19th century, FT Schwann(1810–1882) was, the first neurohistologist, and Claude Bernarde (1813–1878), was pioneer in pathophysiology.

Histopathology

Till the end of the 19th century, the study of morbid anatomy was confined largely to autopsy-based study and thus remained as a retrospective science.

Rudolf Virchow (1821–1905) in Germany **first did the microscopic examination of diseased tissue at cellular level.** This became as the histopathology method of investigation. Virchow hypothesized cellular

theory and is aptly known as "**father of cellular pathology**". According to him:

- All cells come from other cells.
- Disease is an alteration of normal structure and function of these cells.

This led to sound foundation of diagnostic pathology based on microscopy and was followed and promoted by numerous brilliant successive workers. This gave birth to biopsy pathology. Other discoveries by Rudolf Virchow are:

- **Virchow's triad:** Virchow described etiology of thrombosis [(Virchow's triad namely (i) slowing of blood-stream, (ii) changes in the vessel wall and(iii) changes in the blood itself)].
- **Virchow's lymph node:** Metastatic spread of tumor of carcinoma stomach to supraclavicular lymph node.
- **Components and diseases of blood** (fibrinogen, leukocytosis, leukemia).

Frozen section: **Julius Cohnheim** (1839–1884), student of Rudolf Virchow, **described the concept of frozen section** examination while the patient was still on the operation table.

The concept of surgeon and physician as pathologist started in the 19th century and it continued as late as the middle of the 20th century in most clinical departments. During this period, biopsy pathology work was assigned to some faculty member in the clinical department. The notable pathologists of the first half of 20th century had background of clinical training and these include **James Ewing** (1866–1943), **AP Stout** (1885–1967) and **Lauren Ackerman** (1905–1993) in United States of America; **Pierre Masson** (1880–1958) in France, and **RA Willis** in Australia.

Landmarks in Modern Pathology

- **Karl Landsteiner** (1863–1943) **described major human blood groups** in 1900 and is considered "**father of blood transfusion**". He was awarded Nobel prize in 1930.
- **Ruska and Lorries** in 1933 **developed electron microscope**. This helped to identify the ultrastructure of cell and its organelles.
- **George N Papanicolaou** (1883–1962), a Greek-born, American pathologist is known as '**father of Exfoliative cytology**'. In 1930s, he developed **exfoliative cytology for early detection of cervical cancer**.

- **William Boyd** (1885–1979), psychiatrist-turned pathologist was by an **eminent teacher-author in pathology during 20th century**. His textbooks namely 'Pathology for Surgeons' (first edition, 1925) and 'Textbook of Pathology' (first edition, 1932), dominated and inspired the few generations of students of pathology all over the world due to **his flowery language and lucid style**.
- **MM Wintrobe** (1901–1986) **discovered hematocrit technique**.

Modern Pathology

In the latter half of 20th century and during 21st century, advances made it possible to **study diseases at genetic and molecular level**. The evidence based and objective diagnosis may enable the physician to institute targeted therapy. The major benefit due to these advances in molecular biology are in the field of diagnosis and treatment of genetic disorders, immunology and in cancer. Some of the discoveries are as follows:

- **Watson and Crick** in 1953 described **the structure of DNA** of the cell.
- **Tijo and Levan in 1956, identified chromosomes and their correct number in humans** (46 = 23 pairs).
- **Nowell and Hagerford in 1960,** identified **Philadelphia chromosome** t(9;22) in chronic myeloid leukemia. This was the first chromosomal abnormality detected in any cancer.
- **In situ hybridization** (ISH) was introduced in 1969. In this, a labeled probe is used to detect and localize specific RNA or DNA sequences **'in situ'** (e.g., in the original place). Its **later modification uses fluorescence microscopy** (FISH) to detect specific localization of the defect on chromosomes.
- **Recombinant DNA technique** was developed in 1972 using restriction enzymes to cut and paste part/bits of DNA.
- **Kary Mullis** in 1983, introduced **polymerase chain reaction** (PCR). In this multiple copy of DNA fragments can be done and this has revolutionized the diagnostic molecular genetics. PCR analysis is more rapid than ISH, can be automated by thermal cyclers and requires less amount of DNA for the test.
- **Barbara McClintock** invented **flexibility and dynamism of DNA** by for which she was awarded Nobel prize in 1983.

- **Ian Wilmut** and his colleagues at Roslin Institute in Edinburgh in 1997, performed **mammalian cloning**. By using this technique, they performed the somatic cell nuclear transfer to create the clone of a sheep named Dolly. However, reproductive cloning for human beings is very risky and absolutely unethical.
- **Stem cell research:** It started in 2000s by harvesting the primitive stem cells isolated from embryos and maintaining their growth in the laboratory. There are two types of stem cells in humans namely (i) embryonic stem cells and (ii) adult stem cells. Stem cells have many applications in the treatment of many human diseases such as Alzheimer's disease, diabetes, cancer, strokes, etc. In future, stem cell therapy may be able to replace whole organ transplant and stem cells 'harvested' from the embryo may be used as a replacement of damaged tissue/organ. Hematopoietic stem cells are being used for many diseases of the hematopoietic system.
- **Human Genome Project (HGP):** It consisted of a consortium of countries and was completed in April 2003. By HGP, it is possible to read nature's complete genetic blueprint used in making of each human being (i.e., gene mapping). Clinical trials by gene therapy for the treatment of some single gene defects have resulted in some success, especially in hematological and immunological diseases. Future developments in genetic engineering may be able to design new and highly effective individualized treatment options for genetic diseases and also may suggest preventive action against diseases.
- **Uses of Human Genome Project:** It is listed in **Box 1.1.**

Telepathology and Virtual Microscopy

Telepathology is defined as the **practice of diagnostic pathology at a distance**. In this, a remote pathologist (receiving side) utilizes tissue specimens, histological, cytological electronic (digital) images which are transferred over a telecommunication network from a different location (sending side).

Components: The various components of telepathology network include:

- A conventional light microscope
- Devise to capture the image, i.e., a camera mounted on a microscope

BOX 1.1: Uses of Human Genome Project.

The understanding of the genome provides clues for:

- **Etiology** of cancers, Alzheimer's disease, etc.
- Defining the **pathogenesis** of a disease and to study the disease processes at **molecular level**
- **Susceptibility** of an individual to a variety of illnesses, e.g., carcinoma breast, disorders of hemostasis, liver diseases, cystic fibrosis, etc.
- Precise new ways to **prevent** a number of diseases that affect human beings
- To **diagnose and treat** disease. Target genes for treatment and management of diseases.
- Human development and anthropology: Analysis of similarities between DNA sequences from different organisms helps to **study evolution**
- **Researcher:** By visiting the human genome database on the World Wide Web, researcher can examine what other scientists have written about the gene

- Telecommunication link between the sending and receiving sites
- A work station at the receiving site with a high-quality video monitor

Advantages/benefits: Telepathology makes it **faster** and easier to share medical images. Pathologists in different locations can view images simultaneously and discuss diagnoses through teleconferencing. Pathologist can consult pathologists who are specialized in area of concern, such as liver pathology or lung pathology. It allows pathologists, surgeons and radiologists to communicate with each other over diagnostic dilemmas and overcoming the barrier of distance.

Main Categories of Telepathology

The types of telepathology include:

Static image-based systems

In this static telepathology, images are captured from a digital camera connected to a microscope. An image area is selected and transmitted. A limited number of images (1 to 40) are captured and stored in the hard drive of the computer or compact disc-read only memory (CD-ROM) for transmission. It is economical, simple and requires only a standard telephone and internet connection. Disadvantage is that selection of field for images should be done by expert and if done by a non-expert may miss the important areas.

Virtual slide systems

Pathology specimen slides are scanned and high-resolution digital images created for transmission.

Real-time systems

This is also termed robotic interactive telepathology and dynamic pathology. In this, the recipient pathologist remotely guides a robotically controlled motorized microscope by means of a joystick (a device that can be moved forwards, backwards and sideways to control a machine or computer) and view 'real-time' images on a high-resolution monitor. The section can be viewed entirely and this eliminates the inadequate or inappropriate selection of fields. These instruments allow the remote user to move the microscopic field in any direction, to change magnifications, and even to change the focus (particularly useful for cytological preparations). The resolution of the images is practically the same as that obtained with the actual slide under the microscope. This technique is suitable for routine histological preparations, immunostains, cytology preparations, and electron micrographs.

Lesions: Term used for describing the more or less circumscribed pathological changes in tissues and cells produced by disease.

Remission: It is the process of conversion from active disease to quiescence. Some of the chronic diseases are interspersed by periods of quiescence when the patient is relatively in good health.

Relapse: It is the process in which the signs and symptoms of disease reappear.

Normal cell is capable of handling physiological demands and maintains a steady state called homeostasis.

CHAPTER

2

Cell Injury and Adaptation

TYPES OF CELLULAR RESPONSES TO INJURY

Depending on the nature of stimulus/injury, the cellular responses can be mainly divided into four types **(Box 2.1 and Fig. 2.1)**.
Different stages of cellular responses to stress and injurious stimuli are shown in **Flowchart 2.1**.

CELL INJURY

CC: Demonstrate knowledge of the causes, mechanisms, types and effects of cell injury and their clinical significance.

Definition: Cell injury is the effect of stresses due to variety of etiological agents on the cell.

Causes (Etiology) of Cell Injury

Q. Write short note on causes of cell injury.

They range from gross physical trauma (e.g., road traffic accident), to a single gene defect (e.g., enzyme deficiency resulting in specific metabolic disease). Following are the main categories of causes of cell injury.

A. Hypoxia and ischemia: Hypoxia refers to **inadequate oxygenation of tissue**. It is the **most common cause of cell injury**. Hypoxia also results in a deficiency of essential nutrients to the tissue affected and builds up toxic metabolites.

Causes of hypoxia:

- **Decreased blood flow** is called **ischemia** and results from an arterial obstruction. This obstruction may be due to thrombosis, embolism, atherosclerosis or external compression of vessel.
- **Inadequate oxygenation of the blood** (hypoxemia): It may be due to lung disease, decreased perfusion of tissues (e.g., cardiac failure, hypotension shock), decreased oxygen-carrying capacity of the blood (e.g., anemia), severe blood loss, carbon monoxide poisoning.

Mechanism of injury: Hypoxia causes cell injury by reducing aerobic oxidative respiration and decreasing the synthesis of adenosine triphosphate (ATP).

Outcome: Depending on the severity of the hypoxia, cells may adapt, undergo injury, or die.

Hypoxia: Most common cause of cell injury.

Ischemia: Most common cause of hypoxia.

Neurons: Most susceptible to hypoxia and irreversible damage occurs 5 minutes after global hypoxia. First cellular change in hypoxia is decreased oxidative phosphorylation in mitochondria.

Watershed areas: Region between terminal branches of arterial blood supply, where blood supply does not overlap. They are susceptible to hypoxic injury. Watershed areas examples: (1) Cerebral vessels; (2) Mesenteric arteries.

B. Physical agents:

- Mechanical trauma (e.g., blunt/penetrating/crush injuries, gunshot wounds).
- Thermal injury: Extremes of temperature (burns and deep cold).
- Radiation (ionizing radiation and non-ionizing radiation).
- Electric shock.
- Pressure changes: Sudden changes in atmospheric pressure.

C. Chemical agents, toxins and drugs:

- Heavy metals and poisons (e.g., arsenic, mercuric salts or cyanide).
- Simple chemicals (e.g., hypertonic concentrations of glucose or salt).
- Strong acids and alkalies.
- Oxygen at high concentrations is toxic.
- Environmental and air pollutants (e.g., insecticides and herbicides).

- Industrial and occupational hazards (carbon monoxide and asbestos).

BOX 2.1: Types of cellular responses to injury.

1. Cellular adaptations: Hypertrophy, hyperplasia, atrophy and metaplasia
2. Cell injury
   - Reversible cell injury
   - Irreversible cell injury
3. Intracellular accumulations
4. Pathologic calcification

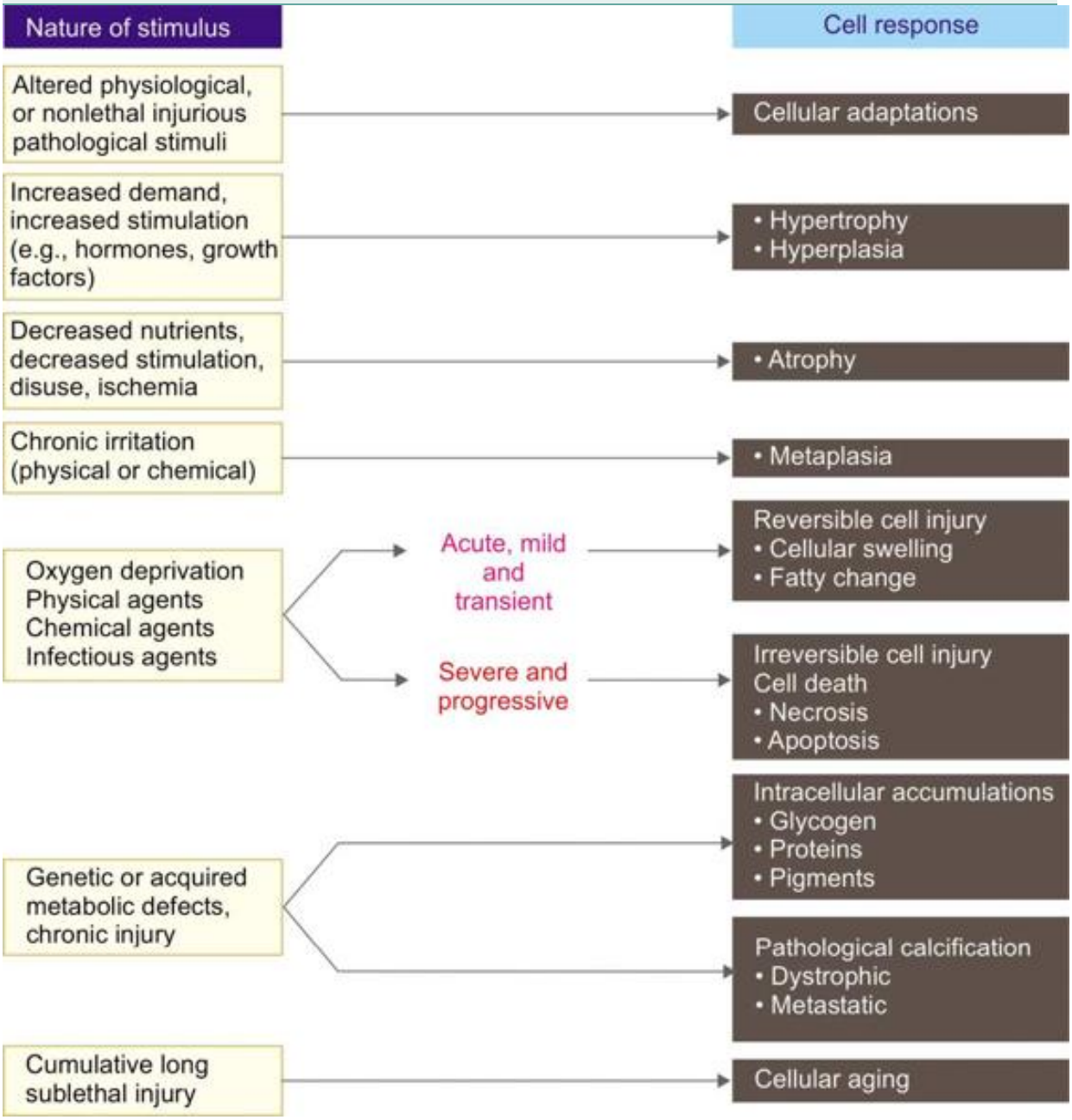
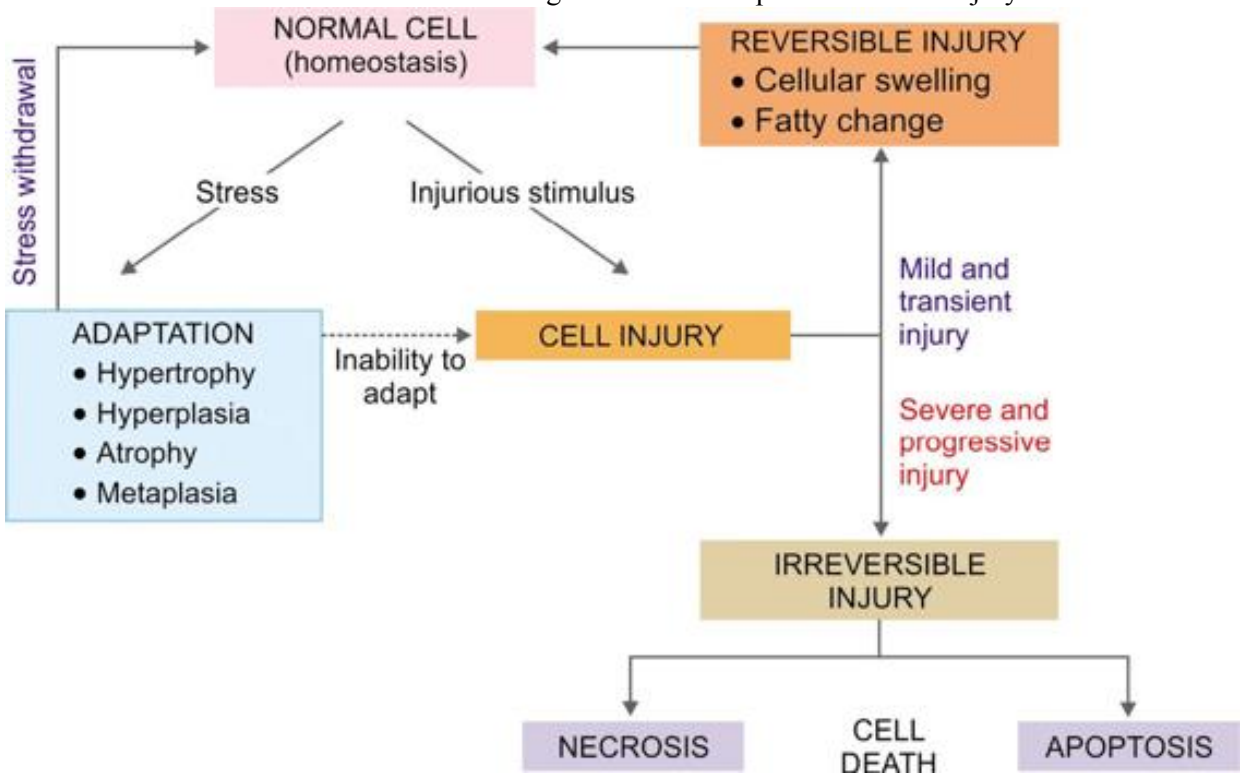


Fig. 2.1: Types of cellular responses to stimuli/injury.

Flowchart 2.1: Different stages of cellular responses to stress/injury.



- Social/lifestyle choices: Addiction to drugs and alcohol and cigarette smoking.
- Therapeutic drugs.

D. Infectious agents: Viruses, bacteria, fungi, rickettsiae and parasites. The mechanism by which these infectious agents cause injury varies.

E. Immunologic reactions:

- **Autoimmunity:** Immune reactions to endogenous self-antigens are responsible for autoimmune diseases.
- **Hypersensitivity reactions and other immune reactions:** Heightened immune reactions to many external agents (e.g., microbes and environmental agents).

F. Genetic derangements: Genetic defects may cause cell injury because of:

- Deficiency of functional proteins (e.g., enzyme defects in inborn errors of metabolism).
- Accumulation of damaged DNA or misfolded proteins.

G. Nutritional imbalances

- **Nutritional deficiencies:**

- Protein-calorie deficiencies
- Deficiencies of specific vitamins.

- **Nutritional excesses:**
  - Excess of cholesterol predisposes to athero-sclerosis.
  - Obesity is associated with increased incidence of several important diseases, such as diabetes and cancer.
  - Hypervitaminosis.

H. Aging: Cellular aging or senescence in aging decreases the ability of cells to respond to stress and, eventually it results in the death of cells and the individual.

I. Idiopathic: Cause is not known.

General Principles of Cell Injury

1. **Cellular response to injury:** It depends on: (1) type of injury, (2) duration of injury and (3) severity of injury.
2. **Consequences of injury:** It depends on: (1) type of cell involved, (2) adaptability of cell, (3) status of cell and (4) genetic make-up of the cell.
3. **Targets and biochemical mechanism of cell injury:** These include: (1) mitochondrial damage/dysfunction, (2) disturbance of calcium homeostasis, (3) damage to cellular membranes and (4) damage to DNA and misfolding of proteins.

Mechanisms of Cell Injury

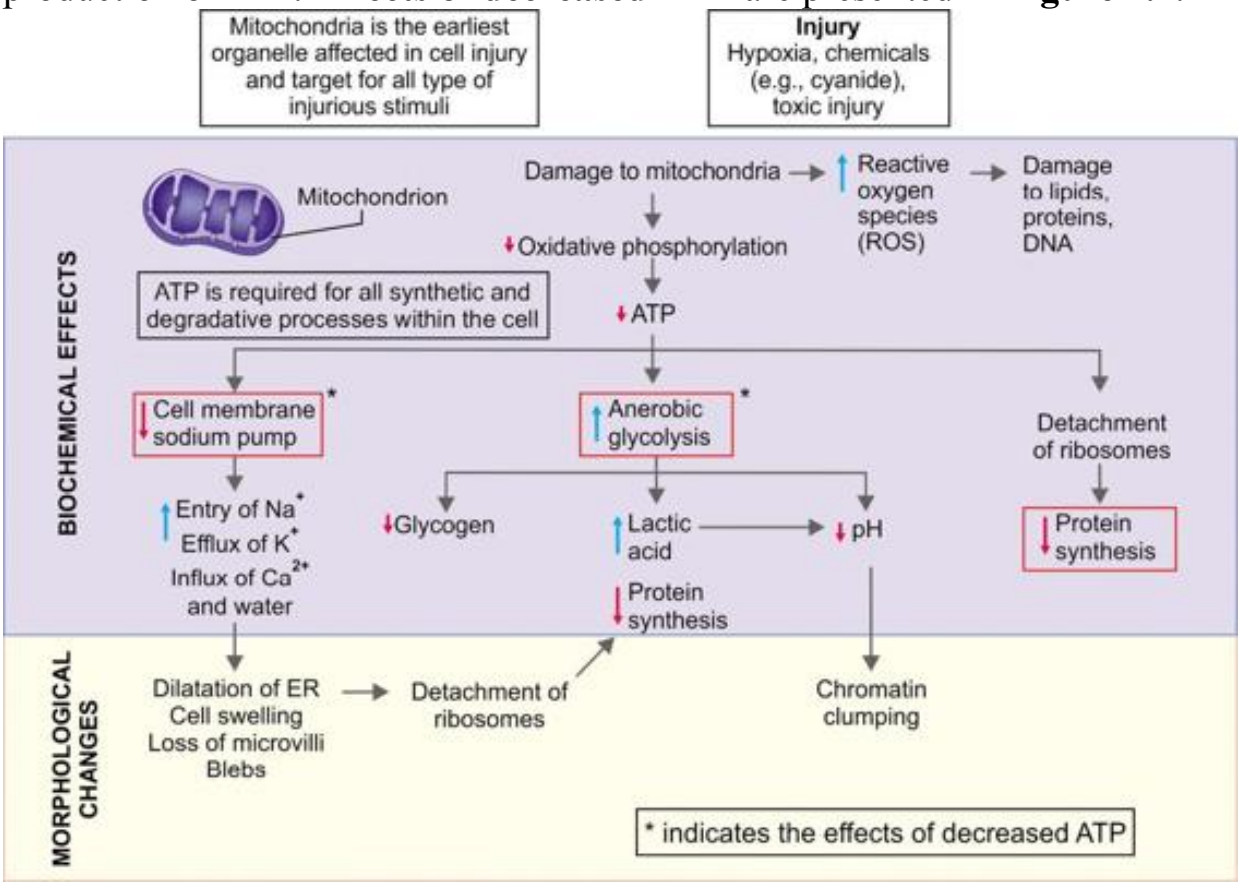
Q. Long essay: Describe the biochemical and molecular mechanism of cell injury.

Q. Write short essay on mechanism (biochemical basis) of cell injury.

Injurious stimuli that cause cell injury lead to complex cellular, biochemical and molecular changes. Certain mechanism is common for most forms of cell injury and cell death.

Decreased Production of Adenosine Triphosphate

Adenosine triphosphate (ATP) is required for all processes within the cell. Injury like hypoxia, chemicals (e.g., cyanide) can cause decreased production of ATP. **Effects of decreased ATP** are presented in **Figure 2.2**.



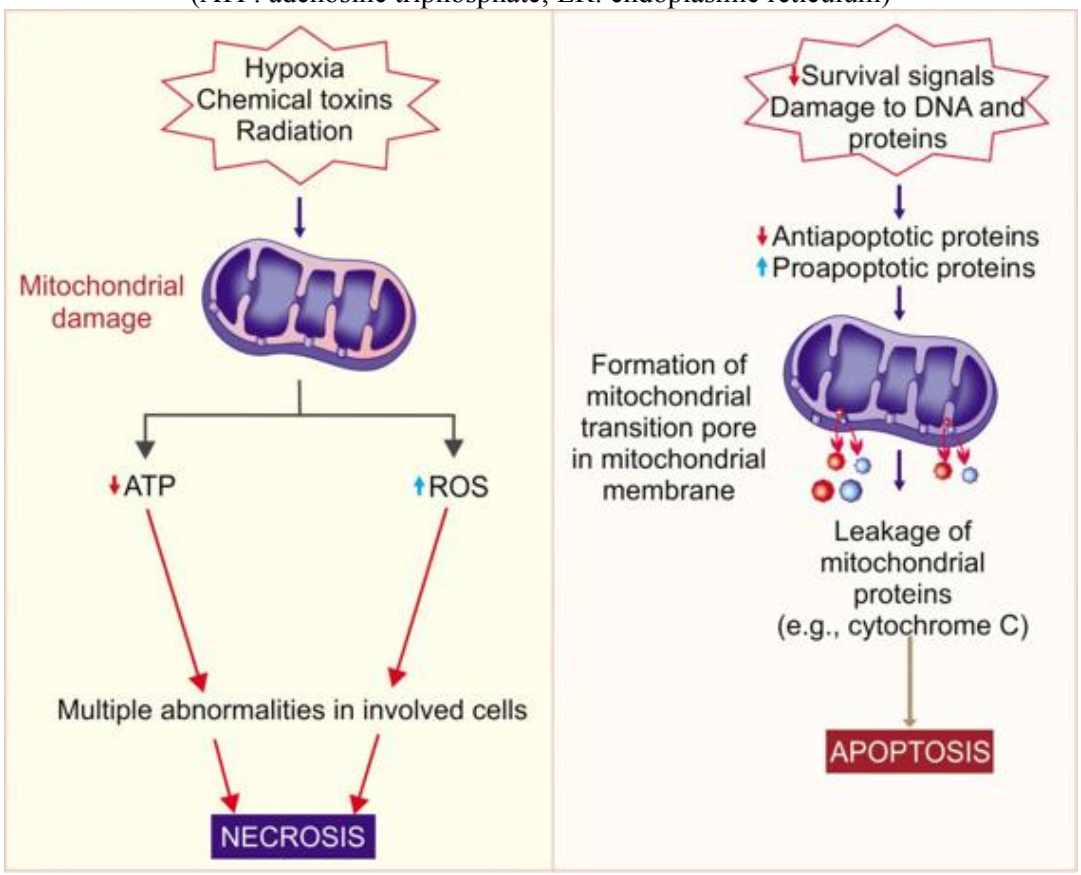
Targets of cell injury:

1. Mitochondrial damage/dysfunction
2. Disturbance of calcium homeostasis
3. Damage to cellular membranes
4. Damage to DNA and misfolding of proteins

Effects of decreased ATP:

1. Failure of the cell membrane sodium pump
2. Increased anaerobic glycolysis
3. Failure of protein synthesis in the ribosomes

Fig. 2.2: Biochemical and morphological changes due to decreased ATP production.
(ATP: adenosine triphosphate; ER: endoplasmic reticulum)



Consequences of mitochondrial damage:
1. Depletion of ATP
2. Formation of reactive oxygen species (ROS)
3. Formation of mitochondrial permeability transition pore
4. Leakage of mitochondrial proteins into cytoplasm

Oxidation: Loss of electrons.
Reduction: Gain of electrons.
Redox reaction: Reduction-oxidation reaction

Reduction-oxidation (redox reaction): A chemical reaction between two substances in which one substance is oxidized and the other reduced.

Fig. 2.3: Effects of mitochondrial damage.

Mitochondrial Damage (Fig. 2.3)

Mitochondria are sensitive to almost all types of injurious stimuli (e.g., hypoxia, toxins).

Consequences of Mitochondrial Damage

1. **Depletion of ATP:** Its effects are mentioned above.
2. **Formation of reactive oxygen species (ROS):** Its effects are mentioned in page 18 **(Fig. 2.4)**.
3. **Formation of mitochondrial permeability transition pore:** It occurs in the mitochondrial membrane. This leads to the **loss of mitochondrial membrane potential, pH changes** and **progressive depletion of ATP** and ultimately **necrosis** of the cell.
4. **Leakage of mitochondrial proteins into cytoplasm:** The mitochondrial membranes contain many proteins **such as cytochrome C and proapoptotic proteins (e.g., BAX and BAK)**. Increased permeability of the mitochondrial membrane may result in leakage of these proteins into the cytosol and induce **apoptosis**.

Influx of Calcium and Loss of Calcium Homeostasis (Fig. 2.5)

Q. Describe the role of cytosolic calcium in cell injury.

Normally, concentration of cytosolic calcium is very low and most of it is sequestered in mitochondria and the endoplasmic reticulum (ER). Ischemia and certain toxins cause an increase in cytosolic calcium. Initially, it is due to the release from intracellular stores and later due to influx across the cell membrane. Increased intracellular calcium stimulates **activation of several damaging enzymes** (e.g., phospholipases, endonucleases and protease) as well as caspases. The **net result is apoptosis**.

Accumulation of Oxygen-derived Free Radicals (Oxidative Stress)

Q. Write short essay/note on free radical injury andits role in cell injury.

Free radicals are **unstable chemical compounds** with a **single unpaired electron in an outer orbit (Fig. 2.5)**. Free radical-mediated cell injury is seen in many types of injury (e.g., chemical and radiation injury, hypoxia, cellular aging, tissue injury caused by inflammatory cells, and ischemia-reperfusion injury).

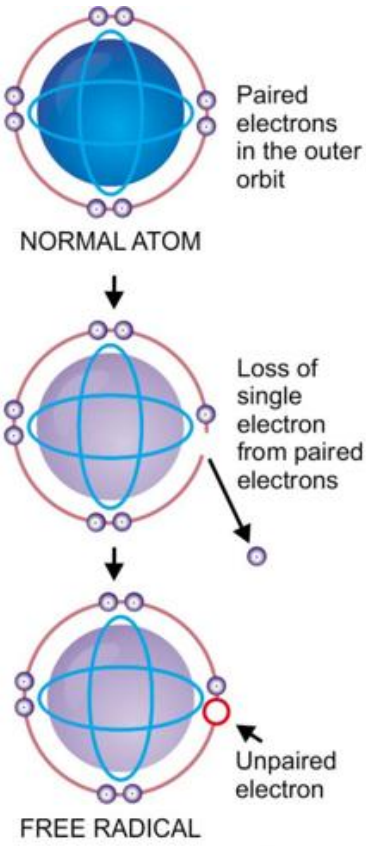
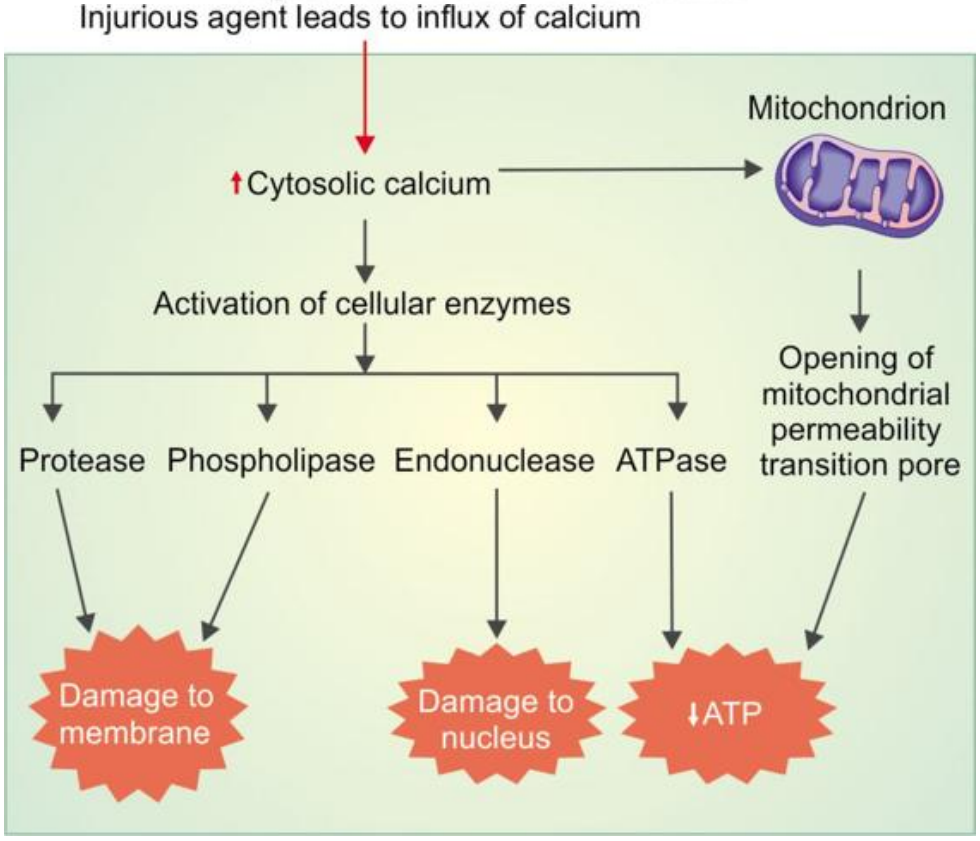


Fig. 2.4: Formation of free radical.



Properties of Free Radicals

- Normally, **free radicals** produced in the cells are **unstable** and are **rapidly destroyed.**
- When free radicals react with any molecules, they convert those molecules into free radicals and thus initiate **autocatalytic reactions.**
- **Excess** of free radicals may be either **due to increased production or ineffective degradation**.

Types of Free Radicals

Q. Write short note on free radical injury (including types of free radicals).

- **Oxygen-derived free radicals:** Reactive oxygen species (ROS) are oxygen-derived free radicals. ROS includes superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl ions (OH).
- **Reactive nitrogen species (nitric oxide derived free radicals):** For example, nitric oxide (NO) is generated by endothelial cells (*refer* **Fig. 3.7**), macrophages, neurons, and other types of cells. NO can act as a free radical and can also be converted to highly reactive peroxynitrite anion ($ONOO^-$), NO_2 and NO_3^-.
- **Free radicals from drug and chemical:** Enzymatic metabolism of exogenous chemicals or drugs can generate free radicals which are not ROS but have similar effects (e.g., CCl_4 can generate CCl_3).

The accumulation of ROS depends on their rates of production and removal.

Mechanism of Production of ROS

1. **In all cells (Fig. 2.6):** ROS are produced normally in **small amounts in the mitochondria**, during the reduction-oxidation (redox) reactions occurring during mitochondrial respiration, and production of energy.
   - During redox reaction, **superoxide (O_2^-)** is produced when oxygen (O_2) is only partially reduced.
   - Superoxide **(O_2^-)** is converted to **hydrogen peroxide (H_2O_2)** spontaneously and by the action of the enzyme superoxide dismutase

(SOD).

- Hydrogen peroxide (H_2O_2) in the presence of metals (e.g., Fe^{2+}) is converted by Fenton reaction to a highly reactive free radical called **hydroxyl radical ($^{\bullet}OH$)**.
- Superoxide **(O_2^-)** is also converted to peroxynitrite ($ONOO^-$) in the presence of nitric oxide (NO).

2. **In phagocytic leukocytes (Fig. 2.7):** ROS is produced in the leukocytes to destroy the ingested microbes and other substances produced during inflammation.
   - During phagocytosis, **ROS** is **produced in the phagosomes and phagolysosomes** of the leukocytes (mainly neutrophils and macrophages) by a process similar to mitochondrial respiration. This process is called as **respiratory burst.**
   - Superoxide **(O_2^-)** is synthesized via NADPH oxidase (nicotinamide adenine dinucleotide phosphate/respiratory burst oxidase) (phagocyte oxidase) present in the phagosome and phagolysosomal membrane of the leukocytes.
   - Superoxide **(O_2^-)** is converted to **hydrogen peroxide (H_2O_2)**.
   - Hydrogen peroxide (H_2O_2) in the presence of myeloperoxidase enzyme is converted to **highly reactive compound hypochlorite (HOCl)**.

Conditions Associated with Increased Generation of Oxygen-derived Free Radicals (Fig. 2.6)

- During inflammation and microbial killing by phagocytes.
- Drugs and chemical injury, including chemical carcinogens.
- Radiation injury (e.g., ultraviolet light, X-rays).
- Reduction-oxidation reactions.
- Ischemia-reperfusion injury induced by restoration of blood flow in ischemic tissue.
- Transition metals such as iron and copper donate or accept free electrons during intracellular reactions and catalyze free radical formation, as in the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH + OH^-$).
- Cellular aging.

Mechanisms of Removal/Neutralization of Free Radicals (Fig. 2.6)

Q. Write short note on antioxidants (mechanisms of removal/neutralization of free radicals).

Serum, tissue fluids and host cells have **antioxidant mechanisms,** which protect against potentially harmful oxygen-derived free radicals **(Table 2.1).** These include:

Fig. 2.5: Effects of increased cytosolic calcium in cell injury.

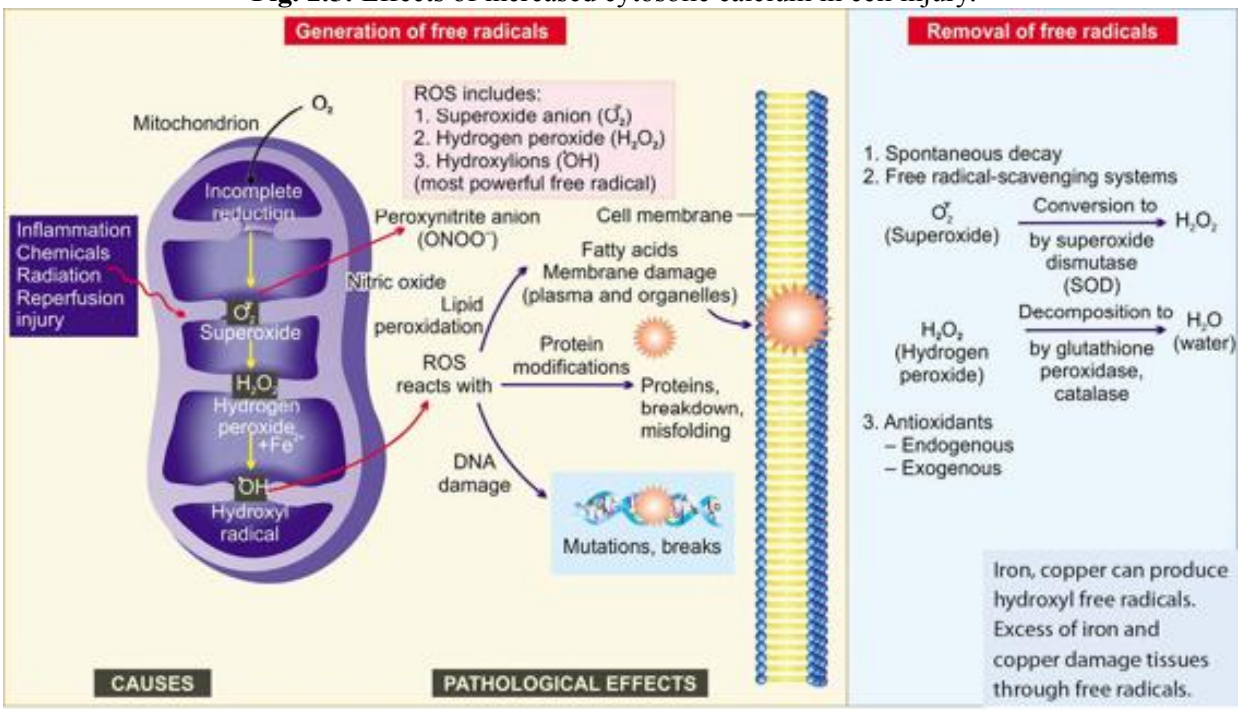


Fig. 2.6: Production and removal of reactive oxygen species (ROS) and their effects in cell injury.

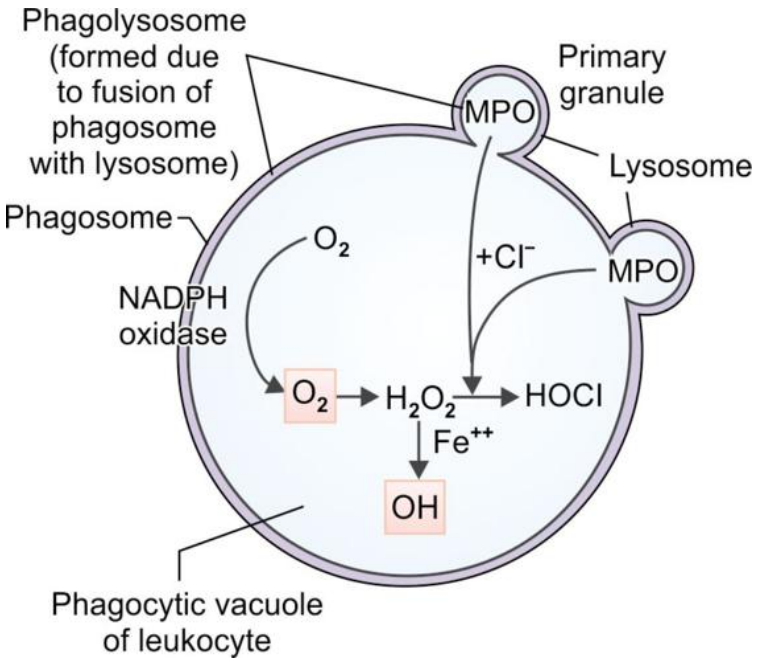


TABLE 2.1: Various types of antioxidants.

Enzymatic antioxidants	*Non-enzymatic antioxidants*
• Superoxide dismutase (SOD) • Catalase • Glutathione peroxidase	• Exogenous: Vitamin E, vitamin A, ascorbic acid and sulfhydryl containing compounds (e.g., cysteine and glutathione) • Endogenous: Storage and transport proteins in the serum, such as transferrin, ferritin, albumin and ceruloplasmin

Enzymatic Antioxidants

- **Spontaneous decay**
- **Free radical-scavenging systems**
  - Enzyme **catalase** neutralizes peroxidase (H_2O_2) free radicals by converting it into water and oxygen.
  - Enzyme **superoxide dismutases** (SODs) neutralizes superoxide free radicals by converting it into hydrogen peroxide.
  - Enzyme **glutathione peroxidase** (enhances glutathione) neutralizes peroxidase (H_2O_2), hydroxyl and acetaminophen free radicals.

Non-enzymatic Antioxidants

- **Exogenous antioxidants** [e.g., vitamin E, vitamin A, ascorbic acid and sulfhydryl containing compounds (e.g., cysteine and glutathione)].
- **Endogenous antioxidants:** Iron and copper are reactive metals, which can catalyze the formation of ROS. Their activities are minimized by binding of these ions to storage and transport proteins (e.g., transferrin, ferritin and ceruloplasmin).

Pathologic Effects of Free Radicals (Fig. 2.6)

Free radicals can cause cell injury in many diseases. Free radicals can activate both **necrosis** and **apoptosis**. Various effects of free radicals are:

- **Lipid peroxidation in membranes** causes extensive membrane damage.

Fig. 2.7: Production of reactive oxygen species in leukocytes inside the phagocytic vacuole of leukocyte.

(Cl^-: chloride ion; Fe^{++}: ferrous ions; HOCl: hypochlorite; H_2O_2: hydrogen peroxide; OH: hydroxyl radical; MPO: myeloperoxidase)

- **Cross-linking and oxidative modification of proteins** damages the enzyme activity and causes abnormal folding of proteins.
- **Damage to DNA.**

Effects of Cell Injury

Defects in Membrane Permeability and Membrane Damage

- **Reversible injury:** In most forms of cell injury, in the early phase there is selective loss of membrane permeability.
- **Irreversible injury:** With the obvious membrane damage, the cell cannot return to normal.

Mechanisms of Membrane Damage

- **Indirect damage:**
  - **Reactive oxygen species (ROS):** It causes injury to cell membranes by **lipid peroxidation**.
  - **Decreased phospholipid synthesis: Hypoxia** through defective mitochondrial function → decreases the production of ATP by **ischemic cells** → leads to decreased phospholipid synthesis in all

cellular membranes (including the mitochondria) and energy-dependent enzymatic activities.

- **Increased phospholipid breakdown:** Severe cell injury increases levels of **cytosolic and mitochondrial Ca^{2+}** → results in **calcium-mediated activation** of endogenous **phospholipases** → which degrades membrane phospholipids → leads to the accumulation of **lipid breakdown products** → cause membrane damage.
- **Cytoskeletal damage:** Cytoskeletal filaments connect the plasma membrane to the cell interior. **Increased cytosolic calcium activates proteases** which may **damage the cytoskeletal elements** and **cell membrane**.

- **Direct damage:** The plasma membrane can also be damaged directly by various bacterial toxins, viral proteins, lytic complement components and a variety of physical and chemical agents.

Consequences of Membrane Damage

Cell injury may damage any membrane, but most important are:

- **Mitochondrial membrane damage:** It results in:
  - Opening of the **mitochondrial permeability transition pore** leading to decreased ATP.
  - Release of proteins that trigger apoptotic death.
- **Plasma membrane damage:** It leads to **loss** of:
  - **Osmotic balance** and influx of fluids and ions
  - Cellular contents.
- **Lysosomal membrane damage:** It leads to:
  - **Leakage of lysosomal enzymes** into the cytoplasm
  - **Activation of lysosomal enzymes** → which results in digestion of proteins, RNA, DNA and glycogen → leads to cell death by necrosis.

Damage to DNA and Proteins

- **Causes of DNA damage:** Exposure to DNA damaging drugs, radiation or oxidative stress.
- **Repair mechanism:** Cells have mechanisms to repair DNA damage. However, if the damage is too severe to be corrected, the cell initiates a suicide program causing death by apoptosis.

Fenton reaction: $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH + OH^-$

Oxidase reactions produce superoxide free radicals.

Free radicals are neutralized by superoxide dismutase, glutathione peroxidase and antioxidants such as vitamin C and E. Vitamin C mainly neutralizes hydroxyl free radicals.

Superoxide dismutase: Enzyme that protects the brain from free radical injury.

Fenton reaction leads to free radical generation when ferrous ions (Fe^{2+}) are converted to ferric ions.

Free radicals steal electrons from neighboring molecules. Free radicals can damage cellular membranes, proteins, and nuclear DNA.

Ischemia-reperfusion Injury

Q. Write short note on ischemia-reperfusion injury.

- **Decreased blood flow** to a tissue or organ is called **ischemia**.
- Depending on the severity and duration of ischemia, the involved tissue may adapt, undergo injury (reversible), or die (irreversible). Therapies to restore blood flow is an important modality of treating ischemia.
- If the involved cells of the tissue are reversibly injured, the restoration of blood flow (reperfusion) is often beneficial. However, under certain circumstances the restoration of blood flow to cells that have been ischemic (reversibly injured) but have not died (irreversibly injured), can paradoxically exacerbate and produce injury at an accelerated pace.
- The damaging process is set in motion during reperfusion and **reperfused tissues undergoes loss of cells** (new damage**) in addition to the cells that are irreversibly damaged (died) at the end of ischemia.** This damaging process is called as **ischemia-reperfusion injury**.
- **Clinical importance:** It contributes to tissue damage following reperfusion in **myocardial infarction** (*refer* page 464) and **cerebral infarction**.

Mechanism of Reperfusion Injury

New damage may be initiated during reoxygenation which includes:

1. **Increased generation of reactive oxygen and nitrogen species:**

- **Increased production of free radicals:** Free radicals may be produced from parenchymal and endothelial cells and from infiltrating leukocytes in reperfused tissue. Free radicals form as a result of mitochondrial damage, causing incomplete reduction of oxygen, or because of the action of oxidases in leukocytes, endothelial cells, or parenchymal cells.
- **Decreased antioxidant mechanism:** Ischemia may result in defective cellular antioxidant defense mechanisms, favoring the accumulation of free radicals.

2. **Inflammation:** Ischemic injury produces cytokines and increased expression of adhesion molecules by hypoxic parenchymal and endothelial cells. They recruit circulating neutrophils to reperfused tissue causing inflammation. The inflammation causes further tissue injury.
3. **Activation of the complement system:** It is an important mechanism of immune-mediated injury. Some IgM antibodies may get deposited in ischemic tissues. When blood flow is restored, complement proteins may bind to the deposited antibodies and complement system may be activated → cause inflammation and more injury to cells.
4. Calcium overload (*refer* page 17)

Free radicals in reperfusion injury are mainly produced by infiltrating leukocytes.

TYPES OF CELL INJURY

CC: Describe the etiology of cell injury (*refer* pages 14-6). Distinguish between reversible-irreversible injury: mechanisms (*refer* pages 16-20); morphology of cell injury.

Two types: **Reversible and irreversible (cell death)**. Reversible injury may progress to a irreversible stage and result in cell death.

Reversible Cell Injury

Reversible injury is the stage of cell injury during which the deranged function and morphology of the injured cells can return to normal if the causative stimulus is removed. If the stimulus is acute and brief (or short

lived) or mild, the cell injury produces changes in the cells which are reversible up to a certain point.

Light microscope features of reversible cell injury: Two patterns of reversible cell injury namely cellular **swelling and fatty change.**

Cloudy Swelling

It was originally applied to the gross appearance of organ involved, but is now applied to the microscopic appearance. It is closely related to **hydropic change, vacuolar degeneration**. They represent disturbances in protein and water metabolism. Being a manifestation of a disturbance in protein metabolism, it is also called **albuminous degeneration**.

Causes: It may be caused by many forms of mild injury but usually due to bacterial toxins, chemical poisons and malnutrition. It is due to changes in ion concentrations and fluid homeostasis. There is an increased flow of water into the cells which results in increased water content of injured cells.

Organs involved: Kidney, liver, heart and muscle.

Gross: Organ affected is slightly enlarged due to swelling of its cells. Organ appears pale because of compression of blood vessels by the swollen cells. Cut surface has a cloudy appearance and opaque.

Microscopy: Best example is cloudy swelling of highly specialized cells of the convoluted tubule of the kidney. The cell is swollen and projects unevenly into the lumen of the tubule. As the process advances the cytoplasm breaks down and its granular material gets discharged into the lumen of the tubule. These granules are proteinaceous in nature.

Steatosis (Fatty Change)

It is explained on pages 22-3.

Irreversible Cell Injury/Cell Death

If the cell is exposed to continuous injurious stimulus or if the injury is severe, the cells undergo cell death. **The mechanism of cell death depends on the nature and severity of the injury.** Two main types of cell death: **Necrosis and apoptosis**.

- **Necrosis:** Always a pathologic process (*refer* pages27-32).

- **Apoptosis:** May be physiological or pathological (*refer* pages 33-7).

INTRACELLULAR ACCUMULATIONS

CC: Intracellular accumulation of fats, proteins, carbohydrates, pigments.

Metabolic derangements in cells may manifest as intracellular accumulation of abnormal amounts of various substances. The abnormal amounts of substances may accumulation either within the cytoplasm (within organelles especially lysosomes) or in the nucleus of the cell. These substances may be normal or abnormal, endogenous (synthesized by the affected cells) or exogenous (produced elsewhere), harmful (causing varying degrees of injury) or harmless (innocuous). Accumulation of the substance can cause reversible cell injury whereas more severe accumulation can lead to irreversible cell injury.

Mechanism of intracellular accumulation (Fig. 2.8): There are four main pathways which can lead to abnormal intracellular accumulations.

1. **Inadequate removal of a normal substance:** This may be secondary to defects in mechanisms of packaging and transport. For example, abnormal metabolism of lipid can cause excessive amounts of intracellular fat as in fatty change (steatosis) in the liver.
2. **Accumulation of an abnormal endogenous substance:** Genetic or acquired defects in folding, packaging, transport, or secretion of an endogenous substance can cause accumulation of this abnormal endogenous substance. For example, certain **mutated forms of α1-antitrypsin.**
3. **Failure to degrade a metabolite:** An inherited deficiency of critical/key enzymes required for breaking down certain endogenous complex compounds/substrates into soluble products can cause accumulation of complex compounds/substrates (that are not metabolized) in lysosomes. For example, in **storage diseases/disorders**.
4. **Deposition and accumulation of an abnormal exogenous substance:** When a cell has neither the enzymatic machinery to degrade the substance (or phagocytosed particles) nor the ability to transport it to other sites, the abnormal exogenous substance can accumulate within the

cells. For example, foreign particulates such as carbon or silica particles or injected tattoo pigments can accumulate in the cells.

Consequences: If the overload can be controlled or stopped, the process of intracellular accumulation is reversible. In inherited storage diseases, there is progressive accumulation and the consequent overload result in injury to cells and can cause death of the tissue involved and the patient.

Lipids

Major classes of lipids are triglycerides, cholesterol/cholesterol esters, and phospholipids. All of them can accumulate in the cells. Phospholipids are major components of the myelin figures observed in necrotic cells. In addition to lipids, abnormal complexes of lipids and carbohydrates accumulate in the lysosomal storage diseases (*refer* pages 250-2).

Steatosis (Fatty Change)

Q. Write short note on causes (etiology), pathogenesis and morphology of fatty change/steatosis liver. Add a note on special stains for fat.

Definition: Abnormal accumulations of triglycerides within cytosol of the parenchymal cells.

Organs involved: Seen in organs involved in fat metabolism namely **liver**. It may also occur in heart, muscle and kidney.

Causes

Causes of steatosis of liver are listed in **Box 2.2**.

BOX 2.2: Causes of hepatic steatosis.

- **Disorders with hepatocyte damage:** Alcoholic abuse, protein malnutrition, starvation, anoxia (anemia, cardiac failure), toxins (carbon tetrachloride, chloroform, etc.) and Reye syndrome. **Alcohol is the most common cause** of fatty change in the liver.
- **Disorders with hyperlipidemia:** Obesity, diabetes mellitus or congenital hyperlipidemia.

Pathogenesis of Fatty Liver

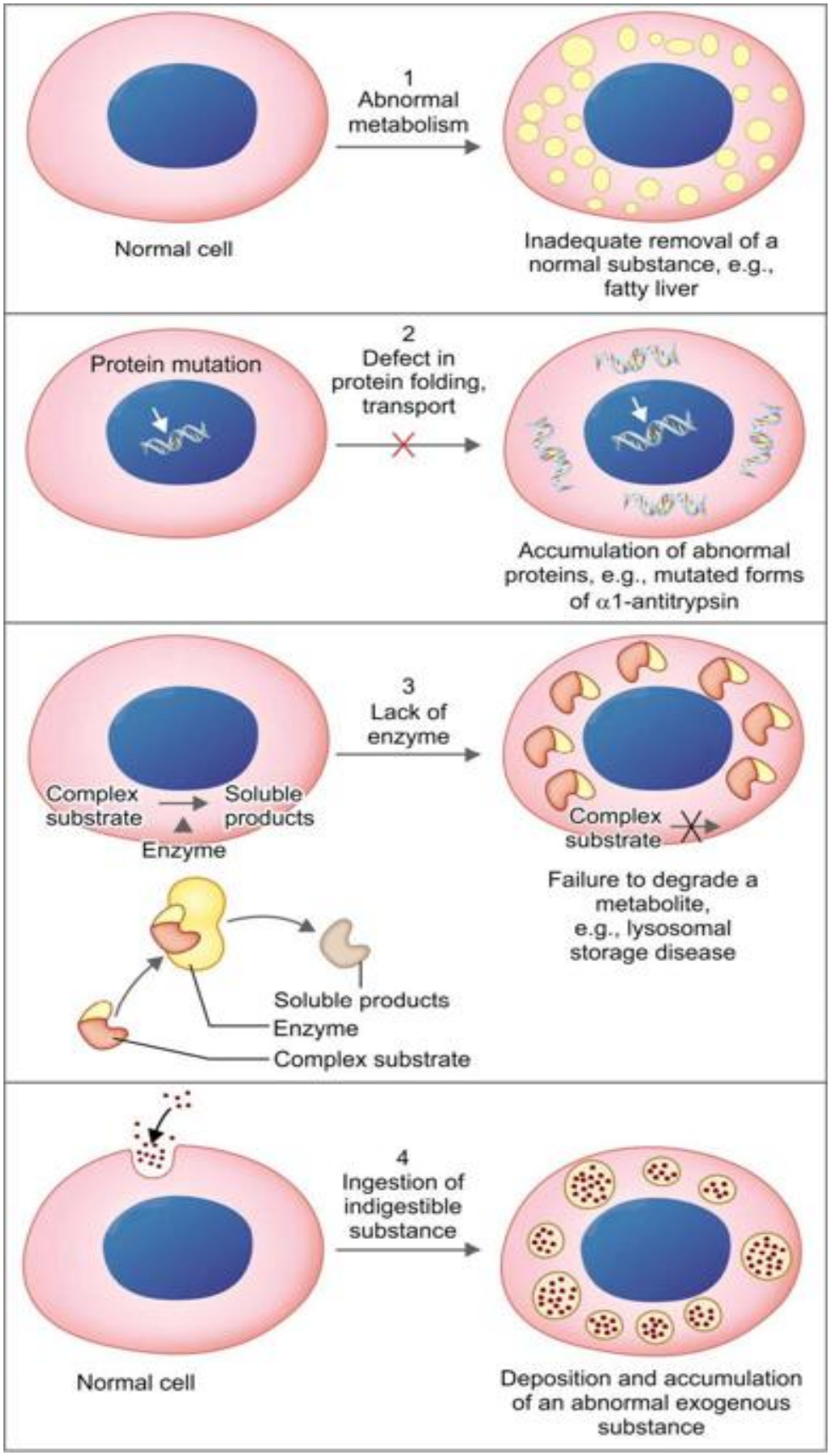

1
Abnormal metabolism
Normal cell
Inadequate removal of a normal substance, e.g., fatty liver
Protein mutation
2
Defect in protein folding, transport
Accumulation of abnormal proteins, e.g., mutated forms of α1-antitrypsin
3
Lack of enzyme
Complex substrate
Soluble products
Enzyme
Complex substrate
Failure to degrade a metabolite, e.g., lysosomal storage disease
Soluble products
Enzyme
Complex substrate
4
Ingestion of indigestible substance
Normal cell
Deposition and accumulation of an abnormal exogenous substance

Fig. 2.8. Mechanisms of intracellular accumulation: (1) Due to abnormal metabolism (e.g., fatty change in the liver). (2) Mutations with alterations in protein folding and transport (e.g., mutated forms of α1-antitrypsin), (3) A deficiency of critical enzymes (e.g., lysosomal storage diseases), (4) An inability to degrade phagocytosed particles (e.g., accumulation of carbon pigment, silica).

Normal hepatocytes contain some amount of fat derived from the uptake of free fatty acids released from adipose tissue (and GI tract). A small amount of fatty acids is also synthesized from acetate in the liver cells. Most of free fatty acid is esterified to triglycerides by the action of α-glycerophosphate and only a small part is changed into cholesterol, phospholipids and ketone bodies. The cholesterol, phospholipids and ketones are used in the body. Most of newly synthesized triglycerides are secreted by the liver as lipoproteins. Various mechanisms are involved in excess accumulation of triglyceride in the liver and one or more mechanism may be responsible for steatosis of liver.

- **Excessive entry of free fatty acids (FFA) into the liver(1 in Fig. 2.9):** From peripheral stores FFA enters into liver during starvation and diabetes.
- **Defective metabolism of lipids:** This may be due to:
  - Increased synthesis of fatty acids from acetate by liver **(2 in Fig. 2.9).**
  - Decreased oxidation of fatty acids into ketone bodies **(3 in Fig. 2.9)** resulting in increased esterification of fatty acids into triglycerides.
  - Decreased synthesis of apoproteins (e.g., in **CCl_4 and protein malnutrition**) causes decreased formation of lipoproteins from triglycerides **(4 in Fig. 2.9)**.
- **Defective excretion of lipoproteins:** Fatty liver may also develop due to defect in excretion of lipoproteins from liver into the blood **(5 in Fig. 2.9).**

MORPHOLOGY

Q. Write a short note on gross and microscopy of fatty liver.

Fatty Liver

- **Gross (Fig. 2.10):** Liver enlarges and becomes **yellow, soft and greasy to** touch.

- **Microscopy:** First, fat is seen as small **vacuoles in the cytoplasm** around the nucleus (macrovesicular steatosis [mi (*refer* **Fig. 19.17A**). Later, the vacuoles coalesce, creating clear spaces [macrovesicular steatosis ((**Fig. 2.11** and *refer* **Fig. 19.17B)**] that displace the nucleus to the periphery of the cell (gives a signet ring appearance—not to be mistaken for signet ring cells seen in signet ring carcinoma—*refer* **Fig. 18.17**).
- **Special stains for fat** sections stained with **Sudan IV or Oil Red-O** give an orange-red color to the fat. **Osmic acid** (is a fixative as well as a stain) and **Sudan black B** gives a black color.



Fig. 2.10: Fatty liver showing liver with yellow colorand sharp border.

Heart

Q. Write short note on heart in fatty change.

Lipid in the cardiac muscle can have **two patterns**:

1. **Alternate involvement:** Prolonged moderate hypoxia (e.g., severe anemia), create grossly apparent bands of involved **yellow myocardium alternating with bands of darker, red-brown, uninvolved myocardium** (**tigered effect, tabby cat appearance**).
2. **Uniform involvement:** More severe hypoxia or some types of myocarditis (e.g., diphtheria infection) show more **uniform involvement of myocardial fibers**.

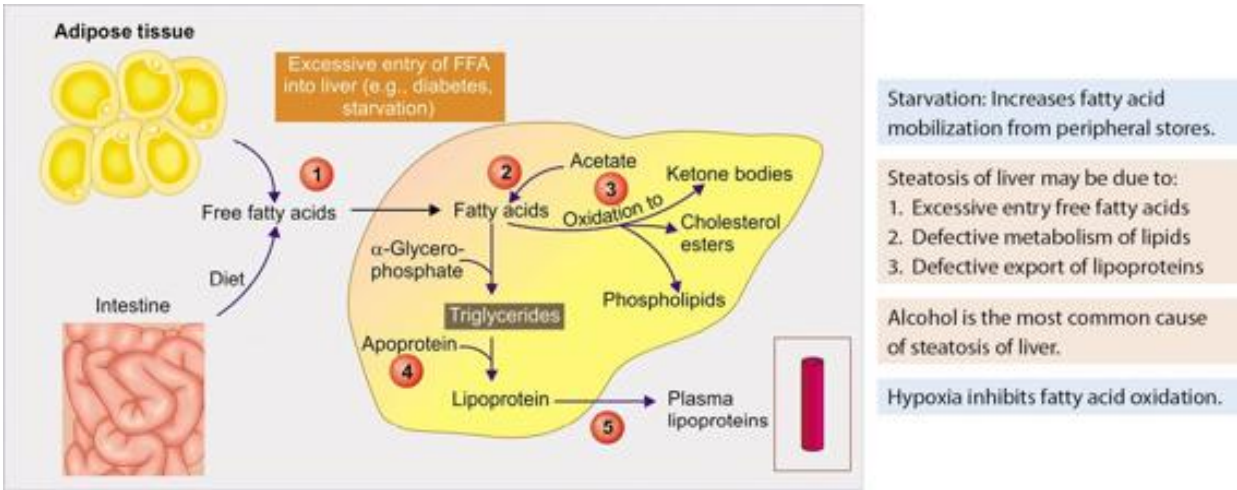
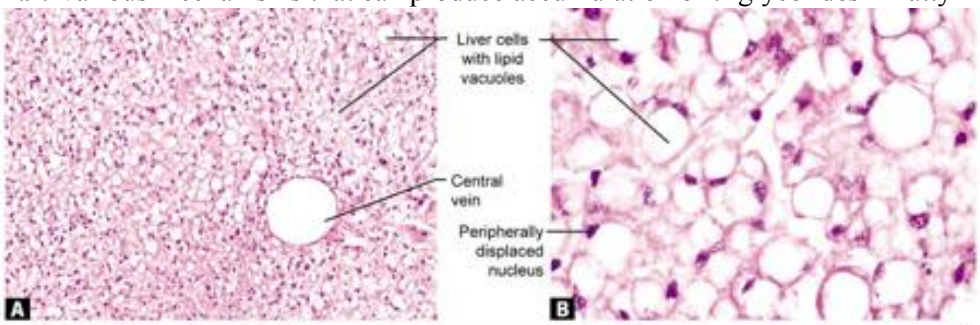


Fig. 2.9: Various mechanisms that can produce accumulation of triglycerides in fatty liver.



Figs. 2.11A and B: (A) Fatty liver in which the hepatocytes show accumulation of fat which appear as clear vacuole in the cytoplasm; (B) Hepatocytes at higher magnification in which the nucleus is displaced to the periphery by accumulated fat.

Cholesterol and Cholesteryl/Cholesterol Esters

Cholesterol metabolism in cells is tightly regulated in such a way that there is normal generation of cell membranes (in which cholesterol is a main component) without significant intracellular accumulation. However, in pathological conditions phagocytic cells may intracellularly accumulate lipid (triglycerides, cholesterol, and cholesteryl esters). It may be either due to increased intake or decreased catabolism of lipids. These pathological conditions are briefly discussed below.

Atherosclerosis: It is a disease of **aorta and large arteries** characterized by the presence of **atherosclerotic plaques composed of smooth muscle cells and macrophages within the intima filled with lipid vacuoles. Most of the lipid is cholesterol and cholesterol esters** (*refer* **page 439**).

Q. Write short note on xanthoma.

Xanthoma: Intracellular accumulation of cholesterol within macrophages is found in acquired and hereditary hyperlipidemic states. **The tumor mass produced by the macrophages filled with cholesterol** is termed xanthomas. The xanthomas are found in the subepitelial connective tissue of the skin and the tendons, producing tumorous masses. Microscopically, it consists of clusters of foamy cells in the subepithelial connective tissue of the skin and in tendons.

Cholesterolosis: It is characterized by the **focal accumulations of cholesterol-laden macrophages in the lamina propria of the gallbladder**. The mechanism is not known.

Niemann–Pick disease, type C: It is a lysosomal storage disease is caused by mutations affecting an enzyme involved in cholesterol trafficking. It is characterized by **accumulation of cholesterol in many organs.**

Proteins

Abnormal pathologic accumulation of protein can occur within the cells. Under light microscopy, abnormal intracellular accumulations of proteins appear as rounded, eosinophilic droplets, vacuoles, or aggregates in the cytoplasm. When viewed under electron microscopy, they can appear as amorphous, fibrillar, or crystalline structures.

Causes of Intracellular Accumulation of Proteins

Excesses morphologically visible abnormal/excessive accumulation of proteins within the cells (intracellular) may occur either due to excessive presentation of proteins to the cells or when the cells synthesize excessive amounts of proteins.

Excessive Presentation of Proteins to the Cells

Reabsorption protein in proximal renal tubules: It occurs in renal diseases associated with loss of protein in the urine (proteinuria). In the kidney, trace (small) amounts of protein (albumin) filtered through the glomerulus are normally reabsorbed in the proximal convoluted tubule by a process called pinocytosis. However, when there is heavy protein leakage (e.g., nephrotic syndrome) through the glomerulus, there is increased/excessive reabsorption of the protein into vesicles. These vesicles

containing this protein appear microscopically as pink hyaline droplets within the cytoplasm of the tubular cell. The process is reversible and with the control of proteinuria, the protein droplets are metabolized and disappear from the cell.

Excessive Synthesis or Accumulation of Proteins

- **Russell bodies:** Plasma cells synthesize immunoglobulins (a type of proteins). Whenever the plasma cells newly synthesize excessive amounts of immunoglobulins they can accumulate within the rough endoplasmic reticulum (RER) present in the cytoplasm of some plasma cells. The RER becomes distended with immunoglobulins and produce large, round, homogeneous eosinophilic (pink hyaline) inclusions called as Russell bodies **(Fig. 2.24B)**.
- **Defective intracellular transport and secretion of critical proteins:** α1-antitrypsin deficiency is a heritable disorder in which, mutations in the gene coding for α1-antitrypsin yield an insoluble protein. The protein produced is significantly slow in folding process and is not easily exported/secreted from the cells. This results in the accumulation of partially folded intermediates which aggregate in the ER of the cytoplasm of the liver cells and appear as eosinophilic globular deposits of a mutant protein. This result in the deficiency of the circulating enzyme and there is excessive digestion of elastic tissues which produce emphysema (*refer* page 493 of Chapter 17). In many of these diseases, the damage is not only due to the loss of protein function but also due to ER stress caused by the misfolded proteins.
- **Accumulation of cytoskeletal proteins**: The internal framework of a cell is composed of protein filaments known as cytoskeletal proteins. They provide structural support to the cells and drive the movement of the cell. There are several types of cytoskeletal proteins and are usually divided into three categories namely microfilaments [thin actin filaments (6–8 nm), thick myosin filaments (15 nm)], intermediate (10 nm) filaments and microtubules (20–25 nm in diameter), depending on the diameter and the composition of filaments. The intermediate filaments provide a flexible intracellular scaffold that organizes the cytoplasm and resists forces applied to the cell. The intermediate filaments are subdivided into five classes: (i) keratin filaments (characteristic of epithelial cells), (ii) neurofilaments (neurons), (iii) desmin filaments (muscle cells), (iv)

vimentin filaments (connective tissue cells), and (v) glial filaments (astrocytes). Accumulations of keratin filaments and neurofilaments are observed in certain types of cell injury.

- **Mallory's body or alcoholic hyaline** (*refer* Fig. 2.24A and page 619): It is an eosinophilic cytoplasmic inclusion seen in liver cells and is characteristic of **alcoholic liver disease**. It appears as amorphous pink masses. They represent intracellular accumulation of intermediate filaments of keratin (cytokeratin).
- **Neurofibrillary tangle** (tau protein): It is found in cortical neurons of the brain of patient with **Alzheimer disease** and consists of neurofilaments and other proteins.

- **Aggregation of abnormal proteins:** Abnormal or misfolded proteins may aggregate and get deposited in tissues and interfere with their normal functions. These deposits may be intracellular, extracellular, or both. These aggregates of proteins may either directly or indirectly cause the pathologic changes.
  - **Intracellular accumulation of misfolded proteins:** It may be due to either increased production of the misfolded proteins or reduce ability to eliminate them.
    - **Prion diseases**: These are neurodegenerative disorders (spongiform encephalopathies) caused by accumulation of abnormally folded prion proteins. Abnormal prion proteins may be due to either inherited mutations or from exposure to the aberrant form of the protein.
    - **Lewy bodies** (α-synuclein): They are observed in neurons of the substantia nigra of patients with Parkinson disease.
  - **Extracellular accumulation of proteins:** Apart from abnormal intracellular accumulation, abnormal proteins deposit can occur primarily in extracellular spaces in some disorders, such as certain forms of amyloidosis (*refer* pages 181-6).

Glycogen

Glycogen is a long-chain polymer of glucose. It is formed and mainly stored in the cytoplasm of liver and to a lesser extent in muscles. Glycogen is a readily available energy source and is depolymerized to glucose and liberated as needed.

Causes: Excessive intracellular deposits/accumulation of glycogen can occur due to an abnormality in either glucose or glycogen metabolism.

- **Glycogen storage disease:** Glycogen is degraded in steps by a series of enzymes. Deficient of any enzymes involved in any of these steps may be observed as an inborn (genetic) error of metabolism called as a glycogen storage disease or glycogenoses.
- **Diabetes mellitus:** Storage of glycogen in cells is normally regulated by blood glucose levels, and hyperglycemic states are associated with increased glycogen stores. Thus, in uncontrolled diabetes mellitus (a disorder of glucose metabolism) there is impaired cellular uptake of glucose, which results in high serum and urine glucose levels. It is accompanied by excessive accumulate of glycogen in hepatocytes, epithelial cells of the renal proximal tubules (renal tubules reabsorb the excess filtered glucose and store it intracellularly as glycogen), β-cells of the islets of Langerhans and cardiac muscle cells.

Morphology: In routine H & E stained sections, glycogen appear as clear vacuoles within the cytoplasm of the affected cells. Glycogen gets dissolved in aqueous fixatives and appear as vacuoles in the cytoplasm. Hence, for its identification, tissues should be fixed in absolute alcohol. Special stains such as Best's carmine or the periodic acid-Schiff (PAS) imparts a rose-to-violet color to the glycogen. Diastase digestion of a parallel section before staining serves as a further control by hydrolysing the glycogen.

Pigments

Q. Write short note on various (exogenous and endogenous) pigments.

TABLE 2.2: Different types of pigments.

Endogenous pigments	*Exogenous pigments*
• Bilirubin	• Carbon (anthracotic)
• Melanin	• Tattooing
• Hemosiderin	• Arsenic
• Hemoglobin derived pigments, lipofuscin	• b-carotene

TABLE 2.3: Causes of hyper- and hypopigmentation.

Generalized hyperpigmentation	***Generalized hypopigmentation***
1. **Addison's disease** 2. **Chloasma:** Hyperpigmentation on the skin of face, nipples, and genitalia during pregnancy 3. **Chronic arsenical poisoning** (raindrop pigmentation of the skin)	**Albinism:** Generalized hypopigmentation due to genetic deficiency of tyrosinase enzyme
Focal hyperpigmentation	***Localized hypopigmentation***
1. **Cäfe-au-lait spots** in neurofibromatosis and Albright's syndrome 2. **Peutz–Jeghers syndrome:** Focal perioral pigmentation 3. **Melanosis coli:** Pigmentation of the mucosa of the colon 4. **Tumors of melanocytes:** Benign (nevi) and malignant (melanoma) tumors 5. **Lentigo:** Premalignant condition	1. **Leukoderma:** Autoimmune disorder with localized loss of pigmentation of the skin 2. **Vitiligo:** Local hypopigmentation of the skin 3. **Acquired focal hypopigmentation:** Leprosy, healing of wounds, DLE, radiation dermatitis, pityriasis alba, pityriasis versicolor, idiopathic guttate hypomelanosis, etc.

Definition: Pigments are **colored substances**, which are either normal constituents of cells (e.g., melanin), or are abnormal, (e.g., carbon) and accumulate in cells.

Different types of pigments are listed in **Table 2.2.**

Melanin

Melanin is an endogenous, brown-black, non-hemoglobin-derived pigment. It is produced by the melanocytes and dendritic cells by the oxidation of tyrosine to dihydroxyphenylalanine by the enzyme tyrosinase. It is stored as cytoplasmic granules in the phagocytic cells namely melanophores. Normally, it is present in the hair, skin, mucosa at some places, choroid of the eye, meninges and adrenal medulla. Various disorders of melanin pigmentation produce generalized and localized hyperpigmentation and hypopigmentation **(Table 2.3)**.

Alkaptonuria

Q. Write short answer on ochronosis.

Homogentisic acid is a pathological black pigment formed in rare metabolic autosomal recessive disorder termed alkaptonuria. It is characterized by deficiency of an oxidase enzyme needed for breakdown of homogentisic acid. This leads to accumulation of homogentisic acid pigment in the skin, connective tissue, cartilage, capsules of joints, ligaments and tendons. The pigment is melanin-like and the pigmentation is known as **ochronosis**. The homogentisic acid is excreted in the urine (homogentisic aciduria). The urine of patients of alkaptonuria, if allowed to stand for some hours in air, turns black due to oxidation of homogentisic acid.

Hemosiderin

Q. Write short note on hemosiderin and hemosiderosis.

Definition: It is a hemoglobin-derived, golden yellow-to-brown, granular or crystalline pigment and is one of the major storage forms of iron.

Causes

Local or systemic excess of iron cause hemosiderin to accumulate within cells.

- **Local excesses:**
  - **Bruise**
  - **Brown induration of lung** in chronic venous congestion of lung (*refer* **Fig. 5.1A**).
- **Systemic excesses: Systemic overload of iron** is known as **hemosiderosis**. The main causes:
  - **Increased absorption of dietary iron**.
  - **Excessive destruction of red cells** (e.g., hemolytic anemias).
  - **Repeated blood transfusions**.

MORPHOLOGY

Site of Accumulation

- **Localized:** Found in the macrophages of the involved area.
- **Systemic:** Initially found in **liver, bone marrow, spleen, and lymph nodes**. Later deposited in macrophages of other organs (e.g., skin, pancreas, kidney).

Microscopy: Appears as a coarse, golden, granular pigment within the cytoplasm.

Special stain: **Prussian blue** (Perl's stain) histochemical reaction in which hemosiderin converts colorless potassium ferrocyanide to blue-black ferric ferrocyanide **(Fig. 2.12).**

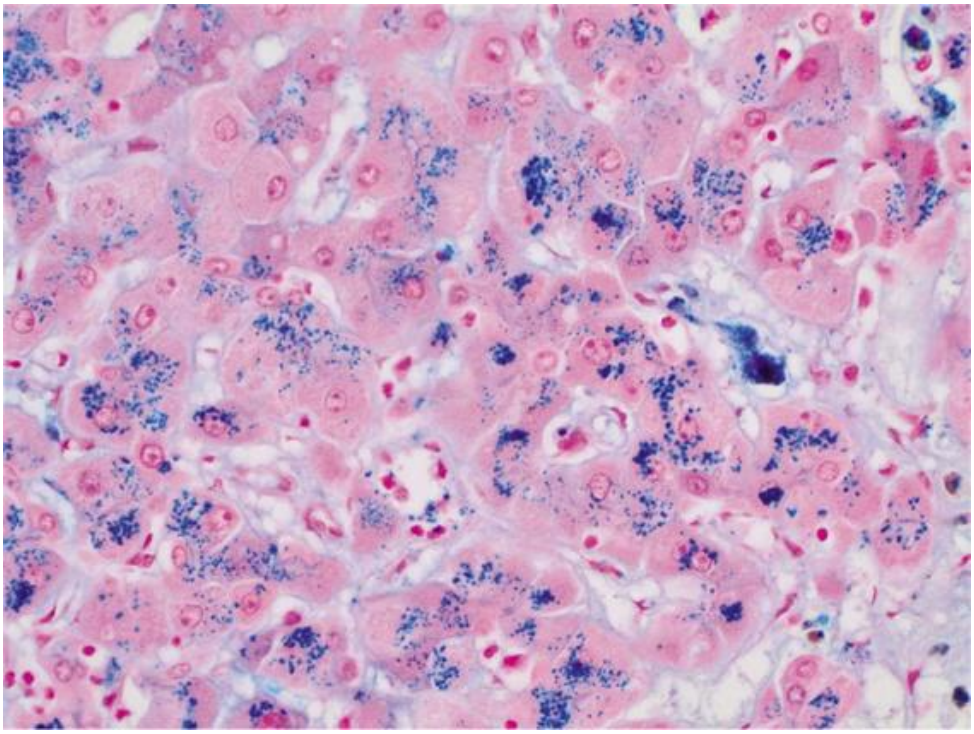


Fig. 2.12: Perl's stain. Liver with hemosiderin (blue) deposits.

Other Pigments

- **Hemochromatosis:** Severe accumulation of iron is associated with damage to liver, heart and pancreas. The triad of cirrhosis of liver, diabetes mellitus (due to pancreatic damage) and brown pigmentation of skin constitute **bronze diabetes**.
- **Hemozoin:** It is a brown-black pigment containing heme in ferric form. This pigment is seen in chronic malaria and in mismatched blood transfusions.
- **Bilirubin** is the normal major pigment found in bile. It is non-iron containing pigment derived from hemoglobin.
- **Lipofuscin.**

Q. Write short note on lipofuscin and brown atrophy of heart.

- Lipofuscin is an **insoluble golden-brown endogenous pigment**. It also called as **lipochrome or wear and tear pigment**.
- **Composition:** It is composed of mixture of lipids, phospholipids and proteins. **It is accumulated by accretion (a gradual increase) of peroxidized unsaturated lipids and oxidized cross-linked proteins**. The term lipofuscin is derived from the Latin word (*fuscus*, brown), and refers to brown lipid.
- **Significance:** It indicates a product of free radical injury and lipid peroxidation. Lipofuscin does not injure cell or its functions. It is observed in cells undergoing slow, regressive changes and is particularly prominent in the **liver and heart (often called brown atrophy of heart)** of aging patients or patients with severe malnutrition and cancer cachexia.
- **Appearance:** Microscopically, it appears as a yellow-brown, finely granular cytoplasmic pigment, often present in the perinuclear region.

Commonly used histochemistry (special stains) in histopathology are listed in **Table 2.4**.

Q. Write a short note on commonly used histochemistry (special stains) in histopathology.

Hemosiderin: Golden brown in color. Degradation product of ferritin.

Lipochrome/lipofuscin: Wear and tear pigment seen in old age, severe malnutrition, and cancer cachexia. Perinuclear in location. Derived through lipid peroxidation.

Lipofuscin: Important indicator of free radical injury.

Pigmentation of liver may be caused by:
1. Lipofuscin
4. Bile pigment
2. Malaria pigment
5. Pseudomelanin
3. Wilson disease

NECROSIS

CC: Describe and discuss cell death—types, mechanisms, necrosis, apoptosis (basic as contrasted with necrosis), autolysis (page 38).

Severe injury (e.g., loss of oxygen and nutrient supply, toxins) cause a rapid and uncontrollable form of death and is called "accidental" cell death. The morphological manifestation of inevitable end result of severe damage is **necrosis**. The necrosis occurs accidentally because the injury is so severe to be repaired and many cellular constituents fail or fall apart. It is not regulated by specific signals or biochemical mechanisms (in contrast to apoptosis).

Q. Define necrosis. Describe the various types of necrosis, causes and pathology of each with suitable examples. Add a note on gangrene.

Definition: Necrosis is the sum of the morphological changes indicative of cell death in a living tissue and caused by the progressive degradative action of enzymes following harmful injury. Necrosis is an **"accidental" and unregulated form of cell death**. It results from damage to cell membranes and loss of ion homeostasis.

The necrotic cells cannot maintain integrity of membrane and their contents including **cellular enzymes** leak out and **ultimately digest the cell.** This bring out acute **inflammatory reaction** in the surrounding tissue (local host reaction). The enzymes which digest the cell are derived from lysosomes of either dying cells themselves or from leukocytes recruited as part of the inflammatory reaction.

MORPHOLOGY (FIG. 2.13)

The general changes occurring in a necrotic cell:

Cytoplasmic Changes

Increased eosinophilia and have glassy, homogeneous appearance. When enzymes have digested cytoplasmic organelles, the cytoplasm becomes vacuolated and gives a "moth-eaten" appearance **(Fig. 2.13)**.

Nuclear Changes

These may take up one of three patterns and are the result of breakdown of DNA and chromatin.

- **Pyknosis:** Shrinkage of nucleus which appears shrunken and deeply basophilic (similar to ink drop).
- **Karyolysis:** Progressive fading of basophilic staining of the nuclei because of digestion of DNA by deoxyribonuclease (DNase) activity and leads to a ghost nuclei. In 1 to 2 days, the nucleus of a dead cell may completely disappear.
- **Karyorrhexis:** The pyknotic nucleus can undergo fragmentation into many smaller fragments. This change is called **karyorrhexis**.

Electron microscopic findings (Fig. 2.13): These include discontinuities in plasma and organelle membranes, marked dilation with the appearance of large amorphous densities in the mitochondria, disruption of lysosomes, and presence of intracytoplasmic myelin figures.

Fates of necrotic cells: After necrosis, these necrotic cells may either persist for some time or may be digested by enzymes and disappear. Dead cells may be replaced by myelin figures, which may be either phagocytosed by other cells or may undergo further degradation into fatty acids. These fatty acids can bind calcium salts and the necrotic cells may become calcified.

TABLE 2.4: Commonly used special stains in histopathology.

Stain	*Substance*	*Interpretation*
Amyloid		
Congo-red under polarizing microscope	Amyloid	Apple green birefringence
Carbohydrates		
Periodic acid-Schiff (PAS)	Glycogen, mucin, mucoprotein, glycoprotein, fungi, basement membranes of glomeruli and tubules	Magenta color
Mucicarmine/Best's carmine	Epithelial mucin	Red color
Alcian blue	Acid mucin	Blue
Lipids		
Sudan III	Lipid	Orange
Oil Red O		Red
Osmium tetroxide		Brown black
Connective tissue		
Van Gieson	Extracellular collagen	Red
Masson's trichrome	Collagen, smooth muscle	Collagen-blue, smooth muscle-red
Phosphotungstic acid hematoxylin (PTAH)	Cross striation of skeletal muscles, glial filaments, fibrin	Dark blue
Verhoeff's elastic	Elastic fibers	Black
Microorganisms		
Gram's stain	Bacteria	Gram +ve = blue Gram –ve = red
Ziehl-Neelsen's (acid-fast) stain	Tubercle bacilli and other acid-fast organisms	Red
Fite-Faraco	Lepra bacilli	Red
Silver methenamine	Fungi	Black
Pigments and minerals		
Prussian blue stain (Perl's stain)	Hemosiderin	Blue
Masson Fontana	Melanin	Black
Von Kossa	Calcium	Black
Alzarine Red S	Calcium	Orange red
Rubeanic acid	Copper	Greenish-black

Most common fixative for light microscopic examination: 10% buffered neutral formalin.

Most common fixative for electron microscopic examination: Glutaraldehyde.

Hematoxylin and eosin (H and E): Routine stain used in histopathology.

Morphological Patterns/Types of Tissue Necrosis

Most of the types of necrosis have distinctive gross appearances (except fibrinoid necrosis) but they can be identified only by microscopic examination.

Coagulative Necrosis

Q. Write short note on coagulative necrosis.

Common type, in which the outline of dead tissues (underlying tissue architecture) is preserved (at least for few days). **Infarct** is a localized area of coagulative necrosis. Coagulative necrosis is characteristic of infarcts (areas of necrosis caused by ischemia) in all solid organs except the brain.

- **Causes: Ischemia** caused by obstruction in a vessel.

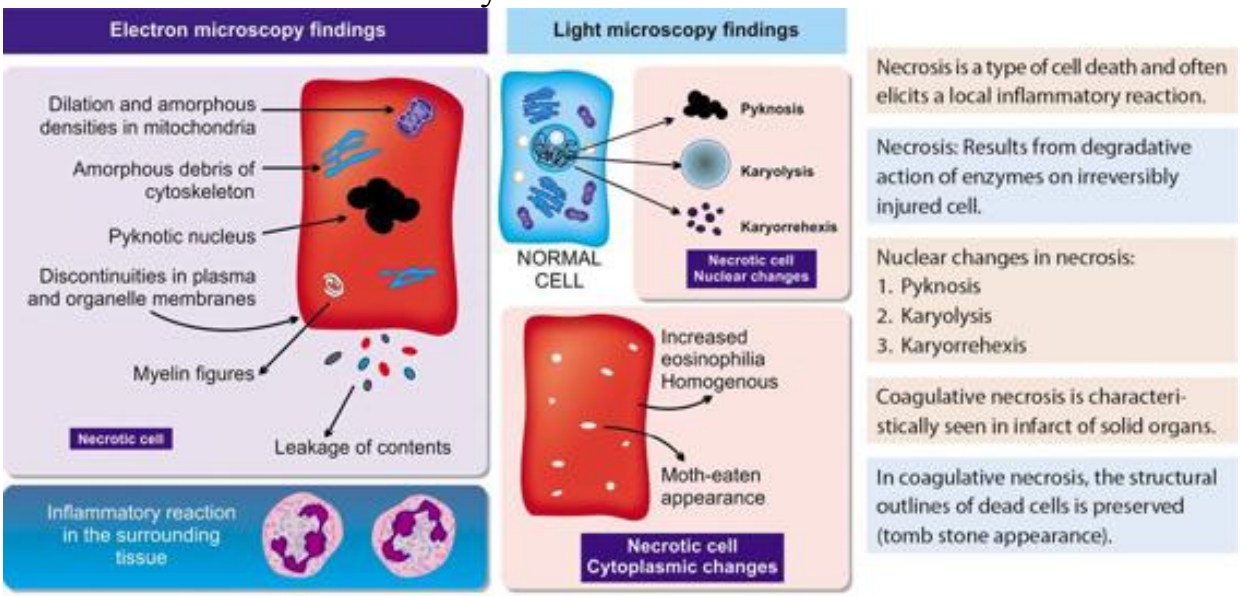


Fig. 2.13: Morphological changes in necrosis.

- **Mechanism:** Ischemia denatures and coagulates structural proteins and enzymes. This blocks the proteolysis of the dead cells.

MORPHOLOGY

Gross

- **Organs affected:** All organs except the brain. More frequent in organs supplied by end arteries with limited collateral circulation such as **heart** (*refer* **Fig. 16.17**), **kidney (Fig. 2.14A), spleen** (*refer* **Fig. 5.20A**) **and limb (dry gangrene)** (*refer* **Fig. 2.20A**).
- **Appearance:** The appearance of necrotic tissue is described as **coagulative necrosis** because it resembles the coagulation of proteins that occurs upon heating. Involved region appear **dry**, **pale**, **yellow and firm. It is wedge-shaped in organs like kidney (Fig. 2.14A) and spleen**.

Microscopy (Figs. 2.14B and C, and *refer*Figs. 16.18 and 20.39)

Indistinct outline of dead tissue. In coagulative necrosis, the normal cells are converted into their 'tomb stones', i.e., outlines of the cells are maintained and the cell type can still be recognized but their cytoplasmic and nuclear details are lost. Nucleus may be either absent or show karyolysis. Leukocytes are recruited to the site of necrosis and the lysosomal enzymes

of the leukocytes digest the necrotic cells. The resulting cellular debris are then removed by phagocytosis by infiltrating neutrophils and macrophages.

Liquefactive Necrosis (Colliquative Necrosis)

Q. Write short note on liquefactive/colliquative necrosis.

In this type of necrosis **dead tissue** rapidly **undergoes softening** and transforms into a liquid viscous mass.

- **Causes:**
  - **Ischemic injury to central nervous system (CNS)**
  - **Suppurative infections:** Infections by bacteria or fungus which stimulate the accumulation of the enzymes of leukocytes and digest ("liquefy") the tissue.
- **Mechanism:** Liquefaction is due to digestive action of the hydrolytic enzymes released from dead cells (autolysis) and leukocytes (heterolysis).

MORPHOLOGY

Gross

The necrotic cells are completely digested and is converted into a viscous liquid that is eventually removed by phagocytes. The involved area appear soft with liquefied center containing necrotic debris. It may later form a cystis space.

Organs affected are:

- **Brain:** Necrotic area is soft and center shows liquefaction **(Fig. 2.15)**. The reason for liquefactive necrosis in brain may be the presence of abundant lysosomal enzymes, or different hydrolases specific to cells of the CNS.
- **Abscess anywhere:** The bacterial infection causes acute inflammatory reaction and the resulting necrosis of tissue produces the liquid viscous material which is frequently creamy yellow and is called **pus. The localized collection of pus is termed abscess.**
- It is also seen in wet gangrene (*refer* **Fig. 2.20B**) and pancreatic necrosis.

Microscopy

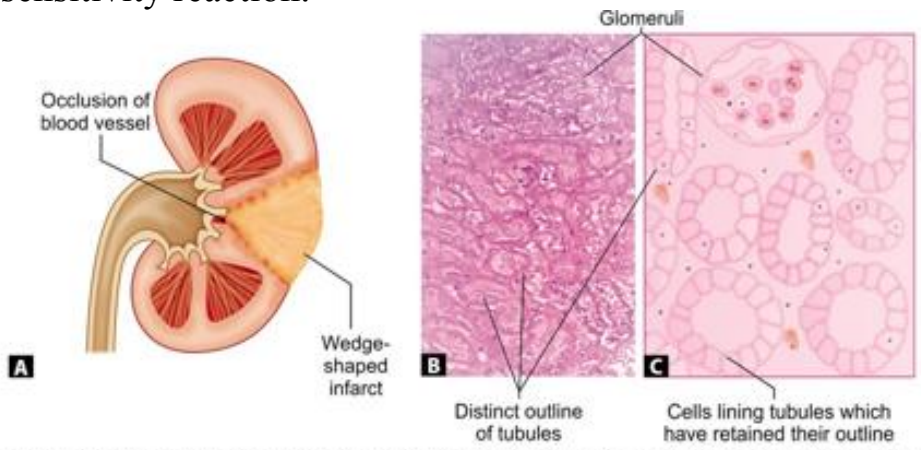
Pus consists of liquefied necrotic cell debris, dead leukocytes and macrophages (scavenger cells).

Caseous Necrosis

Q. Write short note on caseous necrosis.

It is a distinctive type of necrosis which shows **combined features of both coagulative and liquefactive necrosis**.

- **Cause:** Characteristic of **tuberculosis** and is due to the type IV hypersensitivity reaction.

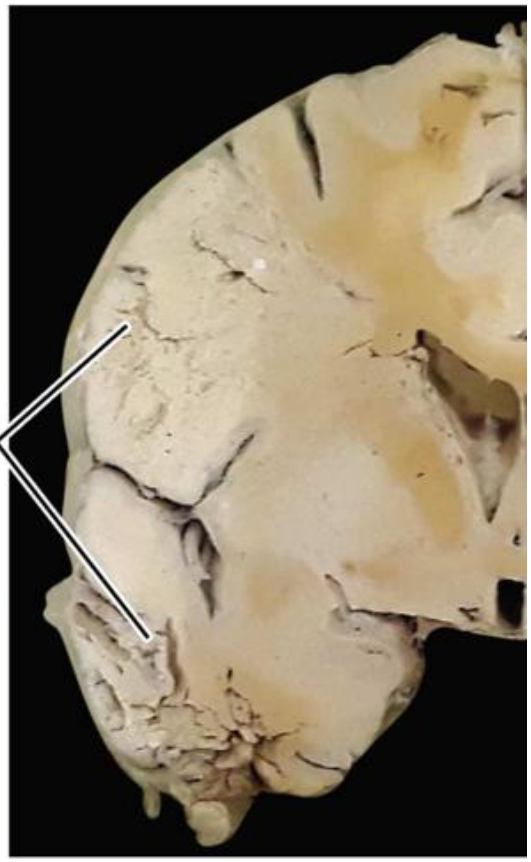


Figs. 2.14A to C: (A) Gross appearance of infarct of kidney; (B) H&E; (C) Diagrammatic microscopic appearance of infarcted area of kidney.

Fate of necrotic cell:

- Digestion by enzymes
- Replacement by myelin figures made up of phospholipids
- Calcification

Ischemic injury to CNS cause liquefactive necrosis and NOT coagulative necrosis.



Liquefactive necrosis in two infarct in the brain. The central area of each infarct is soft and necrotic

Liquefactive necrosis: Characteristically seen in ischemic injury to CNS and suppurative/pyogenic infections.

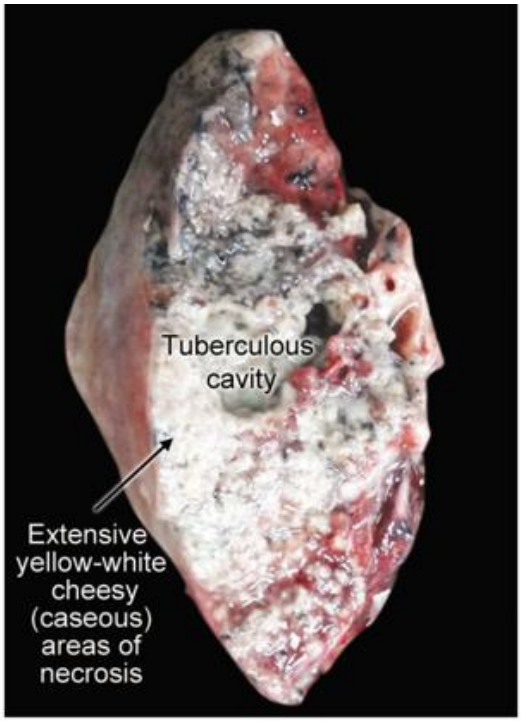


Fig. 2.16: Gross appearance of caseous necrosis. Lung shows cavity with caseous necrosis.

MORPHOLOGY

Gross

- **Organs affected:** Tuberculosis may involve any organ, most common in **lung** and **lymph node**.
- **Appearance:** Necrotic area appears **yellow-white**, **soft, friable, granular** and resembles **dry**, **clumpy cheese**, hence the name caseous (**cheese-like**) necrosis (**Fig. 2.16 and** *refer* **Fig. 17.18).**

Microscopy

- Focal lesion of tuberculosis is a **granuloma (Figs. 2.17, and** *refer* **Figs. 3.9 and 17.17)** which may be **caseating (soft granuloma) or noncaseating (hard granuloma)**.
- **Caseous necrosis** is a collection of fragmented or lysed cells and appears as **eosinophilic, coarsely granular material**. In contrast to coagulative necrosis, there is complete obliteration

of the tissue architecture and it is not possible to identify the cellular outlines. It is surrounded by epithelioid cells; Langhans type giant cells (nuclei arranged in a horse-shoe pattern), lymphocytes and fibroblasts forming granuloma.

- Caseous necrotic material **may undergo dystrophic calcification**.

Fat Necrosis

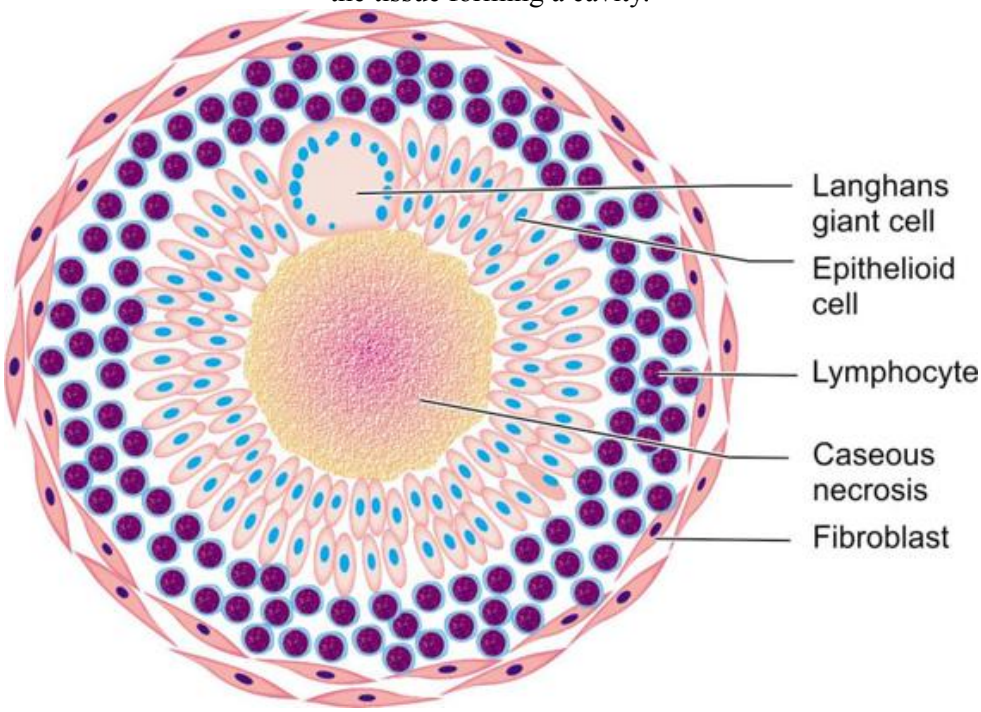
Q. Write short note on fat necrosis.

It refers to **focal areas of fat destruction**, which affects adipose tissue. It occurs mainly at anatomic locations rich in fat.

Types:

1. **Enzymatic fat necrosis:** Occurs in adipose tissue around acutely inflamed pancreas (in **acute pancreatitis**).
   - **Mechanism:** In pancreatitis, the enzymes (one of them is lipase) leak from acinar cells into the substance of the pancreas and the peritoneal cavity and causes tissue damage. Lipase destroys fat cells, splits the triglyceride esters within fat cells and liberates free fatty acids which combine with calcium and form calcium soaps (fat saponification).

Fig. 2.15: Liquefactive necrosis. An infarct in the brain shows soft necrotic area with dissolution of the tissue forming a cavity.



MORPHOLOGY

Gross

Appears as **chalky-white areas (Fig. 2.18A)** which helps both the surgeon and the pathologist to grossly identify the lesions.

Microscopy

The necrotic **fat cells appear pale with shadowy outlines surrounded** by basophilic calcium deposits and an inflammatory reaction **(Fig. 2.18B)**.

2. **Traumatic fat necrosis:** Occurs in tissues with high fat content (like in **heavy, pendulous breast and thigh**) following severe trauma.

Fibrinoid Necrosis

It is a special form of necrosis. Characterized by **deposition of pink-staining (fibrin-like) proteinaceous material** in the tissue matrix with a **staining pattern reminiscent of fibrin**. It obscures the underlying cellular detail.

- **Causes:** Usually seen in **immune-mediated** (deposition of antigen-antibody complexes in the wall of vessels) **vascular injury/vasculitis** (e.g., polyarteritis nodosa), **malignant hypertension, Aschoff bodies** in rheumatic heart disease, placenta in **preeclampsia**, or **hyperacute transplant rejection**.

Microscopy

The fibrinoid necrosis appear as brightly eosinophilic, hyaline-like deposit in the vessel wall **(Fig. 2.19)**. It is better appreciated under phosphotungistic acid hematoxylin (PTAH) stain.

Gangrene (Gangrenous Necrosis)

Q. Define gangrene. Mention its types and differences between them.

Definition: It is **massive necrosis with superadded putrefaction**.

Types: Two types, namely **dry and wet gangrene**. A variant of wet gangrene known as **gas gangrene** is **caused by clostridia** (Gram-positive anaerobic bacteria).

Dry Gangrene

Q. Write a short note on dry gangrene.

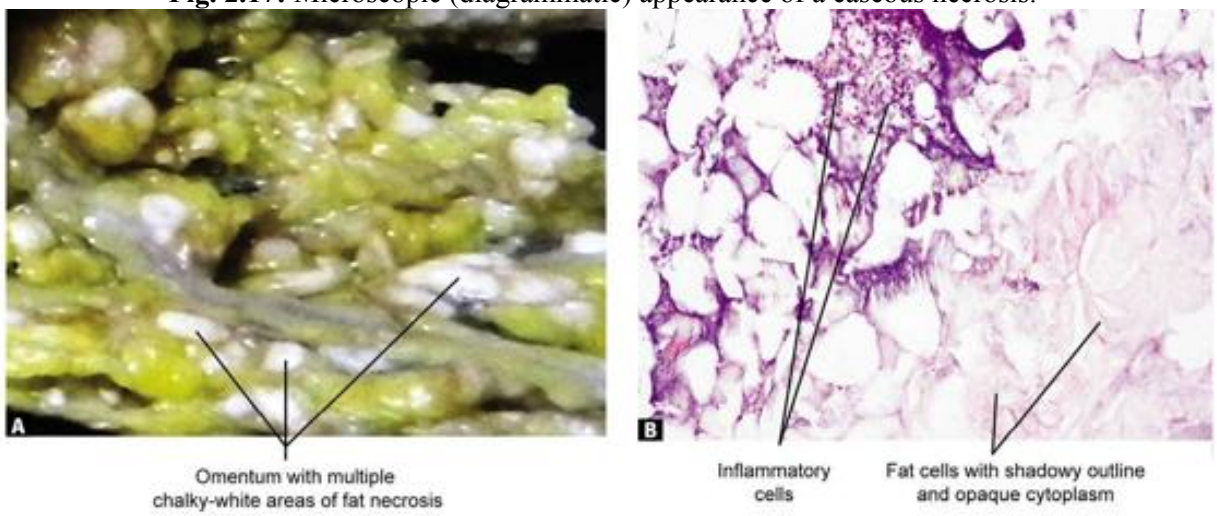
- **Causes: Arterial occlusion** (e.g., atherosclerosis).
- **Sites:** It usually involves a **limb**, generally the distal part of lower limb (leg, foot, and toe).

MORPHOLOGY

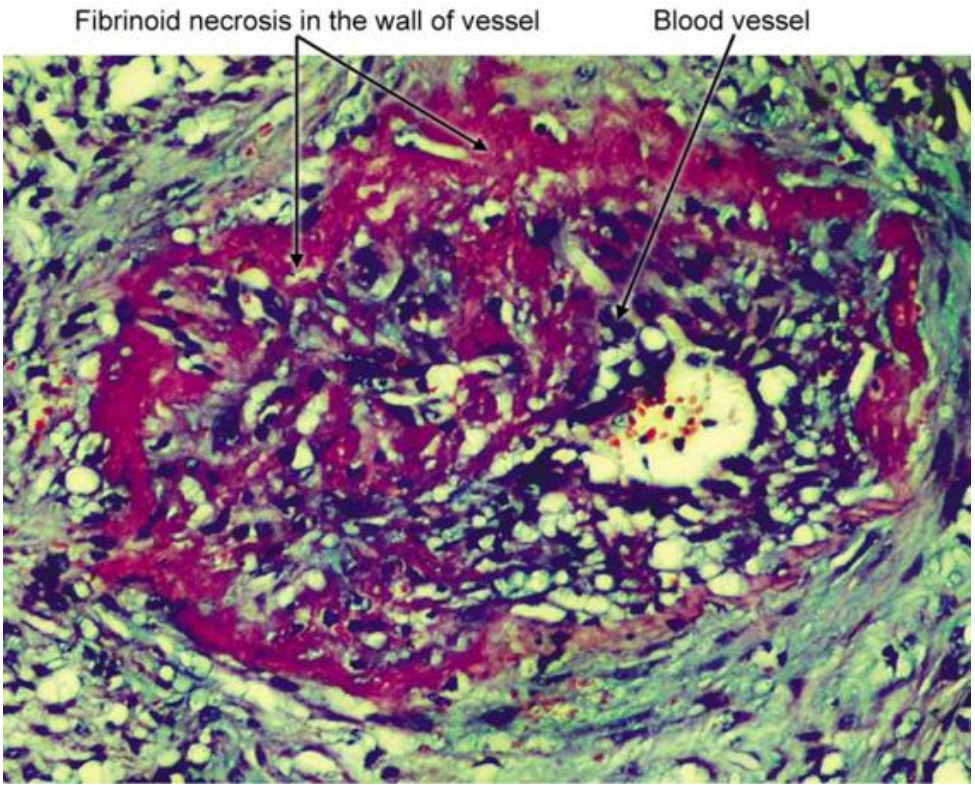
Gross

Affected part is **dry, shrunken** (shriveled) and **dark brown or black** resembling the foot of a **mummy**. The black color is due to the iron sulfide. A **line of demarcation** is seen between gangrenous and adjacent normal area **(Fig. 2.20A)**.

Fig. 2.17: Microscopic (diagrammatic) appearance of a caseous necrosis.



Figs. 2.18A and B: (A) Omentum shows multiple chalky-white areas of fat necrosis caused by acute pancreatitis; (B) Fat necrosis shows necrotic fat cells in the right lower part and inflammatory reaction between normal (left upper area) and area of fat necrosis.



Microscopy

The necrosis (coagulative type) shows smudging of soft tissue and overlying skin. The line of demarcation consists of granulation tissue with inflammatory cells.

Wet Gangrene

Q. Write a short note on wet gangrene.

- **Causes: Due to the venous blockage** (e.g., strangulated hernia, intussusception or volvulus). Diabetic patients may develop both dry (due to atherosclerosis of tibial and popliteal arteries) and wet gangrene. It usually follows a minor injury to toe or foot.
- **Sites:** Occurs in **moist tissues or organs** (e.g., bowel, lung, mouth, etc.).

MORPHOLOGY

Gross

The affected part is soft, swollen, putrid and dark **(Fig. 2.20B)**. No clear line of demarcation.

Microscopy

Liquefactive type of necrosis.

Differences Between Dry and Wet Gangrene (Table 2.5)

Q. List the differences between dry and wet gangrene.

Q. Write a short answer on gas gangrene.

Gas gangrene: Special type of wet gangrene caused by infection with a gas forming anaerobic *Clostridium perfringens*. These organisms enter into the tissues through open contaminated wounds (e.g., muscles, complication of operative procedures on colon). Toxins produced by them cause local necrosis **(Fig. 2.20C)** and edema and are also absorbed causing severe systemic manifestations.

Gummatous Necrosis

The necrotic tissue is firm and rubbery and is usually found in syphilis (*refer* page 92).

Laboratory Detection of Necrosis

Whenever there is necrosis, there is leakage of intracellular proteins through the damaged cell membrane which ultimately reaches the circulation. Thus, it is possible to detect tissue-specific necrosis using blood or serum samples. Examples include (i) cardiac muscle contains a unique isoform of the enzyme creatine kinase and of the contractile protein troponin, (ii) hepatic bile duct epithelium contains the enzyme alkaline phosphatase, and (iii) hepatocytes contain transaminases. Necrosis of these tissues elevate the serum levels of these proteins and are useful markers of tissue damage.

Ultrastructural differences between reversible and irreversible injury is presented in **Table 2.6**.

Liquefactive necrosis: Dead cells are transformed into a liquid viscous mass due to enzymes released from leukocytes accumulated at the site of necrosis.

Caseous necrosis: Cheese-like appearance of the necrotic material.

Caseous necrosis with granuloma is observed in tuberculosis and systemic fungal infections (e.g., histoplasmosis). It is due to the presence of high lipid content in the cell wall in these organisms.

Fig. 2.19: Fibrinoid necrosis in the wall of blood vessel [Phosphotungistic acid hematoxylin (PTAH) stain].



Figs. 2.20A to C: (A) Dry gangrene of left leg shows dry shrunken discolored gangrenous foot separated from adjacent normal area by a line of demarcation; (B) Wet gangrene of small intestine; (C) Gas gangrene in upper limb.

TABLE 2.5: Differences between dry and wet gangrene.

Characteristics	***Dry***	***Wet***
General features		
Common site	Limbs	Bowels
Examples	Gangrene of lower limb due to atherosclerosis	Volvulus, intussusception
Etiological factors		
Cause of ischemia	Arterial obstruction	Commonly venous obstruction
Rate of obstruction	Slow	Abrupt
Gross features		
Appearance of involved part	Shriveled, dry (mummification) and black	Swollen, soft and moist/wet
Line of demarcation	Clear cut between gangrenous and healthy part	Not clear cut
Spread	Slow	Rapid
Prognosis	Fair	Poor due to severe septicemia

Enzymatic fat necrosis is mediated by enzymes whereas traumatic is not mediated by enzymes.

Enzymatic fat necrosis in acute pancreatitis appears as chalky- white areas which help in its gross identification.

Fibrinoid necrosis: Necrotic material appears similar to fibrin and is not fibrin.

Fibrinoid necrosis: Seen in immune-mediated diseases
1. Polyarteritis nodosa
2. Malignant hypertension
3. Autoimmune disorder—systemic lupus erythematosus (SLE)
4. Aschoff bodies in rheumatic fever

Dry gangrene predominantly consists of coagulative type of necrosis.

Wet gangrene predominantly consists of liquefactive type of necrosis. Gas gangrene is a variant of wet gangrene caused by bacteria *Clostridium perfringens* (Gram-positive anaerobic bacteria).

Fournier's gangrene: Seen in scrotal skin.

APOPTOSIS

Q. Write long essay on definition, causes and the mechanism of apoptosis.

Q. Write short note on apoptosis.

Definition: Apoptosis is a **type of (programmed) cell death induced by a tightly regulated suicide program**.

It is characterized by **activation of intrinsic enzymes of the cell that degrade its own nuclear DNA** and **proteins** (both nuclear and cytoplasmic). Fragments of the apoptotic cells (apoptotic bodies) break and fall off from the cells and hence named apoptosis (meaning "falling off").

Causes of Apoptosis

Physiological Situations

- **Removal of excess cells during embryogenesis and developmental processes** (e.g., disappearance of web tissues between fingers and toes).

Q. Describe the ultrastructural changes in reversible cell injury. List the differences between reversible and irreversible injury.

TABLE 2.6: Ultrastructural differences between reversible and irreversible injury.

TABLE 2.6: Ultrastructural differences between reversible and irreversible injury

Structure involved	*Reversible injury*	*Irreversible injury*
Plasma membrane changes	Blebbing, blunting, loss of microvilli	Discontinuities in plasma and organelle membrane
Mitochondrial changes	Swelling and appearance of small amorphous densities	Marked dilatation with appearance of large amorphous densities (precipitated calcium), aggregates of fluffy material (denatures protein)
Endoplasmic reticulum	Dilatation with detachment of polysomes	Swelling and fragmentation
Myelin figure (large intracellular whorled phospholipid masses)	May be present	Usually present
Nuclear changes	Disaggregation of granular and fibrillar elements	Pyknosis, karyolysis and karyorrhexis

Irreversible injury: Large amorphous densities in mitochondria.

- **Elimination of cells after withdrawal of hormonal stimuli** (e.g., endometrial cell breakdown during the menstrual cycle).
- **Elimination of cells after withdrawal of tropic stimuli** (e.g., neutrophils in an acute inflammatory response, lymphocytes after immune response).
- **Elimination of potentially harmful cells:** In immunology, the clones of **self-reactive lymphocytes** that recognize normal self-antigens are deleted by apoptosis.

Pathological Conditions

Apoptosis eliminates cells that are genetically altered or damaged beyond repair. It is responsible for cell loss in many pathologic states:

- **Elimination of cells with damaged DNA:** DNA may be damaged by many injurious agents like radiation, cytotoxic anticancer drugs and hypoxia.
  - ***p53* triggered apoptosis:** Mainly tumor-suppressor gene *p53* recognizes cells with damaged DNA and assesses whether it can be

repaired. If the damage is too severe to be repaired, *p53* triggers apoptosis.

- **Destroying cells with dangerous mutations or with DNA damage** beyond repair by apoptosis prevents the development of cancer.
- In certain cancers, where *p53* is mutated or absent, the apoptosis is not induced in cells with damaged DNA.

- **Elimination of cells with excessively accumulated misfolded proteins:** Mutations in the genes encoding proteins or extrinsic factors (damage due to free radicals) may result in accumulation of unfolded or misfolded proteins.
  - Excessive intracellular accumulation of these abnormally folded proteins in the ER is known as **ER stress**, which results in apoptotic cell death.
  - Apoptosis caused by the accumulation of misfolded proteins is found in several degenerative diseases of the central nervous system (Alzheimer, Huntington, and Parkinson diseases) and other organs.
- **Killing of viral infected cells:** In viral infections, the infected cells are lost mainly due to apoptosis induced either by the virus (as in adenovirus and HIV infections) or by host human response by cytotoxic T lymphocytes (as in viral hepatitis).
- **Elimination of neoplastic cells/rejection of transplant:** The T-cell-mediated mechanism is responsible for apoptosis in tumors and cellular rejection of transplants.
- **Elimination of parenchymal cells in pathologic atrophy:** Obstruction of duct in the **parenchymal** organs like pancreas, parotid gland and kidney can lead to apoptosis of the parenchymal cells.

MORPHOLOGY

Electron Microscope

Q. Write short note on morphology of apoptosis.

The ultrastructural features of apoptosis **(Fig. 2.21)** are:

- **Cell shrinkage:** Cytoplasm becomes dense.
- **Nuclear condensation and fragmentation:** Chromatin aggregates peripherally under the nuclear membrane. The nucleus may break up to produce two or more nuclear fragments.
- **Formation of cytoplasmic blebs and apoptotic bodies:** Cell first shows extensive surface blebbing followed by fragmentation into membrane-bound apoptotic bodies. The

apoptotic bodies are composed of cytoplasm and tightly packed organelles, with or without nuclear fragments.

- **Phagocytosis of apoptotic cells/bodies:** The apoptotic bodies are rapidly ingested by phagocytes **(usually by macrophages)** and degraded by the lysosomal enzymes of phagocytes.

Light Microscopy

The apoptotic cells appear as round or oval mass having intensely **eosinophilic** cytoplasm. The **nuclei appear as fragments of dense nuclear chromatin** and shows pyknosis. Apoptosis **does not elicit an inflammatory reaction** in the host.

Mechanisms of Apoptosis

Q. Write short note on mechanism of apoptosis.

Apoptosis is regulated by biochemical pathway. The survival or apoptosis of many cells depends upon balance between **two opposite sets of signals** namely: **(1) death signal** (proapoptotic) and **(2) prosurvival** (anti-apoptotic) signals. Unlike necrosis, apoptosis engages the cell's own signaling cascades and results in its own death (suicide). Apoptosis results from **activation of enzymes** called as **caspases** (i.e., they are cysteine proteases that cleave proteins after aspartic residues).

Phases of Apoptosis

Divided into (A) initiation phase and (B) execution phase.

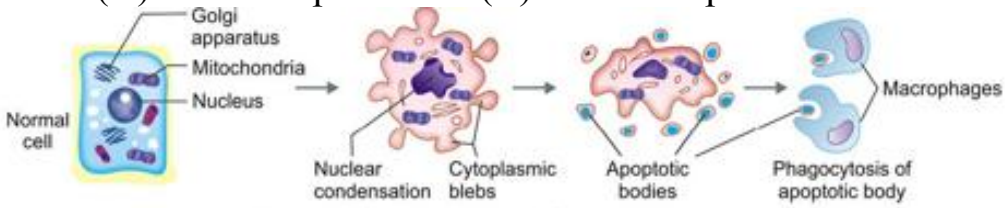


Fig. 2.21: Electron microscopic changes in apoptosis.

A. Initiation Phase

Apoptosis is initiated by signals derived from two distinct pathways activated by distinct stimuli, namely: (1) intrinsic or mitochondrial pathway and (2) extrinsic or death receptor pathway.

1. *Intrinsic (mitochondrial) pathway of apoptosis (Fig. 2.22):*

Mitochondrial damage is the major mechanism in a variety of physiological and pathological apoptosis. It is activated by intracellular signals.

- **Role of mitochondria in apoptosis:**
  - **Mitochondria contain** proteins capable of inducing apoptosis. These include: **cytochrome c and several proapoptotic proteins**.
  - Survival or apoptosis of cell is determined by permeability of mitochondria.
  - **Mitochondrial permeability is controlled by *BCL2* family** of more than 20 **proteins**. This family is named after *BCL2*, which was identified as an oncogene in a **B-cell** lymphoma. These proteins may be broadly divided into proapoptotic or antiapoptotic (prosurvival).
    - **Proapoptotic proteins: *BAX* and *BAK***
    - **Antiapoptotic proteins: *BCL2*, *BCL-XL*, and *MCL1***. They prevent leakage of mitochondrial proteins that trigger apoptosis. Growth factors and other survival signals stimulate production of antiapoptotic proteins.

    If the balance shifts to proapoptotic proteins, the apoptotic cascade is activated.
- **Causes of mitochondrial injury:** The proapoptotic signals include:
  - Deprivation/withdrawal of growth factor or survival signals.
  - DNA damage by radiation, cytotoxic anticancer drugs, hypoxia either directly or through free radical.
  - Accumulation of excessive amount of misfolded proteins (endoplasmic reticulum stress).
  - Increased intracellular free calcium.
- **Steps in intrinsic (mitochondrial) pathway:** Mitochondrial injury causes increased mitochondrial permeability and **release proapoptotic molecules (death inducers) into the cytoplasm**. The different steps are as follows:
  - The above mentioned causes of mitochondrial injury activate a number of sensors of *BCL2* **family** called BH3-proteins (BH = *BCL2* homology). They in turn activate **two critical** proapoptotic ***BCL2* family** effector proteins, namely ***BAX*** and ***BAK***.

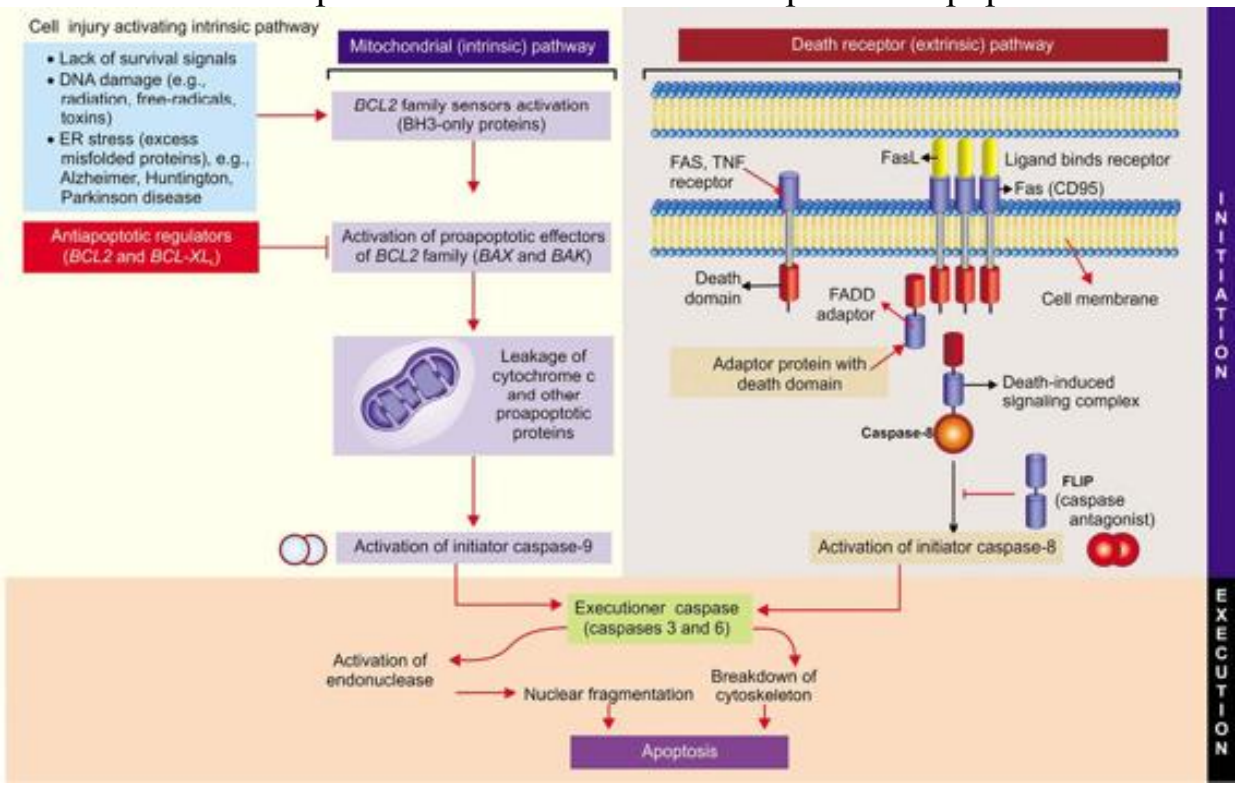
- ***BAX*** and ***BAK*** create channels in the mitochondria that allow release of several **mitochondrial** proteins from the inner mitochondrial membrane to leak out into the cytosol (cytoplasm).
- One of these proteins is **cytochrome c** which binds to a protein called **apoptosis-activating factor-1 (Apaf-1)** and forms an important caspase cascade activator called **apoptosome**. This complex binds to caspase-9, the critical initiator caspase of the mitochondrial pathway which sets in an auto-amplification process.
- The cytoplasm of the normal cells contains proteins which block the activation of caspases and function as physiologic **inhibitors of apoptosis (called IAPs)**. Other mitochondrial proteins may enter the cytoplasm and **neutralize** these IAPs and initiate caspase cascade.
- Sensors of ***BCL2*** **family** namely BH3-only proteins also bind to and **block the function of** protective **antiapoptotic proteins** namely ***BCL2*** and ***BCL-XL***.

2. *Extrinsic (death receptor-initiated) pathway of apoptosis (Fig. 2.22):*

- This pathway is **initiated by extracellular signals**.
- **Many cells express "death-receptors" molecules on the surface of plasma membrane that trigger apoptosis**. Death receptors are **member of the TNF (tumor necrosis factor) receptor family** that contain a cytoplasmic domain called the **death domain** because it is essential for delivering apoptotic signals.
- In the extrinsic (death receptor) pathway, **apoptosis is initiated when** the **death receptors** present gets **activated**.
- The **well-known death receptors are the type 1 TNF receptor (TNFR1) and a related protein called Fas (CD95)**. Fas death receptor is expressed on many cell types and the binding ligand for Fas is called Fas ligand (FasL/CD95L).
- **Functions of extrinsic pathway:** This pathway is **involved in eliminating:**
  - **Self-reactive lymphocytes** thereby avoiding autoimmunity. FasL is expressed on T-cells that recognize self-antigens and function to eliminate self-reactive lymphocytes.
  - **Virus infected cells** through cytotoxic T lymphocytes.

- **Tumor cells** through cytotoxic T lymphocytes.

- Steps in extrinsic pathway:
  - Extrinsic pathway become activated when CD95/Fas binds to its ligand CD95L/FasL.
  - When FasL binds to Fas receptors, their cytoplasmic death domains binds with an **adapter protein**. This adapter protein also contains a death domain and is called Fas-associated death domain (FADD).
  - FADD in turn binds to pro-caspase-8 (an inactive form of caspase-8) via a death domain and generate active caspase-8.
  - Activated caspase-8 mediate the execution phase of apoptosis.



Proapoptotic proteins:
1. *BAX* 2. *BAK*

Antiapoptotic proteins:
1. *BCL2* 2. *BCL-XL*
3. *MCL1*

Proteins that regulate balance between proapoptotic and anti-apoptotic proteins:
1. BAD
4. Puma
2. BIM
5. Noxa

3. BID

Fig. 2.22: Mechanism of apoptosis.

B. Execution Phase of Apoptosis (Fig. 2.22)

- The above mentioned two initiating pathways produce initiator caspases namely: (1) the **mitochondrial pathway activates initiator caspase-9**, and (2) the **death receptor pathway activates the initiator caspase-8**.
- The initiator caspases activate another series of caspases called **executioner caspases (such as caspase-3 and -6)** that mediates the final phase of apoptosis.
- Executioner caspases act on many cellular components and activate DNase, which induces fragmentation of nuclei.
- Caspases also degrade components of nuclear matrix and cytoskeleton resulting in fragmentation of involved cells.

Removal of Apoptotic Cells

Phagocytosis: Apoptotic cells and bodies are engulfed and removed by phagocytic cells (mainly macrophages).The phagocytosis is so efficient that these dead cells and apoptotic bodies disappear within minutes. Even when the apoptosis is extensive their rapid removal prevents release of their cellular contents which may elicit inflammation.

- **Factors favoring phagocytosis:** The apoptotic cells and apoptotic bodies undergo several changes in their membranes and produce signals that favor phagocytosis of these cells/bodies.
  - **Expression of phosphatidylserine:** In healthy cells, **phosphatidylserine** is present on the inner leaflet of the plasma membrane. In cells undergoing apoptosis **phosphatidylserine** turns out and is expressed on the outer layer of the membrane causing easy recognition by receptors present on the macrophage.
  - **Secretion of soluble factors: Apoptotic cells secrete soluble factors** (e.g., **thrombospondin**) that recruit phagocytes.
  - **Natural antibodies and proteins of the complement system** may coat apoptotic bodies which aids in phagocytosis.

Diagnosis/Detection of Apoptosis

1. DNA fragmentation assay is carried out by electrophoresis of genomic DNA. Apoptosis produces "step ladder pattern" in contrast to smeared pattern seen in necrosis.
2. **T**erminal deoxynucleotidyl transferase biotin d-**U**TP **N**ick **E**nd **L**abeling (TUNEL) technique for in vivo detection of apoptosis.
3. Chromatin condensation seen by hematoxylin and eosin, Feulgen and acridine orange staining.
4. Estimation of:
   - Cytosolic cytochrome c
   - Activated caspase
   - Annexin V: Apoptotic cells express phosphatidylserine on the outer layer of plasma membrane because of which these cells are recognized by the dye Annexin.
   - Propidium iodide assay by flow cytometry/fluorescent microscopy.

Disorders Associated with Dysregulated Apoptosis

Disorders with Reduced Apoptosis

It may allow the survival of abnormal cells.

- Cancer
- Autoimmune disease.

Disorders with Increased Apoptosis

This will cause an excessive loss of cells.

- **Neurodegenerative diseases (Alzheimer, Huntigton, Parkinson disease)**.
- **Ischemic injury:** In myocardial infarction and stroke.
- **Death of virus-infected cells:** Many viral infections, important being acquired immune deficiency syndrome (**AIDS**).

Clinical Significance of Apoptosis in Cancers

- Normally, cells with damaged (mutated) DNA are cleared in the body by undergoing apoptosis.
- Apoptosis may be reduced in some cancers. Best established role of *BCL2* in protecting tumor cells from undergoing apoptosis is observed in

follicular lymphoma. In this type of non-Hodgkin lymphoma of B cell origin, there **is translocation (14; 18) (q32; q21)** which causes **over expression of antiapoptotic protein *BCL2*.** This in turn increases the *BCL2*/*BCL-XL* buffer, **protecting abnormal B lymphocytes from undergoing apoptosis** and allows them to survive for long periods.

Q. List the differences between apoptosis and necrosis.

Differences between apoptosis and necrosis are summarized in **Table 2.7**.

Apoptosis: Affects only single or small group of cells. Apoptosis may be physiological or pathological.
Apoptosis: Organelle that plays a pivotal role is mitochondria.
Light microscopic characteristics of apoptosis: • Condensation of nucleus (pyknotic) • Deeply eosinophilic cytoplasm
• Glucocorticoids induce apoptosis • Sex steroids inhibit apoptosis
BCL2 family genes located on chromosome 18.
Apoptosis: Apaf-1 is activated by the release of cytochrome c from mitochondria.
CD 95 (FAS) has a major role in apoptosis and is molecular marker for apoptosis.

TABLE 2.7: Differences between apoptosis and necrosis.

Features	*Apoptosis*	*Necrosis*
Cause	Often physiological, means of eliminating unwanted cells; may also be pathological	Invariably pathological
Biochemical events	Energy-dependent fragmentation of DNA by endogenous endonucleases	Impairment or cessation of ion homeostasis
Lysosomes	Intact	Leak lytic enzymes
Morphology		
Extent	Single or small cluster of cells	Involves group of cells
Cell size	Cell reduced (shrinkage) and fragmentation to form apoptotic bodies with dense chromatin	Cell enlarged (swelling) and undergo lysis
Integrity of cell membrane	Maintained	Disrupted/lost
Nucleus	Fragmentation into nucleosome-size fragments	Pyknosis, karyorrhexis, karyolysis
Cellular contents	Intact; may be released in apoptotic bodies	Enzymatic digestion; may leak out of cell
Adjacent inflammatory response	None	Usual
Fate of dead cells	Ingested (phagocytosed) by neighboring cells	Ingested (phagocytosed) by neutrophil polymorphs and macrophages
DNA electrophoresis	DNA laddering is seen	Shows smearing effect
TUNEL staining	Positive	Negative

Apoptosis: No inflammatory response from adjacent tissue.

Leakage of proteins from the necrotic cells into the circulation is useful for identifying the necrosis using blood and serum samples.

Apoptosis: Extrinsic pathway through TNFRI.

Caspases: Initiators (caspase 8, 9 and 10) and executioners (caspase 3 and 6).

Mechanism of apoptosis has two major steps namely initiation and execution. Apoptosis is mediated by caspases.

Apoptosis is a regulated mechanism of cell death with the least possible reaction by host.

Annexin V on non-permeable cell is indicative of apoptosis. Annexin attaches to cell surface.

Apoptosis: Ladder pattern of DNA electrophoresis is caused by enzyme endonuclease.

OTHER PATHWAYS OF CELL DEATH

Necroptosis

It is a type of cell death that shows features of both necrosis and apoptosis. It is caspase-independent. It resembles morphologically necrosis and mechanistically apoptosis.

Pyroptosis

It is a type of programmed cell death accompanied by the release of fever producing cytokine IL-1 and bears some biochemical similarities with apoptosis.

Autolysis

Autolysis (means self-lysis) is destruction of the cell by its own hydrolytic enzymes released from lysosomes. Autolysis is generally reserved for postmortem change. It develops rapidly in some tissues rich in hydrolytic enzymes such as pancreas and gastric mucosa. It occurs little slowly in tissues such a the heart, liver and kidney; and slow in fibrous tissue. Microscopically, the cellular details are lost and they appears as cells with homogeneous and eosinophilic cytoplasm.

Differences between necrosis and autolysis are listed in **Table 2.8**.

Overview of cell injury in presented in **Flowchart 2.2**.

PATHOLOGIC CALCIFICATION

CC: Describe and discuss pathologic calcifications, gangrene (*refer* pages 31-3).

Q. Write short note on pathologic calcification.

Definition: Abnormal **deposition of calcium salts in tissues other than osteoid or enamel**. It is also associated with deposition of small amounts of iron, magnesium and other minerals.

Types of pathologic calcification are: (1) dystrophic and (2) metastatic.

Dystrophic Calcification

Q. Write short note on dystrophic calcification.

Deposition of calcium salts in **dying or dead tissues**.

Causes

- **Necrotic tissue:** Calcification in **caseous, enzymatic fat necrosis**, in **dead eggs** of *Schistosoma*, cysticercosis and hydatid cysts.

- **Degenerating tissue:**
  - **Heart valves:** Occurs in aging or damaged heart valves
  - **Atherosclerosis, goiter of thyroid, dense old scar, cysts (e.g., epidermal and pilar cysts of skin).**

Q. Write short answer on Monckeberg's medial calcific sclerosis.

- **Monckeberg's medial calcific sclerosis:** Calcification in the media of the muscular arteries **(Fig. 2.23A)** in old people.
- **Psammoma bodies:** Single necrotic cells on which several layers of mineral get deposited progressively to create lamellated shape called **psammoma bodies (Fig. 2.23B).**

Metastatic Calcification

Q. Write short note on metastatic calcification.

Deposition of calcium salts in apparently **normal tissues**. It is associated with hypercalcemia **secondary to deranged calcium metabolism**.

Causes

Q. Write a short answer on causes of metastatic calcification.

- **Increased secretion of parathyroid hormone** (PTH) with subsequent bone resorption-hyperparathyroidism.
- **Destruction of bone tissue:** Secondary to primary tumors of bone marrow (e.g., multiple myeloma, leukemia and metastatic tumors to bone).
- **Vitamin D-related disorders:** Vitamin D intoxication.
- **Renal failure:** Causes retention of phosphate, leading to secondary hyperparathyroidism.
- **Others:** Sarcoidosis and milk alkali syndrome.

TABLE 2.8: Differences between necrosis and autolysis.

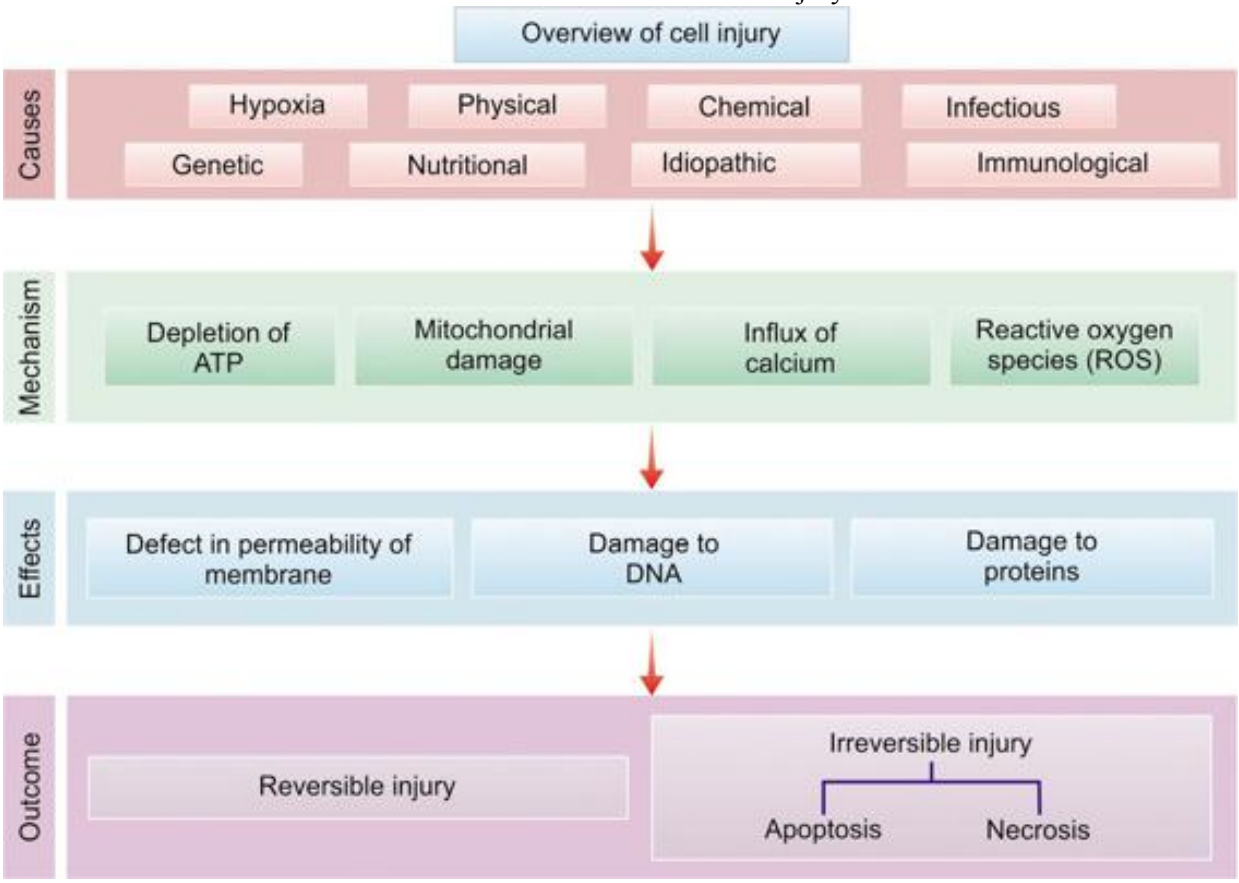
Features	*Necrosis*	*Autolysis*

TABLE 2.8: Differences between necrosis and autolysis.

Features	*Necrosis*	*Autolysis*
Definition	Morphologic changes following cell death in living tissue	Self-digestion or disintegration of cells by enzymes liberated from own lysosomes
Occurrence	In living tissue	After death of cells
Inflammatory reaction	Present	Absent
Calcification	May occur	Absent

Caspase independent programmed cell death is called necroptosis.

Flowchart 2.2: Overview of cell injury.



Q. Write short note on psammoma bodies.

Psammoma body is a dystrophic calcification in which several layers of mineral (calcium) get deposited progressively over a single necrotic cells.

Psammoma body found in:

1. Papillary carcinoma of thyroid
2. Papillary serous cystadenoma of ovary
3. Papillary serous cystadenocarcinoma of ovary
4. Menigioma
5. Papillary carcinoma of the kidney

Sites

- **Lungs: Alveolar septa of the lung**.
- **Kidney: Basement membrane of the renal tubules**.
- **Blood vessels:** On the internal elastic lamina of **systemic arteries and pulmonary veins**.
- **Stomach: Interstitial tissues of the gastric mucosa**.

Common site for metastatic calcification
1. Lungs (most common site)
2. Kidney
3. Blood vessels (e.g., systemic arteries and pulmonary veins)
4. Stomach

MORPHOLOGY OF CALCIFICATION

- **Gross:** Appear as fine, white granules or clumps, feels gritty and sand-like.
- **Microscopy:** Basophilic, amorphous, granular **(Figs. 2.23A and B)**, clumped appearance.

Differences between dystrophic and metastatic calcification are presented in **Table 2.9**.

Pathological calcification:
1. Dystrophic or
2. Metastatic

Dystrophic calcification:
1. Occurs in dead or degenerating tissues
2. Serum calcium level normal
3. Often causes organ dysfunction

ABCDE of dystrophic calcification:

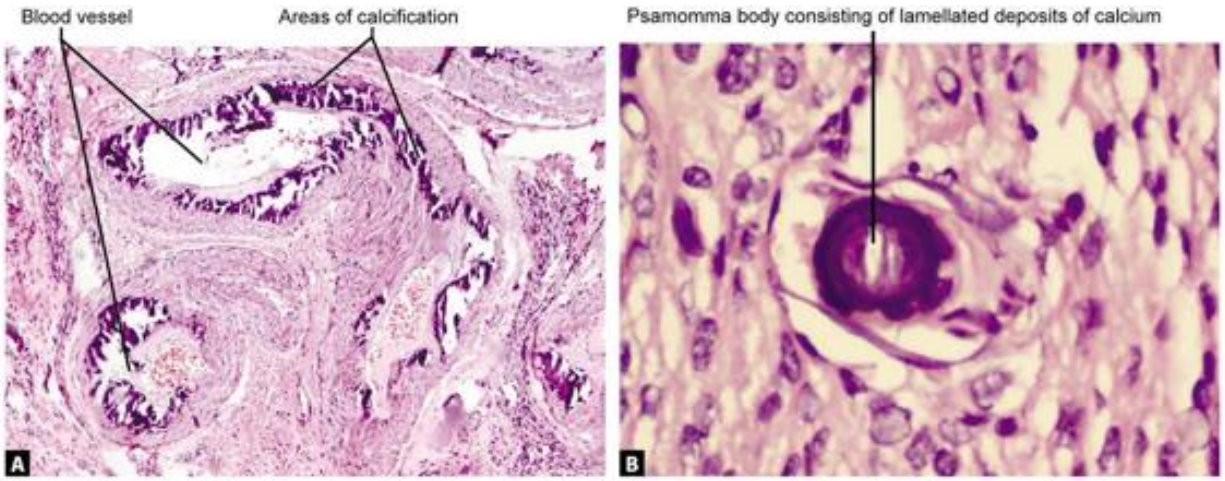
- **A**therosclerosis
- Psammoma **B**odies
- **C**aseous necrosis
- **D**amaged heart valves and dead eggs/parasites
- **E**nzymatic fat necrosis.

Metastatic calcification
1. Occurs in normal living tissues
2. Associated with raised serum calcium
3. Does not cause clinical dysfunction.

Massive deposits of calcium in the kidney is known as nephrocalcinosis and it can lead to kidney damage.

TABLE 2.9: Differences between dystrophic and metastatic calcification.

Features	***Dystrophic calcification***	***Metastatic calcification***
Tissue affected	Dead and degenerated tissue	Living tissue
Calcium metabolism	Normal	Deranged
Serum calcium level	Normal	Raised
Site of calcification	Necrosis (e.g., caseous necrosis), dead parasites, atherosclerotic plaque, etc.	Lung, mucosa of stomach, kidneys and blood vessels



Figs. 2.23A and B: (A) Monckeberg's medial calcific sclerosis in which the tunica media of arteries in the myometrium of uterus show calcification; (B) Photomicrograph of meningioma with psammoma body.

HYALINE CHANGE

Q. Write short note on hyaline change (including definition and types).

Hyaline refers to an alteration within cells or in the extracellular space, which gives a homogeneous, glassy, pink appearance in routine histological sections.

Causes (Table 2.10)

Intracellular Hyaline (Table 2.10)

Q. Write short note on Russel bodies.

- **Mallory body (Fig. 2.24A)** in the liver is alcoholic hyaline **composed of cytoskeletal filaments**.
- **Russell bodies** are **excessive accumulation of immunoglobulins in the rough endoplasmic reticulum of plasma cells (Fig. 2.24B)**.
- **Zenker's degeneration:** Hyaline change of rectus abdominalis muscle (becomes glassy and hyaline) in typhoid fever.

Extracellular Hyaline (Table 2.10)

- Collagenous fibrous tissue in **old scars**.
- Hyaline change in **uterine leiomyoma (Fig. 2.25)**.
- In **chronic glomerulonephritis,** the **glomeruli** show hyalinization.

Q. List the conditions in which Mallory bodies are seen.

Mallory hyaline/body observed in:
1. Alcoholic hepatitis
2. Indian childhood cirrhosis (ICC)
3. Primary biliary cirrhosis
4. Wilson disease
5. Hepatocellular carcinoma
6. Focal nodular hyperplasia

Crooke's hyaline body: Present in basophil cells of pituitary gland in Cushing syndrome.

MUCOID DEGENERATION

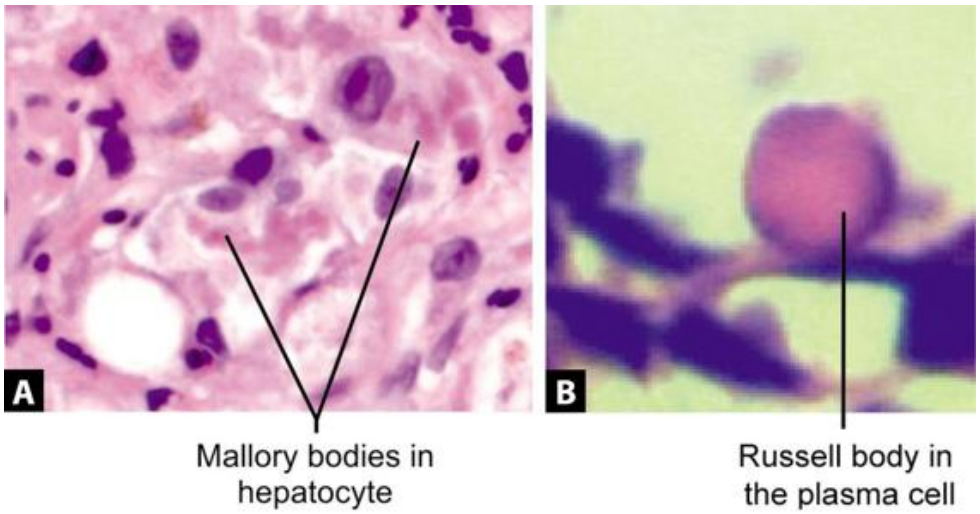
- Mucus is the viscid watery secretion produced by mucous glands. It consists of loose combination of proteins and mucopolysaccharides. Its main constituent is a glycoprotein called mucin.
- Mucus is produced by mitochondria and then they move to Golgi apparatus, where they are converted into mucin. When this process is exaggerated with excessive secretion of mucus it is termed mucoid degeneration.
- Mucin is normally produced by both epithelial cells of mucous membranes and mucous glands and also by certain connective tissue cells, especially in the fetus (e.g., umbilical cord). Mucoid degeneration may involve both epithelial and connective tissue mucin. Usually, connective tissue mucin is termed as myxoid, i.e., mucus like.
- Both epithelial and connective tissue mucin are stained by alcian blue. Epithelial mucin stains positively with periodic acid-Schiff (PAS) and connective tissue mucin is PAS negative and stains positively with colloidal iron.

Epithelial mucin: Examples of excessive epithelial mucin secretion are:

- Catarrhal inflammation of the mucous membrane, e.g., inflammation of mucosa of respiratory tract and gastrointestinal (GI) tract.

TABLE 2.10: Examples of hyaline change.

Intracellular hyaline	***Extracellular hyaline***
1. Mallory bodies 2. Russell bodies (e.g., multiple myeloma) 3. Crooke's hyaline 4. Zenker's hyaline change	1. Collagenous fibrous tissue in scar 2. Hyaline change in uterine leiomyoma 3. Hyaline membrane in newborn 4. Hyaline arteriosclerosis 5. Hyalinization of glomeruli in chronic glomerulonephritis 6. Corpora amylacea in prostate, brain, spinal cord in elderly, old infarct of lung



Figs. 2.24A and B: (A) Mallory bodies; (B) Russell bodies.

- Obstruction, e.g., mucocele of gallbladder, appendix, and oral cavity.
- Cystic fibrosis of pancreas.
- Mucus secreting tumors, e.g., GI tract and ovary.

Connective tissue mucin: Examples:

- Mucoid or myxoid degeneration: Examples of tumors include myxoma, neurofibroma, fibroadenoma and sarcomas.
- Dissecting aneurysm of aorta.
- Myxedema: Myxomatous changes in dermis.
- Myxoid change in ganglion.

CELLULAR ADAPTATIONS

CC: Describe and discuss cellular adaptations: atrophy, hypertrophy, hyperplasia, metaplasia, and dysplasia (*refer* page 197-8).

Q. Write short note on cellular adaptations.

When the cells are exposed to pathological stimuli (that may harm its normal structure and function), the cells can achieve a **new, steady altered state that allows them to survive** and continue to function in an abnormal environment. These reversible changes in the size, number, phenotype, metabolic activity or functions of cells in response to new or altered internal or external conditions or circumstances constitute **cellular adaptations.** The cellular response to persistent, sublethal stress reflects the cell's efforts to

adapt. Cellular stress may be physiological or pathological either due to an increased functional demand or a reversible cellular injury. Physiologic adaptations develop in response to normal stimulation by hormones or endogenous chemical mediators (e.g., enlargement of the breast and uterus during pregnancy), or to the demands of mechanical stress (e.g., muscles). Pathologic adaptations occur to escape injury, but at the expense of normal function (e.g., squamous metaplasia of bronchial epithelium in smokers). Physiologic and pathologic adaptations can take several distinct forms. Types of adaptations are mentioned in **Box 2.1** and **Figure 2.1.**

Hypertrophy

Q. Write short note on (definition, types/examples and mechanism) hypertrophy.

Definition: Increase in the size of the tissue or organ due to increase in the size of cells.

Causes

Increased functional demand/workload. **If the cause/stress is not relieved hypertrophy can progress to functionally significant cell injury.**

Physiological

- **Hypertrophy of skeletal muscle** (e.g., the bulging muscles of body builders and athletes).
- **Hypertrophy of smooth muscle** (e.g., growth of the uterus during pregnancy from estrogenic stimulation).

Pathological

- **Hypertrophy of cardiac muscle** [e.g., left ventricular hypertrophy **(Figs. 2.26A to C)** due to hypertension or damaged valves (aortic stenosis, mitral incompetence)].
- **Hypertrophy of smooth muscle** {e.g., hypertrophy of urinary bladder muscle in response to urethral obstruction [e.g., prostate hyperplasia **(Fig. 2.28 and** *refer* **Fig. 21.4)**], hypertrophy of muscular layer of stomach due to pyloric stenosis)}.

Mechanisms of Cellular Hypertrophy

Hypertrophy is due to increased synthesis of cellular proteins. Steps involved in biochemical mechanisms of myocardial (cardiac muscle) hypertrophy are shown in **Figure 2.27**.

Activation of the Signal Transduction Pathways

Various mechanisms **(Fig. 2.27)** involved are:

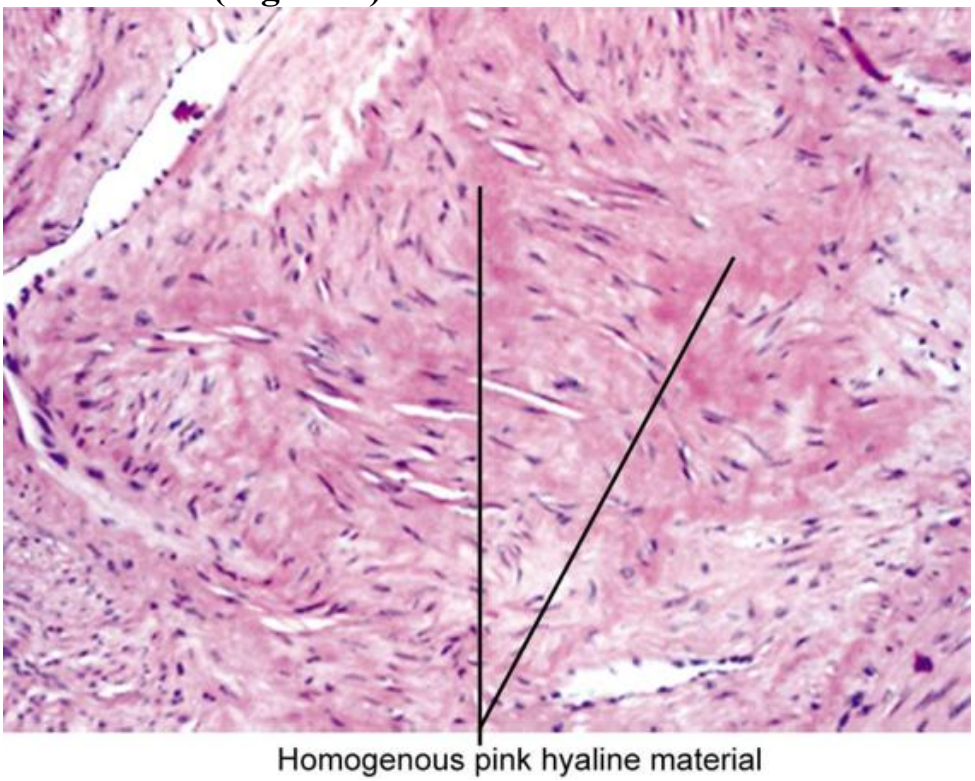
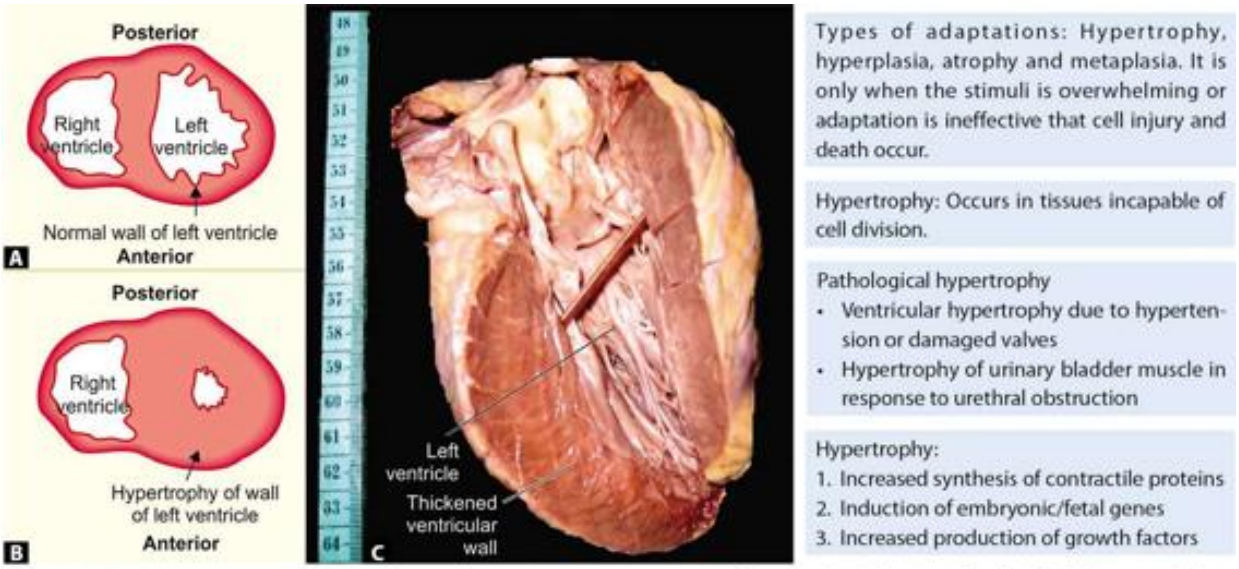


Fig. 2.25: Hyaline change in leiomyoma of uterus.



Figs. 2.26A to C: (A) Transverse section of normal heart; (B) Transverse section of heart with thickening of wall of the left ventricle due to hypertrophy; (C) Longitudinal section of heart with left ventricular hypertrophy.

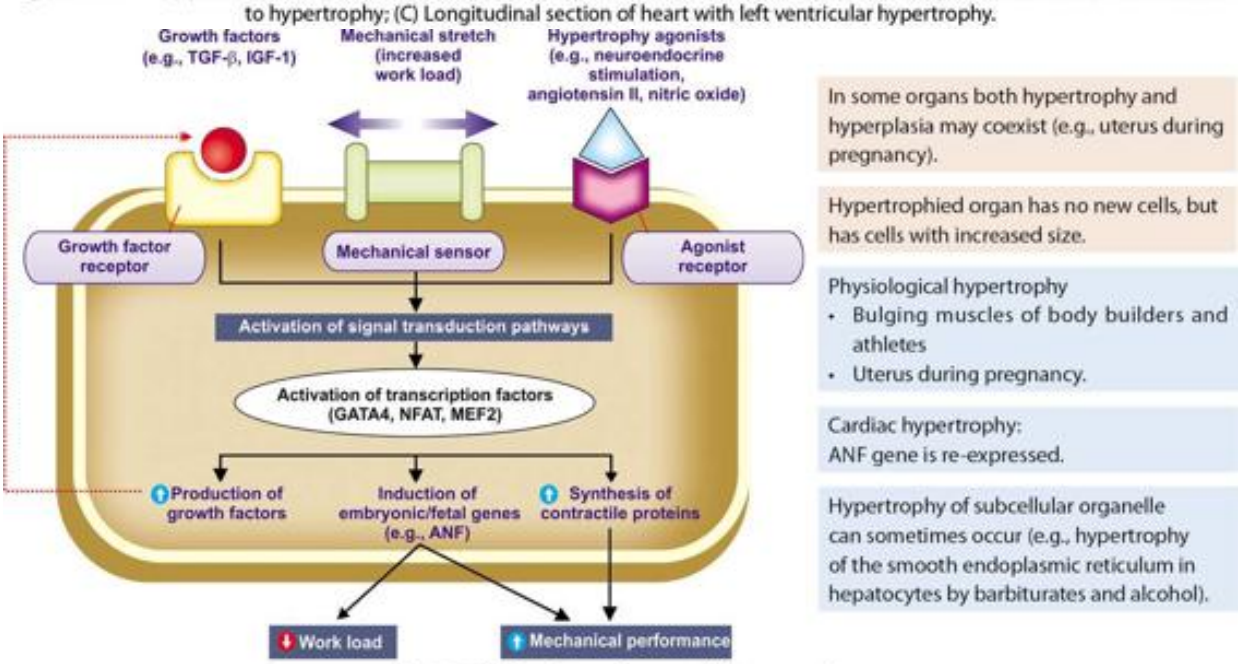


Fig. 2.27: Mechanisms of myocardial hypertrophy.

(ANF: atrial natriuretic factor; IGF-1: insulin-like growth factor; TGF-β: transforming growth factor-β)

Physiological hypertrophy:

Increased workload on the myocardium produces **mechanical stretch** and is the major trigger for physiological hypertrophy.

Pathological hypertrophy:

Growth factors and hypertrophy agonists are involved in pathologic hypertrophy.

- **Growth factors:** These include (TGF-β), insulin-like growth factor-1 (IGF-1) and fibroblast growth factor (FGF).
- **Hypertrophy agonists:** These include α-adrenergic agonists, endothelin-1, angiotensin II, nitric oxide (NO), and bradykinin.

Mechanical sensors also stimulate production of growth factors and agonists. They cause increased synthesis of muscle proteins.

Activation of Transcription Factors

Mechanical stretch, growth factors and hypertrophy agonists activate the signal transduction pathways and transcription factors [e.g., GATA4, nuclear factor of activated T-cells (NFAT) and myocyte enhancer factor 2 (MEF2)]. The activated transcription factors results in:

- **Increased synthesis of contractile proteins:** This is necessary to meet the increased functional demand.
- **Induction of embryonic/fetal genes:** Some genes are normally expressed only during early development of embryo and fetus. They are re-expressed in hypertrophied cells. For example, the gene for atrial natriuretic factor (ANF) is expressed in the embryonic heart, but not expressed after birth. In cardiac hypertrophy, ANF gene is re-expressed. ANF is a hormone that causes salt secretion by the kidney, decreases blood volume and pressure. Its re-expression decreases hemodynamic workload and increases the mechanical performance.
- **Increased production of growth factors.**

MORPHOLOGY

Gross

Involved **organ is enlarged**.

Microscopy

Increase in **size of the cells** as well as the **nuclei**.

Hyperplasia

Q. Write short note on hyperplasia.

Definition: Increase in the number of cells in an organ or tissue, resulting in **increased size/mass**

of the organ or tissue. Increase in number may be **either of differentiated cells or, in few due to less differentiated progenitor cells**.

Causes

Physiological Hyperplasia

Hormonal stimulation or as compensatory process.

- **Hyperplasia due to hormones** (e.g., hyperplasia of glandular epithelium) of the **female breast at puberty, pregnancy and lactation**, hyperplasia of the uterus during pregnancy from estrogenic stimulation.
- **Compensatory hyperplasia** (e.g., in liver following partial hepatectomy).

Pathological Hyperplasia

- Due to **excess** endocrine stimulation or chronic injury/irritation.
- **Excessive hormonal stimulation** [e.g., endometrial hyperplasia (due to estrogen, *refer* **Figs. 22.12 and 22.13**)] and benign prostatic hyperplasia [due to androgens (**Figs. 2.28** and *refer* **Figs. 21.4 to 21.5**)]. Enlargement of the male breast (gynecomastia) may occur in men with excess estrogens [e.g., following estrogen therapy for prostate cancer or in cirrhosis (liver is not capable of metabolizing endogenous estrogens and leads to their accumulation)].
- **Chronic injury/irritation:** Long-standing inflammation or chronic injury may lead to hyperplasia especially in skin or oral mucosa.

Hyperplasia disappears if the signals that initiate it abate. Pathological hyperplasia can progress to cancer, e.g., **endometrial hyperplasia can develop into endometrial cancer**.

Mechanism

- Hyperplasia is characterized by cell proliferation mostly of mature cell mediated **through stimulation by growth factor or hormones**.
- In some cases, the new cells may be derived from tissue **stem cells/less differentiated progenitor cells.**

MORPHOLOGY

Gross

Size of the affected organ is **increased**.

Microscopy

Increased number of cells with increased number of mitotic figures.

Atrophy

Q. Write short note on atrophy.

Definition: Atrophy is the **reduced size of an organ or tissue** resulting from a decrease in cell size and number.

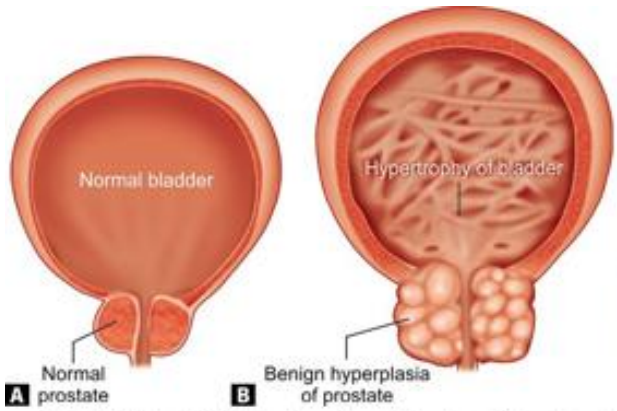
Causes

Physiological atrophy: Common during normal fetal development and in adult life.

- **During fetal development** (e.g., atrophy of embryonic structures such as thyroglossal duct).
- **During adult life** [e.g., involution of thymus, atrophy of brain, gonads, uterus and heart due to aging (senile atrophy)].

Pathological atrophy: Local or generalized.

1. **Local**
   - **Disuse atrophy (decreased workload)**[e.g., atrophy of limb muscles immobilized in a plaster cast (as treatment for fracture) or after prolonged bed rest].
   - **Denervation (loss of innervation) atrophy** [e.g., atrophy of muscle due to damage to the nerves (e.g., poliomyelitis)].
   - **Ischemic (diminished blood supply) atrophy** (e.g., brain atrophy produced by ischemia due to atherosclerosis of the carotid artery).
   - **Pressure atrophy** (e.g., atrophy of renal parenchyma in hydronephrosis due to increased pressure).
   - **Loss of endocrine stimulation** (e.g., endometrium and breast after menopause).
2. **Generalized: Starvation (inadequate nutrition) atrophy** (e.g., protein-calorie malnutrition).



Hyperplasia occurs in cells capable of replication namely labile/stable or stem cells. Not in permanent cells.

Pathological hyperplasia can act as a **fertile soil for cancer.**

Benign hyperplasia of prostate: Due to action of hormone dihydrotestosterone and not testosterone.

Hyperplasia unlike neoplasia can regress, if the stimulation is eliminated.

Figs. 2.28A and B: Cut section of prostate along with urinary bladder: (A) Normal prostate; (B) Enlarged prostate due to nodular hyperplasia. The urinary outflow obstruction results in hypertrophy of bladder muscle.

Mechanisms

Atrophic cells have diminished function. There is **decreased protein synthesis and increased protein degradation** in cells.

MORPHOLOGY

Gross

The **organ is small and often shrunken**.

Microscopy

The **cells are smaller in size** due to reduction in cell organelles.

Differences between atrophy, hypertrophy and hyperplasia are listed in **Table 2.11.**

Q. List the differences between atrophy, hypertrophy and hyperplasia.

Atrophy may be reversible but with irreversible loss of cells and the size of the organ cannot be restored.

In atrophy, cell death is mainly due to apoptosis.

Atrophied cells have increased lipofuscin (wear and tear) pigment.

Metaplasia

Q. Write short note on metaplasia (including types) with examples. What is its significance?

Definition: Metaplasia is a **reversible change** in which **one adult cell type is replaced by another adult cell** type.

Causes

- Metaplasia is usually **fully reversible adaptive response** to **chronic persistent injury**. If the noxious stimulus is removed (e.g., cessation of smoking), the metaplastic epithelium may return to normal.
- Metaplasia is mainly **seen in association with tissue damage, repair and regeneration**.
- The **replacing cell type** is usually **more suited to a change in environment**.

Types of Metaplasia

Epithelial Metaplasia

Squamous metaplasia: Original epithelium is replaced by squamous epithelium.

- **Respiratory tract** [e.g., chronic irritation due to tobacco smoke, the normal ciliated columnar epithelial cells of the trachea and bronchi undergo squamous metaplasia **(Fig. 2.29)**].

TABLE 2.11: Differences between atrophy, hypertrophy and hyperplasia.

	Atrophy	***Hypertrophy***	***Hyperplasia***
Definition	Reduced size of an organ or tissue resulting from a decrease in cell size and number	Increase in the size of the tissue or organ due to increase in the size of cells	Increase in the size/mass of the organ or tissue due to increase in the number of cells
Size of the involved organ	Reduced	Increased/enlarged	Increased
Cells			
Number	Reduced	No change	Increased
Size	Reduced	Increased	No change

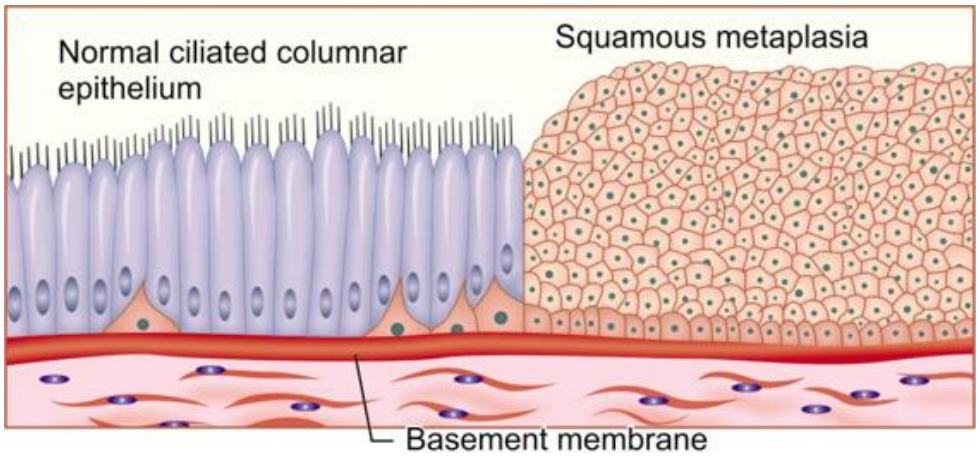
TABLE 2.11: Differences between atrophy, hypertrophy and hyperplasia.

	Atrophy	*Hypertrophy*	*Hyperplasia*
Organelles	Reduced	Increased	No change
Rate of cell division	–	–	Increased
Synthesis of DNA, RNA and protein	–	Increased	Increased

Persistence of stimulus/stress producing metaplasia may predispose to malignant transformation. For example, cigarette smoking initially causes squamous metaplasia in the trachea and bronchi. Later cancers can develop in some of these metaplastic foci.

Metaplastic squamous epithelium can withstand the stimulus/ stress.

Metaplasia is named by the cell which replaces, e.g., in squamous metaplasia, squamous cell replaces the original cells.



Myeloid metaplasia is occurrence of myeloid tissue (with hematopoietic cells) in extramedullary sites (other than bone marrow) such as spleen and liver.

Fig. 2.29: Squamous metaplasia in which columnar epithelium (left) is replaced by squamous epithelium (right).

- **Cervix:** Squamous metaplasia in cervix is associated with chronic infection in the endocervix.

Columnar metaplasia: Original epithelium is replaced by columnar epithelium.

- **Squamous to columnar: In Barrett esophagus, the squamous epithelium of the esophagus replaced by columnar cells** (*refer* **Fig. 18.7**) due to chronic reflux of highly acidic gastric contents into the lower esophagus.
- **Intestinal metaplasia:** The gastric glands are replaced by cells resembling those of the small intestine.

Connective Tissue Metaplasia

Osseous metaplasia: Formation of new bone at sites of tissue injury is known as osseous metaplasia. Bone formation in muscle, known as **myositis ossificans**, occasionally occurs after intramuscular hemorrhage. Other examples include cartilage of larynx and bronchi in elderly individual, scar of chronic inflammation of long duration, fibrous stroma of tumor (e.g., leiomyoma).

Mechanism

Develops due to the reprogramming of precursor cells (i.e., stem cells or undifferentiated mesenchymal cells) that are present in normal tissues. Metaplasia is usually fully reversible. If the noxious stimulus is removed.

Dysplasia is discussed on pages 197-8.

Epithelial metaplasia: Most common type of metaplasia.

Barrett esophagus: Squamous epithelium of the esophagus is replaced by columnar cells.

Connective tissue metaplasia: Myositis ossificans is characterized by bone formation in muscle after trauma.

Hyperplasia/metaplasia in certain cases may progress to dysplasia and neoplasia.

Various types of cellular adaptations are diagrammatically shown in **Figure 2.30**.

CELLULAR AGING

NC: Describe and discuss the mechanisms of cellular aging and apoptosis (*refer* pages 34-6).

Definition: It is the gradual, insidious and progressive decline in structure and function (involving molecules, cells, tissues, organs and organisms) that begins to unfold after the achievement of sexual maturity.

Cellular aging, begins from conception and continues till death. With aging physiological and structural changes develop in almost all systems. Cellular aging is the **result of a progressive decline in the lifespan and functional activity of cells**.

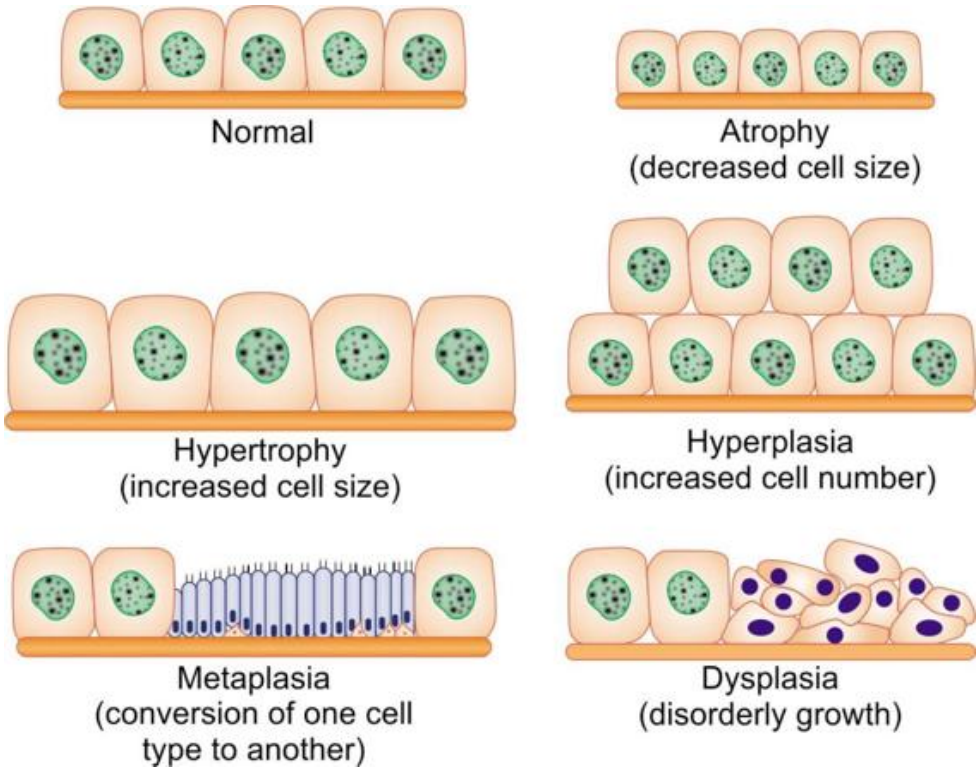


Fig. 2.30: Various types of cellular adaptations.

Causes

Aging is multifactorial and is affected by genetic factors and environmental factors.

- **Genetic abnormalities:** It causes progressive decline in cellular function and viability.
- **Environmental factors:** These include diet, social conditions and development of age-related diseases (e.g., atherosclerosis, diabetes and osteoarthritis). They cause progressive accumulation of sublethal injury over the years at cellular and molecular level.
- Cellular aging may lead to death of the cell or decreased capacity of cells to respond to injury and increasing difficulties in maintaining

physiological homeostasis.

Mechanism of Cellular Aging

Decreased Cellular Replication

Most normal cells (other than stem cells) have a limited capacity for replication. After a fixed number of divisions (about 60–70 cell divisions), all cells become arrested in a terminally nondividing state, known as **replicative senescence.** Aging is associated with progressive replicative senescence of cells.

The following mechanisms may be responsible for progressive replicative senescence of cells and decreased cellular replication in aging.

Telomere Shortening

Telomeres are **protective, short repeated sequences of DNA** (TTAGGG) present **at the end regions of chromosomes.** Telomeres ensure the complete copying of chromosomal ends during the S-phase of the cell cycle. With each cell division in somatic cells, a small section of the telomere is not duplicated and telomeres become progressively shortened **(Fig. 2.29).** When telomeres are sufficiently shortened, ultimately the cells stop dividing (**cell cycle arrest)** leading to a terminally nondividing state. Telomeres represent a 'biological clock', which prevents uncontrolled cell division and cancer. **Telomere shortening** may be one of the mechanisms **responsible for decreased cellular replication. Hayflick limit** is the number of times a normal human cell population will divide until cell division stops.

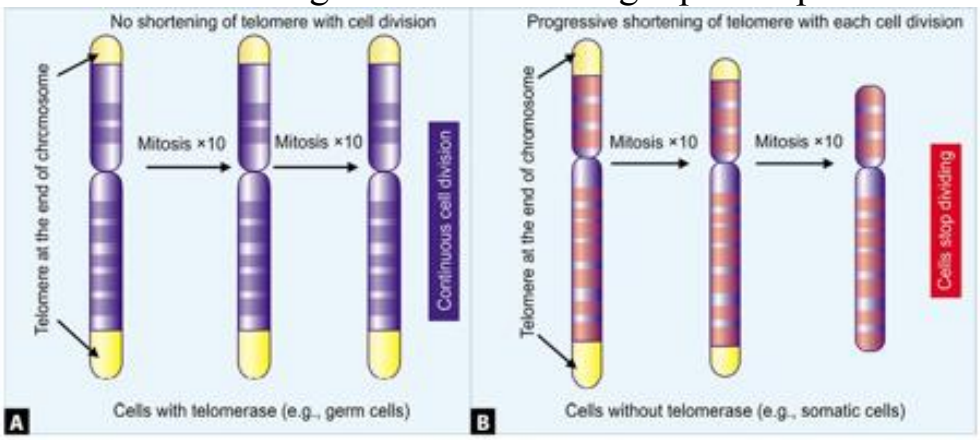
Telomerase

Telomerase is an **enzyme** that **regenerates and maintains telomere length** by addition of nucleotide. Telomerase is a specialized RNA-protein complex which uses its own RNA as a template for adding nucleotides to the ends of chromosomes. Telomerase is absent in most of the somatic cells and expressed in germ cells and is present at low levels in stem cells. Hence, as mature somatic cells age, their telomeres become shorter and they exit the cell cycle. This results in an inability to generate new cells to replace

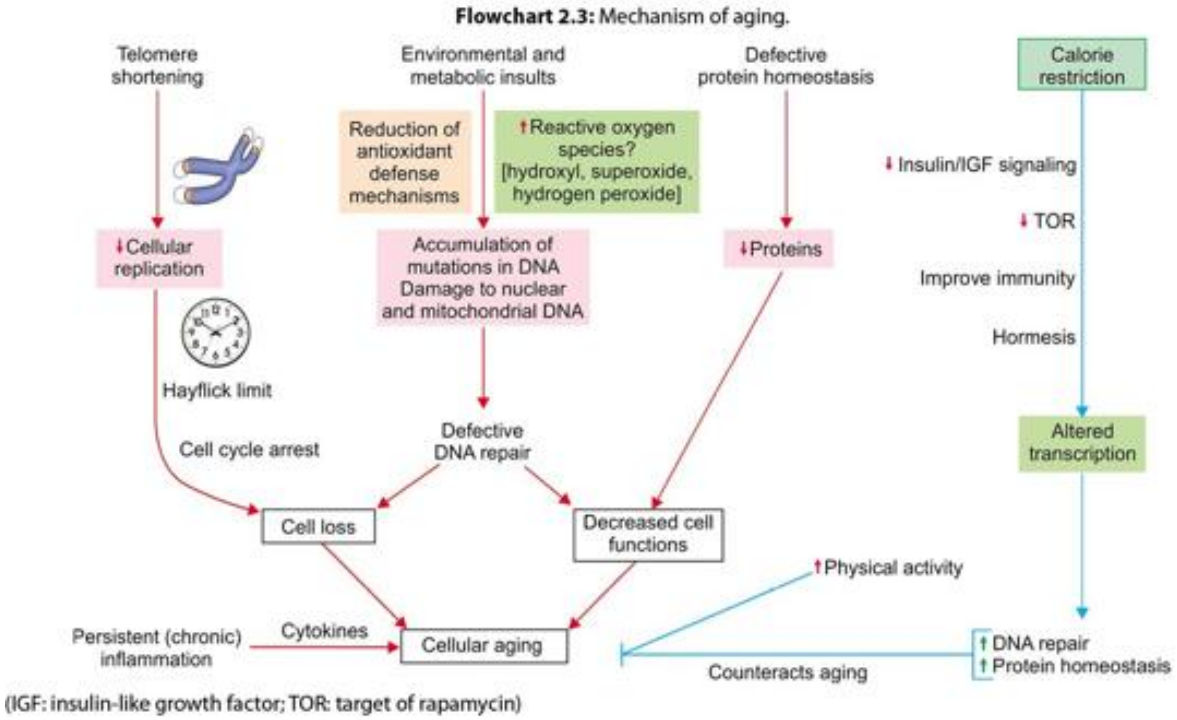
damaged ones. On the other hand, the germ cells (high telomerase activity) have extended replicative capacity **(Figs. 2.31A and B)**. In cancers, the telomerase may be reactivated in tumor cells resulting in maintenance of length of telomeres and allows the cells to proliferate indefinitely. It may be an essential step in formation of cancer. Shortening of telomere also may decrease the regenerative capacity of stem cells.

Environmental and Metabolic Insults (Flowchart 2.3)

Various metabolic insults over time may cause damage to nuclear and mitochondrial DNA. Lifespan of the cell is determined by a balance between cumulative metabolic damage and counteracting repair responses.



Figs. 2.31A and B: Role of telomerase in maintaining chromosomal length.



Flowchart 2.3: Mechanism of aging.

(IGF: insulin-like growth factor; TOR: target of rapamycin)

Metabolic Damage

Reactive oxygen species: One of the toxic products that cause damage to the cells is free radical mainly **reactive oxygen species (ROS)**. ROS may be either produced in excess, or there is reduction of antioxidant defense mechanisms (*refer* pages 18-9).

- **Excessive production of ROS** may be due to environmental influences (toxins and ionizing radiation) and mitochondrial dysfunction. ROS can produce DNA damage associated with aging.
- **Reduction of antioxidant defense mechanisms** may occur with age (e.g., vitamin E, glutathione peroxidase).

The oxidative damage may be an important cause of senescence in aging. Free radicals may damage DNA, causing breaks and genome instability. Damaged cellular organelles also accumulate as the cells age.

Defective Repair Mechanism

Many protective repair responses counterbalance the metabolic damage in cells. One of them is endogenous DNA repair enzymes, which identify the DNA damage and repairs most of it. However, some persists and accumulates as cells age, especially if repair mechanisms become inefficient

over time. DNA repair mechanisms are defective in diseases such as Werner syndrome and ataxia-telangiectasia.
Accumulation of mutations in nuclear and mitochondrial DNA will ultimately compromise the functional activities and survival of cells.

Defective Protein Homeostasis

As age progresses, cells are not able to maintain normal protein homeostasis due to increased turnover and decreased synthesis. This in turn, is caused by reduced translation of proteins and defective activity of chaperones (which promote normal protein folding) and proteasomes (which destroy misfolded proteins). The decrease in intracellular proteins can produce many damaging effects on cell survival, replication, and functions. The accumulation of misfolded proteins and increased loss of functional proteins can trigger apoptosis.

Persistent Inflammation

As age advances there is accumulation of damaged cells, lipids, and other endogenous substances. These may activate the inflammasome (*refer* page 51) pathway, producing persistent low-level inflammation. Persistent inflammation may be responsible for chronic diseases, such as atherosclerosis and type 2 diabetes. Cytokines produced during inflammatory reactions may themselves produce alteration in cells that promote aging. Chronic metabolic disorders may speed up the aging process. Stresses may accelerate aging probably by increased production of glucocorticoids.
Thus, aging can be delayed by either by reducing the metabolic damage or by increasing the repair response to that damage.

Factors that Increases Longevity

Caloric Restriction

It has been found that caloric restriction alters signaling pathways that influence aging and can slow down aging and prolong life. Physical activity can also slow the aging process.

Biochemical changes associated with calorie restriction: These inhibit aging and include:

- **Reduced activation of insulin-like growth factor (IGF) receptor signaling**, which involves a downstream network of kinases and transcription factors. The actions of reduced IGF-1 signaling includes: (i) lowering the rate of cell growth and metabolism, (ii) reduction in errors in DNA replication, (iii) better DNA repair and (iv) improved protein homeostasis.
- **Decreased TOR (target of rapamycin activity):** TOR is a nutrient-sensitive, central controller of cell growth and aging. Decreased TOR activity has been found to slow the aging process and increase life span.
- Improve immunity.

Note: Hormesis is a biological phenomenon whereby a beneficial effect (improved health, stress tolerance, growth or longevity) results from exposure to low doses of an agent that is otherwise toxic or lethal when given at higher doses.

CC (Show how): Identify and describe various forms of cell injuries, their manifestations and consequences in gross and microscopic specimens:

(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

The specimens and slides to be shown include: fatty liver, coagulative necrosis (infarction), liquefactive necrosis (brain infarct), caseous necrosis (tuberculosis), gangrene, calcification, hyaline change (e.g., leiomyoma), left ventricular hypertrophy, nodular hyperplasia of prostate.

CHAPTER

3 Inflammation and Healing

INFLAMMATION

CC: Define and describe the general features of acute and chronic inflammation including stimuli, vascular and cellular events.

Q. Long essay: Define inflammation. Mention the types and causes of acute inflammation. Explain the sequential vascular changes in acute inflammation.

Definition: Inflammation is a complex **local response of the living vascularized tissues to injury (infections and tissue damage)**.

It brings cells (phagocytic leukocytes) **and molecules which are necessary for host defense** (antibodies, and complement proteins) **from the circulation to the site of injury (where they are required), to eliminate the offending injurious agents.** It mainly consists of responses of blood vessels and leukocytes. Sequential steps in an inflammatory reaction are shown in **Flowchart 3.1**.

Inflammation is largely confined to the site of infection or damage but can develop some systemic manifestations (e.g., fever in bacterial or viral infections).

Types of inflammation: Inflammation may be divided into **acute or chronic**.

Q. Mention the types of inflammation. List the differences between acute and chronic inflammation.

Differences between acute and chronic inflammation are listed in **Table 3.1**.

Sometimes, the term subacute inflammation is used to describe the inflammation between acute and chronic.

Cardinal Signs of Inflammation

Q. Mention the cardinal signs of inflammation and its mechanism.

- The four cardinal signs of inflammation as mentioned by Roman encyclopedist **Celsus (Aulus Cornelius Celsus)** are listed in **Table 3.2**.
- A fifth clinical sign, **loss of function** (functio laesa), was later added by Rudolf Virchow.

Flowchart 3.1: Sequential steps in an inflammatory reaction.

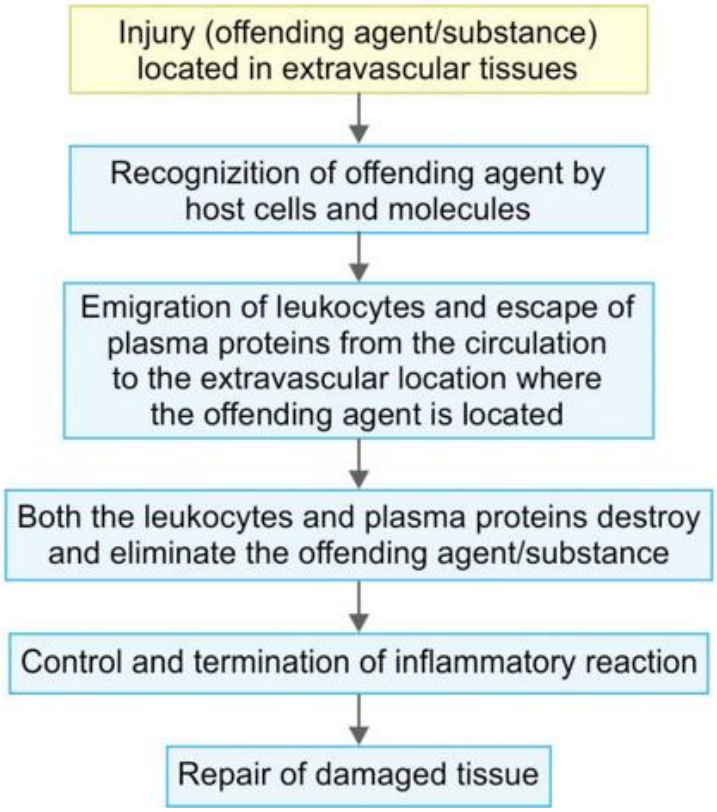


TABLE 3.1: Differences between acute and chronic inflammation.

	Acute inflammation	*Chronic inflammation*
Onset	Rapid in onset (usually in minutes or hours)	May follow acute inflammation or be slow in onset (days)
Duration	Short duration. Lasts for hours or a few days	Longer duration; may be months
Predominant cells	Neutrophils (also called polymorphonuclear leukocytes)	Lymphocytes, monocytes/macrophages and sometimes plasma cells
Characteristics	Exudation of fluid and plasma proteins (edema) and the emigration of leukocytes	Inflammatory cells associated with the proliferation of blood vessels, tissue destruction and fibroblast proliferation
Injury/damage to tissue and fibrosis	Usually mild and self-limited and can progress to a chronic phase	Usually severe and progressive with fibrosis and scar formation
Signs: Local and systemic	Prominent	Less prominent

Inflammation and the accompanying repair process is a beneficial host response in most instances, but can sometimes be harmful.

TABLE 3.2: Cardinal signs of inflammation (Celsus).

Cardinal sign	***Mechanism***
Rubor (redness)	Increased blood flow and stasis
Calor (heat)	Increased blood flow
Tumor (edema/swelling)	Increased vascular permeability causing escape of a protein-rich fluid from blood vessels
Dolor (pain)	Chemical mediators: Prostaglandins and kinins

Causes of (Stimuli for) Inflammation (Box 3.1)

Q. Mention the various causes of acute inflammation.

BOX 3.1: Causes of acute inflammation.

- **Infections** (bacterial, viral, fungal, and parasitic) and **microbial toxins**.
- **Tissue necrosis:**
  - **Ischemia** (e.g., myocardial infarction)
  - **Physical agents**
    - Mechanical trauma (e.g., blunt/penetrating/crush injuries)
    - Thermal injury (e.g., burns or frostbite)
    - Radiation
    - Electric shock
    - Sudden changes in atmospheric pressure
  - **Chemical injury** (e.g., strong acids and alkalies, insecticides, and herbicides)
- **Foreign bodies** (e.g., sutures, talc)
- **Immune reactions:**

- Hypersensitivity reactions
- Autoimmune diseases

Russian zoologist Elie Metchnikoff: Phagocytosis.

Sir Thomas Lewis: Triple response.

Julius Cohnheim first described emigration of leukocytes through microvasculature walls in inflammation.

RECOGNITION OF MICROBES AND DAMAGED CELLS

Pattern-Recognition Receptors

Recognition of microbes, necrotic cells and foreign substances: The first step in inflammatory responses is the recognition of microbes, necrotic cells and foreign substances**. This recognition is done by receptors** on cell surface (cellular receptors) **and circulating proteins.**

Cellular Receptors for Microbes

The presence of infectious pathogens (microbes) is recognized through receptors present on cells. These cells include phagocytes, dendritic cells (cells in epithelia and all tissues which are involved in capturing microbes), and many other cells. There are many cellular receptors for microbes which detect pathogens or the damage induced by them (*refer* page 142). These receptors recognize simple molecules and regular patterns of molecular structure called **pathogen-associated molecular patterns (PAMPs)** that are part of many microorganisms but not of the host body's own cells. PAMPs structures include mannose-rich oligosaccharides, peptidoglycans, and lipopolysaccharides of the bacterial cell wall, as well as unmethylated CpG DNA commonly found in many pathogens. Hence, such cellular receptors for microbes are known as "**pattern recognition receptors (PRRs)**". The well-known of these PRRs are transmembrane proteins that belong to the family of *Toll-like receptors (TLRs).*

Q. Write a short note on Toll-like receptors (TLRs) and its importance.

Toll-like Receptors (Fig. 3.1)

They are named after the founding member, *Toll*, a gene that was discovered in Drosophila. TLRs are **located in plasma membranes and endosomes**. They are capable of detecting PAMPs derived from extracellular and ingested microbes like bacterial lipopolysaccharide (LPS, or endotoxin) or bacteria taken into phagosome by phagocytosis. There are 10 expressed TLR genes in humans. Each TLR recognizes a distinct set of molecular patterns. TLRs are single-pass transmembrane proteins. Their extracellular region is composed of **leucine-rich repeat (LRR)** such as CD14.

- **Recognition of PAMPs**: Microbes have few highly conserved common molecular structures shared by entire classes of pathogens. These structures are called pathogen associated molecular patterns (PAMPs) and are essential for the infectivity of these pathogens. TLRs recognize these PAMPs.

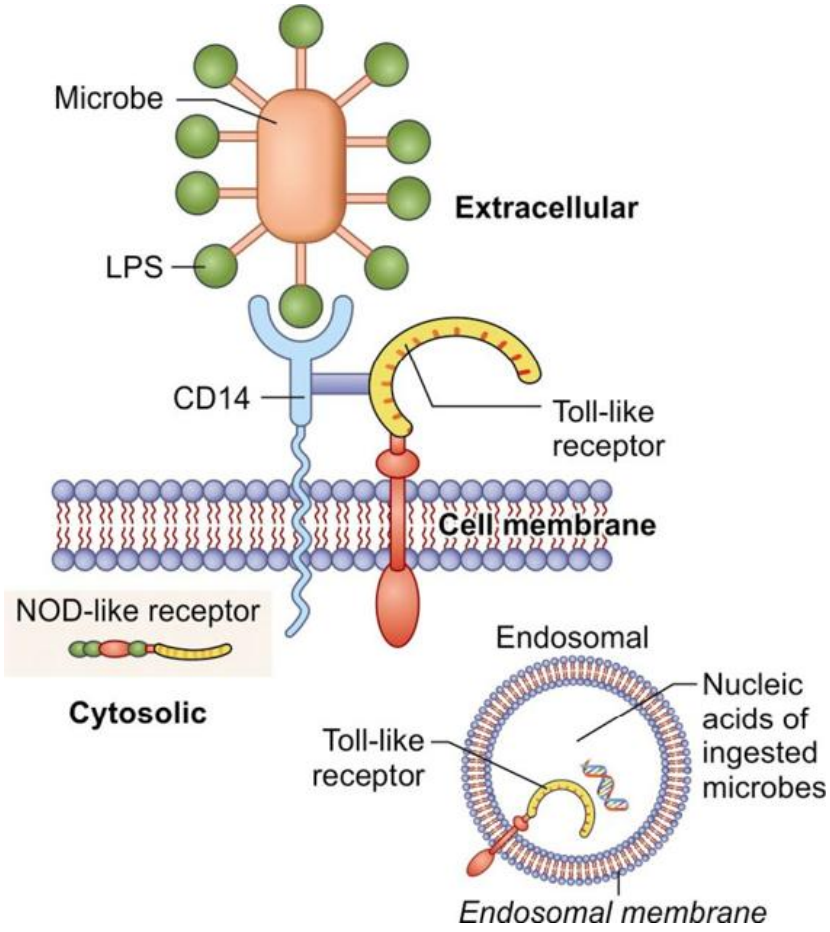


Fig. 3.1: Toll-like receptor.
[LPS: lipopolysaccharide; CD14: a member of the family of leucine-rich repeat (LRR) proteins].

- **Protein production**: Once the microbes are recognized by these receptors, they stimulate the production and expression of many secreted and membrane proteins. These proteins include cytokines that induce inflammation, anti-viral cytokines (interferons), and cytokines and membrane proteins that promote lymphocyte activation and immune responses.

Sensors of Cell Damage

All cells contain cytosolic receptors which recognize molecules that are liberated or altered due to cell damage. These are called **damage-associated molecular patterns (DAMPs)**. Examples of these molecules include uric acid (a product of DNA breakdown), ATP (released from damaged mitochondria), decreased intracellular K^+ concentrations (due to loss caused by injury to plasma membrane injury), DNA (when it is liberated into the cytoplasm and not sequestered in nuclei, as it should be normally), etc. The

cytosolic receptors activate a multiprotein cytosolic complex called the *inflammasome*. **Inflammasome** can recognize products of dead cells (e.g., uric acid, microbial products). It stimulates the production of the cytokine interleukin-1 (IL-1). In turn, IL-1 recruits leukocytes and thus induces inflammation. The recruited leukocytes, phagocyte and destroy dead cells. Gain-of-function mutations in the cytosolic receptors produce rare diseases known as *autoinflammatory syndromes* which are characterized by spontaneous inflammation. IL-1 antagonists are effective in the treatment of these disorders.

- **Inflammasome and associated disorders:** Inflammasome is also responsible for inflammatory reactions to urate crystals (cause of gout), cholesterol crystals (in atherosclerosis), lipids (in metabolic syndrome and obesity-associated diabetes), and amyloid deposits in the brain (in Alzheimer disease). IL-1 plays a role in atherosclerosis and obesity-associated type 2 diabetes mellitus.

Circulating Proteins

Many plasma proteins recognize microbes and function to destroy blood-borne microbes and stimulate inflammation at the site of tissue infection.

- **Complement system**: It reacts against microbes and activated complement products act as mediators of inflammation.
- **Mannose-binding lectin:** It is a circulating protein which recognizes microbial sugars and promotes ingestion of microbes and activates the complement system.
- **Collectins:** These proteins also bind to microbes and promote phagocytosis of microbes.

Inflammasome: Multiprotein complex which can recognize products of dead cells (e.g., uric acid, microbial products).

ACUTE INFLAMMATION

Q. Explain the sequential vascular changes/reactions of blood vessels/hemodynamic changes in acute

inflammation.

Sequence of events in acute inflammation: Acute inflammation has two major components namely: **(1) reactions of blood vessels (vascular changes)** and **(2) reactions of leukocytes (cellular events)**.

REACTIONS OF BLOOD VESSELS(VASCULAR CHANGES)

Q. Long essay: Define inflammation. Describe the vascular changes (reactions of blood vessels) in acute inflammation.

Purpose: To deliver the circulating cells, fluids and plasma proteins from the circulation to sites of infection or tissue injury.

The reactions of blood vessels in acute inflammation **(Figs. 3.2 and 3.3)** consist of: **changes in the vascular flow and caliber** and **increased vascular permeability**.

Changes in Vascular Flow and Caliber

- **Vasodilatation:** It is the earliest feature of acute inflammation; sometimes, it follows a transient constriction of arterioles.
  - **Effect:** Result is increased blood flow → local heat and redness.
  - **Chemical mediators involved:** Histamine, prostaglandins, kinins and nitric oxide (NO).
- **Increased permeability of the microvasculature:** It leads to escape of protein-rich fluid from the circulation into the extravascular tissues.
  - **Chemical mediators involved:** Histamine, leukotrienes, neuropeptides and kinins.
- **Slowing of blood flow:** It leads to concentration of RBCs in small vessels and increased viscosity of the blood.
- **Stasis:** It is responsible for localized redness.
- **Leukocyte events:** Described later on pages 53-7.

Increased Vascular Permeability (Vascular Leakage)

Q. Write short essay on mechanism of increased vascular permeability (vascular leakage) in inflammation.

Exudation is defined as the process of escape of fluid, proteins and circulating blood cells from the vessels into the interstitial tissue or body cavities.

Escape of a protein-rich fluid **causes edema** and is one of the cardinal signs of inflammation. Differences between transudate and exudate are listed in **Table 3.3**.

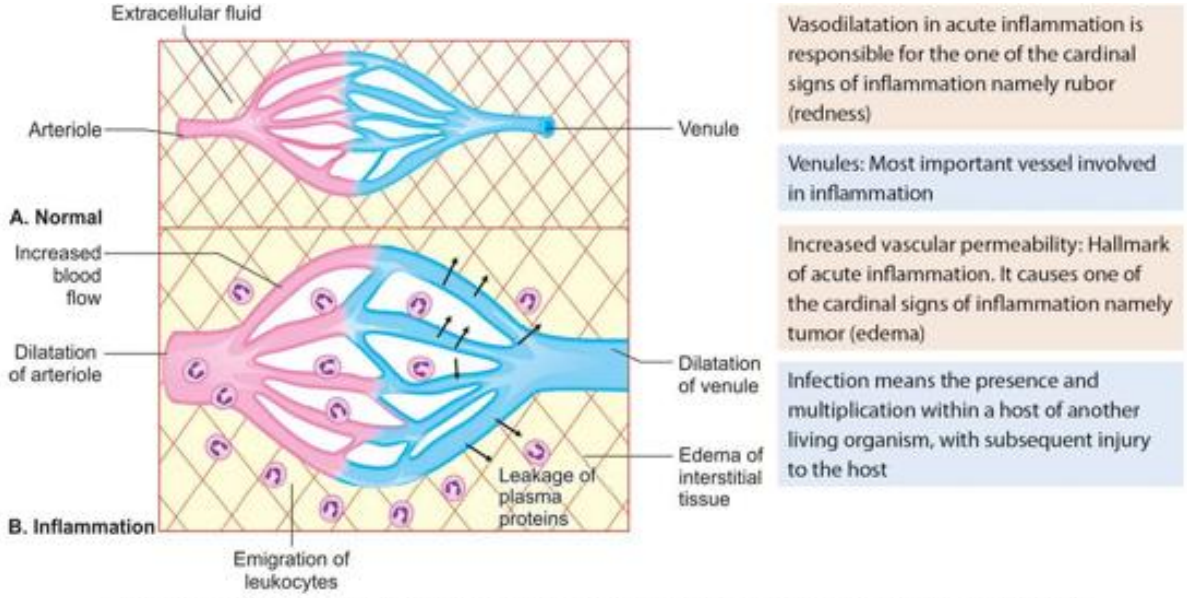
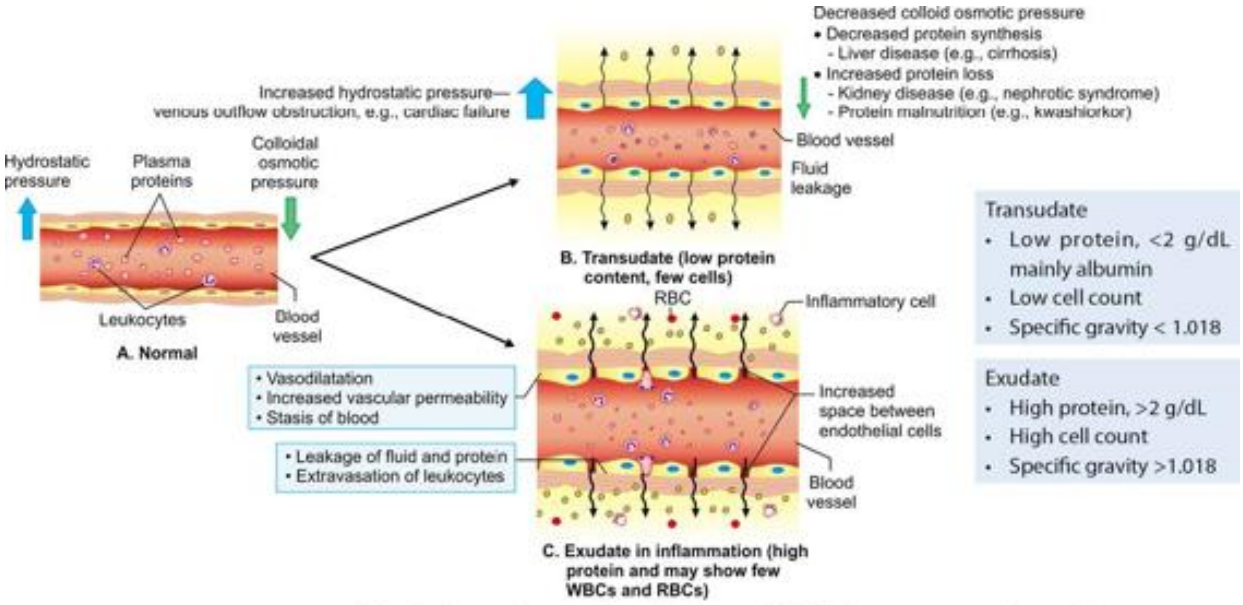


Fig. 3.2: Local features of acute inflammation, compared to normal are vasodilatation, increased blood flow, leakage of plasma fluid and proteins, and emigration of leukocyte.



Figs. 3.3A to C: (A) Normal fluid exchange between blood and extracellular fluid; (B) Formation of transudate; (C) Formation of exudate in inflammation.

Mechanism of Increased Vascular Permeability

Q. Describe the mechanism of increased vascular permeability.

Several mechanisms can cause increased vascular permeability:

1. **Contraction of endothelial cells:**
   - Most common mechanism of vascular leakage.
   - Occurs immediately after injury and is usually short-lived (15–30 minutes) and hence called as **immediate transient response**.
   - **Chemical mediators involved:** Histamine, bradykinin, leukotrienes, the neuropeptide substance P.
2. **Direct endothelial injury** (e.g., burns or infection by microbes). It is called as immediate **sustained response**.
3. **Leukocyte-mediated vascular injury:** The leukocyte (mainly neutrophils) which adheres to the endothelium during inflammation may themselves injure the endothelial cells.

Q. Mention the differences between transudate and exudate.

TABLE 3.3: Difference between transudate and exudate.

TABLE 3.3: Difference between transudate and exudate.

Characteristics	*Transudate*	*Exudate*
Cause	Noninflammatory process	Inflammatory process
Mechanism	Ultrafiltrate of plasma, due to increased hydrostatic pressure with normal vascular permeability	Increased vascular permeability
Appearance	Clear, serous	Cloudy/purulent/hemorrhagic/chylous
Color	Straw yellow	Yellow to red
Specific gravity	<1.018	>1.018
Protein	Low, <2 g/dL, mainly albumin	High, >2 g/dL
Fibrin content and clot formation	Fibrin content id low and does not coagulate	Fibrin content is high and coagulates/clot spontaneously
Cell count	Low	High
Type of cells	Few lymphocytes and mesothelial cells	Neutrophils in acute and lymphocytes in chronic inflammation
Bacteria	Absent	Usually present
Lactate dehydrogenase (LDH)	Low	High
Examples	Seen in congestive cardiac failure	Pus
Character of edema	Pitting type	No pitting

4. **Increased transcytosis:** Process of transport of fluids and proteins through the channels called vesiculo-vacuolar organelle is increased in number during inflammation.
5. **Leakage from new blood vessels:** During repair new blood vessels are formed (angiogenesis). These vessels are leaky till the endothelial cells mature.

Increased vascular permeability and chemotaxis: Occurs predominantly in venules (except in lungs, where it occurs in capillaries).

Cellular events in acute inflammation:
- Leukocyte recruitment

- Leukocyte activation

LEUKOCYTIC/CELLULAR EVENTS

Q. Long essay: Define inflammation.

Q. Describe the leukocyte/cellular changes/events in acute inflammation.

This process **delivers leukocytes** capable of phagocytosis (neutrophils and macrophages) **to the site of injury**. Neutrophils are produced in the bone marrow. They are rapidly recruited to sites of inflammation and are short-lived. Macrophages (discussed on page 68) are slow to respond but are long-lived. Leukocyte events can be divided into: **leukocyte recruitment and leukocyte activation**. The most important leukocytes are the phagocytic neutrophils and macrophages. They ingest and destroy bacteria and other microbes.

Leukocyte Recruitment/Extravasation

Normally, leukocytes move rapidly in the blood, and during inflammation, they slow down and escape to the site of injury/causative agent in the extravascular space.

Definition: Leukocyte recruitment to sites of inflammation (extravasation) is the process of migration of leukocytes from the lumen of the vessel to the site of injury in the extravascular tissues.

The extravasated leukocytes perform the main function of eliminating the causative agents.

Steps in Leukocyte Recruitment/Extravasation (Fig. 3.4)

The journey of leukocytes from the lumen of blood vessel to the tissue is a multistep process. This is mediated and controlled by adhesion molecules and cytokines.

In the Vascular Lumen

Normally, leukocytes move rapidly in the flowing blood which prevents them from attaching to the vascular endothelium. In inflammation, they should be stopped and then brought to the offending agent or the site of tissue damage, outside the vessels.

1. **Margination:** During inflammation when the blood flow slows down (stasis), **leukocytes** (mainly neutrophils) **move towards the peripheral column** and accumulate along on the endothelial surface of vessels. This process of leukocyte redistribution is termed margination.
2. **Rolling:** Marginated leukocytes are able to detect and react to changes in the endothelium. Initially, they attach weakly to the endothelium, detach and bind again with a mild jumping movement. It causes rolling of leukocyte along the endothelial surface.

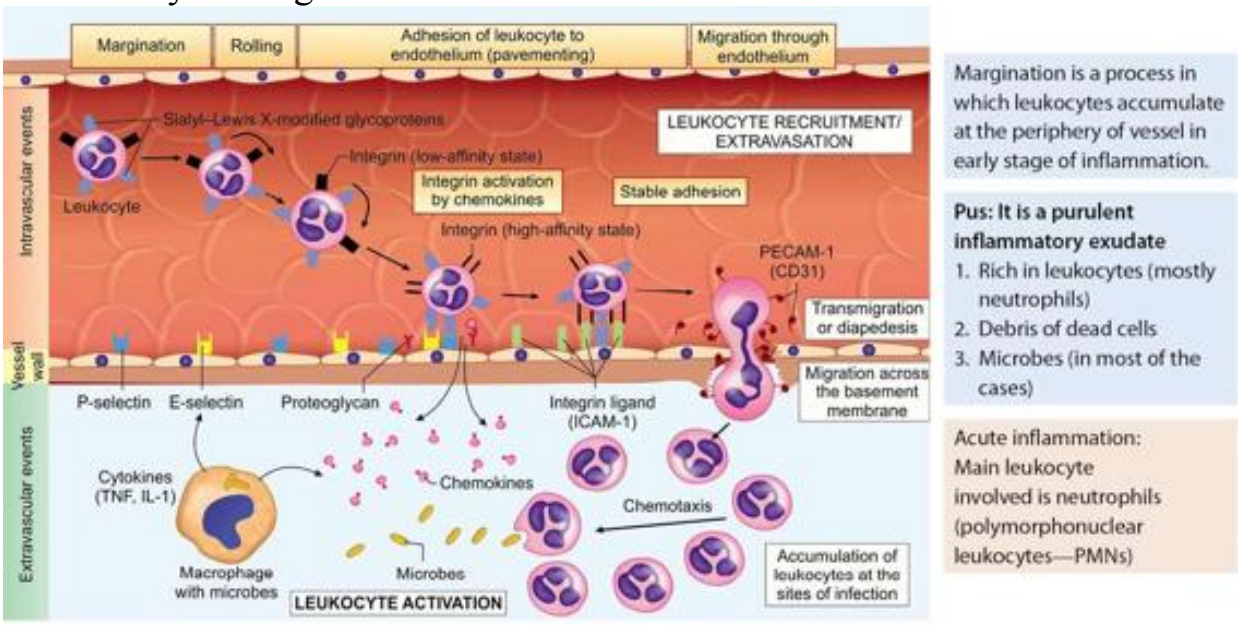


Fig. 3.4: Various steps of leukocyte migration through blood vessels. The leukocytes first roll, and then firmly adhere to endothelium, followed by transmigration across the endothelium. Leukocytes pierce the basement membrane, and migrate toward chemoattractants from the source of injury. Selectins are involved in rolling; integrins in adhesion; and CD31 (PECAM-1) in transmigration.

Q. Describe the role of selectins and integrins in acute inflammation.

TABLE 3.4: Selectins and complimentary selectin ligands involved in rolling.

Type of selectin	*Distribution*	*Ligand and their expression*
L-selectin (CD62L)	Neutrophils, monocytes, T cells and B cells	Sialyl-Lewis X/PNAd on GlyCAM-1, CD34, MAdCAM-1

TABLE 3.4: Selectins and complimentary selectin ligands involved in rolling.

Type of selectin	*Distribution*	*Ligand and their expression*
E-selectin (CD62E)	Endothelium activated by cytokines (TNF, IL-1)	Sialyl-Lewis X (e.g., CLA) on glycoproteins; expressed on neutrophils, monocytes, T-cells
P-selectin (CD62P)	Endothelium activated by cytokines (TNF, IL-1), histamine, or thrombin	Sialyl-Lewis X on PSGL-1 and other glycoproteins; expressed on neutrophils, monocytes, T-cells

(GlyCAM-1: glycan-bearing cell adhesion molecule-1; MAdCAM-1: mucosal adhesion cell adhesion molecule-1; TNF: tumor necrosis factor; IL-1: interleukin-1; CLA: cutaneous lymphocyte antigen-1; PSGL-1: P-selectin glycoprotein ligand-1)

Selectins are either not present or expressed at low levels in unactivated endothelial cells.

- **Molecules involved:** Selectin family of adhesive molecules and its complementary ligands **(Table 3.4)**. ***Selectins*** **mediate the initial weak interactions between leukocytes and endothelium**.
- **Chemical mediators involved: Cytokines such as (1) tumor necrosis factor (TNF), (2) interleukin-1 (IL-1)** and **(3) chemokines (chemoattractant cytokines)**. Cytokines are secreted by cells in tissues, in response to microbes and other injurious stimulus. The endothelial selectins (E-selectins) are activated after stimulation by cytokines and other mediators.

3. **Adhesion of leukocyte to endothelium:** Endothelium gets activated and leukocytes bind more firmly and finally come to rest at some point. They resemble as pebbles over the endothelium (pavementing) and a bloodstream runs without disturbing them.
   - **Molecules involved: Integrins** (family of leukocyte surface proteins) **and corresponding ligands(Table 3.5).**
   - **Chemical mediators involved:**
     - Endothelial cells are activated by cytokines namely: TNF and IL-1 and increase the expression of ligands for integrins on leukocyte **(Table 3.5)**. Drugs that block TNF leukocyte recruitment are used for the treatment of chronic inflammatory diseases.
     - Chemokines are chemoattractant cytokines that cause leukocyte activation and **conversion of low-affinity** integrins **on leukocyte to high-affinity state** resulting in firm adhesion of the leukocytes to the endothelium.

TABLE 3.5: Integrins and complimentary ligands involved in endothelial-leukocyte adhesion.

Type of integrins	*Distribution*	*Complimentary ligands expressed on endothelium*
β1 integrin VLA-4 (CD49aCD29)	Monocytes, T-cells	VCAM-1 (CD106)
β2 integrins LFA-1 (CD11aCD18)	Neutrophils, monocytes, T-cells	ICAM-1 (CD54), ICAM-2 (CD102)
β2 integrins MAC-1 (CD11bCD18)	Monocytes, dendritic cells	ICAM-1 (CD54), ICAM-2 (CD102)

(ICAM: intercellular adhesion molecule; VCAM: vascular cell adhesion molecule)

Across the Vessel Wall and the Endothelium

Q. Write short note on leukocyte transmigration.

1. **Transmigration or diapedesis:** Leukocytes migrate through the vessel wall by squeezing through the intercellular junctions between the endothelial cells.
   - **Molecules involved:** These include a **member of the immunoglobulin superfamily called CD31 or PECAM-1 (platelet endothelial cell adhesion molecule).**
2. **Migration across the basement membrane:** Leukocytes penetrate the basement membrane of the vessel by secreting collagenases.

Outside the Vessel Wall

Q. Define and write short note on chemotaxis.

1. **Chemotaxis**.

Definition: Chemotaxis is defined as process of **migration of leukocytes toward the inflammatory stimulus** in the **direction of the gradient** of locally produced chemoattractants.

Chemoattractants: These are produced by microbes and by host cells in response to infections and tissue damage and during immunologic reactions. These may be:

- **Exogenous:** Bacterial products (e.g., N-formylmethionine terminal amino acid).
- **Endogenous:**
  - Cytokines, mainly chemokine family (e.g., **IL-8**)

- Complement components: **C5a**, **C3a**
- Arachidonic acid metabolites of lipoxygenase pathway: **Leukotriene B_4 (LTB_4)**.

2. **Accumulation of leukocytes at the sites of infection and injury:** This is achieved by binding of leukocytes to the extracellular matrix proteins through **integrins and CD44**.
   - **Type of leukocytes infiltrates:**
     - **Neutrophils:** Predominantly during the first 6–24 hours.
     - **Monocytes:** Neutrophils are replaced by monocytes in 24–48 hours.

Clinical Importance of Leukocyte Adhesion Molecules

- Three main types of leukocyte adhesion deficiency (LAD) have been identified.
- All are transmitted as autosomal recessive disease.
- Characterized by the inability of neutrophils to exit the circulation to sites of infection, leading to **leukocytosis** and **increased susceptibility to infection.**

Process of loose binding and detachment of leukocytes to endothelial cells is termed rolling. **Selectins** and its complimentary ligands are responsible for **rolling**. During inflammation, the endothelial cells at the site of inflammation gets activated and express high-levels of selectins.

Integrins are responsible for firm adhesion of leukocytes with endothelial cells.

Chemotaxis is the **unidirectional movement of leukocytes** towards injurious agent.

Acute inflammation: Neutrophils predominate in early stage and are replaced by monocytes after 24 hours.

Pseudomonas infection: Neutrophils predominate over 2 to 4 days.

Genetic deficiencies of leukocyte adhesion molecules cause recurrent bacterial infections.

Leukocyte adhesion deficiency type 1 (LAD1):
- Integrin defects
- Recurrent infections
- Persistent leukocytosis

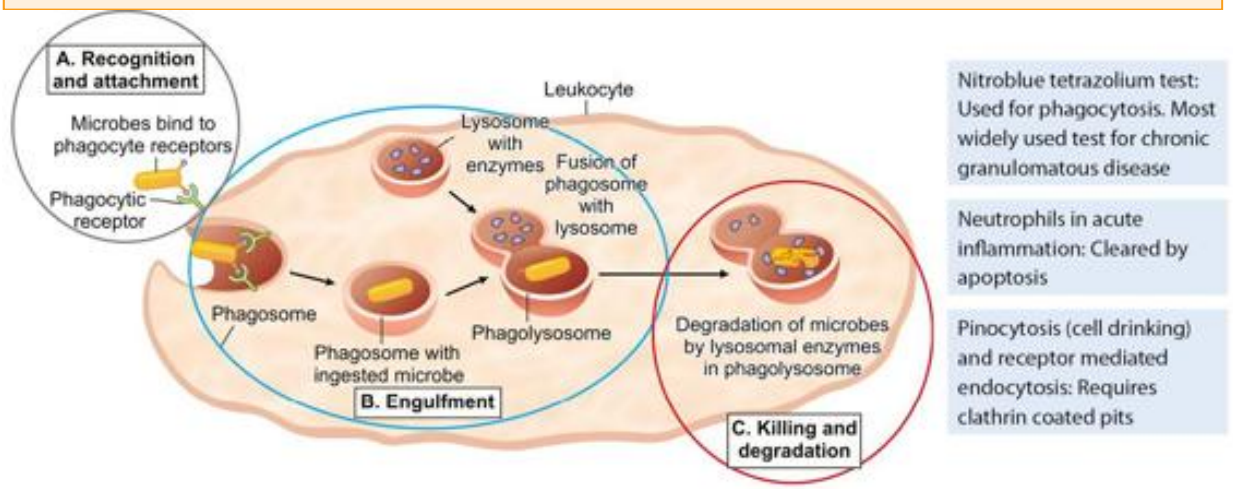
- Delayed separation of umbilical stump

Leukocyte adhesion deficiency type 2 (LAD2):
- Selectin defects
- Recurrent infections
- Bombay blood group
- Mental retardation

Leukocyte adhesion deficiency type 3 (LAD3):
- Caused by mutations in the gene *FERMT3*
- Impaired integrin activation
- Increased susceptibility to infection
- Leukocytosis, and petechial hemorrhage

Leukocyte Activation

Definition: Recognition of microbes or dead cells by the leukocyte receptors initiates several responses in leukocytes together known as **leukocyte activation**. The most important functional responses of leukocyte activation is **phagocytosis and intracellular killing**.



Figs. 3.5A to C: Different steps in phagocytosis. (A) Recognition and attachment which involves binding to receptors on the leukocyte membrane to injurious agent (e.g., bacteria); (B) Engulfment, formation of phagosome and fusion of lysosomes with phagocytic vacuoles to form phagolysosome; (C) Killing/degradation of ingested particles within the phagolysosomes by lysosomal enzymes and by reactive oxygen and nitrogen species.

Phagocytosis and Clearance of the Offending Agent (Figs. 3.5A to C)

Q. Write short note on phagocytosis and its sequence of events.

Definition: Many leukocytes **recognize**, **internalize, and digest** foreign material, microorganisms,

or cellular debris by a process termed phagocytosis.

Steps in phagocytosis: It consists of three steps **(Figs. 3.5A to C):**

1. Recognition and attachment
2. Engulfment
3. Killing or degradation of the ingested material.

Recognition and Attachment

Receptors on the surface of phagocytic cells recognize components of microbes and necrotic cells. Leukocytes express several receptors that recognize external stimuli. These include (1) receptors for microbial products (e.g., Toll-like receptors-TLRs), (2) G protein-coupled receptors (recognize N-formyl methionie residues), (3) receptors for cytokines (for IFN-γ), (4) mannose receptors (binds to terminal mannose and fucose residues of glycoproteins and glycolipids present on microbial cell walls), (5) scavenger receptors (bind and ingest low-density lipoprotein (LDL) particles and a variety of microbes), and (6) receptors for opsonins (described below).

Q. Write short note on opsonins and their role in inflammation.

- **Receptors for opsonins (phagocytic receptor):** The phagocytosis is enhanced when leukocyte receptors recognize microbes coated by specific host proteins known as **opsonins**. The major opsonins are IgG antibodies, the C3b breakdown product of complement, and certain plasma lectins (notably mannose-binding lectin) called collectins **(Table 3.6)**.

Clinical significance of opsonins:

After exposure to antigen, B cells get activated and mature into plasma cells, which produces immunoglobulins (IgG).

Bruton disease: Defect in maturation of the B-cells leading to absence of immunoglobulin production. Hence, there is **defective opsonization** in Bruton disease.

Engulfment

Next step in phagocytosis is engulfment and formation of a phagocytic vacuole. Phagocytosis is dependent on polymerization of actin filaments present in the phagocytic cell.

- **Phagosome:** Extensions of the **cytoplasm of leukocyte form pseudopods surrounding the particle to be ingested** and forms a vesicle or vacuole called a phagosome.
- **Phagolysosome:** The membrane of **phagosome fuse**s with membrane of **lysosome to form a phagolysosome**. Lysosomal **granules are discharged** into this phagolysosome.

TABLE 3.6: Different opsonins and their corresponding receptors on leukocyte.

Opsonin	***Receptor on leukocyte***
IgG antibodies	Fc receptor (FcγRI)
Complement components C3	Type 1 and 3 complement receptor (CR1 and CR3)
Collectins	C1q

During engulfment, the phagocyte also may release some contents of its granule into the extracellular space. They may damage innocent bystander normal cells.

Clinical significance of defects in phagolysosome function:

Chédiak-Higashi syndrome: It is a autosomal recessive condition characterized by:

- **Increased susceptibility to infections: Due to defective fusion of phagosomes with lysosomes in phagocytes.**
- Leukocyte abnormalities include:
  - Neutropenia (decreased numbers of neutrophils)
  - Defective degranulation and delayed microbial killing
  - Peripheral blood smear: **Leukocytes** contain **giant granules**, due to aberrant phagolysosome fusion.
- Gene associated with this syndrome encodes a **large cytosolic protein** called **LYST** (Lysosomal Trafficking Regulator), which **regulates lysosomal trafficking**.
- Albinism: Due to abnormalities in melanocytes.
- Nerve defects.
- Bleeding disorders due to defect in platelets.

Killing and Degradation

Killing and degradation of ingested microbial agents/particles occurs within neutrophils and macrophages. Most important microbicidal agents are: (1) reactive oxygen species (2) reactive nitrogen species-derived from nitric oxide (NO), (3) lysosomal enzymes and (4) granule enzymes and other proteins.

Reactive oxygen species (ROS):

Q. Write short note on free radicals and acute inflammation.

Types of ROS **(also called reactive oxygen intermediates)** are:

- Superoxide anion (O_2 one electron)—**weak**
- Hydrogen peroxide (H_2O_2, two electrons)—**weak**
- Hydroxyl ions ($^{\bullet}OH$), three electrons—**highly reactive.**

Mechanism of production (*refer* pages 17-9)**:** In the phagocytic vacuole of leukocyte, rapid activation of NADPH oxidase (also called **phagocyte oxidase**), oxidizes NADPH (reduced nicotinamide-adenine dinucleotide phosphate) to NADP. During the process oxygen is reduced to superoxide anion (O_2). In neutrophils, this oxidative reaction occurs during phagocytosis and is called as *respiratory burst*.

- $O_2$is converted into hydrogen peroxide (H_2O_2) by spontaneous dismutation (a process of simultaneous oxidation and reduction).

  $O_2 + 2H \rightarrow H_2O_2$

- Amount of H_2O_2 produced is insufficient to kill most of the microbes by itself but the enzyme **myeloperoxidase** (MPO) present in the azurophilic granules of neutrophils (*refer* Fig. 1.9) can convert it into a powerful ROS. MPO in the presence of a halide such as Cl^-, converts H_2O_2 to hypochlorous radical ($HOCl^{\bullet}$), which is a potent oxidant and antimicrobial agent. Hypochlorite ($HOCl^{\bullet}$) destroys microbes either by **halogenation** (chemical reaction that involves the addition of halogens, e.g., chlorine) or by proteins and lipid peroxidation (oxidative degradation of lipids).

- H_2O_2 is also converted to hydroxyl radical ($^{\bullet}OH$) which is also powerful destructive agent.

Oxygen-derived radicals may be released extracellularly from leukocytes and may produce tissue damage during inflammation. Serum, tissue fluids, and host cells have *antioxidant mechanisms* which can protect tissue damage caused by these potentially harmful oxygen-derived radicals (*refer* pages 17-9).

***Reactive nitrogen species** (**Fig. 3.7** and page 62):*

Q. Write short note on nitric oxide in inflammation.

NO ***(Nitric Oxide)*** is a soluble gas, which is produced from L-arginine by the action of nitric oxide synthase (NOS) and can kill microbes similar to ROS (for source, types and actions *refer* page 61). Inducible (iNOS) type (one of the three types) is involved in microbial killing. iNOS is expressed when macrophages are activated by cytokines (e.g., IFN-γ) or microbial products. The iNOS produces NO within macrophages.

In macrophages, $^{\bullet}NO$ reacts with superoxide (O_2^-) and produces highly reactive free radical peroxynitrite ($ONOO^{\bullet}$). Similar to ROS, they attack and damage the lipids, proteins, and nucleic acids of microbes and host cells.

Lysosomal enzymes:

Acid hydrolases of lysosomes degrade the dead microorganisms. Elastase within lysosomes can kill bacteria.

Granule enzymes and other proteins:

Neutrophils and monocytes have granules containing enzymes and anti-microbial proteins. **These granules are** actively secreted and are thus **different from classical lysosomes**. They can **degrade microbes and dead tissues and may also damage tissue**.

- **Neutrophils:** They contain two main types of granules namely: **smaller specific (or secondary) granules and larger azurophil (or primary) granules** (*refer* page 62).

- **Monocytes/macrophages:** Contents of their granules is mentioned on page 68.

Phagocytic vesicles containing engulfed material (or microbial) may fuse with granules of leukocytes and also with lysosomes. They will destroy the ingested material. If the initial leukocytic infiltration goes unchecked, the enzymes in these granules can damage the normal tissues. Normally, these harmful enzymes (especially proteases) are controlled by a system of **anti-proteases** in the serum and tissue fluids. One of important anti-protease is α_1-anti-trypsin, which is the major inhibitor of neutrophil elastase. A deficiency of α_1-anti-trypsin may lead to sustained action of leukocyte proteases, and damage the tissue (e.g., emphysema).

Phagocytosis: Process by which recognition, internalization and digestion of foreign material, microorganisms, or cellular debris occurs.

Opsonization: Process of coating of a particle (e.g., microbe), by opsonins to increase its **phagocytosis**.

Opsonins include:
- Antibodies
- Complement fragment C3b
- Acute phase proteins (e.g., CRP)
- Collectins
- Mannose-binding lectins

Phagocytosis (cell eating): Requires polymerization of actin filaments in the leukocytes.

Oxygen dependent killing in leukocytes is done **through generation of ROS** by NADPH oxidase present in the leukocytes.

Oxygen dependent MPO system is the **most powerful microcidal mechanism**.

Hypochlorite ($HOCl^{\bullet}$)
1. Active component of bleach
2. It is an end product of oxygen dependent MPO system

Phagocytosis by leukocytes can destroy or remove the microbes and dead cells.

Chediak-Higashi syndrome: No formation of phagolysosome. Leukocytes have giant granules due to aberrant fusion of organelle.

Neutrophil secrets cathepsin G.

Neutrophil Extracellular Traps

Definition: Neutrophil extracellular traps (NETs) are extracellular fibrillar networks produced by neutrophils in response to infectious pathogens (bacteria and fungi) and inflammatory mediators (e.g., chemokines, cytokines, and complement proteins).

Mechanism: During the formation of NET, the nuclei of the neutrophils are lost, leading to a distinctive form of death of the neutrophils (sometimes termed NETosis). NETs are also formed in the blood during sepsis.

Composition: NETs consist of a viscous meshwork of nuclear chromatin (includes histones and associated DNA) that binds and concentrates granule proteins such as anti-microbial peptides and enzymes.

Function: NETs **concentrate antimicrobial substances at sites of infection and prevent the spread of the microbes** by trapping them in the fibrils. It is a non-phagocytic **mechanism of killing microbes**.

Significance: The nuclear chromatin in the NETs may be a source of nuclear antigens in systemic autoimmune diseases (e.g., lupus). In these patients, there is immunological reaction against their own DNA and nucleoproteins.

Inherited Defects in Microbicidal Activity

1. **Chronic granulomatous disease (CGD):** Group of congenital (inherited) disorders characterized by defects in bacterial killing.
    - **Decreased oxidative burst:** It is due to **defects in** the genes encoding components of **phagocyte oxidase (NADPH oxidase)** which generates superoxide anion (O_2^-). Variants of phagocyte oxidase defect are:
        - X-linked defect: Defect in the gene coding membrane component of NADPH/phagocyte oxidase.
        - Autosomal recessive: Defect in the gene coding cytoplasmic component of NADPH/phagocytic oxidase.
    - Susceptible to recurrent bacterial infection.
    - Disease is named granulomatous because the initial neutrophil defense is inadequate and there is **chronic inflammatory reaction rich macrophage** that tries to control the infection. These collections of activated macrophages try to wall off the microbes, forming aggregates called granulomas.

- **Diagnosis of CGD:**
  - **Nitroblue-tetrazolium (NBT) test:** This test depends on the direct reduction of NBT by superoxide anion (O_2^-) to form an insoluble formazan. It is positive in normal individuals (with NADPH oxidase), but negative in CGD.
  - **Dihydrorhodamine (DHR) test:** In this test, whole blood is stained with DHR, incubated and stimulated to produce superoxide anion (O_2^-). This free radical reduces DHR to rhodamine in cells with normal NADPH oxidase.
  - **Cytochrome C reductase assay:** This is an advanced test that quantifies the amount of superoxide anion (O_2^-) that can be produced by patient's phagocytes.

2. **MPO deficiency:** Decreased microbial killing because of defective MPO —H_2O_2 system.

Acquired Defects of Leukocyte Functions

- **Decreased production of leukocytes** [e.g., bone marrow suppression (tumors, radiation, and chemotherapy)].
- **Defect in leukocyte adhesion and chemotaxis** (e.g., diabetes, malignancy, sepsis, chronic dialysis).
- **Defects in phagocytosis and microbicidal activity** (e.g., leukemia, anemia, sepsis, diabetes, malnutrition).

Chronic granulomatous disease (CGD) is characterized by absence of NADPH oxidase and respiratory burst (Repeated infections by catalase positive organisms, bacterial infections by *Staphylococcus aureus* and fungal due to *Candida*).

Agents that block TNF (major cytokines in leukocyte recruitment) are most successfully used for the treatment of chronic inflammatory diseases.

Examples of leukocyte-induced injury:
- Acute: Acute respiratory distress syndrome, glomerulonephritis
- Chronic: Rheumatoid arthritis, atherosclerosis.

Termination of the AcuteInflammatory Response

Inflammation is terminated after the elimination of the offending agent. It is brought out by the following:

- Acute inflammatory process is reduced after the causative agents are removed. This is because of the following properties of the **chemical mediators of inflammation chemical mediators:**
  i. Are produced only as long as the stimulus persists
  ii. Have short half-life span
  iii. Are degraded after their release
- **Neutrophils also have short life span** and die by apoptosis within hours to a day or two after leaving the bloodstream.
- There is variety of **termination mechanisms** which stops the inflammatory process. These include:
  i. A switch in the type of metabolite produced from arachidonic acid namely proinflammatory leukotrienes to anti-inflammatory lipoxins (*refer* page 61).
  ii. The liberation of anti-inflammatory cytokines [e.g., transforming growth factor-β (TGF-β) and IL-10] from macrophages and other cells.

Termination of inflammation occurs when the offending agent is eliminated.

CHEMICAL MEDIATORS OF INFLAMMATION

CC: Enumerate and describe the mediators of acute inflammation.

Q. List chemical mediators of inflammation. Discuss the role chemical mediators in inflammation.

Numerous chemical mediators are responsible for inflammatory reactions.

General Features of Chemical Mediators

- **Source of mediators:** Mediators are derived either from cells or from plasma proteins **(Table 3.7).**
  - **Cell-derived mediators:**
    - Present either as preformed molecules (e.g., histamine in mast cell granules) or are synthesized de novo (e.g., prostaglandins, cytokines) in response to a stimulus.
    - Produced usually by platelets, neutrophils, monocytes/macrophages, and mast cells.

- **Plasma-derived mediators:** Produced mainly in the **liver** and present in the circulation as **inactive precursors, which require activation** (e.g., complement proteins, kinins).

- **Tightly regulated actions**.
- **Inter-related:** One mediator can stimulate the release of other mediators. The secondary mediators may have the similar, different or even opposite actions.
- Most act by **binding to specific receptor** on target cells.
- **Diverse targets:** Target cell type varies depending on the type of mediator. They can act on one or few or many diverse targets, or may have different effects on different types of cells.
- **Short-lived:** Most of these mediators have a short-lifespan.

Main mediators involved in the inflammatory reaction are listed in **Table 3.7**.

Cell-Derived Mediators

Q. Write short note on cell-derived mediators of inflammation.

Vasoactive Amines: Histamine and Serotonin

Histamine and serotonin are the first mediators to be released during inflammation, which are stored as preformed molecules in cells.

1. **Histamine:** It is a preformed vasoactive mediator. Responsible for immediate transient response.

Source: Mast cells (richest source), **blood basophils and platelets**.

- **Stimuli:**
  - **Physical injury** (e.g., trauma, cold, heat).

Q. Name the cell-derived mediators of inflammation.

Q. Name the plasma-derived mediators of inflammation.

TABLE 3.7: Main chemical mediators of acute inflammation.

Cell-derived	*Plasma protein-derived*

TABLE 3.7: Main chemical mediators of acute inflammation.

Cell-derived	*Plasma protein-derived*
• **Vasoactive amines** – Histamine – Serotonin	• **Complement components** – C3a – C5a – C3b – C5b-9 (MAC)
• **Arachidonic acid (AA) metabolites** – Prostaglandins – Leukotrienes	• **Kinins** – Bradykinin – Kallikrein
• **Platelet-activating factor (PAF)**	• **Coagulation/fibrinolytic system**
• **Reactive oxygen species (ROS)**	
• **Nitric oxide (NO)**	
• **Cytokines (TNF, IL-1) and Chemokines**	

(IL-1: interleukin-1; T NF: tumor necrosis factor; MAC: membrane attack complex).

- **Immune reactions** in which antibodies bind to mast cells (e.g., **allergic reactions**)
- **Other chemical mediators:** C3a and C5a, leukocyte-derived histamine-releasing proteins, neuropeptides (e.g., substance P), cytokines (IL-1, IL-8).

Actions: (1) **Dilation of arterioles** and (2) **increase of the vascular permeability**.

2. **Serotonin (5-hydroxytryptamine):** It is a preformed vasoactive mediator.

- **Source: Platelets,** some **neurons** and **enterochromaffin cells** in the gastrointestinal tract.
- **Stimulus:** Platelet aggregation and antigen-antibody complexes.
- **Actions:** Similar to those of histamine.

Arachidonic Acid Metabolites (Prostaglandins, Leukotrienes, and Lipoxins)

Q. Write short note on role of arachidonic acid metabolites in inflammation.

Arachidonic Acid (AA)

- **Source:** Derived from cell membrane phospholipids mainly by the enzyme phospholipase A_2.
- **Stimuli:** Mechanical, chemical, and physical stimuli or other mediators (e.g., C5a).
- **AA metabolism: Occurs along two major enzymatic pathways (Fig. 3.6)**. These are **cyclooxygenase pathway** (produce prostaglandins) and **lipoxygenase pathway** (produces leukotrienes and lipoxins).

A. Products of cyclooxygenase pathway: Most important products of cyclooxygenase pathway involved in inflammation are PGE_2, PGD_2, PGI_2 (prostacyclin), and TXA_2 (thromboxane A_2).

Q. Write short note on role of prostaglandin in acute inflammation. Add a note on thromboxane A_2.

- **Mechanism:** They are produced from AA by the actions of two **cyclooxygenases,** COX-1 and COX-2.
- **Local effects:**
  - **Thromboxane A2 (TxA_2): Vasoconstriction** and promotes **platelet-aggregation**
  - **Prostacyclin (PGI_2): Vasodilation** and **inhibits platelet aggregation**
  - **PGD_2 and PGE_2: Vasodilation and increased permeability**. PGD_2 is also a chemoattractant for neutrophils.
- **Systemic effects:**
  - Prostaglandins are responsible for **pain and fever** in inflammation.

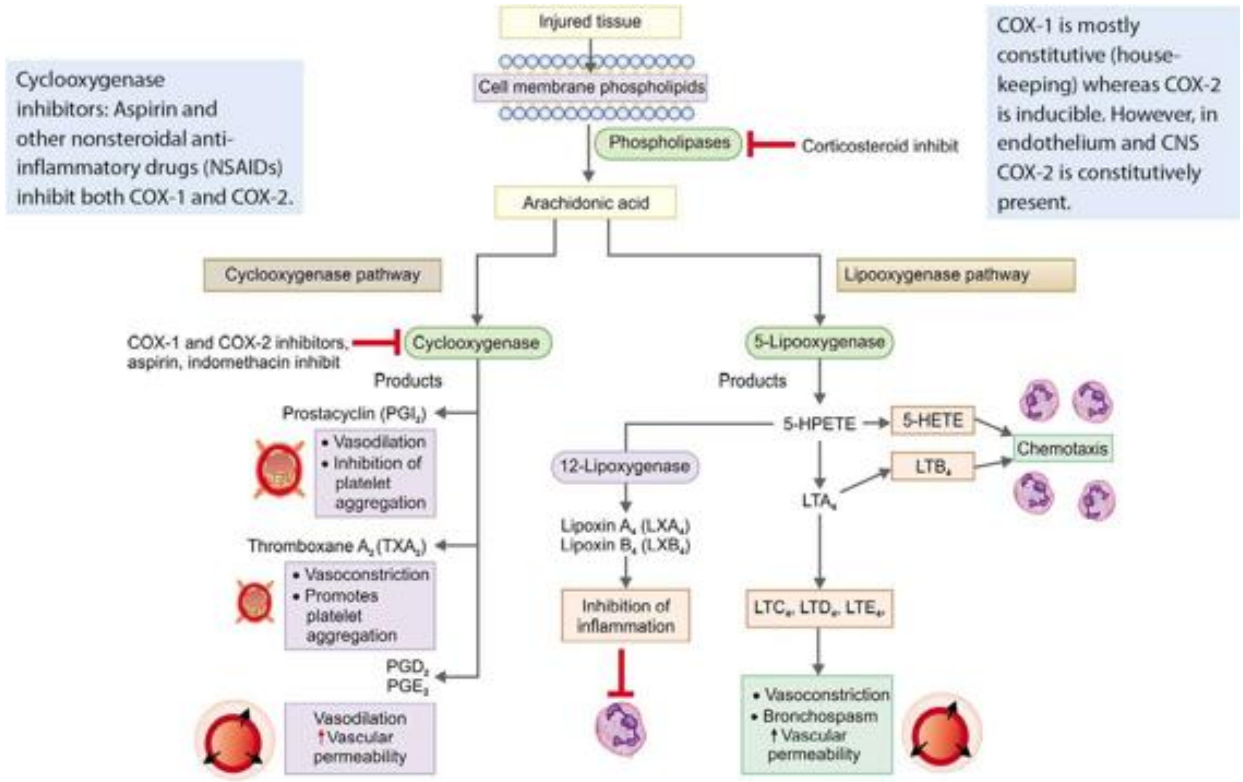


Fig 3.6: Arachidonic acid metabolites involved in inflammation. The cyclooxygenase pathway generates prostaglandins (PGIs) and thromboxane (TXA_2). The lipoxygenase pathway forms lipoxins (LXs) and leukotrienes (LTEs)
(COX: cyclooxygenase; HETE: hydroxyeicosatetraenoic acid; HPETE: hydroperoxy eicosatetraenoic acid)

- PGE_2 causes cytokine-induced **fever** during infections.

B. Products of lipoxygenase pathway: (1) Leukotrienes and (2) lipoxins.

1. **Leukotrienes: Products and their actions:**
   - **5-hydroxyeicosatetraenoic acid (5-HETE):** Chemotactic for neutrophils, and is the precursor of the leukotrienes.
   - **LTB_4**
     - **Chemotactic agent**
     - Neutrophil activation causing adhesion to endothelium, generation of ROS, and release of lysosomal enzymes.
   - **Leukotrienes C_4, D_4, and E_4** (LTC_4, LTD_4, LTE_4)
     - Vasoconstriction
     - Bronchospasm (in asthma)
     - **Increased vascular permeability**.
2. **Lipoxins (LXs):**
   - **Actions: Inhibit inflammation**
     - **Inhibit neutrophil chemotaxis** and recruitment
     - **Inhibit leukocyte adhesion** to endothelium

Main actions of arachidonic acid metabolites (eicosanoids) involved in inflammation are presented in **Table 3.8**.

Platelet-activating Factor (PAF)

Q. Write short note on platelet-activating factor.

Source: Platelets, basophils, mast cells, neutrophils, macrophages, and endothelial cells.

Action: Multiple inflammatory effects:

- **Vascular reactions:** Vasoconstriction and bronchoconstriction, and at low concentrations it induces vasodilation and **increased** vascular permeability.
- **Cellular reactions: Increased leukocyte adhesion** to endothelium and **chemotaxis**.
- **Platelet aggregation**.

Reactive Oxygen Species

Q. Write short note on free radicals and acute inflammation.

Reactive oxygen species (ROS) are chemically reactive oxygen-derived free radical. Normally, they are rapidly inactivated. But increased production can cause cell injury.

Cell of origin: Leukocytes (neutrophils and macrophages).

Mechanism of production: Leukocytes during phagocytosis (after exposure to microbes, chemokines, and immune complexes) generate oxygen-derived free radicals (*refer* **Figs. 2.4 and 2.6**).

Types: Superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$).

$O_2^{\bullet-}$ can combine with NO to form reactive nitrogen species (peroxynitrite $ONOO^-$).

Actions:

- **Physiologic function:** ROS in leukocytes **destroys** phagocytosed **microbes and necrotic cells**.
- **Pathological actions:**
  - Endothelial cell damage, which causes increased vascular permeability.
  - Injury to other cells (e.g., tumor cells, parenchymal cells and red blood cells).
  - Inactivation of antiproteases, such as α_1-antitrypsin, e.g., destruction of elastic tissues in emphysema of lung.

Nitric Oxide

Q. Write short note on nitric oxide in inflammation.

Nitric oxide (NO) is a soluble, free radical gas which causes vasodilation (was known as endothelium-derived relaxing factor).

Source: Many cells such as endothelial cells, macrophages and neurons in the brain.

Synthesis: Synthesized from L-arginine, molecular oxygen, and NADPH by the enzyme nitric oxide synthase (NOS).

Types: Three isoforms/types of NOS: type I neuronal (nNOS), type II inducible (iNOS) and type III endothelial (eNOS).

Action (Fig. 3.7): It acts in a paracrine manner on target cells.

- **Vasodilatation** by relaxing vascular smooth muscle cells.
- **Controls inflammatory responses** by inhibiting leukocyte recruitment and adhesion.
- **Reduced platelet adhesion, aggregation and degranulation.**
- **Microbicidal activity**.

Cytokines and Chemokines

Q. Write short note on cytokines.

These are polypeptides which function as mediators in **immune responses** and in **inflammation (acute and chronic)**.

Source: Cytokines are secreted by many types of cell (activated lymphocytes and macrophages, endothelial, epithelial, and connective tissue

cells). Important cytokines involved in inflammation are discussed below.

Tumor Necrosis Factor and Interleukin-1

These are the two major cytokines involved in inflammation.

Source: Activated macrophages.

TABLE 3.8: Main actions of arachidonic acid metabolites (eicosanoids) in inflammation.

Action	*Arachidonic acid metabolites (eicosanoid)*
Vasodilation	PGI_2 (prostacyclin I_2), PGE_2, PGD_2
Increased vascular permeability	Leukotrienes C_4, D_4, E_4
Chemotaxis, leukocyte adhesion	Leukotriene B_4, HETE (hydroxyeicosatetraenoic acid)

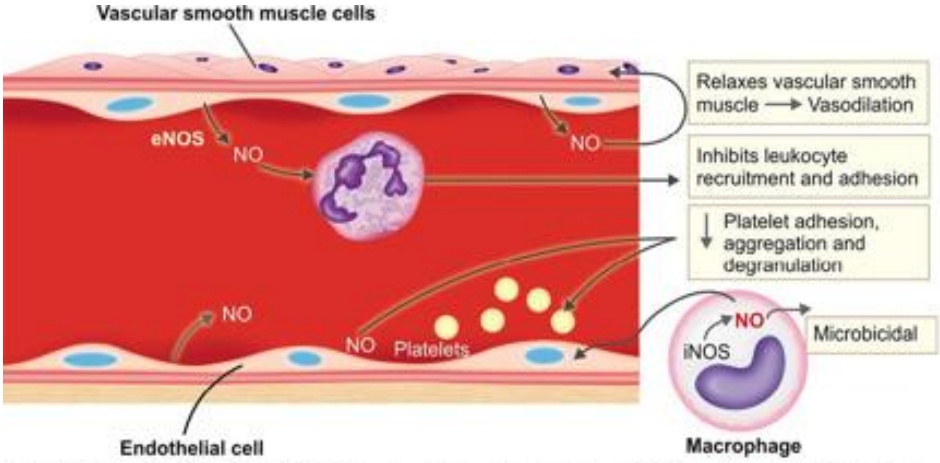


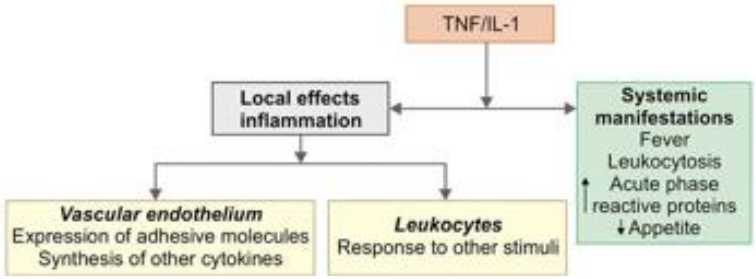
Fig. 3.7: Role of nitric oxide (NO) in blood vessels and macrophages. NO is produced by NO synthase enzymes. It causes vasodilation, and NO-derived free radicals are microbicidal.

Nitric oxide: Synthesized from the amino acid L-arginine

NO produce vasodilatation and kills microbes

Inhibitors of inflammation:
1. Nitric oxide (NO)
2. Lipoxins

Flowchart 3.2: Important local and systemic effects of tumor necrosis factor (TNF) and interleukin-1 (IL-1).



Cytokines include:

- Interleukins
- Chemokines
- Colony-stimulating factors
- Interferons
- Tumor necrosis factors

IL-1: Most important cytokine responsible for systemic effects of inflammation

Stimuli: Endotoxin and other microbial products, immune complexes, physical injury, and many inflammatory stimuli.

Actions in inflammation (Flowchart 3.2):

- **Local effects:**
  - **Endothelium:** Endothelial activation and increased expression of **endothelial adhesion molecules**.
  - **Leukocytes:** TNF increases the responses of neutrophils to other stimuli (e.g., bacterial endotoxin).
  - **During repair:** Proliferation of fibroblasts and increased synthesis of collagen.
- **Systemic effects:**
  - Fever
  - Leukocytosis
  - Systemic acute-phase reactions
  - Suppresses appetite: TNF contributes to cachexia seen in some chronic infections.

Chemokines

Chemotactic cytokines or chemokines are small proteins, which selectively attracts various leukocytes to the site of inflammation.

Classification: Chemokines are classified into four major groups namely—(1) C-X-C chemokines, (2) C-C chemokines, (3) C chemokines and (4) CX3C chemokines.

Action: Chemotaxis of monocytes, eosinophils, basophils, and lymphocytes except neutrophils. They activate leukocyte and promote their recruitment to the sites of inflammation.

Other Cytokines in Acute Inflammation

- IL-6 produced by macrophages and other cells is involved in local and systemic reactions.
- IL-17 produced by T lymphocytes promotes neutrophil recruitment.

Lysosomal Constituents of Leukocytes

Neutrophils

Types of granules:

1. **Smaller specific (or secondary) granules:** They contain lysozyme, collagenase, gelatinase, lactoferrin, plasminogen activator, histaminase, and alkaline phosphatase.
2. **Larger azurophil (or primary) granules:** They **contain myeloperoxidase**, bactericidal factors (lysozyme, defensins), acid hydrolases, and a variety of neutral proteases (elastase, cathepsin G, nonspecific collagenases, proteinase 3).

Monocytes and Macrophages

They also contain acid hydrolases, collagenase, elastase, phospholipase, and plasminogen activator. These are active mainly in chronic inflammation.

Neuropeptides

- These are small peptides, such as substance P and neurokinin A.
- **Source:** Secreted by sensory nerves and various leukocytes.
- **Action:** Vasodilation and increased vascular permeability.

Chemical mediators: Most of them have short-lifespan.

Vasoactive amines namely—histamine and serotonin cause **vasodilatation and increased vascular permeability**.

Chemical mediators:
1. Cell-derived or
2. Plasma protein-derived

Histamine and serotonin are preformed vasoactive cell-derived mediators.

Arachidonic acid: Can be enzymatically converted into prostaglandins and leukotrienes (both together called as eicosanoids).

Many anti-inflammatory drugs act by inhibiting the synthesis of eicosanoids.

PGI_2: Inhibition of platelet aggregation (I = inhibition)
TXA_2: Platelet aggregation (A = aggregation).

$PGF_{2\alpha}$: Vasoconstrictor
PGD_2 and PGE_2: Vadodilators

Arachidonic acid products: Can mediate almost every step of inflammation.

Broad-spectrum inhibitors: Corticosteroids reduce the transcription of genes encoding COX-2, phospholipase pro-inflammatory cytokines (such as IL-1 and TNF), and iNOS.

Lipoxygenase inhibitors: Drugs which inhibit leukotriene production (e.g., Zileuton) or block leukotriene receptors (e.g., Montelukast) are used in the treatment of asthma.

ROS: Cause killing of microbes and tissue damage.

Cytokines are soluble proteins that mediate immune responses and inflammation.

Cytokines exert their effect by binding to specific receptors on target cells.

Cytokines play multiple roles in inflammation. Causes endothelial activation and fever.

Some chemokine receptors (CXCR-4, CCR-5) act as coreceptors involved in binding and entry of the human immunodeficiency virus into lymphocytes.

IL-10 and TGF-β: Possess anti-inflammatory action. TGF-β is the most important fibrogenic agent.

Main cytokines involved in acute inflammation are: **TNF, IL-1 and IL-6**.

Chemokines are chemotactic and also cause leukocyte activation.

Lysosomal enzymes:
- Microbial killing
- Tissue injury

Plasma-derived Mediators

Q. Name the plasma-derived mediators of inflammation.

Chemical mediators derived from plasma proteins belong to three interrelated systems:

1. Complement
2. Kinin
3. Clotting systems.

Complement System

Q. What are the three methods of complement activation and its effector function in acute inflammation?

Q. Write a short note on role of complements in inflammation.

The complement system is a group of plasma proteins synthesized in the liver, and are numbered C1 to C9.

Pathways of complement system activation (Fig. 3.8): The decisive step in complement activation is the proteolysis of the third component, C3.

Cleavage of C3 can occur by any one of the three pathways:

1. **Classical pathway:** It is activated by **antigen-antibody** (Ag-Ab) complexes.
2. **Alternative pathway:** It is triggered by microbial surface molecules (e.g., endotoxin, or LPS), complex polysaccharides, cobra venom, and other substances, **in the absence of antibody**.
3. **Lectin pathway:** It directly activates C1 when plasma mannose-binding lectin binds to mannose on microbes.

Functions of Complement

(a) Anti-infective functions:

1. **Leukocyte activation, adhesion and chemotaxis:** C5a causes **leukocyte activation and adhesion.** C3a and C5a are **powerful chemotactic** agents for neutrophils, monocytes, eosinophils, and basophils.

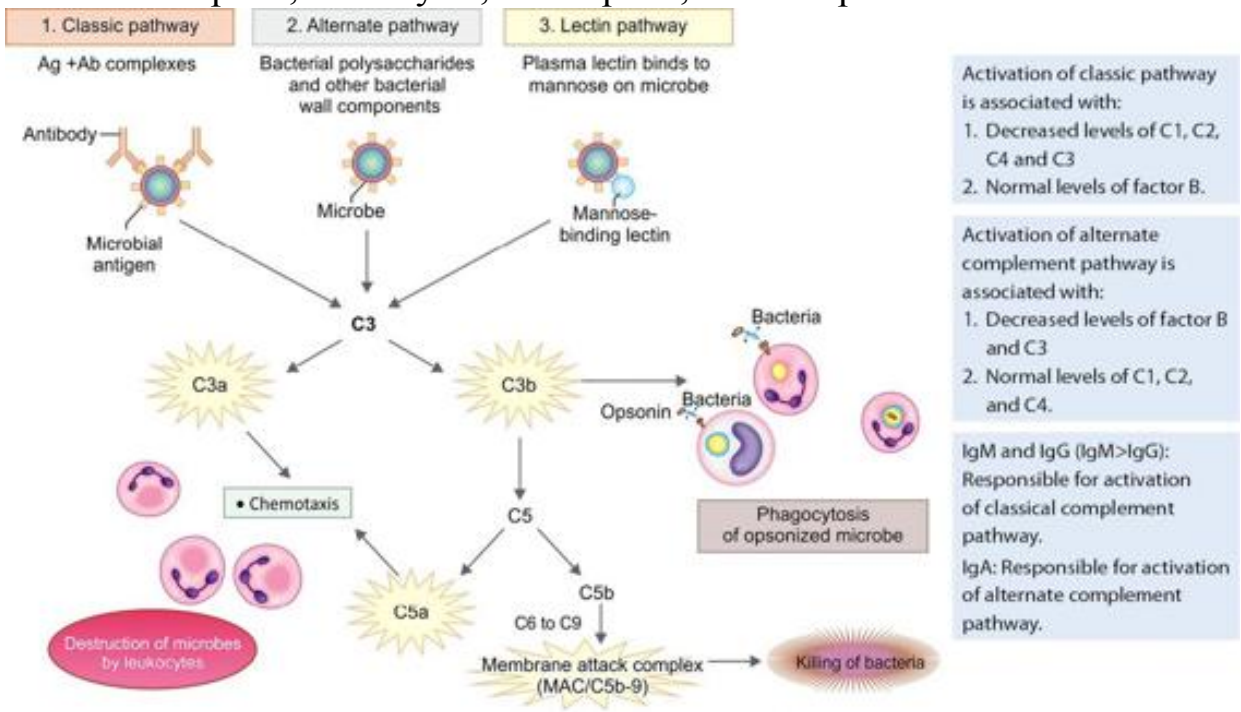


Fig. 3.8: Different pathways of activation and functions of the complement system. All pathways of activation lead to cleavage of C3.

2. **Opsonization and promote phagocytosis:** C3b and its cleavage product iC3b (inactive C3b) act as opsonins. They promote phagocytosis by neutrophils and macrophages through surface receptors for these complement fragments.
3. **Cell and bacterial lysis:** The deposition of the membrane attack complex—MAC (C5b-C9) on cells and bacterial wall creates pores. This allow water and ions to enter into the cells and results in death (lysis) of the cells and bacteria.
4. **Increased vascular permeability:** C3a, C5a complement components stimulate histamine release from mast cells. This in turn increase vascular permeability and cause vasodilation. C3a and C5a are called anaphylatoxins, because their actions are similar to mast cell mediators involved in anaphylaxis.

5. **Activation of AA:** C5a activates the lipoxygenase pathway of AA metabolism in neutrophils and monocytes, thereby causing release of more chemical mediators.

(b) Interplay between innate and adaptive immune system: Complements play a role in defense against microbes through innate and adaptive immunity.

(c) Other functions: These include clearance of:

- Immune complexes (Clq, C3)
- Apoptotic cells (Clq, C3).

The activation of complement is controlled by cell-associated and circulating regulatory proteins (e.g., C1 inhibitor, decay accelerating factor (DAF) and CD59).

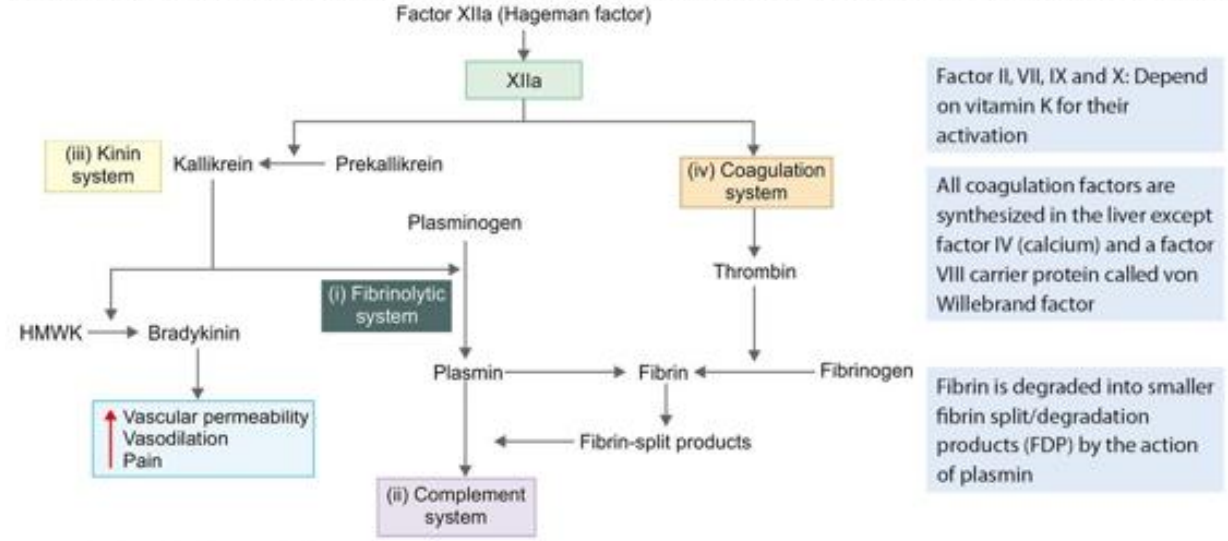
Coagulation and Kinin Systems

Inflammation and clotting system are intertwined with each other.
Activated Hageman factor (factor XIIa) activate the four systems involved in the inflammatory response**(Flowchart 3.3)**.

1. **Activation of fibrinolytic system:** Factor XIIa stimulates fibrinolytic system by converting plasminogen to plasmin. The role of fibrinolytic system in inflammation are:
   - **Activation of complement system.**
   - **Fibrin split products:** Plasmin degrades fibrin to form fibrin split products, which may **increase vascular permeability**.
2. **Activation of the kinin system**
3. **Activation of the alternative complement pathway:** Factor XIIa can activate alternate complement pathway.
4. **Activation of the coagulation system:** Factor XIIa activates coagulation system and forms thrombin, which has inflammatory properties.

Deficiency of C2:
1. Most common complement deficiency.
2. Associated with streptococcal septicemia and lupus like syndrome in children

Flowchart 3.3: Interrelationships between the four plasma-derived chemical mediator systems. Activation of factor XII (Hageman factor) is a key event leading to (i) conversion of plasminogen to plasmin, resulting in generation of fibrin split products and (ii) active complement products. (iii) Activation of kallikrein produces kinins and (iv) activation of the coagulation system results in fibrin formation.



(HMWK: high-molecular-weight kininogen)

Kinins System

Kinins are vasoactive peptides derived from plasma proteins.

- **Mechanism of production:** Factor XIIa converts prekallikrein to kallikrein, which in turn, cleaves high-molecular-weight kininogen to produce bradykinin.
- **Actions of bradykinin:**
  - **Increases vascular permeability**
  - **Pain** when injected into the skin.
- **Actions of kallikrein:**
  - Potent activator of Hageman factor
  - Chemotactic activity: Directly converts C5 to the chemoattractant product C5a.

Most important mediators involved in acute inflammation are summarized in **Table 3.9**.

C3 is the complement component that can be activated by(1) classical (Ag+Ab complexes), (2) alternate pathway and(3) lectin pathway.

C1 inhibitor: Blocks activation of C1. Inherited deficiency of C1 inhibitor is associated with hereditary angioedema (edema at multiple sites including the larynx).

Complement components can cause chemotaxis (C3a C5a), opsonization (C3b) and killing (MAC) and increased vascular permeability.

Critical step in complement system: Activation of C3.

C3a and C5a are called anaphylotoxins, because their actions are similar to mast cell mediators involved in anaphylaxis.

Activation of complement is controlled by cell-associated and circulating regulatory proteins. These include: C1 inhibitor, decay-accelerating factor (DAF), and factor H.

Activated factor XII (XIIa) triggers activation of:
1. Coagulation system
2. Kinin system
3. Complement system
4. Fibrinolytic system

Bradykinin mediates **increased vascular permeability** and **pain**.

Genetic or acquired defects in leukocyte function: Recurrent infections.

In genetic deficiency of MPO, the increased susceptibility to infection is due to: Inability to produce hydroxyl-halide radicals.

OUTCOMES OF ACUTE INFLAMMATION (FLOWCHART 3.4)

Q. Write short note on outcomes of acute inflammation.

1v **Resolution:** It is the process of complete **return** of tissue architecture **to normal** following acute inflammation. It occurs:
- n When the injury is limited or short-lived
- n With no or minimal tissue damage
- n When injured tissue is capable of regeneration.

2v **Organization/healing by fibrosis:** Process of **replacement of dead tissue by living tissue,** which matures to form scar tissue is known as **organization**. It occurs:

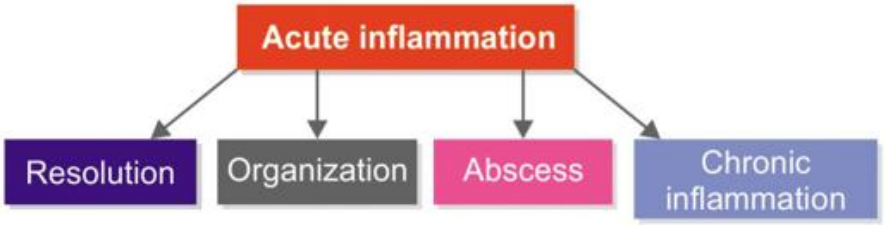
Q. Write short note on role of different mediators in different reactions of inflammation.

TABLE 3.9: Important mediators involved in acute inflammation.

Bacterial products (e.g., *N*-formyl methyl peptides)	Bacteria	
Fever	IL-1 TNF	Macrophages, endothelial cells, mast cells
	Prostaglandins	Mast cells, leukocytes
Pain	Prostaglandins	
	Bradykinin	Plasma protein
Tissue damage	Lysosomal enzymes	Leukocytes
	Reactive oxygen species	
	Nitric oxide	Endothelium, macrophage

(IL-1: interleukin-1; IL-6: interleukin-6; TNF: tumor necrosis factor).

Flowchart 3.4: Outcomes of acute inflammation: (1) resolution, (2) organization (healing by fibrosis and scarring), (3) abscess or (4) chronic inflammation.



- When there is plenty of fibrin exudation in tissue or serous cavities (pleura, peritoneum) which cannot be removed or cleared.
- In presence of significant tissue destruction.
- With inflammation in tissues incapable of regeneration.

This process involves growing of connective tissue into the area of tissue damage or exudate, and is converted into a mass of fibrous tissue (scar).

3. **Abscess:** Localized collection of pus is called abscess. If the area of acute inflammation is walled off by inflammatory cells and fibrosis, neutrophil products destroy the tissue and form an abscess.
4. **Progression to chronic inflammation:** Chronic inflammation may follow acute inflammation, or it may be chronic from the beginning itself. Acute inflammation may progress to chronic when the acute inflammatory response cannot be resolved. This may be due to:
   - Persistence of the injurious agent or
   - Abnormality in the process of healing.

   Examples:

- Bacterial infection of the lung may begin as acute inflammation (pneumonia). But when it fails to resolve, it can cause extensive tissue destruction and form a cavity with chronic inflammation known as lung abscess.
- Acute osteomyelitis, if not treated properly may progress to chronic osteomyelitis.
- Chronic inflammation with a persisting stimulus results in peptic ulcer of the duodenum or stomach, which may persist for months or years.

MORPHOLOGICAL TYPES/PATTERNS OF ACUTE INFLAMMATION

Q. Write short note on morphological types/patterns of acute inflammatory reaction with suitable examples.

Gross and microscopic appearances can often provide clues about the cause.

Serous Inflammation

- Characterized by marked outpouring of a thin serous fluid.
- Serous exudate or effusion is yellow, straw-like in color and microscopically shows either few or no cells.
- **Example:**
  - Skin blister formed in burns or viral infection.
  - Inflammation of synovium (synovitis).
  - Pleural effusion as a complication of lobar pneumonia.

Effusion: Accumulation of fluid in serous cavities (peritoneal, pleural, and pericardial).

Fibrinous Inflammation

- **Marked increase in vascular permeability** leads to escape of large molecules like fibrinogen from the lumen of the vessel into the extravascular space and forms fibrin. The **exudate rich in fibrin** is called fibrinous exudate.

- A fibrinous exudate is mostly observed with inflammation in the lining of body cavities, such as the meninges, pericardium and pleura. When a fibrinous exudate develops on a serosal surface, such as the pleura or pericardium, it is known as fibrinous pleuritis or fibrinous pericarditis respectively.
- Microscopically, fibrin appears as an eosinophilic or pink meshwork of threads or pink amorphous coagulum.
- For example, fibrinous pericarditis (*refer* **Fig. 16.30**) is seen in rheumatic fever and classically known as "**bread and butter**" pericarditis.

Suppurative or Purulent Inflammation: Abscess

Q. Write a short note on abscess.

- It is characterized by the production of large amounts of pus or purulent exudate.
- Microscopically, shows neutrophils, liquefactive necrosis, and edema fluid. Bacteria (e.g., staphylococci), which produce localized suppuration are called as **pyogenic** (pus-producing) bacteria. For example, acute appendicitis.
- **Abscesses:** It is the **localized collections of purulent inflammatory exudates in a tissue**, an organ, or a confined space. Abscesses have a central necrotic focus (consisting of necrotic leukocytes and necrotic parenchymal cells) surrounded by a zone of preserved neutrophils (e.g., boil caused by *Staphylococcus aureus*). If pus accumulates in hollow organs or pleural cavity, it is known as empyema.

Hemorrhagic Inflammation

When inflammation is associated with severe vascular injury or deficiency of coagulation factors, it causes hemorrhagic inflammation, e.g., acute pancreatitis (*refer* **Fig. 19.39**) due to proteolytic destruction of vascular walls.

Catarrhal Inflammation

Acute inflammation of a mucous membrane is accompanied by excessive secretion of mucus and the appearance is described as catarrhal, e.g., common cold.

Membranous Inflammation

In this type, epithelium is covered by membrane consisting of fibrin, desquamated epithelial cells and inflammatory cells, e.g., pharyngitis or laryngitis due to *Corynebacterium diphtheriae*.

Pseudomembranous Inflammation

- It is characterized by superficial mucosal ulceration covered by sloughed mucosa, fibrin, mucus and inflammatory cells.
- For example, pseudomembranous colitis due to *Clostridium difficile* colonization of the bowel, usually following broad-spectrum antibiotic treatment.

Necrotizing (Gangrenous) Inflammation

The combination of necrosis and bacterial putrefaction is **gangrene** (*refer* pages 31-2 and **Fig. 2.20**), e.g., gangrenous appendicitis.

Ulcer

Q. Write short note on ulcer.

An ulcer is defined as a local defect, or excavation, of the surface of an organ or tissue. Common sites:

1. Mucosa of the mouth, stomach [(e.g., peptic ulcer of the stomach or duodenum (*refer* **Fig. 18.12**)], intestines, or genitourinary tract.
2. Skin and subcutaneous tissue of the lower extremities (e.g., varicose ulcers).

Terminology

Bacteremia: It is defined as condition characterized by the presence of small number of bacteria in the blood. They cannot be detected by direct microscopic examination of blood and are detected by blood culture (e.g., typhoid infection caused by *Salmonella typhi*).

Septicemia: It is defined as the presence of rapidly multiplying, highly pathogenic bacteria in the blood (e.g., pyogenic cocci/bacilli). It is usually associated with systemic effects such as toxemia and neutrophilic leukocytosis.

Pyemia: It is the dissemination of small septic emboli in the blood which produce their effects at the site of their lodgment. Thus, it can lead to pyemic abscesses or septic infarcts.

Cellulitis: It is the term used for diffuse inflammation of the soft tissues due to organisms, produced from spreading effects of substances like hyaluronidase released by some bacteria.

CHRONIC INFLAMMATION

CC: Define and describe chronic inflammation including causes, types, non-specific and granulomatous; and enumerate examples of each.

Definition: Chronic inflammation is defined as **inflammation of prolonged duration** (weeks or months) in which inflammation, tissue damage, and healing occurs at same time, in varying combinations.

Chronic inflammation may:

1. **Follow an acute inflammation,** which does not resolve (e.g., chronic osteomyelitis) or
2. **Begin as insidious, low-grade, chronic,** response without any acute inflammatory reaction.

Causes of Chronic Inflammation

Q. What are the causes of chronic inflammation?

1. **Persistent infections:** Microbes that are difficult to eradicate elicit delayed-type of hypersensitivity and produce chronic inflammation, e.g., mycobacteria, and certain viruses, fungi, and parasites. Some agents may cause a **distinct pattern of chronic inflammation** known as **granulomatous reaction**.
2. **Immune-mediated inflammatory (hypersensitivity) diseases:**
   - **Autoimmune diseases** (e.g., rheumatoid arthritis).
   - **Allergic reactions** (e.g., bronchial asthma)
   - **Unregulated immune response** (e.g., inflammatory bowel disease)
3. **Prolonged exposure to toxic injurious agents:**
   - **Exogenous: Silica** is a nondegradable inanimate exogenous material. If persons are exposed to silica particles for long time, it causes an inflammatory lung disease called **silicosis**.
   - **Endogenous**: **Atherosclerosis** is a disease of arterial intima, probably represents a chronic inflammatory process partly due to endogenous toxic plasma **lipid components**.

Morphologic Features

Q. Mention the morphological/histological features of chronic inflammation.

Chronic inflammation is characterized by:

- **Mononuclear cells infiltrate**: Macrophages, lymphocytes, and plasma cells.
- **Tissue destruction** caused by the persistence of causative agent or by the inflammatory cells.
- **Healing by fibrosis.**

Chronic Inflammatory Cells and Mediators

Q. Write short note on cells of chronic inflammation.

Macrophages

Macrophage is the predominant cell in chronic inflammation.

Macrophage Events in Inflammation

Q. Mention the role of macrophages in chronic inflammation.

- **Monocytes** also emigrate into extravascular tissues early in acute inflammation, and **within 48 hours**, they are the **predominant cell type**.
- **On reaching extravascular tissue**, the monocyte is **transformed into** a larger phagocytic cell known as tissue **macrophage**.

Macrophage Activation

Tissue macrophages are activated by **two major pathways:**

1. *Classical macrophage activation*

- **Mediators of activation:** It is brought out mainly by
  - Microbial products (e.g., endotoxin)

- T cell-derived signals: Mainly cytokines (e.g., IFN-γ)
- Foreign substances (e.g., crystals and particulate matter).

- **Products of activated macrophages**
  - Lysosomal enzymes
  - Nitric oxide
  - Reactive oxygen species (ROS)
- **Function:** Phagocytosis and killing/elimination of ingested microbes.

2. *Alternate macrophage activation*

- **Mediators of activation:** It is brought out mainly by **cytokines** IL-4 and IL-13 produced by T-cells and other cells.
- **Function:** Initiation of the tissue repair, (they are not bactericidal).

Functions of Macrophages in Inflammation

- **Phagocytosis:** Ingestion and elimination of microbes and necrotic tissue.
- **Initiation of the tissue repair**
- **Secretion of mediators of inflammation**: These include cytokines (TNF, IL-1, chemokines, etc.) and arachidonic acid metabolites.
- **Display signal to T-cells and respond signals from T-cells:** This is responsible for the feedback loop for defense against many microbes by cell-mediated immune response.

Lymphocytes

- **B and T-lymphocyte:** They are found in both antibody-mediated and cell-mediated immune reactions.
- **B lymphocytes:** They may develop into plasma cells and produce antibodies either against foreign or self-antigens at in the inflammatory site.
- **T lymphocytes:** Important being **$CD4^+$ helper T** cells which has **3 subtypes** namely:

Q. Write short note on T helper cell.

- **T_H1:** Produce IFN-ϒ and activates macrophage in the classical pathway.

- **T_H2:** Produce IL-4, IL-5 and IL-13 which recruit and activate eosinophils and activate macrophages through alternate pathway. Involved in defense against helminthic infestation and allergic reaction.
- **T_H17:** Produce IL-17 and other cytokines which recruit neutrophils and monocytes.

$CD4^+$ T lymphoytes secrete cytokines and promote inflammation and influence the nature of the inflammatory reaction.

Other Cells

- **Plasma cells:** They are derived from activated B lymphocytes and produce antibodies either against foreign or self-antigens. Activated B lymphocytes and antibody-producing plasma cells are seen at the site of chronic inflammation (e.g., syphilis). In some chronic inflammatory reactions, the lymphocytes, antigen-presenting cells, and plasma cells form lymphoid follicles similar to those found in lymph nodes. Examples include in synovium of patients with rheumatoid arthritis, in the thyroid in Hashimoto thyroiditis, and in the gastric mucosa in *Helicobacter pylori* infection.
- **Eosinophils:** They are seen in immune reactions mediated by IgE and in parasitic infections. A **chemokine**, which **attracts eosinophil recruitment is eotaxin**. The granules of eosinophils contain **major basic protein** which is toxic to parasites and also destroy the epithelial cells.
- **Mast cells:** They are distributed in connective tissues and participate in both acute and chronic inflammatory reactions. They are seen in allergic reactions to foods, insect venom, or drugs.
- **Neutrophils:** These are **characteristic of acute inflammation**. However, in many forms of chronic inflammation that lasts for months, continue to show many neutrophils. These may be because of either persistence of microbes or cytokines and other mediators produced by activated macrophages and T lymphocytes. For example in **chronic bacterial infection of bone (osteomyelitis)**, a neutrophilic exudate can persist for many months. Some times, neutrophils are seen along with chronic inflammation and this pattern of inflammation is called as **acute on chronic**.

Causes of chronic inflammation:

1. Persistent infections
2. Immune-mediated inflamamtory (hypersensitivity) diseases
3. Prolonged exposure to toxic agents

Sequelae: Chronic inflammation can cause disabling tissue damage, e.g., rheumatoid arthritis, tuberculosis, and atherosclerosis.

Most common cause of chronic inflammation: Persistent infection.

Tissue macrophage: Derived from hematopoietic stem cells in the bone marrow and from progenitors in the embryonic yolk sac and fetal liver during early development.

Main cytokines involved in chronic inflammation:
(1) IL-12 (2) IFN-γ (3) IL-17

$CD4^+$ helper T cells are of 3 types: (1) T_H1 (2) T_H2 (3) T_H17.

Chronic inflammation: Infiltration by lymphocytes, macrophages and plasma cells, often with significant fibrosis. In chronic endomeritis, there are plasma cells.

Viral infections: Lymphocytes are first cells to arrive at the site of inflammation.

TYPES OF CHRONIC INFLAMMATION

It can be divided into (1) chronic-nonspecific inflammation and (2) granulomatous inflammation.

Granulomatous Inflammation

Q. Define and classify granuloma. Write a short note on granulomatous lymphadenitis.

Definition: A **granuloma** is defined as a **distinctive type of chronic inflammation** characterized by **microscopic aggregation of activated macrophages** (that are transformed into epithelium-like/epithelioid cells) with scattered lymphocytes.

Older granulomas in addition show rim of fibroblasts and connective tissue as the outermost layer.

Structure of Granuloma

Q. Write short note on epithelioid cell and granuloma.

- **Epithelioid cells:** These are **modified macrophages which resemble epithelial cells**.
  - They have a **pale pink granular cytoplasm** with indistinct cell borders, often appearing to merge into one another.
  - The **nucleus is oval or elongate**, and may show folding of the nuclear membrane. The nucleus is less dense than that of a lymphocyte.
- **Giant cells: Epithelioid cells** frequently **fuse to form giant cells** and are found in the periphery or sometimes in the center of granulomas. These giant cells may attain diameters of 40–50 μm and have many small nuclei. Nuclei may be as many as 20 or more which are and may be arranged either peripherally (Langhans-type giant cell) or haphazardly (foreign body–type giant cell).
- **Lymphocytes:** As a cell-mediated immune reaction to antigen, lymphocytes form an integral part of granulomatous inflammation. Some types may be accompanied by plasma cells.
- **Necrosis: Sometimes granulomas are associated with central necrosis** (e.g., tuberculosis). However, the granulomas in Crohn disease, sarcoidosis, and foreign body reactions does not have necrotic centers and are called as *noncaseating (hard) granulomas*.
- **Fibrosis:** Granulomas may heal by producing extensive fibrosis.

Types of Granulomas

Depending on the pathogenesis they are of two types:

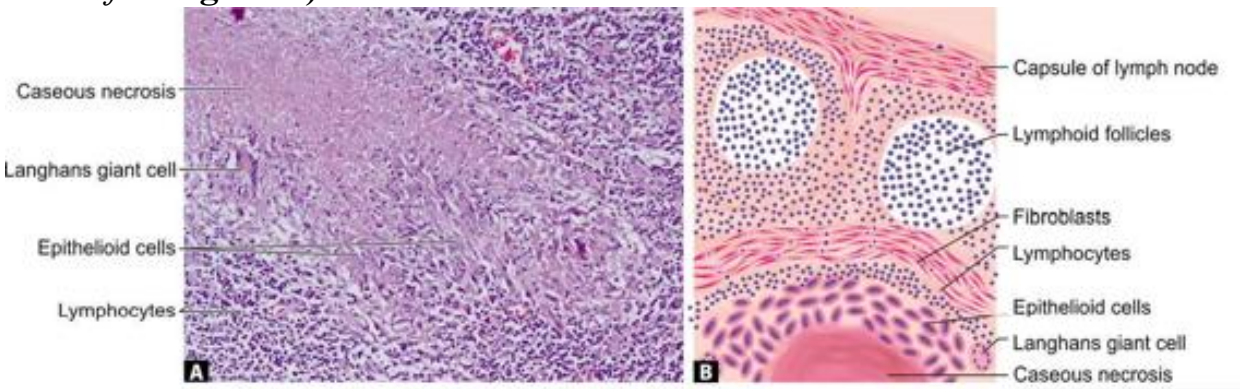
1. Foreign Body Granulomas

- It develops against relatively inert foreign bodies which do not incite any specific inflammatory or immune response (absence of T-cell-mediated immune responses).
- The foreign body which elicit granuloma include suture materials, talc (associated with intravenous drug abuse), or other fibers that are large enough to be phagocytosed by a macrophage. Epithelioid cells and giant cells are apposed to the surface of these foreign bodies.
- The foreign material can usually be found in the center of the granuloma, particularly if seen with polarized light (appears refractile).

2. **Immune Granulomas**

- These are caused by agents/microbes which are capable of inducing a persistent T-cell–mediated immune response.
- Immune granulomas usually develop when the inciting agent is difficult to eradicate, such as a persistent microbe (e.g., *Mycobacterium tuberculosis*) or a self-antigen. In these granulomas, macrophages activate T cells to produce cytokines, such as IL-2. This in turn activates other T cells, perpetuating the response, and IFN- γ, which activates the macrophages.
- Granuloma in tuberculosis **(Figs. 3.9A and B)** is referred to as a tubercle and usually shows central caseous necrosis (due to a combination of hypoxia and free radical–mediated injury) and is rare in other granulomatous diseases. Sometimes, it may be necessary to perform additional tests/investigations to identify the etiologic agent.
  - Special stains, e.g., acid-fast stains for tubercle bacilli
  - Culture methods, e.g., in tuberculosis and fungal diseases
  - Molecular techniques, e.g., the polymerase chain reaction in tuberculosis.
  - Serologic studies, e.g., in syphilis.

One of the cause of granulomatous lymphadenitis is tuberculosis **(Fig. 3.9 and *refer* Fig. 6.11).**



Figs. 3.9A and B: (A) Tuberculous granuloma showing an area of caseous necrosis surrounded by epithelioid cells, Langhans-type giant cell, and lymphocytes; (B) Diagrammatic appearance of granuloma (only part of it is shown) in tuberculous lymphadenitis.

Caseous necrosis:
- Tuberculosis
- Histoplasmosis

TABLE 3.10: Examples of granulomatous inflammation.

Disease	*Cause*	*Tissue reaction*

TABLE 3.10: Examples of granulomatous inflammation.

Disease	***Cause***	***Tissue reaction***
Tuberculosis (*refer* **Fig. 3.9**)	*Mycobacterium tuberculosis*	Caseating granuloma (tubercle): central necrosis with amorphous granular debris surrounded by epithelioid cells, rimmed by lymphocytes, histiocytes and fibroblasts. Occasional Langhans giant cells, presence of acid-fast bacilli
Leprosy **(Fig. 4.1B and C)**	*Mycobacterium leprae*	Acid-fast bacilli in macrophages; noncaseating granulomas
Syphilis	*Treponema pallidum*	Gumma: microscopic to grossly visible lesion. Consists of histiocytes; plasma cell infiltrate; central necrotic cells without loss of cellular outline (coagulative necrosis)
Cat-scratchdisease	Gram-negative *Bacillus*	Rounded or stellate granuloma containing central granular debris and neutrophils; giant cells rare
Sarcoidosis (*refer* **Fig. 17.21**)	Unknown etiology	Noncaseating granulomas with plenty of activated macrophages
Crohn disease (inflammatorybowel disease) (*refer* **Fig. 18.33**)	Immune reaction against intestinal bacteria, self-antigens	Dense chronic inflammatory infiltrate with occasional noncaseating granulomas in the wall of the intestine

Examples of granulomatous inflammation are listed in **Table 3.10**.

Eosinophils are observed in:
- Hypersensitivity reactions
- Parasitic infestations

Granulomatous inflammation: Distinctive pattern of chronic inflammation. It is produced by few infectious as well as noninfectious conditions and involves immune reactions.

Epithelioid cells: Modified macrophages and morphologically resemble epithelial cells. Macrophages are activated by IFN-g secreted by $CD4^+$ T-cells.

Giant Cell

Q. Write short note on various types of giant cells and the conditions associated with it.

Definition: Cell with more than one nucleus is called as giant cell or multinucleated cell.

Types of giant cells (Fig. 3.10): Various types of giant cells and its associated conditions are metioned in **Box 3.2**.

Granulomatous Diseases

Mycobacterium is bacteria, which appear as slender aerobic rods that grow in straight or branching chains. Mycobacteria have a waxy cell wall composed of mycolic acid, which is responsible for their **acid fast nature**. Mycobacteria are weakly Gram-positive. They are discussed on pages 85-8 and 511-8.

Acid fast means that mycobacteria retain stains even on treatment with a mixture of acid and alcohol.

SYSTEMIC EFFECTS OF INFLAMMATION

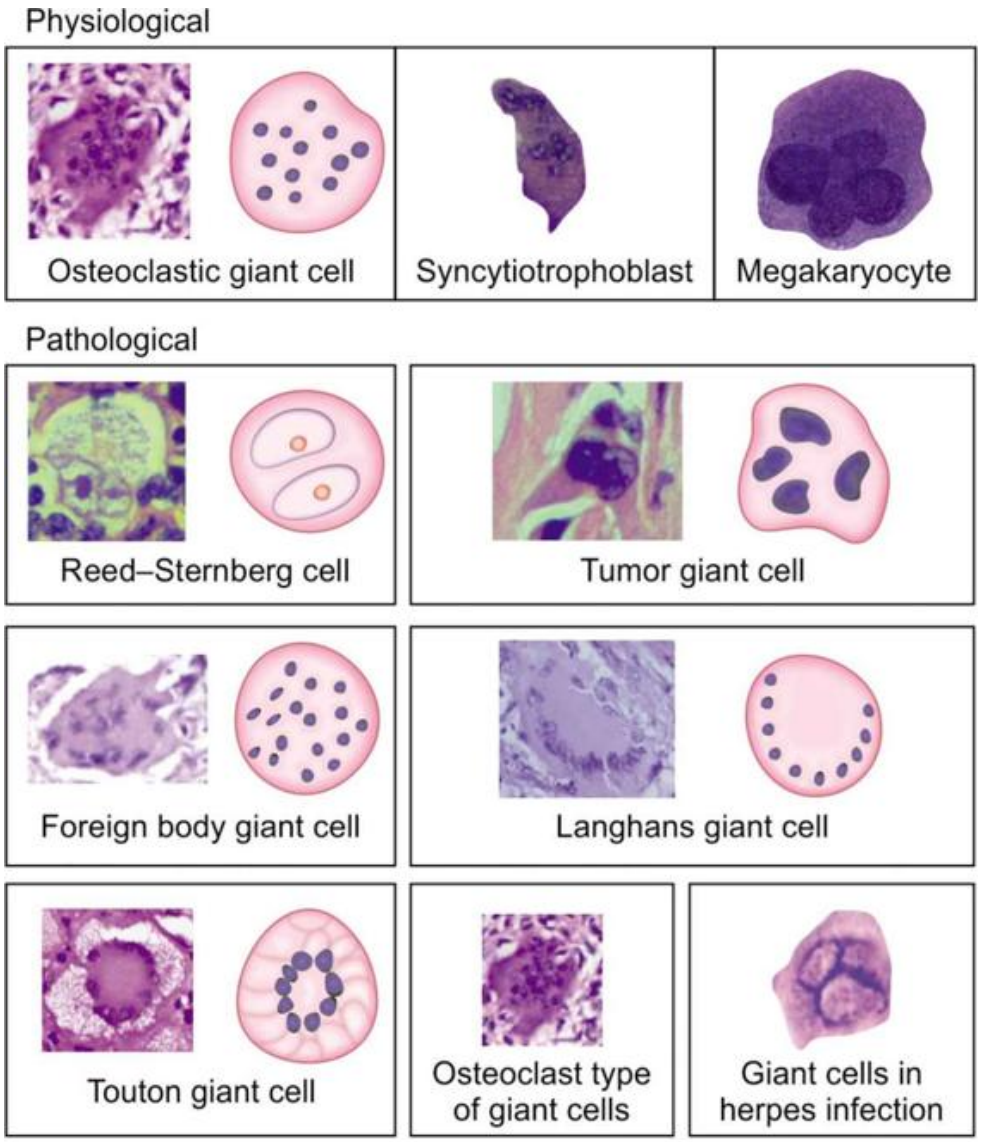


Fig. 3.10: Various types of giant cells.

BOX 3.2: Types of giant cells (Fig. 3.10).

PHYSIOLOGICAL

- *Osteoclast*
- *Syncytiotrophoblast*
- *Megakaryocyte*

PATHOLOGICAL

Damaged muscle fibers

- Regenerating sarcolemmal cells in damaged skeletal muscles

Tumor giant cells: They have hyperchromatic nuclei of varying size and shape

- Giant cell tumors: Bone (osteoclastoma)
- Reed Sternberg cells: Hodgkin lymphoma

- Giant cell variants of many malignant tumors, e.g., carcinoma of lung

Giant cells resulting from fusion of cells

- Viral infection
  - Epithelial giant cells, e.g., herpes virus infection (molding of nuclei to one another)
  - Connective tissue, e.g., Warthin–Finkeldey giant cells in measles

Fused macrophages

- Foreign body giant cells: These have multiple uniform nuclei scattered throughout the cytoplasm.
  - Reaction to exogenous insoluble material (e.g., suture material, talc, etc.)
  - Reaction to insoluble endogenous material [e.g., keratin (dermoid cyst of ovary, epidermal cyst), cholesterol, urate crystals (in gout)]
- Touton giant cells: These cells have vacuolated cytoplasm due to lipid, e.g. in xanthoma
- Reaction to certain organisms [e.g., tuberculosis (Langhans giant cells in which nuclei are arranged in a horseshoe pattern), fungal infections, syphilis]
- Fusion of cardiac histiocytes: Aschoff giant cells in rheumatic heart disease.

Q. Write short note on systemic effects of inflammation.

Systemic changes in acute inflammation are collectively known as **acute-phase response**, or the **systemic inflammatory response syndrome** (SIRS).

Causes: Due to cytokines produced by leukocytes, in response to infections or immune reactions. Most important cytokines are TNF, IL-1, and IL-6.

The clinical and pathologic changes of acute-phase response are discussed below.

Fever

- **Pyrogens:** These are molecules that cause fever. It may be **exogenous (**bacterial products, like **LPS)**, which stimulate leukocytes to release **endogenous** pyrogens **(cytokines such as IL-1 and TNF)**. The cytokines increase the enzymes cyclooxygenases resulting in conversion of arachidonic acid (AA) into **prostaglandins**.
- Pyrogens and prostaglandins may act on hypothalamic thermoregulatory center causing fever.

Raised Plasma Levels of Acute-phase Proteins

Q. Write short note on acute phase proteins/reactants.

Acute-phase proteins are plasma proteins synthesized in the liver and may be markedly raised in response to inflammatory stimuli.

Types of Acute-phase Proteins

(1) C-reactive protein (CRP), (2) fibrinogen, (3) serum amyloid A (SAA) protein. Their synthesis by hepatocytes is increased by cytokines, especially IL-6 (for CRP and fibrinogen) and IL-1 or TNF (for SAA).

Actions/Functions

- Many acute-phase proteins (CRP and SAA) bind to microbial cell walls and **may act as opsonins**.
- Fibrinogen binds to red cells to form stacks (**rouleaux**) and responsible for **raised erythrocyte sedimentation rate** (ESR).
- During acute inflammation, acute-phase proteins have beneficial effects but prolonged production (especially SAA) like in chronic inflammation causes **secondary amyloidosis**.

C-reactive Protein (CRP)

Q. Write short note on C-reactive protein.

CRP is an acute phase reactant synthesized mainly by the liver. Its synthesis is stimulated by a number of inflammatory mediators (mainly by cytokines, e.g., IL-6) acting on liver cells. CRP augments the innate immune response by binding to microbial (bacteria) cell walls or may act as opsonins and activate the classical complement cascade. They also bind chromatin and helps in clearing necrotic cell nuclei.

Significance

- Raised serum levels of CRP is a **marker for increased risk of myocardial infarction in patients with coronary artery disease**. Probably inflammation involving atherosclerotic plaques in the coronary arteries may predispose to thrombosis and subsequent myocardial infarction.
- Plasma CRP is a **strong, independent marker of risk for myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac**

death, even in healthy individuals.

- CRP is also a useful marker for assessing the effects of risk reduction measures, such as cessation of smoking, weight loss, exercise, and statins; each one of these reduce CRP levels.

Changes in the Leukocytes

Leukocytosis (refer Box 12.1)

Total leukocyte count more than 11,000/µL are termed as leukocytosis. Common in inflammatory reactions, especially those caused by bacterial infections.

- **Leukocyte count: May be increased up to 15,000 or 20,000 cells/µL**. Sometimes, it may be extremely high reaching 40,000 to 100,000/µL associated with more immature neutrophils in the blood (shift to the left) and are called as **leukemoid reactions** (*refer* page 329), similar to the white cell counts found in leukemia. It is important to distinguish it from leukemia, which is a malignant disease.
- **Cause:** It is due to increased release of leukocytes from the bone marrow caused by cytokines, including colony stimulating factors (CSFs), TNF and IL-1.
- **Bacterial infections** cause an increase in the blood neutrophil count known as **neutrophilia** (*refer* **Box 12.3**).

Lymphocytosis (refer Box 12.6)

It is seen in **viral infections** (e.g., infectious mononucleosis, mumps, and German measles).

Eosinophilia (refer Table 12.3)

It is seen in **bronchial asthma, allergy, and parasitic infestations**.

Leukopenia (refer Box 12.4)

Decreased number of circulating white cells is associated with few infections like **typhoid fever and some viruses, rickettsia, and certain protozoa**.

Other Features of the Acute-phase Response

It includes:

- Increased pulse and blood pressure
- Anorexia and malaise, probably due to cytokines acting on brain cells.
- In severe bacterial infections (sepsis), cytokines (mainly TNF and IL-1) may be produced in large quantities and can result in disseminated intravascular coagulation and cardiovascular failure.

Fever is produced by exogenous or endogenous pyrogens.

Endogenous pyrogens: Cytokines (IL-1, TNF) stimulate production of prostaglandins in hypothalamus.

Exogenous pyrogen: Bacterial products (e.g., LPS).

NSAIDs inhibit prostaglandin synthesis and thereby reduce fever.

C-reactive protein (CRP): Marker of necrosis and disease activity.

Infections are associated with **raised ESR**.

Leukocytosis and **neutrophilia** are characteristically observed in **bacterial infections.**

Lymphocytosis: In viral infections, e.g., infectious mononucleosis, mumps, and German measles.

Eosinophilia: In bronchial asthma, allergy, and parasitic infestations.

Leukopenia: Associated with few infections like typhoid fever and some viruses, rickettsia, and certain protozoa.

Polyclonal gammopathy: Indicates chronic inflammation.

HEALING

CC: Define and describe the process of repair and regeneration including wound healing and its types.

Q. Define the term healing, regeneration and repair.

Injury to cells and tissues **results in loss of cells and tissues.** It sets in inflammation (restrict the tissue damage) and initiate replacement of lost

tissue by living tissue.

Definition: Healing is a process of replacement of dead tissue by living tissue.
It can be broadly divided into regeneration and repair.

1. **Regeneration:** It is a process in which **lost tissue is replaced by tissue of similar type**. It results in the complete restoration of lost or damaged tissue by proliferation of residual uninjured cells and replacement from stem cells.
2. **Repair:** It is defined as a process in which **lost/damaged tissue is replaced by fibrous tissue or scar**.

Factors Deciding the Pattern of Healing

The proportion of regeneration and repair process in healing depends on:

Proliferative Capacity of the Tissue

According to proliferative capacity of the cells, the tissues of the body can be divided into three groups:

1. **Labile (continuously dividing) tissues:** The cells of labile tissues proliferate throughout life, replacing the lost cells from stem cells. Examples:
   - Hematopoietic cells of the bone marrow
   - Surface epithelia of the skin, oral cavity, vagina, and cervix
   - Columnar epithelium of the gastrointestinal tract and uterus.
2. **Stable (quiescent) tissues:** Cells of stable tissue normally do not proliferate; but can **proliferate in response to injury or loss of tissue**. Examples:
   - **Parenchymal cells** of **liver**, kidneys, and pancreas
   - **Mesenchymal cells:** Fibroblasts, vascular endothelial cells, smooth muscle cells, chondrocytes, and osteocytes.
3. **Permanent (nondividing) tissues:** Cells of these tissues **cannot proliferate after birth**. In these tissues, repair is by scar formation. Example:
   - **Neurons: Damaged neurons** are **replaced by** the proliferation of the **glial cells**
   - **Skeletal muscle** cells
   - **Cardiac muscle** cells.

However, limited stem cell replication and differ-entiation can occur in some areas of the adult brain, and heart muscle cells can proliferate after myocardial necrosis.

Extent of Tissue Injury

- **Mild and short duration:** The damaged tissue is healed by regeneration without significant scarring.
- **Severe and chronic:** Healing occurs by fibrous tissue forming scar.
  - Severe tissue injury damages both parenchymal cells and the extracellular matrix (ECM) framework
  - Chronic inflammation

Replacement of lost tissue may occur by regeneration with complete restoration or by replacement by connective tissue to form scar.

Repair is a healing process, but it may itself cause tissue dysfunction (e.g., in pathogenesis of atherosclerosis).

Labile tissues with regenerative capacity
- Hematopoietic cells
- Epithelium of skin and gastrointestinal (GI) tract.

Stable tissues: Proliferate in response to injury or loss of tissue, e.g., parenchymal cells of liver and kidney.

Permanent tissues: Cells cannot proliferate after birth, e.g., neurons, cardiac muscle.

STEM CELLS

Q. Write short note on stem cells. What are its subtypes?

Definition: Stem cells are characterized by their **ability of self-renewal and capacity to generate differentiated cell lineages**.

Properties

1. **Self-renewal capacity** and capacity to generate differentiated cell lineages.
2. **Asymmetric replication:** This is characterized by division of stem cell into two cells:
   i. One daughter cell which gives rise to mature cells.
   ii. Other cell remains as undifferentiated stem cell which retains the self-renewal capacity.

Types

1. **Embryonic stem cells:** During development of embryo, the blastocysts contain **undifferentiated pluripotent stem cells**, which are called as embryonic stem cells or ES cells. These cells **can form cells of all three germ cell layers**.
   - Normal function: To give rise to all cells of the body.
2. **Adult (somatic) stem cells:** Adult stem cells are less undifferentiated than ES cells found in adults. They are found among differentiated cells within a tissue. They have **more limited capacity to generate different cell types than ES cells.** They usually differentiate into particular tissue.
   - Normal function: Tissue homeostasis.
3. **Induced pluripotent stem cells** (iPS cells): This is achieved by transferring the nucleus of adult cells to an enucleated oocyte.
   - **Use:** For **therapeutic cloning** in the treatment of human diseases.

Sites of Stem Cells

1. **Bone marrow:** It contains two types of stem cells namely:
   i. **Hematopoietic stem cells** (HSCs)**:**
      - They can generate all of the blood cell lineages, and are used for the treatment of hematologic diseases.
      - They can be collected directly from the bone marrow, from umbilical cord blood, and from the peripheral blood.
   ii. **Marrow stromal cells** (MSCs)**:** They can generate chondrocytes, osteoblasts, adipocytes, myoblasts, and endothelial cell precursors depending on the tissue to which they migrate.
2. **Intestinal epithelium:** Stem cells may be located immediately above Paneth cells in the small intestine or at the base of the crypt in the colon.

3. **Liver:** The liver contains stem cell in the canals of Hering, which are capable of differentiating into hepatocytes and biliary cells.
4. **Cornea:** Stem cells are located in the limbus region between the conjunctiva and the cornea.
5. **Skin:** Stem cells are located in the bulge area of the hair follicle, in the sebaceous glands, and in the lower layer of the epidermis.

Stem cell:
- Self-renewal capacity
- It is an dormant phase of cell cycle
- Asymmetric replication
- Capacity to generate differentiated cell lineages

Types of stem cells: (1) embryonal, (2) adult and (3) induced

Embryonal cells are pluripotent cells capable of forming cells of all three germ cell layers.

Stem cells reside in special microenvironments called niches.

Stem cells: Used in bone marrow transplantation in the treatment of various types of leukemia and lymphoma.

CELL CYCLE AND CELL PROLIFERATION

Definition: Cell cycle proliferation is a regulated process, which involves activators and inhibitors, as well as checkpoints.

- Inflammation is the primary response of living tissue to injury. With inflammation, there will be damage or loss of tissue, which has to be replaced by living tissue. This replacement is done by transient increase in cellularity due to proliferation of cells by either regeneration and/or by repair.
- Proliferation of cells is characterized by DNA replication and mitosis. The **sequence of events that control DNA replication and mitosis is known as the cell cycle**.

Phases of Cell Cycle (Fig. 3.11)

- G_1 (presynthetic)
- S (DNA synthesis)

- G_2 (premitotic)
- M (mitotic) phase.

Checkpoints: They check whether there is any damage to DNA and chromosomes in the replicating cells. These checkpoints make sure that only normal cells complete replication. There are two checkpoints:

1. **G_1/S checkpoint monitors the integrity of DNA before replication**.
2. **G_2/M checkpoint checks DNA after replication** and monitors whether the cell can safely enter mitosis.

Growth Factors

Q. Write short note on growth factors.

Definition: Growth factors stimulate the activity of proteins which are needed for cell survival **growth and division**.

Mechanism of action: Growth factors induce cell proliferation by **binding to specific receptors**, and deliver positive growth signals to the target cells. These signals stimulate the **expression of genes** whose products have several functions which includes:

- Activation of cell cycle
- Relieve blocks which prevent cell cycle progression
- Prevention of apoptosis
- Increases the synthesis of cellular components (nucleic acids, proteins, lipids, carbohydrates) needed for a mother cell to produce two daughter cells.

Various growth factors involved in wound healing and regeneration are listed in **Table 3.11**.

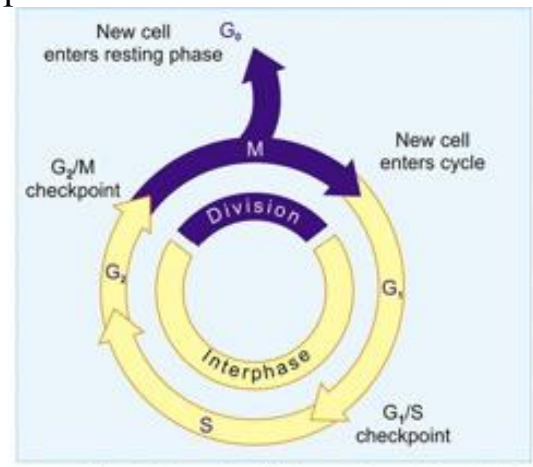
Signaling Mechanisms of GrowthFactor Receptors

Q. Write short note on different types of signaling.

The **receptor-mediated signal transduction** process is activated by the binding of ligands (e.g., growth factors and cytokines) to specific receptors. Receptor activation leads to expression of specific genes.

Modes of signaling (Figs. 3.12A to C): Depending on the source of the ligand and the location of its corresponding receptors (i.e., in the same,

adjacent, or distant cells), the modes of signaling can be divided into three types:



Cell cycle is a tightly regulated process and has checkpoint controls which prevent the proliferation of abnormal cells

Two checkpoints in cell cycle:
1. G_1/S checkpoint
2. G_2/M checkpoint

Quiescent cells, which have not entered the cell cycle, are in the G_0 state

G_1 phase: Most variable phase in cell cycle

G_1 to S phase: Most critical phase in the cell cycle

Major action of growth factors is to stimulate genes that control growth. Many of them are called as proto-oncogenes

Fig. 3.11: Cell cycle showing different phases (G_0, G_1, G_2, S, and M). Cells from labile tissues (e.g., epidermis) may remain in cycle continuously; stable cells (e.g., liver cells) are quiescent but can enter the cell cycle; permanent cells (e.g., neurons) have lost the capacity to proliferate and cell cycle arrests in the G_1 phase or exit the cycle and are in G_0 phase.

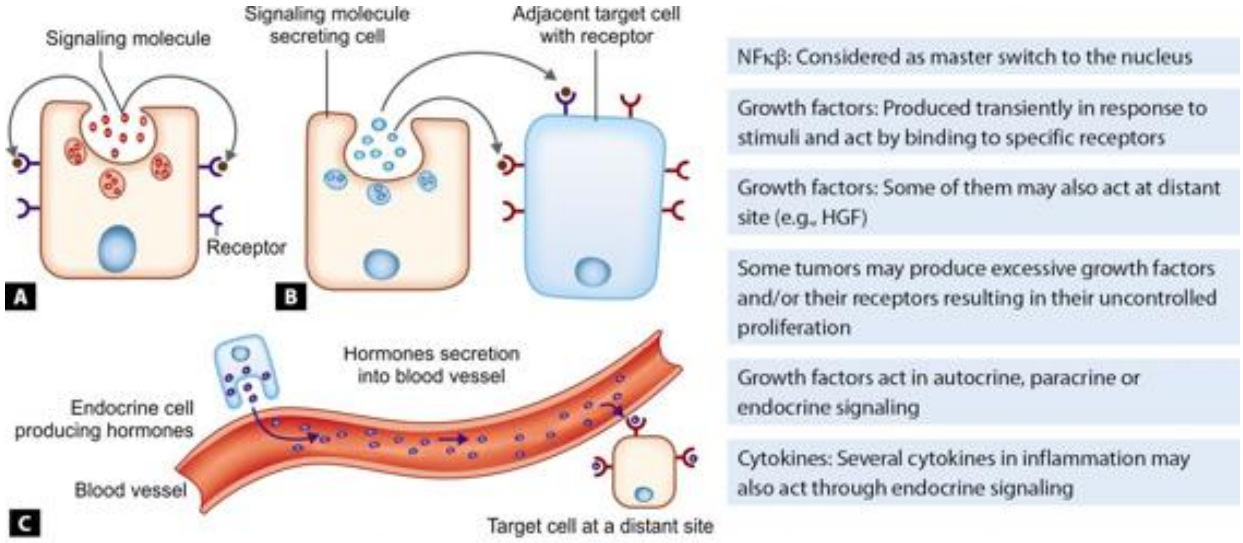
TABLE 3.11: List of growth factors and cytokines involved in wound healing and regeneration.

A. GROWTH FACTOR

Type of growth factor	*Receptor*	*Functions*
EGF family 1. Epidermal growth factor α (EGF-α) 2. Transforming growth factor α (TGF-α)	EGFR 1. EGFR1 (ERBB1) 2. ERBB2 (HER-2 or HER2/Neu)	Keratinocyte migration Formation of granulation tissue
Hepatocyte growth factor/scatter factor (HGF/SF)	c-MET	Proliferation of epithelial cells, hepatocytes, and endothelial cells Increases cell motility Keratinocyte replication
Platelet-derived growth factor (PDGF) • Isoforms A, B, C, D	PDGFR α and β	Chemotaxis and activation of PMNs, macrophages Activation and proliferation of fibroblasts, smooth muscle cells and endothelial cells Stimulates production of ECM
Vascular endothelial cell growth factor (VEGF) • Isoforms A, B, C, D	VEGFR-1, VEGFR-2, and VEGFR-3	Increases vascular permeability; mitogenic for endothelial cells Angiogenesis

TABLE 3.11: List of growth factors and cytokines involved in wound healing and regeneration.

A. GROWTH FACTOR		
Type of growth factor	***Receptor***	***Functions***
Fibroblast growth factor (FGF) family Keratinocyte growth factor (FGF-7)	FGFRs 1–4	Wound repair-epithelialization [FGF-2 and KGF (FGF-7)] Angiogenesis (FGF-2)
Transforming growth factor β (TGF-β) and related growth factors TGF-β isoforms (TGF-β1, TGF-β2, TGF-β3)	TGF-β receptors (types I and II)	Growth inhibitor for most epithelial cells Potent fibrogenic agent Strong anti-inflammatory effect
B. CYTOKINES		
• Tumor necrosis factor (TNF) and IL-1 participate in wound healing • TNF and IL-6 are involved in liver regeneration	TNF receptor (TNFR), or death receptor, for TNF, interleukin-1 receptor (IL-1R) for IL-1 and interleukin 6 receptor (IL-6R) also known as CD126 (Cluster of differentiation 126) for IL6	TNF activates macrophages; regulates other cytokines and has multiple functions



Figs. 3.12A to C: Modes of signaling: (A) Autocrine; (B) Paracrine; (C) Endocrine signaling.

1. **Autocrine signaling:**
   - Signaling molecules **act on the cells which secretes them**.
   - Examples: Liver regeneration, proliferation of antigen-stimulated lymphocytes, tumors.
2. **Paracrine signaling:**

- Signaling molecule is produced by one cell type, that acts on adjacent target cells (usually of a different type) which expresses the appropriate receptor.
- Example: **Healing by repair**: Factor produced by macrophage (one cell type) has growth effect on fibroblast (adjacent target cells of different type).

3. **Endocrine signaling:**
   - **Hormones:** These are produced by cells of endocrine organs, are usually carried by the blood and act on **target cells that are at a distant from the site of its synthesis**.

HEALING BY REGENERATION

Regeneration returns an injured or lost tissue to its original state. The regeneration of injured cells and tissues involves proliferation of cells. This proliferation is brought out by growth factors.

Requirements for regeneration: Regeneration of damaged tissue requires survival or **integrity of the basement membrane, extracellular matrix, architecture and tissue stem cells** (or progenitor cells that can replicate and differentiate).

Cells that proliferate during healing: There are many types of cells that proliferate during healing. These include the **remaining cells of the injured tissue** (to restore normal structure), **vascular endothelial cells** (to generate new vessels for supplying the nutrients required for the healing process), and **fibroblasts** (needed for the formation of fibrous tissue to fill defects that cannot be healed regeneration).

Type of cells or tissue capable of regeneration: Tissue regeneration can occur in parenchymal organs composed of cells that are capable of proliferation. Regeneration in tissues or organs containing **labile cells** (e.g., hematopoietic cells in the bone marrow, surface epithelia) or **stable cells** (e.g., liver, kidney, endothelial cells, fibroblasts), The new cells are derived from tissue stem cells and rapidly proliferating immature progenitors. However, in permanent tissues (e.g., neurons and cardiac muscle cells), the cells cannot regenerate and healing is only by forming scar.

HEALING BY REPAIR, SCAR FORMATIONAND FIBROSIS

Healing may be either by **regeneration or repair or combination of both.** With mild and transient injury, there is regeneration. If the tissue injury or damage persists, inflammation becomes chronic, resulting in excessive deposition of connective tissue known as **fibrosis** (repair).

Steps in Healing by Repair (Scar Formation)

Q. Write short note on steps of wound healing.

Inflammation

Whenever there is tissue injury, inflammatory reaction begins which tries to limit the damage and remove the injured tissue. At the same time, it also promotes the deposition of ECM components at the site of injury and stimulates angiogenesis.

Cell Proliferation

Several cell types such as epithelial cells, endothelial and other vascular cells, and fibroblasts, proliferate and migrate to close the wound. Each cell type has unique functions.

Epithelial cells: In response to locally produced growth factors, epithelial cells migrate over the wound to cover it.

Endothelial and other vascular cells: They proliferate and form new blood vessels and this process is termed as angiogenesis (*refer* below).

Fibroblasts: They proliferate and migrate into the site of injury and produce collagen fibers which forms scar.

Granulation Tissue

Q. Write short note on granulation tissue.

The first 24 to 72 hours of the repair process begins with proliferation of fibroblasts and vascular endothelial cells. A specialized type of tissue unique and **hallmark (characteristic) of healing wounds** is formed and is called

granulation tissue **(Fig. 3.13A)**. This term derives from its pink, soft, granular gross appearance of granulation tissue observed below the scab of a skin wound.

Microscopy **(Figs. 3.13B and C)**: Granulation tissue consists of:

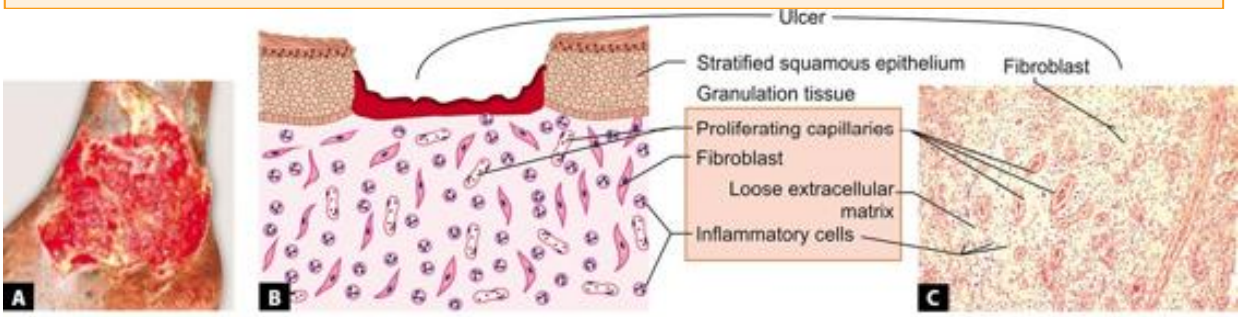
- **Proliferating fibroblasts**
- **New small blood vessels** (angiogenesis): These are thin-walled, delicate, leaky capillaries, that allow the passage of plasma proteins and fluid into the extravascular space. This leakage of plasma proteins and fluid is responsible for edema often seen in granulation tissue.
- **Loose connective tissue**: The capillaries of granulation tissue are surrounded by a loose extracellular matrix.
- **Scattered chronic inflammatory cells** mainly macrophages.

Amount of granulation tissue formed depends on the (i) **size of the tissue deficit** created by the wound and (ii) **intensity of inflammation**.

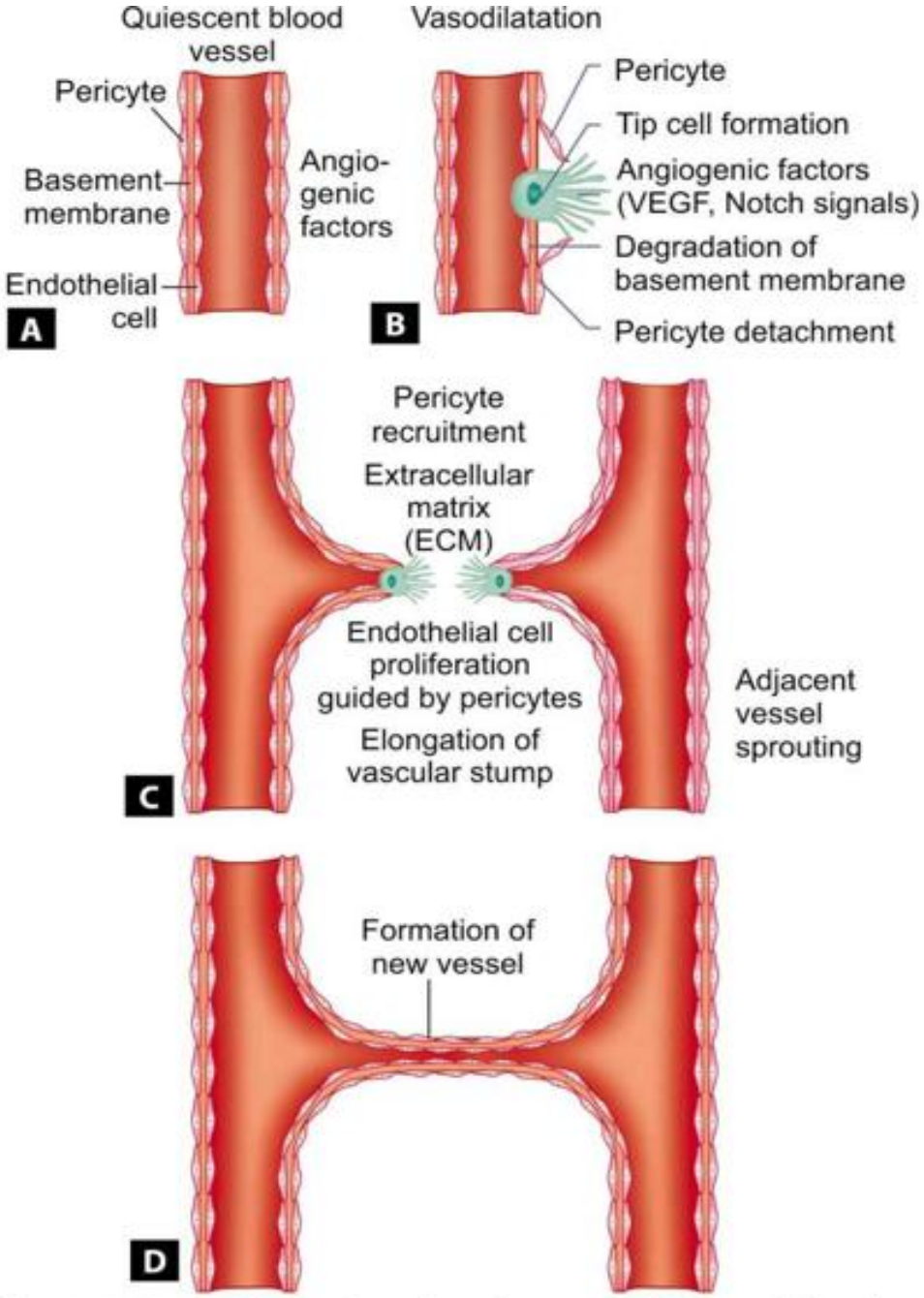
Angiogenesis

Q. Write short note on angiogenesis in repair.

Definition: Angiogenesis is the process of formation of new blood vessels from existing vessels.



Figs. 3.13A to C: (A) Pink, soft, granular appearance of granulation tissue on the surface of healing wounds; (B) Diagrammatic; (C) H&E. Microscopy of granulation tissue consisting of numerous blood vessels, fibroblasts and edema.



Figs. 3.14A to D: Mechanism of angiogenesis. (A) Normal blood vessel; (B) Vasodilatation: First, pericytes separate followed by mobilization and proliferation of endothelial cells from the existing vessel to form capillary sprouts towards the site of injury (angiogenic stimuli); (C) Endothelial cells proliferate, loosely following each other, and are presumably guided by pericytes. Maturation of vessel (stabilization) involves the recruitment of pericytes and smooth muscle cells to form the periendothelial layer; (D) Finally, blood-vessel sprouts will fuse with other sprouts to build new circulatory systems.

Steps in angiogenesis (Figs. 3.14A to D):

- **Vasodilatation** in response to nitric oxide and **increased permeability of the pre-existing vessel due** to VEGF.
- **Separation of pericytes** from the abluminal surface of blood vessel. Breakdown of the basement membrane to facilitate formation of a vessel sprout.
- **Migration and proliferation of endothelial cells** toward the site of injury by fibroblast growth factors (FGFs), mainly FGF-2.
- **Maturation of endothelial cells** and remodeling into capillary sprouts/tubes.
- **Formation of mature vessel:** It involves recruitment of pericytes and smooth muscle cells to form the periendothelial layer.
- **Suppression of endothelial proliferation** and migration, and deposition of basement membrane.

Scar Formation

- The leukocytes, edema, and angiogenesis disappear, accomplished by the **increased accumulation of collagen.** The **granulation tissue scaffolding is converted into a pale, avascular scar.**
- **Components of scar:** It is composed of spindle-shaped fibroblasts, dense collagen, fragments of elastic tissue, and other ECM components.
- **Connective tissue special stains:** To identify different protein constituents of scars and fibrotic tissues, special stains are useful.
  - **Trichrome stain:** It contains three stains (hence its name), which stain red cells orange, muscle red, and collagen blue. Thus, it detects collagen fibers in the scar tissue.
  - **Elastin stain:** It identifies delicate fibers of elastin, which is the major component of flexible elastic tissue.
  - **Reticulin stain:** Reticulin is one of extracellular matrix protein of the connective tissue stroma of normal organs and is also present in early scars. It is composed of type III collagen.
- **By the end of the first month,** the scar consists of acellular connective tissue without inflammatory infiltrate.

Connective Tissue Remodeling

- **After the formation of scar, there is remodeling to increase its strength and contract it. The outcome of the repair depends on the balance between synthesis and degradation of ECM proteins.** TGF-β is a potent fibrogenic agent which stimulates ECM synthesis. On the other hand, the degradation of collagens and other ECM components is performed by a family of matrix *metalloproteinases (MMPs)*. They are named so because they are dependent on metal ions (e.g., zinc) for their activity. The connective tissue in the scar continues to be modified and remodeled. Remodeling of the connective tissue framework is an important feature. It is the long-lasting phase of tissue repair.
- Remodeling indicates that the equilibrium/balance between ECM synthesis (collagen deposition) and degradation has been restored.

Role of Macrophages in Repair

Q. Write short note on role of macrophages in inflammation/repair.

Macrophages are important cells involved in repair. Their functions in repair include:

- Clear the offending agents and dead tissue.
- Provide growth factors for the proliferation of various cells.
- Secret cytokines that stimulate fibroblast proliferation and connective tissue synthesis and deposition.

Proliferation of cells occur when quiescent cells enter the cell cycle.

Growth factors: Multiple effects and include cell proliferation, survival, migration, contractility, differentiation, and angiogenesis.

In most healing processes, a combination of repair and regeneration occurs.

Angiogenesis is the process of formation of new blood vessels from existing vessels.

Growth factors involved in angiogenesis: Most important are vascular endothelial growth factor (**VEGF)** and fibroblast growth factor (FGF-2).

The term granulation tissue is derived from its pink, soft, granular appearance on the surface of healing wounds.

Microscopically, granulation tissue consists of:

1. New small blood vessels
2. Fibroblasts.

Granulation tissue is essential for repair.

CUTANEOUS WOUND HEALING

Q. Long essay: Define the terms "healing", "regeneration" and "repair". Describe the healing of a clean surgical wound/healing by first intention.

Healing by Primary Union or by First Intention

Definition: Healing of a **clean, uninfected surgical incision** in the skin joined with surgical sutures is known as healing by **primary union or by first intention**.

Surgical incision causes **death of a minimum number** of epithelial and connective tissue **cells**. The **disruption of epithelial basement membrane** continuity is also **minimal**. Re-epithelialization occurs by regeneration and there is a **relatively thin scar**. This is simplest type of cutaneous wound healing.

Stages in the Healing by First Intention (Figs. 3.15A to D)

First 24 Hours

- **Formation of blood clot:** It is formed in the space between sutured margins. Blood clot contains not only trapped red cells but also fibrin, fibronectin and complement components. Clot stops bleeding and acts as a scaffold for migrating and proliferating cells. **Dehydration at the external surface of the clot** leads to formation of a **scab** over the wound.
- **Neutrophil infiltration:** Within 24 hours of wound, **neutrophils** appear at the margins of the incision. Neutrophils use the scaffold produced by the

fibrin clot for its migration. They **release proteolytic enzymes which clean out debris**.

- **Epithelial changes:** At the cut edges of the wound, the **basal cells of the epidermis** begin to show **mitotic activity**. Epithelial cells from both the edges of wound proliferate and migrate across the wound along the dermis.

Two Days

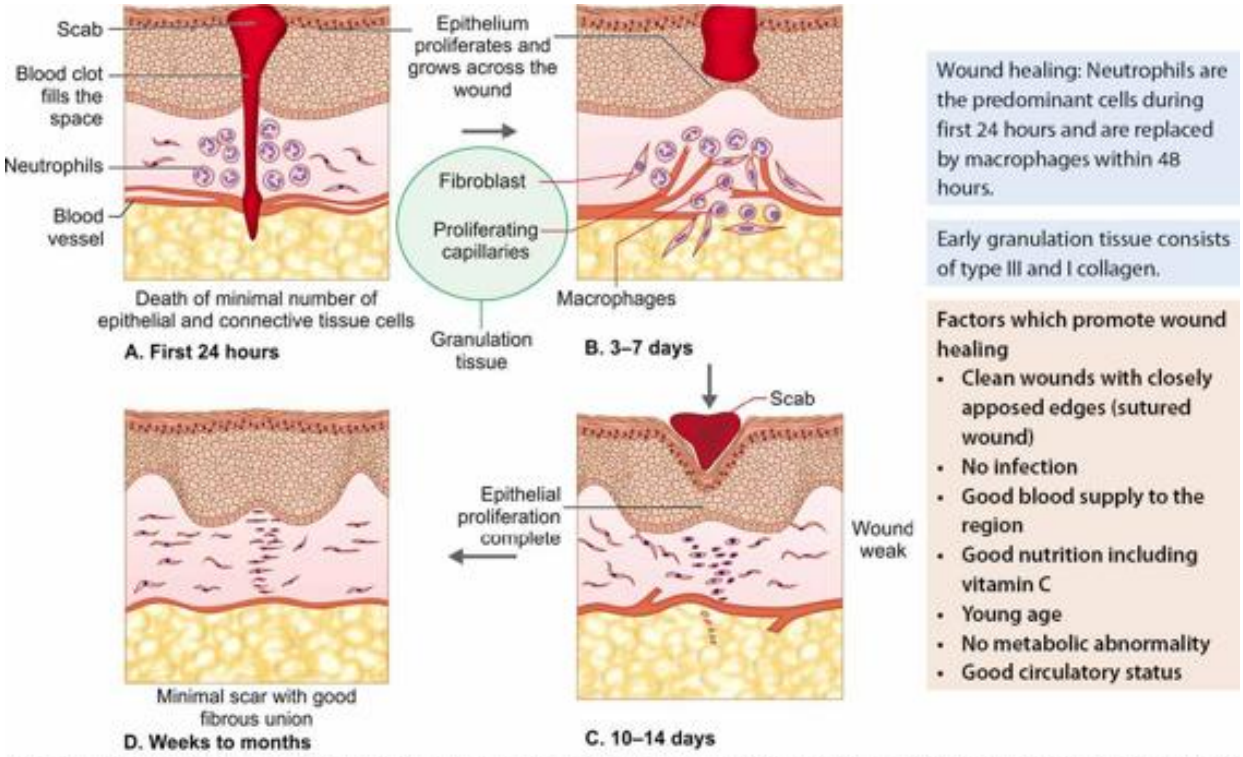
- **Neutrophils are replaced by macrophages**. **Macrophages play a central cellular role in the repair process**.
- The **epithelial cells fuse in the midline below the surface scab** and epithelial continuity is re-established in the form of a thin continuous surface layer.

Three to Seven Days

- **Granulation tissue** begins to invade incision space. It progressively grows into the **incision space/wound and fills the wound area** by 5–7 days. Collagen is progressively laid down.
- **Surface epidermis** achieves its **normal thickness** and differentiation. It matures with surface keratinization.
- Acute **inflammatory response** begins to **subside**.

Ten to Fourteen Days

- Leukocytic infiltration, edema, and angiogenesis disappear during the second week.
- **Increased accumulation of collagen** and **regression of vascular channels**. The **granulation tissue scaffolding is converted into a pale, avascular scar.** Wound normally gains about 10% strength of normal skin. Further fibroblast proliferation occurs with collagen deposition.



Figs. 3.15A to D: Healing by primary intention. (A) A wound with closely apposed edges and minimal tissue loss. The blood clots and fills the gap between the edges of the wound; (B) Epithelium at the edges proliferates. Minimal amount of granulation tissue is formed; (C) The epithelial proliferation is complete and the wound is weak; (D) Fibrosis with a small scar.

Weeks to Months

- The **scar** appears as **acellular** connective tissue covered by intact epidermis and without inflammatory infiltrate.
- Collagen deposition along the line of stress and **wound gradually achieves maximal 80% of tensile strength** of normal skin.

Healing by Secondary Union or by Second Intention

Q. Describe the mode of healing of wound by second intention.

Definition: When injury produces **large defects** on the skin surface with **extensive loss of cells and tissue**, the healing process is more complicated. Healing in such cutaneous wound is referred to as healing by **secondary union or by second intention**.

Features of Healing by Secondary Intention (Figs. 3.16A to D)

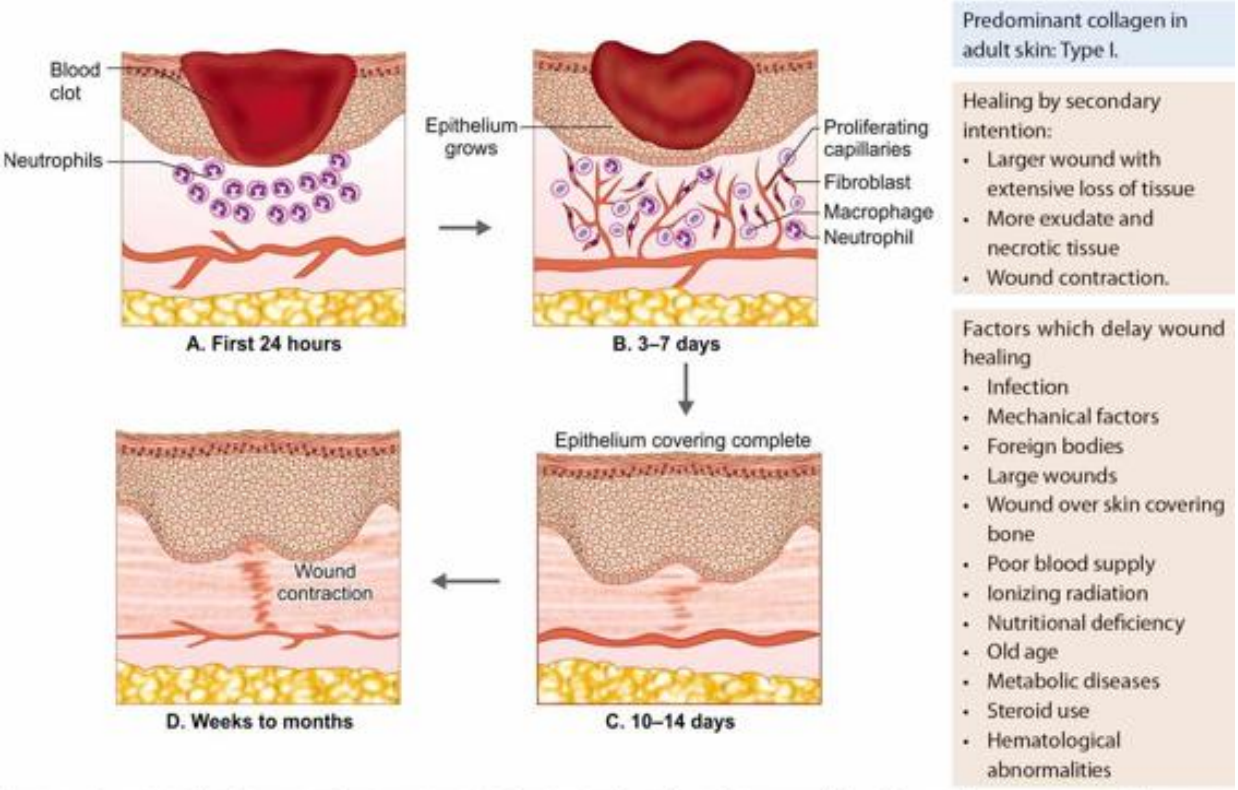
- Larger wounds show **more exudate and necrotic tissue**. The **clot or scab** formed at the surface of wound is **large**. Full epithelialization of the wound surface is slow because of the larger gap.
- **Severe inflammatory reaction** because of larger defect and greater necrotic tissue.
- The **larger defect requires more** amount of (abundant) **granulation tissue**.
- Extensive deposition of collagen with substantial scar formation.
- **Wound contraction:** Wound contraction generally occurs in large surface wounds and is an important feature in healing by secondary union.

Myofibroblasts of granulation tissue **have ultrastructural features of smooth muscle cells**. They contract in the wound tissue and are responsible for wound contraction.

Wound Strength

Major portion of the connective tissue in repair is fibrillar collagens (mostly type I collagen) and are responsible for the development of strength in healing wounds.

Time for a Skin Wound to Achieve its Maximal Strength



Figs. 3.16A to D: Healing by secondary intention: (A) There is significant loss of tissue and the edges are far apart. Acute inflammation develops both at the edges and base; (B) The cell proliferation starts from the edges and large amount of granulation tissue is formed; (C) The wound is covered on the entire surface by the epithelium. The collagen fibers are deposited; (D) Granulation tissue is replaced by a large scar. There is significant wound contraction.

TABLE 3.12: Differences between healing by primary and secondary intention.

Feature	***Primary intention***	***Secondary intention***
Nature of wound	Clean surgical wound	Unclean
Margins	Surgical clean margin	Irregular
Sutures	Used for apposition of margins	Cannot be used
Infection	Absent	May be infected
Amount of granulation tissue	Scanty at the incised gap and along suture track	Abundant and fill the gap
Outcome	Neat linear scar	Irregular contracted scar
Complications	Rare	Infection and suppuration

- **At the end of the first week:** When sutures are removed from an incisional surgical wound, **wound strength** is about **10%** that of normal unwounded skin.

- **Four weeks:** Wound strength quickly increases over the next 4 weeks, and then slows down.
- **Three months:** Wound strength **reaches a 70–80% of the tensile strength** of unwounded skin.

Differences between healing by primary and secondary intention is discussed in **Table 3.12**.

Q. Compare/tabulate the differences between wound healing by primary and secondary intention with suitable diagrams. Discuss the factors promoting and delaying the process.

Basic mechanisms of healing by primary (first intention) and secondary (second intention) union are similar.

Wound contraction is an important feature of healing by secondary intention and is mediated by myofibroblasts.

Advantages of wound contraction:
- Decreases the gap between the dermal edges of the wound
- Reduces the wound surface area

Wound strength:
- 10% after 1st week
- Rapidly increases during next 4 weeks
- 70% at the end of 3rd month

FACTORS THAT INFLUENCE WOUND HEALING

Q. List the factors that influence wound healing.

Q. List the factors which promote healing.

Q. List the factors which delay healing.

Local Factors

Factors that Delay Wound Healing

1. **Infection:** It is the single most important cause for delay in healing. Infection causes persistent tissue injury and inflammation.
2. **Mechanical factors: Movement of wounded area** may compress the blood vessels and separate the edges of the wound and can result in delayed healing.
3. **Foreign bodies:** Unnecessary sutures or foreign bodies (fragments of steel, glass), or even bone can delay healing.
4. **Size and type of wound**: Large excisional wounds or wounds caused by blunt trauma.
5. **Location of injury:** Wound over the skin covering bone with little intervening tissue prevents wound contraction (e.g., skin over the anterior tibia). The edges of skin lesions (e.g., burns) in such locations cannot be apposed.
6. **Blood supply:**
   - **Varicose veins** of the legs decrease the venous drainage and **can cause nonhealing ulceration**.
   - **Bed sores (decubitus ulcers**) result due to prolonged, localized, pressure, which diminishes both arterial and venous blood flow.
7. Ionizing radiation decreases repair process.
8. Complications may delay wound healing.

Factors that Favor Wound Healing

1. **Size and type of wound**: Small surgical incisional or other injuries heal quickly with less scar formation.
2. **Blood supply:** Wounds in areas with **good blood supply,** such as the face, heal **faster than** those with **poor blood supply**, such as the foot.

Systemic Factors

1. **Nutritional deficiencies:** Delays wound healing and these include:
   - Protein deficiency (e.g., malnutrition).
   - Vitamin C deficiency: Inhibits collagen synthesis and retard healing.
   - Trace elements: Copper and zinc deficiency.
2. **Age:** Wound healing is rapid in young compared to in aged individuals.

3. **Metabolic status: Diabetes mellitus** is associated with delayed healing due to microangiopathy.
4. **Circulatory status: Inadequate blood supply** (due to arteriosclerosis) or venous abnormalities (e.g., varicose veins) that retard venous drainage, delay healing.
5. **Hormones: Glucocorticoids have anti-inflammatory effects** and also inhibit collagen synthesis, thereby **impair wound healing**.
6. **Hematological abnormalities:** Quantitative or qualitative defects in neutrophils and bleeding disorders may slow the healing process.

Infection is the **most common cause** of **delay in wound healing**.

Vitamin C:
1. Essential for synthesis of collagen
2. Antioxidant
3. Reducing agent

Vitamin C deficiency:
1. Decreases cross-linking of trophocollagen
2. Decreases tensile strength of wound

Zinc: Acts as a cofactor in collagenase.

COMPLICATIONS OF WOUND HEALING

Q. Mention the complications of wound healing.

Inadequate Granulation Tissue Formation

Inadequate formation of granulation tissue or a deficient scar formation can cause **wound dehiscence** and **ulceration**.

1. **Dehiscence** (the wound splitting open) or rupture of a wound is most common life-threatening complication after abdominal surgery. It is due to increased abdominal pressure/mechanical stress on the abdominal wound from vomiting, coughing, or ileus.
2. **Ulceration:**
   - Wounds can ulcerate due to inadequate angiogenesis during healing. For example, wounds in the leg of patients with atherosclerotic peripheral vascular disease or varicose veins usually ulcerate.

- Nonhealing wounds also develop in regions devoid of sensation. These **neuropathic or trophic ulcers** may be seen in diabetic peripheral neuropathy and nerve damage from leprosy.

3. **Incisional hernia** resulting from weak scars of the abdominal wall due to a defect caused by prior surgery. They are due to insufficient deposition of extracellular matrix or inadequate cross-linking in the collagen matrix.

Excessive Scar Formation

Excessive formation of the components of the repair process can result in:

1. **Hypertrophic scar:** The accumulation of excessive amounts of extracellular matrix, mostly collagen may give rise to a raised scar at the site of wound known as a hypertrophic scar. They usually develop after thermal or traumatic injury, which involves the deep layers of the dermis.
2. **Keloid:** If the scar tissue grows/progress beyond the boundaries of the original wound and does not regress, it is called a keloid **(Fig. 3.17)**. Thus, keloid is an exuberant scar that recurs with still larger keloid after surgical excision.

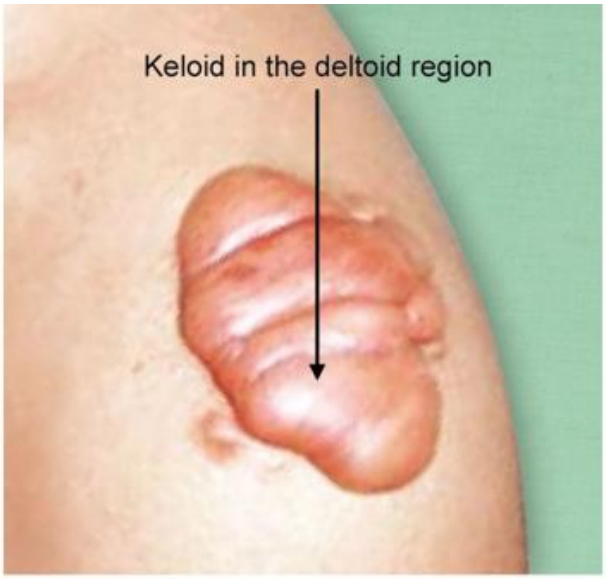


Fig. 3.17: Keloid in the upper arm.



Fig. 3.19: Wound contracture—severe contracture of a wound on neck and the right side of neck.

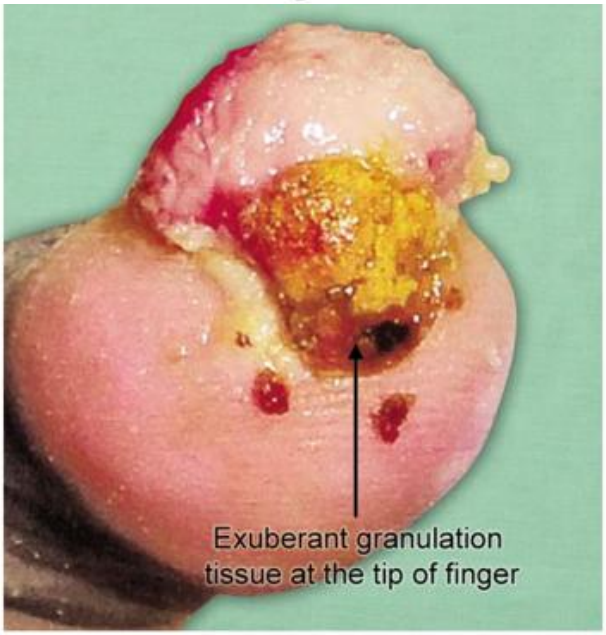


Fig. 3.18: Exuberant granulation tissue at the tip of the finger (B diagrammatic).

3. **Exuberant granulation:**
   - **Pyogenic granuloma or granuloma pyogenicum (Fig. 3.18)**:
     - This consists of the localized formation of excessive amounts of granulation tissue.

- Such exuberant granulation tissue projects above the level of the surrounding skin and prevents re-epithelialization. This mass formed is often named as **proud flesh.**

- **Desmoids or aggressive fibromatoses**:
  - Incisional scars or traumatic injuries may be followed by excessive proliferation of fibroblasts and other connective tissue elements.
  - They are known as desmoids, or aggressive fibromatoses, which may recur after excision.

Excessive Contraction

- A decrease in the size of a wound due to myofibroblasts is known as **contraction**.
- An exaggeration of this contraction is termed **contracture** and results in deformities of the wound and the surrounding tissues.
- Consequences of contractures:
  - **Compromise movements** [e.g., contractures that foll-ow severe burns can compromise the movement of the involved region **(Fig. 3.19)** and joint movements].
  - **Obstruction** [e.g., in GI tract contracture (stricture) can cause intestinal obstruction].

Others

1. **Infection** of wound by microbes.
2. **Epidermal cysts** can develop due to persistence of epithelial cells at the site of wound healing.
3. **Pigmentation** may develop due to either colored particle left in the wound or due to hemosiderin pigment.
4. **Neoplasia** (e.g., squamous cell carcinoma may develop in Marjolin's ulcer, which is the scar that follows burns in skin).

Fibrosis

- Normal wound healing is associated with deposition of collagen.
- The excessive deposition of collagen and other ECM components in a tissue is termed as fibrosis. It is usually observed in chronic inflammation.
- **TGF-β is an important fibrogenic agent**.

- Examples of disorders with f**ibrosis**: Cirrhosis of liver, pneumoconioses, chronic pancreatitis and glomerulonephritis.

Complications of wound healing is shown in **Flowchart 3.5.**

Wound dehiscence: Most common life-threatening complication of wound that develops after abdominal surgery.

Keloid: More frequent among dark-skinned persons. It is one of the complication of wound healing and is due to excessive deposition of type III collagen.

Proud flesh: Exuberant granulation tissue also known as pyogenic granuloma or granuloma pyogenicum.

Flowchart 3.5: Complication of wound healing.



Pyogenic granuloma: Excessive granulation tissue must be removed for restoration of the continuity of the epithelium.

Desmoid is an aggressive fibromatosis usually develops in the anterior abdominal wall.

Contracture: Exaggeration of wound contraction. Common sites for contractures are palms, the soles and the anterior aspect of the thorax.

TGF-β: Important fibrogenic agent.

Complications of wound healing:
A. Deficient scar formation
B. Excessive formation of the repair components
C. Formation of contractures
D. Others

CC (Show how): Identify and describe various forms of cell injuries, their manifestations and consequences in gross and microscopic specimens.

(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Gross and microscopic specimens to be shown include: granulation tissue, acute appendicitis, lobar pneumonia, tuberculosis, leprosy and actinomycosis/mycetoma.

CHAPTER 4

Infections and Infestations

LEPROSY

CC: Define and describe the pathogenesis and pathology of leprosy.

Leprosy (Hansen disease—after the discovery of the causative organism by Hansen), is a **chronic**, **granulomatous**, slowly progressive, destructive infection caused by ***Mycobacterium leprae***.

Sites of involvement: Mainly involves the **peripheral nerves**, **skin and mucous membranes** (nasal) and results in disabling deformities.

Leprosy is one of the oldest human diseases and lepers were isolated from the community in the olden days.

Mycobacterium leprae

- Slender, **weakly acid-fast** intracellular bacillus. It closely resembles *Mycobacterium tuberculosis* but is less acid-fast.
- **Proliferates at low temperature** of the human skin.
- **Cannot be cultured** on artificial media or in cell culture.
- **Experimental animals:** Lepra bacilli grow at sites where the temperature is below that of the internal organs. Examples: **Foot pads of mice**, ear lobes of hamsters, rats, and other rodents.
- **Experimentally transmitted to nine branded armadillos** (they have low body temperature ranging from 32–34°C).
- **Antigen in lepra bacilli:** The bacterial cell wall contains mainly 2 antigens namely *M. leprae*-specific phenolic glycolipid (PGL-1) and lipoarabinomannan (LAM).

Mode of transmission: It has comparatively low communicability.

1. **Inoculation/inhalation:** Likely to be transmitted from person to person through aerosols from asymptomatic lesions in the upper respiratory tract. Inhaled *M. leprae*, is taken up by alveolar macrophages and disseminates through the blood, but replicates only in relatively cool tissues of the skin and extremities.
2. **Intimate contact:** For many years with untreated leprosy patients. They shed many bacilli from damaged skin, nasal secretions, mucous membrane of mouth and hair follicles.

Source of infection: *M. leprae* is present in nasal secretions or ulcerated lesions of patients suffering from leprosy.
Incubation period: Generally 5–7 years.

Classification

Q. Classify leprosy.

A. Ridley and Jopling (1966) Classification

It depends on the clinicopathological spectrum of the disease, which is **determined by the immune resistance of the host (Flowchart 4.1)**. Leprosy is classified into five groups with two extremes or polar forms, namely— tuberculoid and lepromatous types.

1. **Tuberculoid leprosy (TT):** It is the polar form that has maximal immune response.
2. **Borderline tuberculoid (BT):** In this type, the immune response falls between BB and TT.
3. **Borderline leprosy (BB):** It exactly falls between two polar forms of leprosy.
4. **Borderline lepromatous (BL):** It has the immune response that falls between BB and LL.
5. **Lepromatous leprosy (LL):** It is the other polar form with least immune response.

Variants of Leprosy

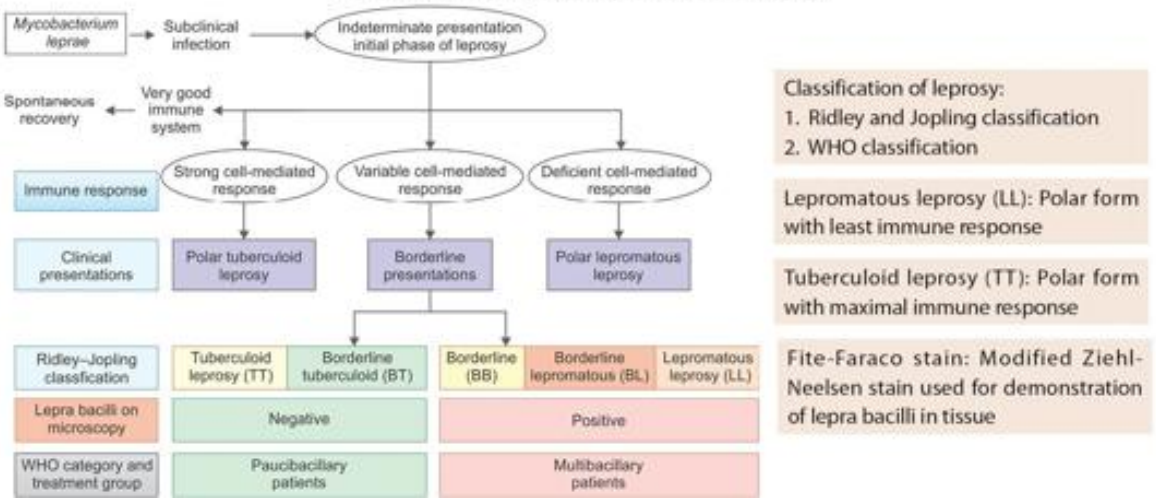
Q. Write a short note on indeterminate leprosy

- **Indeterminate leprosy:** It is an initial nonspecific stage of any type of leprosy. Pure neural leprosy with neurologic involvement is the main feature. The skin lesions of leprosy are not seen. Indeterminate leprosy and tuberculoid lesions are paucibacillary and their diagnosis is made together with clinical evidence (for microscopy *refer* page 88).
- **Histoid leprosy:** It is a variant of lepromatous leprosy in which the skin lesions grossly resemble nodules of dermatofibroma (a soft tissue tumor involving skin) and microscopically shows numerous lepra bacilli.

B. WHO Classification

- Paucibacillary: All cases of tuberculoid leprosy and some cases of borderline type.
- Multibacillary: All cases of lepromatous leprosy and some cases of borderline type.

Flowchart 4.1: Ridley–Jopling classification of leprosy.



Pathogenesis

- *Mycobacterium leprae* does not secrete any toxins, and its virulence depends on properties of its cell wall (similar to that of *M. tuberculosis*) and immunization with BCG may provide some protection against*M. leprae* infection.

- Cell-mediated immunity is reflected by delayed-type hypersensitivity reactions to dermal injections of a bacterial extract called **lepromin**.
- The **T-helper (T_H1) lymphocyte response** to *M. leprae*, determines whether an individual develops tuberculoid or lepromatous type of leprosy.
  - **Tuberculoid leprosy** patients have a T_H1 response which secretes IL-2 and IFN-γ. The later (IFN-γ) is essential for an effective host macrophage response.
  - **Lepromatous leprosy** patients have a weak T_H1 response and, in some a relative increase in the T_H2 response → results in a poor cell-mediated immunity → proliferation of lepra bacilli. Sometimes antibodies may be produced against *M. leprae* antigens, but they are usually not protective. These can form immune complexes with free antigens and lead to erythema nodosum, vasculitis, and glomerulonephritis.

MORPHOLOGY

Q. Write short note on morphology of tuberculoid leprosy.

Two extremes or polar forms of the diseases are the tuberculoid and lepromatous types.

1. Tuberculoid Leprosy

It is the **less severe form** of leprosy. It is **very slow in its course** and most patients die with leprosy.

Lesion in Skin

- **Number of lesions**: Single or very few lesions.
- **Site:** Usually on the **face, extremities, or trunk**

- **Type: Localized, well-demarcated, red or hypopigmented, dry, elevated,** skin **patches (Fig. 4.1A)** having raised outer edges and depressed pale centers (central healing). As they progress they develop irregular shapes with induration.

Nerve Involvement

- **Dominating feature** in tuberculoid leprosy.
- Nerves are surrounded by granulomatous inflammatory reactions and, may destroy small (e.g., the peripheral twigs) nerves.

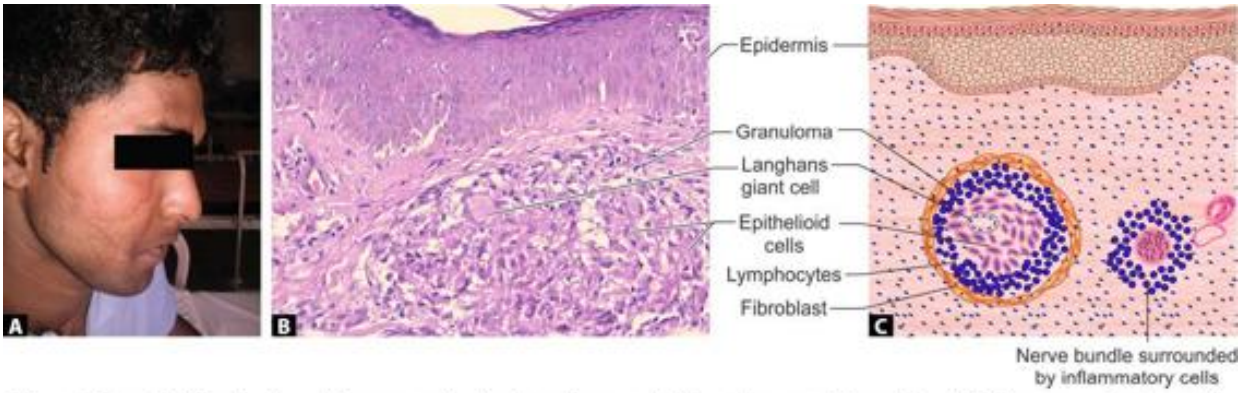
- Nerve involvement → causes **loss of sensation** in the skin → atrophy of skin and muscle. These affected parts are liable to trauma, and lead to the development of chronic skin ulcers.
- **Consequences:** It may lead to **contractures, paralyses, and autoamputation** of fingers or toes. Involvement of facial nerve can lead to paralysis of the eyelids, with keratitis and corneal ulcerations.

Microscopy (Figs. 4.1B and C)

- **Granuloma:** These are well-formed, circumscribed and **non-caseating** (no caseation). Seen in all involved sites and in the dermis of skin. Termed tuberculoid leprosy because the granulomas resemble those found in tuberculosis. Granulomas are composed of epithelioid cells (modified macrophages), Langhans giant cells, and lymphocytes.
- **Absence of Grenz zone:** Granulomas in the dermis extend to the basal layer of the epidermis (without a clear/Grenz zone).
- **Fite-Faraco** (modified Z-N stain for demonstration of lepra bacillus) stain generally **does not show lepra bacillus**, hence the name "**paucibacillary**" leprosy.
- **Perineural (surrounding nerve fibers) inflammation**: By lymphocytes **(Fig. 4.1C).**
- **Strong T-cell immunity:** It is responsible for granulomas formation, without lepra bacilli.

2. Lepromatous Leprosy

It is the **more severe form** and is also called **anergic leprosy**, because of the unresponsiveness (anergy) of the host immune system.



Figs. 4.1A to C: (A) Localized, well-demarcated, red or hypopigmented skin patches on the face; (B and C) Microscopy of tuberculoid leprosy with circumscribed non-caseating granulomas; (B) Photomicrograph; (C) Diagrammatic.

Q. Write short note on morphology of lepromatous leprosy.

Sites Involved

Lesion in skin

- **Thickening of skin** and **multiple, symmetric, macular, papular, or nodular lesions**. The nodular skin lesions may ulcerate. Most skin lesions are **hypoesthetic or anesthetic**.
- More severe involvement of the **cooler areas of skin** (e.g., earlobes, wrists, elbows, knees, and feet), than warmer areas (e.g., axilla and groin).
- With progression, the nodular lesions in the face and earlobes may coalesce to produce a lion like appearance known as **leonine facies (Fig. 4.2)**. This may be accompanied by loss of eyebrows and eyelashes.

Peripheral nerves

- Particularly the **ulnar and peroneal nerves** are symmetrically invaded with mycobacteria.
- **Loss of sensation and trophic changes** in the hands and feet may follow the damage to the nerves.

Testes

Usually, severely involved, leading to destruction of the seminiferous tubules → sterility.

Other sites

- Anterior chamber of the eye: **Blindness**.
- Upper airways: Chronic nasal discharge and voice change.

Microscopy of Skin Lesion (Figs. 4.3A to C)

- **Flattened epidermis**: Epidermis is thinned and flattened (loss of rete ridges) over the nodules.
- **Grenz** (clear) **zone:** It is a characteristic, **narrow, uninvolved dermis** (normal collagen) which separates the epidermis from nodular accumulations of macrophages.

Q. Write short note on lepra cell.

- **Lepra cells:** The nodular lesions contain large aggregates of lipid-laden foamy macrophages (lepra cells, Virchow cells), filled with aggregates ("globi") of acid-fast lepra bacilli(*M. leprae*).

- **Fite-Faraco (acid-fast) stain:** It shows **numerous lepra bacilli** ("red snappers") within the foamy macrophages. They may be arranged in a parallel fashion like **cigarettes in a pack**.
- Due to the presence of numerous bacteria, lepromatous leprosy is also referred to as **"multibacillary"**.

In advanced cases, *M. leprae* may be present in sputum and blood.

Some individuals may have with intermediate forms of disease, called **borderline leprosy**.

3. Borderline Leprosy

Borderline tuberculoid (BT) shows epithelioid cells and numerous lymphocytes with a narrow clear subepidermal zone. Lepra bacilli are few and found in nerves.

Borderline lepromatous (BL) shows predominantly histiocytes, few epithelioid cells and lymphocytes. Numerous lepra bacilli are found.

Mid-borderline (BB) or dimorphic form shows sheets of epithelioid cells without any giant cells. Few lymphocytes are found in the perineurium. Lepra bacilli are seen mostly in nerves.

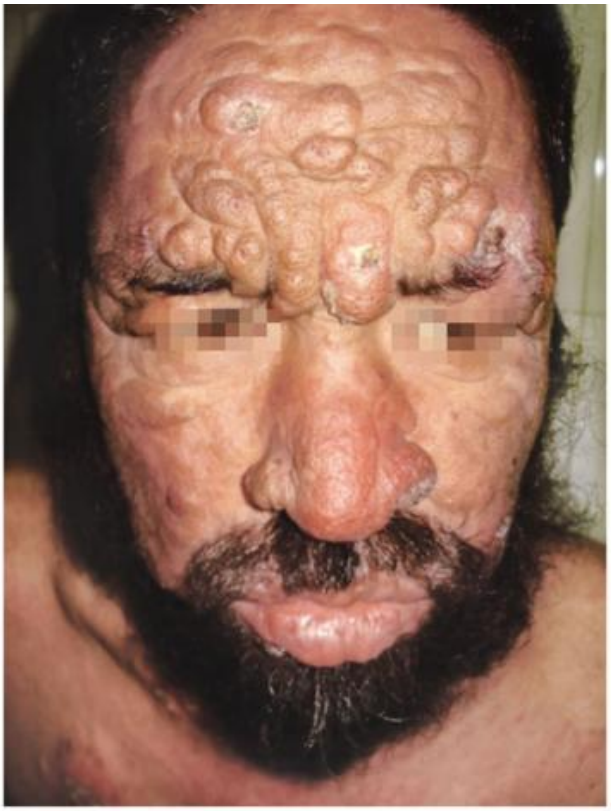
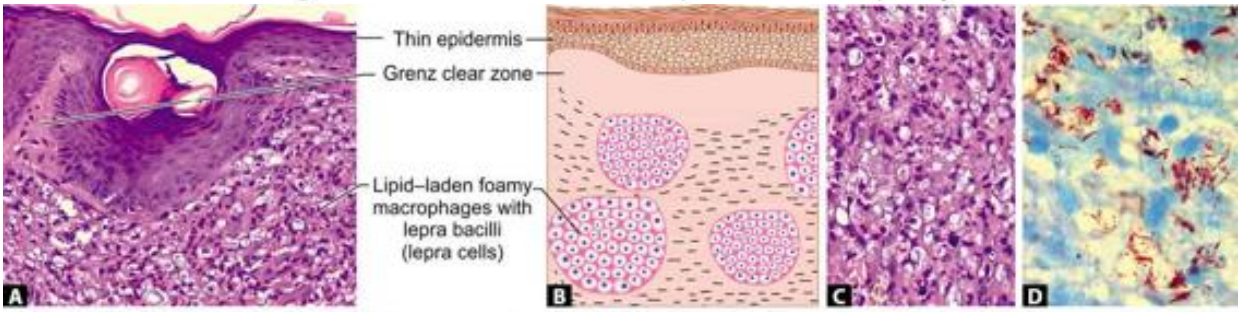


Fig. 4.2: Leonine facies of lepromatous leprosy.



Figs. 4.3A to C: Microscopic appearance of lepromatous leprosy. (A) Photomicrograph; (B) Diagrammatic. The epidermis is thinned and the dermis shows dense collections of lepra cells. The epidermis is separated from the collections of lepra cells by an uninvolved Grenz zone; (C) Photomicrograph. High power view showing foamy macrophages; (D) Acid-fast lepra bacilli within macrophages (Fite–Faraco stain).

4. Indeterminate Leprosy

Microscopically, features are nonspecific and few findings help in suspecting leprosy. These include: (1) local infiltration of lymphocytes or mononuclear cells surrounding the skin adnexa (e.g., hair follicles and sweat glands) or around blood vessels, (2) involvement of nerve (if seen strongly favors the diagnosis) and (3) finding of lepra bacilli (which confirms the diagnosis).

Lepromin Test

It is **not a diagnostic test** for leprosy. It is **used for classifying** the leprosy based on the immune response.

- **Procedure:** An antigen extract of *M. leprae* called lepromin is intradermally injected.

Q. Write short note on lepromin test/Mitsuda reaction.

- **Reaction**:
  - An **early positive reaction** appearing as an indurated area in 24–48 hours is called **Fernandez reaction**.
  - A **delayed granulomatous reaction** appearing after 3–4 weeks is known as **Mitsuda reaction**.
- **Interpretation:**
  - **Lepromatous leprosy**—shows **negative lepromin test** due to suppression of cell-mediated immunity.
  - **Tuberculoid leprosy—**show **positive lepromin test** because of delayed hypersensitivity reaction.

Reactions in Leprosy

The immunity in leprosy may change spontaneously or following treatment.

Type I Reaction

Borderline leprosy is the **most unstable form of leprosy** where **immune status may shift up or down**. These are called as type I reaction, which may be of two types:

1. Upgrading reactions: If immunity improves, the disease may shift towards tuberculoid leprosy.
2. Downgrading reaction: If the immunity decreases, the disease moves towards lepromatous leprosy.

Type II Reaction or Erythema Nodosum Leprosum

- It **occurs mostly in lepromatous** leprosy, particularly when on treatment.
- **Clinical features:** (1) Tender red plaque or nodules and (2) fever, malaise and arthralgia.
- **Microscopy:**
  - Necrotizing vasculitis
  - Lepra bacilli in the foamy macrophages.

Differences between lepromatous and tuberculoid leprosy are presented in **Table 4.1.**

Diagnosis of Leprosy

1. **Clinical examination:**
   - **Sensory testing**
   - Examination of peripheral nerve
2. **Demonstration of acid-fast bacilli:**
   - Skin smears prepared by **slit and scrape method**
   - *Mycobacterium leprae* can be demonstrated in tissue sections, in split skin smears by splitting the skin, and in nasal smears by the following techniques:
     - Acid-fast (Ziehl–Neelsen) staining.
     - Fite–Faraco staining procedure is a modification of ZN procedure and is considered better for more adequate staining of tissue sections **(Fig. 4.3C)**.
     - Gomori methenamine silver (GMS) staining can also be employed.
   - **Nasal swabs stained by Ziehl–Neelsen (ZN) method**. The staining procedure is similar to that procedure employed for *M. tuberculosis* but can be decolorized by lower concentration (5%) of sulfuric acid (less acid-fast).
3. **Skin biopsy:** Fite–Faraco staining procedure is a modified ZN procedure and is better for tissue sections.
4. **Nerve biopsy**
5. **Molecular method: Polymerase chain reaction (PCR).**

IgM antibodies to PGL-1 antigen: Found in 95% of patients of lepromatous leprosy and in 60% of tuberculoid leprosy.

M. leprae: Grows best in cooler tissues: (1) Skin, (2) Peripheral nerves, (3) Anterior chamber of eye, (4) Upper respiratory tract and (5) Testis.

Mycobacterium leprae: Only bacterium that invades peripheral nerves.

TABLE 4.1: Differences between lepromatous and tuberculoid leprosy.

Characteristics	*Lepromatous leprosy*	*Tuberculoid leprosy*
Clinical features		
Skin lesions	Symmetrical, multiple, ill-defined, macular, nodular	Asymmetrical, hypopigmented, well-defined macular
Disfigurement	Leonine facies, loss of eyebrows, pendulous earlobes, claw-hands, saddle nose	Minimal disfigurement
Nerve involvement	Seen, but with less severe sensory loss than tuberculoid	Common with sensory disturbances
Microscopy of skin lesions		
Type of lesion	Nodular or diffuse collections of lepra cells within dermis	Noncaseating granulomas composed of epithelioid cells and giant cells
Grenz/clear zone between inflammatory cells and epidermis	Present	Absent
Lepra bacilli	Plenty within the lepra cells as globular masses (globi)	Rare if any
Bacillary index	4 or 5	0
Other features		
Immunity	Suppressed-low resistance	Good immunity-high resistance
Lepromin test	Negative	Positive
Infectivity	High	Low
Complications	Erythema nodosum leprosum (ENL) may cause vasculitis, glomerulonephritis and nerve damage	Nerve damage can lead to sensory disturbance, paralysis

Diagnosis of Leprosy
- Staining of smears or skin biopsy
  - Acid fast (Ziehl–Neelsen) stain
  - Fite–Faraco stain
- Molecular method—PCR

Morphological index (MI):
- Measure of number of acid-fast bacilli (AFB) in skin scrapings that stain uniformly bright
- Correlates with viability of AFB

Bacteriological index (BI): Quantifies *M. leprae* in tissue or smears. It scored from 1+ to 6+ (range from 1 to 10 bacilli per 100 fields to > 1000 per field) as multibacillary leprosy whereas BI of 0 + is termed paucibacillary.

Leprosy WHO classification: Paucibacillary and multibacillary.

Tuberculoid leprosy has a strong T_H1 response compared to weak T_H1 response in lepromatous leprosy.

Tuberculoid leprosy:
1. Good immune response
2. Lepromin test positive
3. Noncaseating granuloma in the skin
4. Nerve involvement

Lepromatous leprosy:
- Grenz zone is a narrow, uninvolved dermis that separates epidermis from macrophages
- Lepra cells are large lipid-laden macrophages filled with *M. leprae*.

Virchow (lepra/foam) cells are diagnostic of LL.

Lepromatous leprosy:
1. Leonine facies
2. Low resistance
3. Thinned epidermis
4. Grenz zone
5. Lepra cells filled with acid-fast bacilli
6. Lepromin test negative

Uses of lepromin test:
1. Classification of leprosy
2. Evaluation of cell-mediated immunity status in patient
3. Know the prognosis

SYPHILIS

Introduction: Spirochetes are gram negative, slender corkscrew-shaped bacteria covered in a membrane called an outer sheath, which may mask its antigens from the host immune response.

Syphilis (lues) is a **chronic, sexually transmitted** disease **caused by spirochete *Treponema pallidum***.

Etiology

Treponema pallidum (Fig. 4.4A)

- It is a **thin, delicate, corkscrew-shaped spirochete**, measures about 10 µm long with tapering ends and has about 10 regular spirals.
- Actively motile, showing rotation round the long axis, backward and forward motion.
- Cannot be grown in artificial media.
- **Staining:** It does not stain with ordinary bacterial stains and is too slender to be seen in Gram stain. It can be visualized by **silver stains, dark-field examination, and immunofluorescence techniques**.
- **Source of infection:** An open lesion of **primary or secondary syphilis**. Lesions in the mucous membranes or skin of the genital organs, rectum, mouth, fingers, or nipples.
- **Mode of transmission:**
  - **Sexual contact:** It is the usual mode of spread.

- **Transplacental transmission**: From mother with active disease to the fetus (during pregnancy) → congenital syphilis.
- **Blood transfusion.**
- **Direct contact:** With the open lesion is rare mode of transmission.

Basic Microscopic Lesion

Irrespective of stage, the basic microscopic lesion of syphilis consists of:

- **Mononuclear inflammatory infiltrate:** Predominantly of **plasma cells and lymphocytes**.
- **Obliterative endarteritis:** It is a characteristic obstructive vascular lesion in which mononuclear infiltrates surround small arteries and arterioles (**periarteritis**).

Stages of Syphilis (Flowchart 4.2)

Q. Write a short note on stages of syphilis.

Treponema pallidum passes from the site of inoculation to regional lymph nodes and enters to the systemic circulation, and disseminate throughout the body. Syphilis can be **(1) congenital or (2) acquired**. The course of acquired syphilis is divided into three stages: (i) primary syphilis, (ii) secondary syphilis and (iii) tertiary syphilis.

Primary Syphilis

Develops about **3 weeks after contact** with an infected individual and the lesion is primary chancre.

Primary Chancre

Q. Write short note on primary chancre.

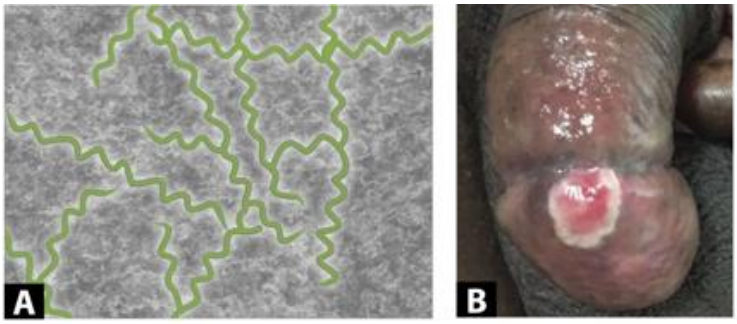
It is the classical lesion of primary syphilis.

- **Sites: Penis (Fig. 4.4B) or scrotum** in men and **cervix, vulva** and **vaginal** wall in women. It may also be seen in the anus or mouth.

MORPHOLOGY

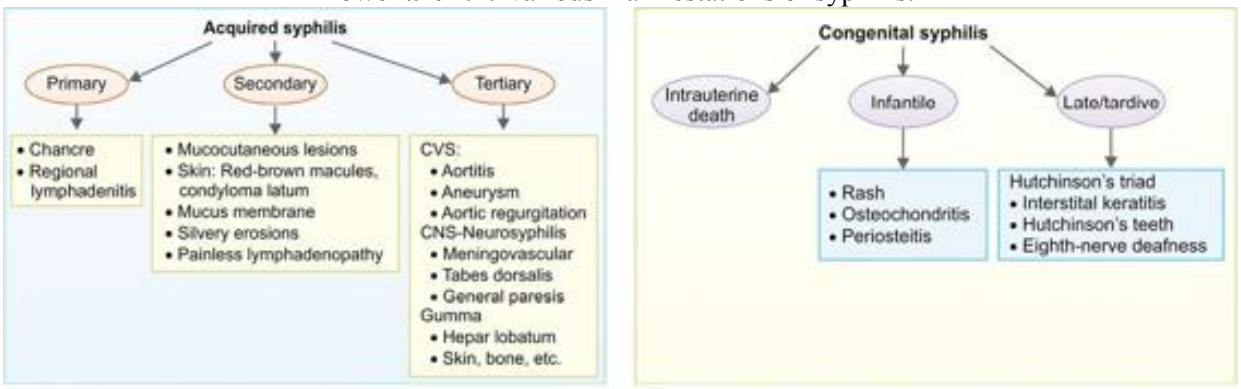
Gross Features

- It is single, firm, **nontender (painless)**, slightly raised, **red papule** (chancre) up to several centimeters in diameter. It erodes to create a clean-based shallow ulcer. Because of the induration surrounding the ulcer, it is designated as **hard chancre (Fig. 4.4B).**



Figs. 4.4A and B: (A) Diagrammatic appearance of *Treponema pallidum* under Dark-field examination; (B) Primary chancre on glans penis.

Flowchart 4.2: Various manifestations of syphilis.



(CVS: cardiovascular system; CNS: central nervous system)

- **Demonstration of treponema**: Plenty of treponemes can be demonstrated in the chancre by (1) silver stains (e.g. Warthin-Starry stain) or (2) immunofluorescence techniques or (3) Dark-field examination.

Microscopy

- **Mononuclear infiltration:** Consisting of **plasma cells,** with scattered macrophages and lymphocytes. These cells are also seen surrounding the blood vessels (periarteritis).
- **Blood vessels with endarteritis:** It is characterized by endothelial cell proliferation which progresses to intimal fibrosis.

Regional Lymphadenitis

It is due to nonspecific acute or chronic inflammation.

- Treponemes may spread throughout the body by blood and lymphatics even before the appearance of the chancre.
- **Symptoms:** Usually, painless and often unnoticed.

Fate: It heals in 3–6 weeks with/without therapy.

Secondary Syphilis

Q. Write short note on secondary syphilis.

It develops 2–10 weeks after the primary chancre in approximately 75% of untreated patients. Its manifestations are due to systemic spread and proliferation of the spirochetes within the skin and mucocutaneous tissues.

LESIONS OF SECONDARY SYPHILIS

Q. Write short note on anogenital syphilis.

Mucocutaneous Lesions

These are painless, superficial lesions and contain spirochetes and are infectious.

Skin Lesions

- **Skin rashes:** Consist of **discrete red-brown macules** less than 5 mm in diameter, but it may be scaly/pustular/annular. They are more frequent on the **palms** of the hands **(Fig. 4.5A)**, or **soles** of the feet.
- **Condylomata lata (Fig. 4.5B):** These are **broad-based, elevated plaques** with numerous spirochetes. They are seen in moist areas of the skin, such as the **anogenital region (perineum, vulva, and scrotum),** inner thighs, and axillae.

Mucosal Lesions

- Usually occurs in the mucous membranes of **oral cavity or vagina as silvery-gray superficial erosions**. These lesions contain numerous *T. pallidum* and are the **highly infectious**.
- **Microscopy:** Similar to primary chancre, i.e., infiltration by plasma cells and endarteritis obliterans.

Painless Lymphadenopathy

Especially involves **epitrochlear nodes** and shows plenty of spirochetes.

Symptoms: Mild fever, malaise, and weight loss are common in secondary syphilis, which may last for several weeks. The lesions subside even without treatment.

Tertiary Syphilis

Q. Write short note on tertiary syphilis and its manifestations.

- After the lesions of secondary syphilis have subsided patients enters an asymptomatic latent phase of the disease.
- The latent period may last for 5 years or more (even decades), but spirochetes continue to multiply.
- This stage is rare if the patient gets adequate treatment, but can occur in about one-third of untreated patients.
- Focal ischemic necrosis due to obliterative endarteritis is responsible for many of the processes associated with tertiary syphilis.

Manifestations: Three main manifestations of tertiary syphilis are: **cardiovascular syphilis, neurosyphilis, and so-called benign tertiary syphilis.** These may occur alone or in combination.

Cardiovascular Syphilis

NC: Describe the etiology, pathophysiology, pathology features and complications of syphilis on the cardiovascular system.



Figs. 4.5A and B: (A) Symmetrical skin rashes of secondary syphilis on palm; (B) Condyloma lata of vulva and perineum composed of broad-based elevated plaques.

A

B

Most **frequently involves the aorta** and known as syphilitic aortitis.

MORPHOLOGY

- **Syphilitic aortitis:** Accounts for more than 80% of cases of tertiary disease, and affects the **proximal aorta**.
- **Saccular aneurysm and aortic valve insufficiency:**
  - **Occlusion of the vasa vasorum** due to endarteritis leads to **necrosis and scarring of the aortic media,** causing a loss of elasticity, strength and resilience.
  - Gradual weakening and slow progressive **dilation of the aortic root and arch**, causes **aortic valve insufficiency** and **aneurysms of the proximal aorta**. Syphilitic aneurysms are saccular and seen in the ascending aorta, which is unusual site for the more common atherosclerotic aneurysms.
  - On gross examination, the aortic intima appears rough and pitted (**tree-bark appearance**).
- **Myocardial ischemia**: Narrowing of the coronary artery ostia (at the origin from aorta) caused by subintimal scarring may lead to myocardial ischemia/infarction.

Neurosyphilis

It may be asymptomatic or symptomatic.

- **Asymptomatic neurosyphilis:** It is detected by CSF examination, which shows pleocytosis (increased numbers of inflammatory cells), elevated protein levels, or decreased glucose. Antibodies can also be detected in the CSF, which is the most specific test for neurosyphilis.
- **Symptomatic disease:** Takes one of several forms
  - **Chronic meningovascular disease:** Chronic meningitis → involves base of the brain, cerebral convexities and spinal leptomeninges.
  - **Tabes dorsalis:** It is characterized by **demyelination of posterior column, dorsal root and dorsal root ganglia**.
  - **General paresis of insane:** Shows generalized brain parenchymal disease with **dementia**; hence called as general paresis of insane.

Benign Tertiary Syphilis

It is characterized by the formation of nodular lesions called **gummas** in any organ or tissue. Gummas reflect development of delayed hypersensitivity to

the spirochete. Gummas are very rare and may be found in patients with acquired immune deficiency syndrome (AIDS).

SYPHILITIC GUMMAS

Q. Write short note on gumma.

- May be single or multiple.
- **White-gray and rubbery**.
- Vary in size from microscopic lesions to large tumor-like masses.

Site

They occur in most organs but mainly involve

- **Skin, subcutaneous tissue** and the mucous membranes of the upper airway and mouth.
- **Bone and joints:** It causes local pain, tenderness, swelling, and sometimes pathologic fractures.
- In the **liver,** scarring due to gummas may cause a distinctive hepatic lesion known as **hepar lobatum**.

Microscopy

Center of the gummas show **coagulative necrosis** → surrounded by plump, **palisading macrophages**, fibroblasts and plenty of **plasma cells**. Treponemes are scant in gummas.

Congenital Syphilis

Q. Write short note on congenital syphilis.

Transplacental Transmission

- *T. pallidum* can cross placenta and spread from infected **mother to the fetus** (during pregnancy).
- Transmission occurs, when **mother is suffering from primary or secondary syphilis** (when the spirochetes are abundant). Because routine serologic testing for syphilis in done in all pregnancies, congenital syphilis is rare.

Manifestations

It can be divided into:

1. Intrauterine Death and Perinatal Death.

Congenital syphilis may result in intrauterine and perinatal death.

2. Early (Infantile) Syphilis

It occurs in the **first 2 years of life** and often manifested by **nasal discharge** and congestion (**snuffles**).

- A **desquamating or bullous eruption/rash** can lead to epidermal sloughing of the skin, mainly in the hands, feet, around the mouth and anus.
- **Skeletal abnormalities:**
  - **Syphilitic osteochondritis:** Inflammation of bone and cartilage is more distinctive in the nose. Destruction of the vomer causes collapse of the nasal bridge → produces characteristic **saddle nose deformity**.
  - **Syphilitic periostitis:** It involves the tibia and causes excessive new bone formation on the anterior surfaces and leads to anterior bowing, or **saber shin**.

Q. Write short note on hepar lobatum.

- **Liver:** Diffuse fibrosis in the liver called as hepar lobatum.
- **Lungs:** Diffuse interstitial fibrosis → lungs appear pale and airless (**pneumonia alba**).

3. Late (Tardive) Syphilis

Manifests **2 years after birth**, and about 50% of untreated children with neonatal syphilis will develop late manifestations.

Q. Write short note on components of Hutchinson's triad.

- **Manifestations:** Distinctive manifestations in **Hutchinson's triad** are:
  - Interstitial keratitis.
  - **Hutchinson's teeth:** They are like small screwdrivers or peg-shaped incisors, with notches in the enamel.
  - Eighth-nerve deafness.

Laboratory Diagnosis

- **Immunofluorescence of exudate** from the chancre is important for diagnosis in primary syphilis.
- Microscopy and polymerase chain reaction (PCR) are also useful.
- **Serological tests: Nontreponemal antibody tests—**These tests measure antibody to cardiolipin, a phospholipid present in both host tissues and *T. pallidum*.

Q. Write short note on false-positive VDRL test.

- These antibodies are detected by the rapid plasma reagin and venereal disease research laboratory (VDRL) tests.
- False-positive VDRL test: Found in certain acute infections, collagen vascular diseases (e.g., systemic lupus erythematosus), drug addiction, pregnancy, hypergammaglobulinemia of any cause, and lepromatous leprosy.
- Antitreponemal antibody tests: These measure antibo-dies, which react with *T. pallidum*. These include:
  - Fluorescent treponemal antibody absorption test (FTA)
  - Microhemagglutination assay for *T. pallidum* antibodies.

Jarisch–Herxheimer reaction:

- Treatment of syphilitic patients having a high bacterial load, by antibiotics can cause a massive release of endotoxins, and cytokine that may manifest with high fever, rigors, hypotension, and leukopenia.
- This **syndrome** is called the Jarisch–Herxheimer reaction, which **can develop not only in syphilis but also in other spirochetal diseases**, such as Lyme disease.

Syphilis: Caused by spirochete *Treponema pallidum*.

Primary syphilis: Chancre is the painless lesion seen in the external genitalia with regional lymphadenitis.

Secondary syphilis:
1. Mucocutaneous lesions
2. Generalized lymphadenopathy

Treponema pallidum: It can be identified in lesions of primary or secondary syphilis.

Tertiary syphilis: Involves mainly CVS, CNS and focal lesions called gumma.

Cardiovascular syphilis: Involves proximal aorta and lead to saccular aneurysm of aorta and aortic valve incompetence. **Syphilis never causes aortic stenosis**.

Neurosyphilis:
1. Chronic meningovascular disease
2. Tabes dorsalis
3. General paresis of insane

Syphilitic gumma: Central area of coagulative necrosis surrounded by plump, palisading macrophages, fibroblasts and plenty of plasma cells.

Congenital syphilis: Caused by maternal transmission of *T. pallidum*. May lead to intrauterine death or infantile syphilis with widespread injury to skin, liver, bone and lungs.

Hutchinson's triad: (1) Interstitial keratitis (2) Hutchinson's teeth(3) Eighth-nerve deafness.

TUBERCULOSIS

Refer pages 511-8 of Chapter 17.

NC: Define and describe the pathogenesis and pathology of common bacterial, viral, protozoal and helminthic diseases.

BACTERIAL DISEASES

Pyogenic Bacteria

- **They produce suppurative/purulent inflammation.** Pyogenic inflammation is a type of inflammation characterized by increased vascular permeability and leukocytic infiltration, mainly of neutrophils. The neutrophils accumulate at the site of infection due to release of chemoattractants from the "pyogenic" (pus-forming) bacteria. These bacteria are mostly extracellular gram-positive cocci and gram-negative rods.
- **Gross:** Size of the lesion depends on the location of the lesion and the organism involved. The size of purulent lesions may range from tiny microabscesses to diffuse involvement of the involved organ or tissue.
- **Microscopy:** It consists of pus, which is formed by masses of dying and dead neutrophils and liquefactive necrosis of the involved tissue.

- **Consequences:** Depends on the site and causative organism. Examples include:
  - Pneumococcal infection of lung usually spares alveolar walls and cause lobar pneumonia that resolves completely.
  - Staphylococci and *Klebsiella* infection of lung destroy alveolar walls and form abscesses that heal with scar formation.
  - Bacterial pharyngitis resolves without sequelae.
  - Untreated acute bacterial infection of a joint destroys the joint.
- **Bacteremia:** It is an invasion of the bloodstream by bacteria and occurs when bacteria enter the blood. It occurs commonly as an integral part of some infections.
- **Pyemia:** Septicemia is the presence of infective agents in the bloodstream. Pyemia is **septicemia due to pyogenic organisms**. Pyemia occurs when pathogenic organisms enter into the bloodstream and form small aggregates (microemboli). They result in either pyemic abscesses or septic infarct in various organs.

Diphtheria

- Diphtheria is caused by *Corynebacterium diphtheria*.
- *Corynebacterium diphtheria* is a slender gram-positive rod with clubbed ends.
- **Mode of spread:** From person-to-person in respiratory droplets (respiratory diptheria) or skin exudate (cutaneous diptheria).
- **Incubation period:** Commonly 3–4 days.
- **Types:** Common types include:
  - **Respiratory diphtheria:** It causes pharyngeal infection. Inhaled *C. diphtheria* is carried in respiratory droplets proliferate at the site of attachment on the mucosa. It produces exotoxin which causes necrosis of the epithelium and formation of a dense fibrinosuppurative exudate. The coagulation of this exudate on the ulcerated necrotic surface creates a tough, dirty gray to black, superficial membrane known as **pseudomembrane** (because it is not formed by viable tissue). When the membrane sloughs off, there is bleeding and may lead to asphyxia. With control of the infection, the pseudomembrane is either coughed-up or dissolved by enzymatic digestion. The toxin also damages the heart, nerves, and other organs.

- **Cutaneous diphtheria:** It causes chronic ulcers with a dirty gray membrane without any systemic damage.

- **Immunization with diphtheria toxoid (formalin-fixed toxin):** It stimulates production of toxin-neutralizing antibodies which protect persons from the lethal effects of the toxin.

Gram-negative Bacterial Infections

Whooping Cough or Pertussis

Pertussis or whooping cough is a highly communicable acute bacterial disease of childhood, caused by the gram-negative coccobacillus ***Bordetella pertussis***. The wide use of DPT (**D**iphtheria, **P**ertussis and **T**etanus) vaccine has reduced the prevalence of whooping cough.

Incubation period: About 1–2 weeks.

Pathogenesis

- *Bordetella pertussis* has strong affinity for the bronchial epithelium and also invades macrophages. *B. pertussis* proliferates and stimulates the bronchial epithelium to produce abundant tenacious mucus causing **laryngotracheobronchitis**.
- In severe cases, it causes bronchial mucosal erosion, hyperemia, and copious mucopurulent exudate.
- The toxin produced by the organism inhibits neutrophils and macrophages and paralyze cilia. Within 7–10 days after exposure, catarrhal stage of whooping cough begins and is the most infectious stage.
- Peripheral blood shows **marked lymphocytosis** (up to 90%) and enlargement of lymphoid follicles in the bronchial mucosa and peribronchial region.

Clinical features: It is characterized by **paroxysms of violent coughing** followed by a characteristic **loud inspiratory "whoop."** Other features include low-grade fever, rhinorrhea, conjunctivitis and excess tear production. The condition is self-limiting but may cause death due to asphyxia in infants.

Bacillary dysentery (*refer* pages 568-9).

VIRAL DISEASES

Poliovirus Infection

- Poliovirus causes an acute systemic viral infection. It can produce a wide range of clinical manifestations ranging from mild, self-limited infections to paralysis of limb muscles and respiratory muscles.
- Poliovirus is a spherical, unencapsulated **RNA virus** belonging to the enterovirus genus.
- **Mode of infection:** Poliovirus infects only humans and is transmitted by the **fecal-oral route**.
- The vaccines [Salk formalin fixed (killed) vaccine and the Sabin oral, attenuated (live) vaccine] have eradicated polio in India.

Pathogenesis

- **Incubation period:** About 10 days.
- The polio virus is ingested and replicates in the mucosa of the pharynx, tonsils and gut (Peyer patches in the ileum).
- From the mucosa, it spreads through lymphatics to lymph nodes and eventually the blood. Most poliovirus infections are asymptomatic.
- In nonimmunized patients poliovirus infection causes a subclinical or mild gastroenteritis. In about 1% of infected patients, poliovirus secondarily invades the CNS and replicates in motor neurons of the spinal cord (spinal poliomyelitis) or brain stem (bulbar poliomyelitis).

MORPHOLOGY

Acute cases show mononuclear cell perivascular cuffs in the **anterior horn motor neurons of the spinal cord**.

Clinical Features

- **Spinal cord involvement:** When polio affects the motor neurons of the spinal cord, it destroys motor neurons and leads to paralysis.
- **CNS infection:** It causes meningeal irritation and shows features of aseptic meningitis.

Herpes Virus Infections

- Herpes viruses are large encapsulated viruses with double-stranded DNA genome.
- Herpes viruses cause acute infection followed by latent infection. During latent infection, the viruses persist in a noninfectious form with periodic reactivation and shedding of infectious virus. Reactivation of the virus causes dissemination of the infection and tissue injury.
- There are eight types of human herpes viruses, belonging to three subgroups: α-group (e.g., HSV-1, HSV-2); β-group (e.g., CMV, human herpesvirus-6) and the γ-group (EBV and KSHV/HHV-8).

Herpes Simplex Viruses

There are two types of Herpes simplex viruses (HSV) namely—**HSV-1 and HSV-2.** They **differ serologically but are closely related genetically and cause a similar set of primary and recurrent infections.**

1. **Mucocutaneous lesions**
   - HSV-1 and HSV-2 cause self-limited cold sores and gingivostomatitis.
   - **Blisters or cold sores:** Both viruses replicate at the site of entry of the virus (namely—the skin and the mucous membranes usually oropharynx or genitals). They cause vesicular lesions of the epidermis. Fever **blisters or cold sores** are observed in the facial skin around mucosal orifices (lips and nose).
   - **Gingivostomatitis:** It is usually observed in children and is caused by HSV-1.
   - The viruses spread to sensory neurons and are transported along axons to the neuronal cell bodies, where they establish latent infection.
   - **Genital herpes** is more often caused by HSV-2 than by HSV-1.
   - In immunocompetent individuals, primary HSV infection resolves, although the virus remains latent in nerve cells. During latency period, the viral DNA remains within the nucleus of the neuron. Reactivation of HSV-1 and HSV-2 may occur with or without symptoms, and this reactivation causes the spread of virus from the neurons to the skin or to mucous membranes.
2. **Ophthalmic lesions:** Two forms of **corneal lesions may be produced: (1) herpes epithelial keratitis and(2) herpes stromal keratitis** (can lead to corneal blindness).

3. **Nervous system:** HSV-1 can also produce encephalitis.

MORPHOLOGY

- HSV-infected cells contain large, pink to purple **intranuclear inclusions** (Cowdry type A).
- HSV also produces inclusion-bearing multinucleated syncytia.

Rabies

Rabies is a viral disease causing **severe encephalitis** and transmitted to humans by the **bite of a rabid animal (usually a dog)**.

Rabies virus is sensitive to and killed by ethanol, iodine preparations, and soap detergents.

MORPHOLOGY

Gross

External examination of the brain shows edema and vascular congestion.

Microscopy

- It shows widespread neuronal degeneration and an inflammatory reaction which is most severe in the brainstem.
- **Negri bodies:** These are the **pathognomonic** microscopic features. They are cytoplasmic, round to oval, eosinophilic inclusions that can be found in pyramidal neurons of the hippocampus and Purkinje cells of the cerebellum. Rabies virus can be identified within the Negri bodies by ultrastructural and immunohistochemical methods.

Clinical Features

Rabies virus enters the CNS by ascending along the peripheral nerves from the wound site (bite of a rabid animal).

Incubation period usually ranges from **1 to 3 months** and it depends on the distance between the wound and the brain.

Symptoms

- **Nonspecific symptoms:** Rabies presents with nonspecific symptoms such as malaise, headache, and fever. **Local paresthesia around the wound** along with above symptoms is diagnostic.
- **CNS excitability:** As the infection progresses, patient develops severe CNS excitability. The slightest touch is painful and causes violent motor

responses or even convulsions. Signs of meningeal irritation and flaccid paralysis may develop as the disease progresses.

- **Hydrophobia:** Contracture of the pharyngeal muscles on swallowing produces foaming at the mouth. This causes aversion/fear to swallow even water (hydrophobia).
- Produces coma and death from respiratory failure.

Measles (Rubeola)

Q. Write short note on measles.

- Measles is an **acute viral infection** caused by measles (rubeola) virus.
- It **affects multiple organs** and causes a wide range of disease ranging from mild, self-limited infections to severe systemic manifestations. It can produce severe disease in patients with defects in cellular immunity (e.g., with HIV or hematologic malignancy).
- Measles can be prevented by vaccine. Epidemics of measles occur among unvaccinated individuals.
- Diagnosis is by clinical features, by serology, or by detection of viral antigen in nasal exudates or urinary sediments.

Pathogenesis

- Measles virus is a single-stranded RNA virus of the paramyxo virus family.
- **Mode of transmission:** Measles virus is transmitted by respiratory droplets.
- **Incubation period:** 9–11 days.
- Measles can replicate in many cell types such as epithelial cells and leukocytes. Initially, virus multiplies within the respiratory tract and then spreads to local lymphoid tissues. Replication of the virus in lymphoid tissue is followed by viremia and systemic dissemination to many tissues. The tissues involved are conjunctiva, skin, respiratory tract, urinary tract, small blood vessels, lymphatic system, and CNS.
- Most children develop T-cell–mediated immunity to the virus which control the viral infection and produces the measles rash. Hence, the measles rash is less common in patients with deficiencies in cell-

mediated immunity. In malnourished, it may cause croup, pneumonia, diarrhea and protein-losing enteropathy, keratitis (producing scarring and blindness), encephalitis, and hemorrhagic rashes ("black measles").

- Measles can cause transient immunosuppression, resulting in secondary bacterial and viral infections.
- Antibody-mediated immunity to measles virus protects against reinfection.

MORPHOLOGY

- **Measles rash:** It is blotchy, reddish-brown and observed on the face, trunk, and proximal extremities. It is produced by dilated skin vessels, edema, and a mononuclear perivascular infiltrate.
- **Koplik spots:** These are pathognomonic of measles and consist of ulcerated mucosal lesions in the oral cavity near the opening of the Stensen (parotid) ducts. They appear as small, irregular, bluish-white dots measuring 1 mm in diameter surrounded by erythema.

- **Lymphoid organs—Warthin–Finkeldey cells:** Lymphoid organs show marked follicular hyperplasia, enlarged germinal centers, and randomly distributed multinucleate giant cells, called **Warthin–Finkeldey cells** (pathognomonic of measles). These giant cells have eosinophilic nuclear and cytoplasmic inclusion bodies. These are also found in the lung and sputum.

RICKETTSIAL INFECTIONS

Rickettsiales are vector-borne obligate intracellular bacteria. These organisms have the structure of gram negative, rod-shaped bacteria. However, they stain poorly with Gram stain.

Diseases Produced

They cause epidemic and scrub typhus, spotted fevers (*Rickettsia rickettsia*), ehrlichiosis, and anaplasmosis.

- **Epidemic typhus**
  - Caused by *Rickettsia prowazekii.*

- **Mode of transmission:** Transmitted from person-to-person by body lice.
- **Clinical features:** Include macular rash that progresses to a petechial, maculopapular rash on the entire body except the face, palms, and soles.

- **Scrub typhus**
  - Caused by *Orientia tsutsugamushi*.
  - **Mode of transmission:** By chiggers.
  - **Clinical features:** Include fever, headache, myalgia and cough and associated lymphadenopathy from the chigger bite.
- **Rocky Mountain spotted fever**
  - Caused by *Rickettsia rickettsia*.
  - **Mode of transmission:** By dog ticks.
  - **Clinical features:** Begins as a nonspecific severe illness with fever, myalgias, and gastrointestinal distress. As the disease progresses widespread macular then petechial rash involving the palms and soles develop.

Pathogenesis

- The manifestations of rickettsial infections are mainly due to infection of endothelial cells (especially those in the lungs and brain) which results in endothelial dysfunction and injury.
- Widespread endothelial dysfunction/injury can cause shock, peripheral and pulmonary edema, and disseminated intravascular coagulation, renal failure and CNS manifestations (e.g., coma).

MORPHOLOGY

Characterized by small vessel lesions and focal areas of hemorrhage and inflammation in many organs and tissues.

Diagnosis

- Rickettsial diseases are usually diagnosed clinically.
- Diagnosis is confirmed by serology or immunostaining of the organisms.

CHLAMYDIAL INFECTIONS

Chlamydia Trachomatis

It is a small gram negative bacterium and exists in two forms during its unique life cycle.

- **Elementary body:** It is infectious, metabolically inactive form and has a spore like structure. Host cells take up the elementary body by endocytosis and form endosome. The bacteria prevent fusion of the endosome and lysosome.
- **Reticulate body:** Inside the endosome of the host cell, the elementary body differentiates into a metabolically active form, called the reticulate body. The reticulate body replicate and forms new infectious elementary bodies.

Diseases produced: *C. trachomatis* infection cause—(1) urogenital infections, (2) inclusion conjunctivitis (3) lymphogranuloma venereum, (4) ocular infection of children, trachoma.

Genital infection by *C. trachomatis* is the most common sexually transmitted bacterial infection in women.

MORPHOLOGY

- *C. trachomatis* **urethritis** appears identical to gonorrhea. It produces mucopurulent discharge containing mainly neutrophils.
- **Lymphogranuloma venereum** contains a mixed granulomatous and neutrophilic inflammatory response. Chlamydial inclusions are seen in the cytoplasm of epithelial cells or inflammatory cells. Regional lymphadenopathy is common and shows granulomatous inflammatory reaction associated with irregularly shaped foci of necrosis containing neutrophils (**stellate abscesses**). With time, extensive fibrosis may cause local lymphatic obstruction, lymphedema, and strictures.

FUNGAL INFECTIONS AND OPPORTUNISTIC INFECTIONS

Fungi grow as multicellular filaments (mold) or individual cells alone or in chains (yeast). They have cell wall which gives them a shape.

- **Yeasts** are round to oval and reproduce by budding. Some yeasts (e.g., *Candida albicans*) produce buds that fail to detach and become

elongated, producing a chain of elongated yeast cells called **pseudohyphae**.

- **Molds** are thread-like filaments (hyphae) that grow and divide at their tips. They can produce round cells known as **conidia**, which can easily become airborne, disseminating the fungus.
- Many fungi are dimorphic, i.e., they exist as yeast or molds, depending on environmental conditions (yeast form at human body temperature and a mold form at room temperature).

Types of Fungal Infections

Fungal infections are also known as *mycoses*. Human fungal infections are divided into four major types:

1. **Superficial and cutaneous mycoses:** Common and limited to the skin, hair, and nails.
2. **Subcutaneous mycoses:** Involve the skin, subcutaneous tissues, and lymphatics.
3. **Endemic mycoses:** Caused by dimorphic fungi and can produce serious systemic illness in healthy individuals.
4. **Opportunistic mycoses:** They can cause life-threatening systemic diseases in individuals who are immunosuppressed.

Diagnosis

1. Histological examination.
2. **Culture:** Definitive identification of some requires culture.

Yeast

Q. Write short note on candidiasis.

Candidiasis

Live as commensals: *Candida* species reside normally in the skin, mouth, gastrointestinal tract, and vagina. They usually live as commensals and seldom produce disease in healthy individuals.

Infection: Most *Candida* infections occur when the normal commensal flora breach the skin or mucosal barriers. There are different strains of *Candida* of which *C. albican* is the most frequent cause of human fungal

infections. These infections may be either limited to the skin or mucous membranes or may disseminate widely.

Pathogenesis: A single strain of *Candida* can live as commensal or may become pathogenic. *Candida* can shift between different phenotypes and phenotypic switching is the mechanism by which *Candida* adapts to changes in the host environment (e.g., produced by antibiotic therapy, the immune response, or altered host physiology).

- *Candida* produce many **adhesins** (e.g., integrin-like protein) which helps them to adhere to host cells and are important determinant of virulence.
- *Candida* produce many **enzymes** that contribute to invasiveness (e.g., aspartyl proteinases).

MORPHOLOGY

C. albicans may appear as yeast, pseudohyphae, and, less commonly, true hyphae, defined by the presence of septae. The pseudohyphae form consists of chain of budding yeast cells joined end to end at constrictions (**Fig. 4.6** Inset). They represent an important diagnostic clue.



Fig. 4.6: Candidiasis (arrow) in a patient with AIDS. Inset shows pseudohyphae of Candida.

Superficial Candidiasis

Diabetic and burn patients are susceptible to superficial candidiasis.

- Most commonly candidiasis takes the form of a superficial infection on mucosal surfaces of the **oral cavity (thrush) (Fig. 4.6)**. Florid proliferation of the fungi creates gray-white, dirty-looking pseudomembranes. This form of candidiasis is found in newborns, debilitated people, children receiving oral steroids for asthma, following a course of broad-spectrum antibiotics (destroy competing normal bacterial flora) and HIV-positive patients.
- **Candida esophagitis** is seen in AIDS patients and in those with hematolymphoid malignancies.
- **Candida vaginitis** is common and is seen in women who are diabetic, pregnant, or on oral contraceptive pills or on antibacterial agents (which alter the vaginal flora).
- **Cutaneous candidiasis:** It may present as infection of the nail (onychomycosis), nail folds (paronychia), hair follicles (folliculitis), moist, intertriginous skin, such as armpits or webs of the fingers and toes (intertrigo), and penile skin (balanitis). **In healthy** individual *Candida* can cause **vaginiti**s and diaper rash (cutaneous candidal infection in the perineum of infants, the region in contact with wet diapers).

Invasive/Disseminated Candidiasis

It is caused by dissemination of *Candida* into the bloodstream and thereby spreading into various tissues or organs. It can develop especially in patients with indwelling intravenous lines or catheters, or undergoing peritoneal dialysis. Severe disseminated candidiasis develops mostly in patients with neutropenia (e.g., due to acute leukemia, after chemotherapy, or hematopoietic stem cell transplantation). It may lead to shock and disseminated intravascular coagulation.

Cryptococcosis

Two species of *Cryptococcus* namely—*C. neoformans* and *C. gattii* can cause disease in humans. Both of them grow as encapsulated yeasts.

Species of Cryptococcus

1. *Cryptococcus neoformans*

Source: *Cryptococcus neoformans* is present in the soil and in bird (particularly pigeon) droppings. Mode of infection in human is through inhalation.

Susceptibility: Though *C. neoformans* can cause meningoencephalitis in healthy individuals, more frequently it causes an opportunistic infection in patient with AIDS, leukemia, lymphoma, systemic lupus erythematosus, or sarcoidosis, and in immunosuppressed transplant recipients. Many of these patients receive high-dose corticosteroids, which is a major risk factor for *C. neoformans* infection.

2. *Cryptococcus gattii*

Source: *C. gattii* is found in soil, and is acquired by inhalation. It causes disease in immunologically normal individuals and usually present as large lesions producing mass effects or may mimic the radiologic appearance of a neoplasm.

Pathogenesis

Virulence factors of *Cryptococcus* which helps it in evasion of host defenses are:

- **Polysaccharide capsule** of Cryptococci prevents its phagocytosis by alveolar macrophages, and also leukocyte migration, and recruitment of inflammatory cells.
- **Melanin production**: Laccase in the yeast catalyzes melanin formation which (1) has antioxidant properties, (2) reduces phagocytosis, (3) counteracts the effects of antifungal agents, (4) binds iron, (5) and provides cell wall integrity.
- **Enzymes:** Such as serine proteinase may help in tissue invasion; mannitol dehydrogenase (produces mannitol) causes osmotic edema and inhibits killing of the yeast by neutrophils and reactive oxygen species.

Q. Write a short note on morphology of Cryptococcus.

MORPHOLOGY

Cryptococcus has yeast form and has no pseudohyphal or hyphal forms.

- The cryptococcal yeast measures 5–10 μm and has a highly characteristic thick gelatinous capsule containing a polysaccharide **(Fig. 4.6)**.
- **Special stains: In tissues, the polysaccharide stains intensely red with periodic acid-Schiff and mucicarmine** and is detected with antibody-coated beads in an agglutination assay. India ink preparations produces a negative image, and the thick capsule as a clear halo within a dark background.

Sites Involved

- **Lung is the primary site** of infection and it is **usually mild and asymptomatic**
- **Central nervous system (CNS): The major lesions produced by *Cryptococcus* are in the CNS, mainly involving the meninges, cortical gray matter, and basal nuclei.**

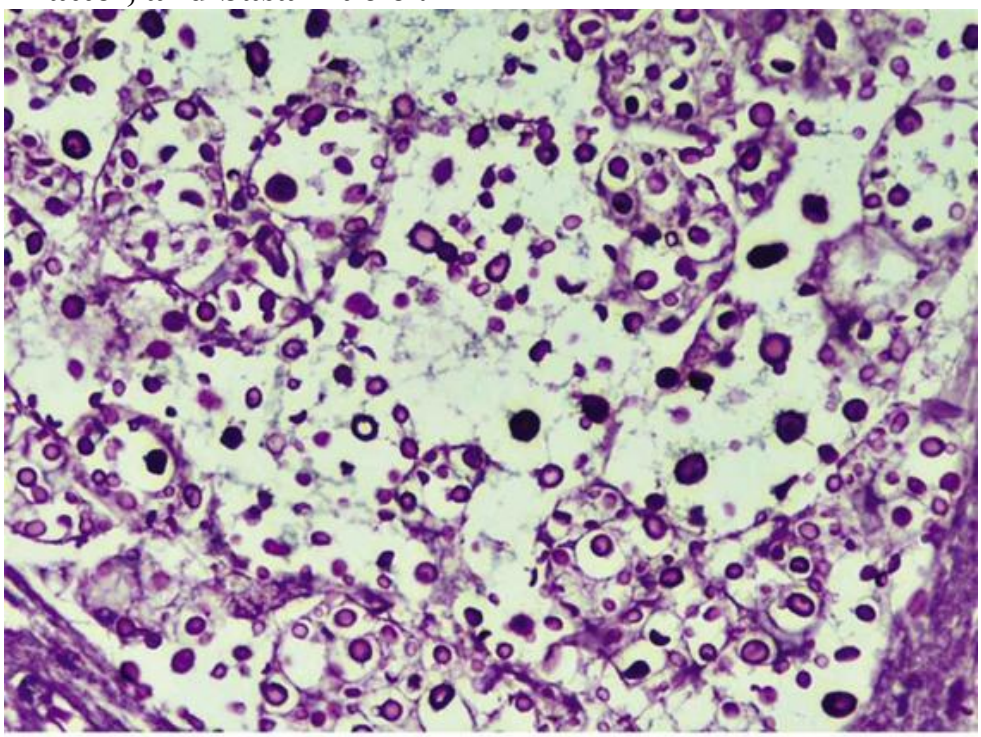


Fig. 4.7: Cryptococci forming gelatinous masses expanding the perivascular Virchow–Robin spaces within the gray matter of cerebral cortex producing the soap-bubble lesions in a patient with AIDS.

- **Host response** to cryptococci is varies and depends on the immunity of the host.

- In immunosuppressed patients, there may not be any inflammatory reaction and gelatinous masses of fungi can grow in the meninges or expand the perivascular Virchow–Robin spaces within the gray matter, producing the soap-bubble lesions **(Fig. 4.7)**.
- In severely immunosuppressed patients, *C. neoformans* may widely disseminate to the skin, liver, spleen, adrenals, and bones.
- In nonimmunosuppressed individual or in those with protracted disease, the fungi produces a chronic granulomatous reaction.

Molds

Aspergillosis

***Aspergillus* is a ubiquitous mold.** *Aspergillus fumigatus* is the most common pathogenic species of the fungus.

- It causes allergies (allergic bronchopulmonary aspergillosis) in healthy individuals.
- It produces serious sinusitis, pneumonia, and invasive disease in immunocompromised individuals.

Predisposing factors: These include conditions causing neutropenia and use of corticosteroids.

Mode of transmission: *Aspergillus* species are transmitted as airborne conidia.

Pathogenesis: Lung is the major portal of entry. The small sized (about 2–3 μm) spores of *A. fumigatus* reach the alveoli. Alveolar macrophages recognize conidia through TLR2 and the lectin dectin-1 receptors. Both receptors activate phagocytes to ingest and kill the conidia. In immunosuppressed states, conidia can produce hyphae and these hyphae invade tissues. Invasive aspergillosis is highly associated with neutropenia and impaired neutrophil defenses.

Virulence factors: *Aspergillus* produces many virulence factors. These include adhesins, antioxidants (e.g., melanin pigment, mannitol, catalases, and superoxide dismutases), **enzymes** (phospholipases, proteases), **and toxins.**

Lesions: The aflatoxin produced by *Aspergillus* species growing on the surface of improperly stored peanuts is carcinogen and may cause **liver cancer**. Sensitization to *Aspergillus* spores can cause **allergic alveolitis**.

Allergic bronchopulmonary aspergillosis, associated with hypersensitivity due to superficial colonization of the bronchial mucosa, may develop in patients with asthma.

MORPHOLOGY

Colonizing Aspergillosis (Aspergilloma)

It refers to growth of the fungus within the cavities of lung with minimal or no invasion of the tissues (the nose also is usually colonized). Lung cavities are usually the result of prior tuberculosis, bronchiectasis, old infarcts, or abscesses. Proliferating masses of hyphae form brownish "fungus balls" seen lying free within these lung cavities. Patients with aspergillomas usually present with recurrent hemoptysis.

Invasive Aspergillosis

It is an opportunistic infection which develop in immunosupp-ressed patients. The primary lesions are usually in the lung and appear as necrotizing pneumonia. They are sharply delineated, rounded, gray foci having hemorrhagic borders; they are called as target lesions.

Aspergillus has a tendency to invade blood vessels and may spread to involve the heart valves and brain.

***Aspergillus* forms fruiting bodies (usually in lung cavities) and septate filaments, 5–10 µm thick, branching (Fig. 4.8) at acute angles (40°).**

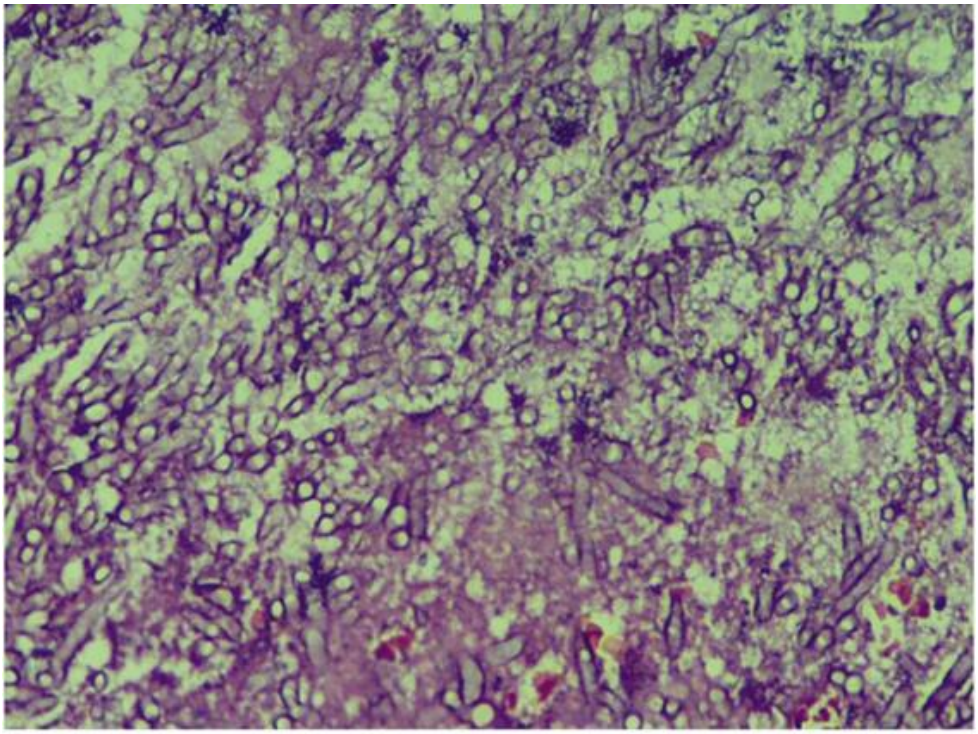


Fig. 4.8: *Aspergillus* consisting of septate hyphae with acute-angle branching.

Mucormycosis (Zygomycosis)

Mucoromycotina are widely found in nature and does not harm immunocompetent individuals. But they can infect immunosuppressed individuals causing mucormycosis.

Mucormycosis (formerly zygomycosis) is an opportunistic fungal infection caused by bread mold fungi. These mold fungi include *Mucor, Rhizopus, Lichtheimia (*formerly *Absidia),* and *Cunninghamella*.

Predisposing factors: These include conditions causing neutropenia, use of corticosteroids, diabetes mellitus, iron overload, and breakdown of the skin barrier (e.g., in burns, surgical wounds, or trauma).

Pathogenesis: Mode of transmission: Mucormycotina are transmitted by airborne asexual spores. However, infection can occur through skin or ingestion.

Lesions: Inhaled spores usually produce infection in the nasal sinuses and the lungs.

MORPHOLOGY

Mucormycetes form nonseptate hyphae of variable width (ranging from 6 to 50 μm). They exhibit frequent right angle branching, distinct from *Aspergillus* hyphae.

Sites involved: The three main sites of invasion are: (i) the nasal sinuses, (ii) lungs, and (iii) gastrointestinal tract, depending on mode of transmission (i.e., inhalation or ingestion). In diabetic patients, the fungus may spread from nasal sinuses to the orbit and brain, giving rise to **rhinocerebral mucormycosis**.

Lung involvement with mucoromycotina may be secondary to rhinocerebral disease, or it may be primary in patients with severe immunodeficiency.

Dimorphic Fungi

The important dimorphic fungi include blastomyces, histoplasma, and coccidioidomyces.

T**hey grow as yeast in human tissue but as a hyaline mold when cultured in laboratory at room temperature.**

MYCETOMA

Q. Write a short note on Madura foot/mycetoma foot and its gross features.

Definition: Mycetoma is defined as a chronic suppurative infection involving a limb, shoulder or other tissues and is characterized by draining/discharging sinuses. The discharging grains (material) consists of colonies of fungi or bacteria.

Types: Mycetomas can be divided into two main types depending on the causative agent namely (i) **Eumycetoma** (fungal disease and comprises about 40% of cases) and (ii) **Actinomycetoma** (higher bacteria and comprises about 60% of cases).

Eumycetoma

- **Etiology:** It is a chronic granulomatous fungal disease caused by true fungi, *Madurella mycetomatis* or *Madurella grisea*.

- **Mode of infection**: The organisms are inoculated directly from soil into bare feet during agricultural work, from carrying of contaminated sacks on the shoulders, and into the hands from infected vegetation.

MORPHOLOGY

It involves skin and subcutaneous tissue. After several months of infection, the affected site, most commonly foot, manifests as an open area or break in the skin. The affected part is swollen and when foot is affected, it is termed mycetoma pedis (mycetoma of the foot) or 'Madura foot' **(Fig. 4.9).** This was initially named **Madura foot**, after its discovery in the region called Madura in India. The infection extends deeply into the subcutaneous tissues, along the fascia and eventually invade the bones. They drain serosanguinous fluid containing granules through sinus tracts. The size, color and degree of hardness of granules depend on the etiologic species and are characteristic of mycetoma. The surrounding tissue shows granulomatous reaction. The fungi stain positively with Gomori's methenamine silver (GMS) stain.

Actinomycosis

Q. Write short note on actinomycosis.

- It is a chronic suppurative disease caused by anaerobic bacteria, *Actinomycetes israelii.* It is not a fungus.
- The organisms are commensals in the oral cavity, gastrointestinal (GI) tract and vagina.
- **Mode of infection:** Infection is always endogenous in origin and not due to personal contact.
- Break in mucocutaneous continuity, diminished immunity due to some underlying disease favors the organism to invade, proliferate and disseminate.

MORPHOLOGY

Depending on the anatomic location of lesions, actinomycosis is divided into four types:

1. **Cervicofacial actinomycosis:**
   - It is the most common form (60%) and has best prognosis.
   - Infections gain through tonsils, carries teeth, periodontal diseases or trauma following extraction of tooth.



Fig. 4.9: Mycetoma (Madura foot) showing numerous sinuses discharging serosanguinous fluid.

- In the beginning, a firm swelling develops in the lower jaw (i.e., lumpy jaw). Later, the mass breaks down and form abscess and sinuses. Typically, the sinus discharges yellow sulfur granules. The infection may spread into the adjacent soft tissues and may destroy the bone.

2. **Thoracic actinomycosis:**
   - The infection of lung is as a result of aspiration of organism from the oral cavity or extension of infection from abdominal or hepatic lesions.
   - Initially, lung lesions resemble pneumonia but as the disease progresses it spreads to the whole lung, pleura, ribs and vertebrae.
3. **Abdominal actinomycosis:**
   - The common sites are appendix, cecum and liver.
   - The infection occurs as a result of swallowing of organism from oral cavity or as an extension from thoracic cavity.
4. **Pelvic actinomycosis:** It develops as a complication of intrauterine contraceptive devices (IUCDs).

Microscopy (Figs. 4.10A and B)

Following features are seen irrespective of the location of actinomycosis:

- **Granulomatous reaction with central suppuration:** There is formation of abscesses in the center of lesions and the periphery of the lesions show chronic inflammatory cells, giants cells and fibroblasts.
- The **central abscess** contains bacterial colony (Sulfur granule) characterized by radiating filaments (was called as ray fungus) surrounded by hyaline, eosinophilic, club-like ends which represent immunoglobulins.
  - **Special stains for bacteria:** The organisms are gram-positive filaments and nonacid-fast (except nocardia). They stain negatively with Gomori's methenamine silver (GMS) stain.

PARASITIC DISEASES

Protozoa

Protozoa are unicellular eukaryotic organisms. The protozoa are transmitted by insects or by the fecal-oral route.

MALARIA

CC: Define and describe the pathogenesis and pathology of malaria.

- Malaria, caused by the intracellular parasite *Plasmodium*.
- *Plasmodium falciparum* (causes severe cerebral malaria) and the four other malaria parasites that infect humans (*P. vivax, P. ovale, P. knowlesi,* and *P. malariae*) are all transmitted by female anopheles mosquitoes.
- **Incubation period:** It varies with the type of species:
  - *P. vivax*—14 days (ranges 8–17 days)
  - *P. falciparum*—12 days (ranges 9–14 days)
  - *P. malariae*—28 days (ranges 18–40 days)
  - *P. ovale*—17 days (ranges 16–18 days).

Life Cycle and Pathogenesis (Fig. 4.11)

The life cycles of the *Plasmodium* species are similar.

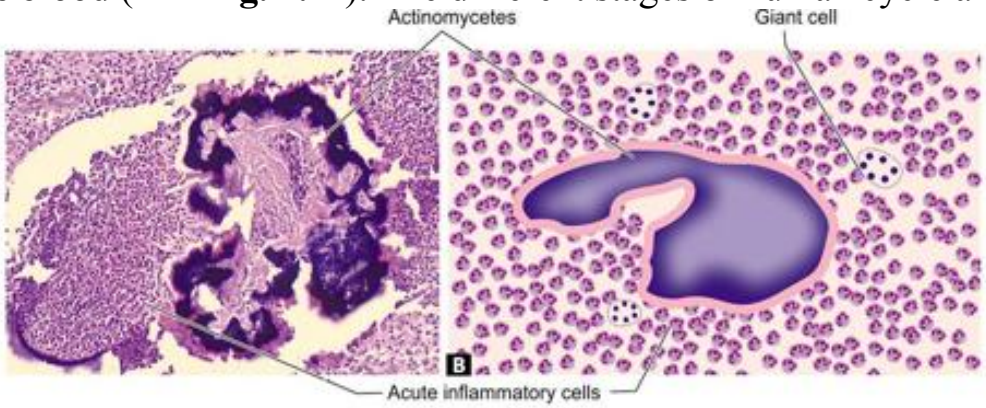
- ***P. vivax, P. ovale, P. knowlesi, and P. malariae:*** They produce **low levels of parasitemia**, mild anemia.
- ***P. falciparum* infection:** It **produces high levels of parasitemia** and may lead to severe anemia, cerebral symptoms, renal failure, pulmonary edema, and death.

The life cycle of *Plasmodium* species is simple because it involves only humans and mosquitoes. However, the development of the parasite is complex, because it passes through several morphologically distinct forms. Malarial parasites pass their life cycle in two different hosts namely— (1) human (intermediate host) and (2) female anopheles mosquito (definitive host). Notable feature of the life cycle is the alternation of sexual and asexual phases in the two hosts. The asexual phase, called schizogony,

occurs in the human. In the mosquito, first a sexual phase (gamogony) occurs; followed by another asexual reproductive phase (sporogony).

Human Cycle

It starts with the introduction of infectious stage of *Plasmodium*, the *sporozoite* by the bite of infected female anopheles mosquito. The mouthparts of male anopheles mosquito cannot penetrate human skin; hence, they feed solely on plant juices. The sporozite is slender, elongated, and measure about 10–55 mm in length and about 1 mm in diameter. The *sporozoite* is found in the salivary glands of mosquitoes. During mosquito bite, the mosquito takes a blood meal and sporozoites are released into the human's blood (1 in **Fig. 4.11**). The different stages of human cycle are:



Figs. 4.10A and B: Microscopy of actinomycosis showing central bacterial colony surrounded inflammatory cells. (A) Hematoxylin and eosin; (B) Diagrammatic.

A

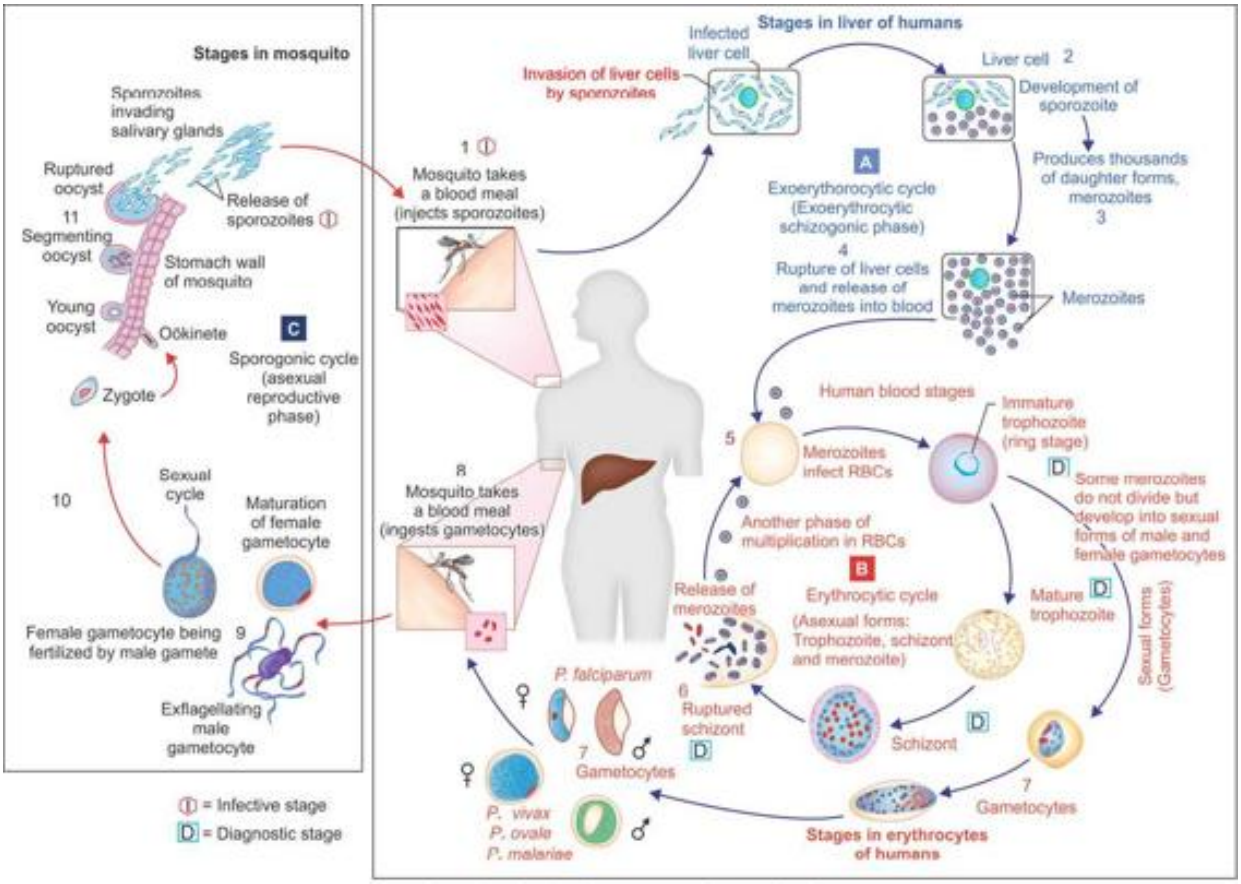


Fig. 4.11: Life cycle of malarial parasites (*refer* text for description).

Exoerythrocytic Stage (Pre-erythrocytic Schizogony)

This stage occurs in liver. Within minutes of entry of sporozites (infectious stage) into the human blood, they attach to and invade liver cells (2 in **Fig. 4.11**). Within liver cells, malaria parasites multiply, and the spindle-shaped sporozoites become rounded and transform into trophozoites (feeding stage of the parasite). These **trophozoite** undergoes several nuclear divisions **(schizogony)** and transforms into pre-erythrocytic schizont. **Pre-erythrocytic schizont** contains several merozoites (3 in **Fig. 4.11**). About 30,000 *merozoites* (asexual, haploid forms) are released when each infected liver cells ruptures (4 in **Fig. 4.11**).

- During *P. falciparum* infection, rupture of liver cells usually occurs within 8 to 12 weeks.
- In contrast, *P. vivax* and *P. ovale* releases weeks to months after initial infection.

The infection of the liver and development of merozoites is referred to as the exoerythrocytic stage. This stage is asymptomatic. Only few liver cells/hepatocytes are infected by *Plasmodium*. Hence, liver damage does not occur in malaria. Some sporozoites of *P. vivax* and *P. ovale* do not develop further and may remain in liver as hypnozoites. However, they can cause relapse of malaria after many years.

Erythrocytic Stage (Fig. 4.11)

Once released from the liver, *Plasmodium* merozoites bind to the surface of red cells (5 in **Fig. 4.11**) and invade red blood cell (RBC) by penetration of the membrane. Within the red cells (erythrocytic stage) the parasites grow and hydrolyze hemoglobin through its enzymes.

Asexual forms (6 in **Fig. 4.11**)**:** In the red blood cells, parasite passes through the stages of asexual forms namely—trophozoite, schizont and merozoite. These asexual forms of parasite can be demonstrated in the thick blood smears.

- *Trophozoite* is the first stage of the parasite in the red cell and is characterized by the presence of a single chromatin mass. First immature trophozoites (ring forms) are formed followed by mature trophozoites.
  - **Ring form:** Early trophozoite form is called as ring form **(Fig. 4.11)**. It appear as a signet ring containing a central vacuole and peripheral thin rim of cytoplasm and a nucleus. Ring forms are the first asexual form that can be demonstrated in the peripheral blood. Ring form occupies one-third of RBC except in *P. falciparum*, where it occupies one-sixth of RBC. *Plasmodium* feeds on hemoglobin. The undigested product of hemoglobin metabolism such as hematin, excess protein and iron porphyrin combine to form **malarial pigment (hemozoin pigment)**. The appearance of malarial pigment varies. Mostly it is brown black and numerous (except in *P. vivax* it is yellowish-brown and in *P. falciparum*, it is few in number).
  - **Late trophozoite:** Ring form enlarges and appear more irregular due to amoeboid movement and transforms into late trophozoite or amoeboid form.
- *Schizont:* It is the next stage. Late trophozoites become compact, vacuoles disappear, pigments scatter throughout cytoplasm and nucleus becomes

larger and lies at the periphery. This form is called **schizont**. It shows multiple chromatin masses, each of which develops into a merozoite.

- *Merozoites*: Schizont undergoes multiple nuclear divisions (schizogony or merogony) and produces 6–30 daughter merozoites arranged in the form of rosette. Lysis of the red cell containing merozoites occurs and the new merozoites infect additional red cells. The characteristic clinical features of malaria such as paroxysmal fever, chills, and rigors develop during the release of these merozoites into the blood. The periodicity of such paroxysms (every 48–72 hours) varies with the species of the malarial parasite. Ruptured RBCs release the daughter merozoites, malarial pigments and toxins into the circulation. This responsible for malarial paroxysm of fever at the end of each erythrocytic cycle.

Sexual forms (7 in **Fig. 4.11**)**:** Most malaria parasites within the red cells develop into merozoites. However, some parasites after entering into RBCs, under specific conditions instead of developing into trophozoites transform into sexual forms called gametocytes. The gametocytic development occurs in the blood vessels of organs such as spleen and bone marrow and only the mature gametocytes appear in the peripheral blood. The gametocytes are round in shape (except in *P. falciparum* in which they are crescent or banana shaped). They are of two types namely (i) male gametocyte (or microgametocyte) and (ii) female gametocyte (or macrogametocyte). Microgametocytes are smaller in size, lesser in number, their cytoplasm stains pale blue, and nucleus is larger, stains red and diffuse. In contrast, macrogametocytes are larger, numerous, their cytoplasm stains deep blue, nucleus is small, red and compact. Gametocytes neither cause any clinical illness nor they divide. Patient harboring gametocytes are considered as carriers or reservoirs of infection. They play an important role in the transmission of the disease. These gametocytes infect the mosquito when mosquito bites the infected human and takes its blood meal.

Malaria is more severe when caused by *Plasmodium falciparum* than the other *Plasmodium* species.

Mosquito Cycle (8 to 12 in Fig. 4.12)

A female anopheles mosquito during its blood meal from (8 in **Fig. 4.11**) an infected patient ingests both sexual and asexual forms of parasite, but it is only the mature sexual forms (gametocytes) that are capable of development

(hence considered as infective form of the parasite to mosquito). The asexual forms get digested. Nucleus of the male gametocytes divides into eight flagellated actively motile bodies called as **microgametes**. Microgametes protrude out as thread-like filaments, lash out for some time and then, break free. This process is called as **exflagellation** (9 in **Fig. 4.11**). Female gametocytes do not divide and do not undergo exflagellation but it matures to form one **macrogamete or female** gamete. The female gametocyte gets fertilized by male microgamete and forms nonmotile round **zygote** (10 in **Fig. 4.11**). The zygote transforms into vermicular motile elongated form with an apical complex (the **ookinete** stage). Till this stage, the development takes place in the midgut of the mosquito. The ookinete penetrates into the stomach wall of the mosquito and lies just beneath the basement membrane. It becomes rounded and form **oocyst** (11 in **Fig. 4.11**). Oocysts undergo sporogony (meiosis) and produce thousands of spindle- shaped **sporozoites**. The mature oocyst rupture and release the sporozoites. These **sporozoites migrate to salivary gland** (12 in **Fig. 4.11**). Mosquito is considered as infective when the sporozoites are present in salivary gland. Once infected, mosquito remains infective throughout its life.

Mixed infection: Different species of *Plasmodium* can infect the same mosquito. This can transmit mixed infection to human beings.

MORPHOLOGY

Q. Write a short note on morphology of Plasmodium falciparum in a peripheral blood smear.

- **Peripheral blood smear:** Diagnosis of malaria infection can be made by examination of a Giemsa/Leishman-stained peripheral blood smear **(Fig. 4.12)**. It **shows the asexual stages of the parasite within infected red cells.**
- **Spleen:** *Plasmodium falciparum* infection produces **splenomegaly**. In chronic infections, the spleen becomes fibrotic and brittle, with a thick capsule. The parenchyma appears gray or black because of the granular, brown-black, faintly birefringent hemozoin pigment.

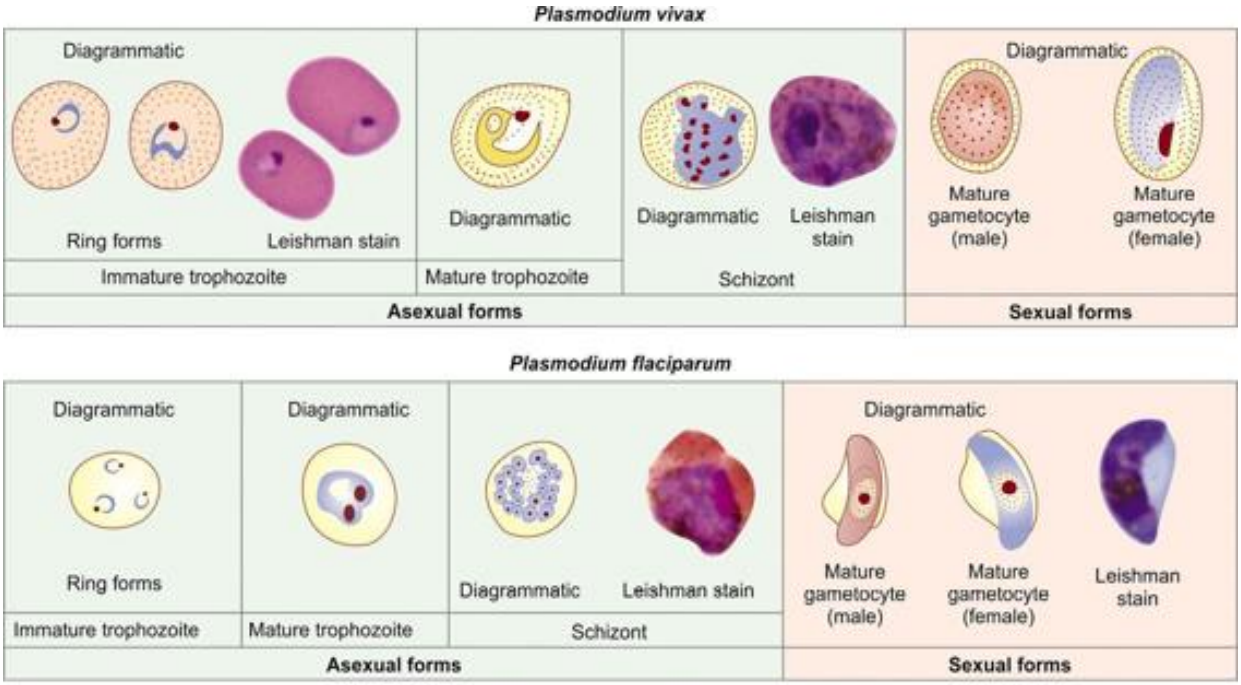


Fig. 4.12: Appearance of malarial parasite *(Plasmodium vivax and falciparum)* in human.

- **Liver:** It is enlarged and pigmented. Kupffer cells contain malarial pigment, parasites, and cellular debris.
- **Brain:** In **cerebral malaria** caused by *P. falciparum*, blood vessels of the brain show plugging of parasitized red cells. Around the vessels there are ring hemorrhages and small focal inflammatory reactions called as **malarial** or **Dürck granulomata**.

Tapeworms (Cestode Parasites)

CC: Define and describe the pathogenesis and pathology of cysticercosis.

Cysticercosis

- Cysticercosis is infection caused by larvae that develop after ingestion of eggs of tapeworm namely—***Taenia solium*** (pork tapeworm/armed tape worm). **Cysticercus is the resting stage of larva in the intermediate host**.
- *Taenia solium* has a complex life cycle and requires two mammalian hosts: (1) a definitive host (in which the worm reaches sexual maturity—humans) and (2) an intermediate host (in which the worm does not reach sexual maturity—humans and pigs).

Mode of infection

T. solium can be transmitted to humans in two ways with distinct outcomes.

1. Ingestion of larval cysts called cysticerci (present in uncooked pork) or
2. Ingestion of eggs present in contaminated food/water.

Pathogenesis (Fig. 4.13)

- **Ingestion of cysticerci** (larval cysts)**:** When larval cysts are ingested in undercooked pork by human beings, they attach to the intestinal wall and develop into mature adult tapeworms. They can grow to many meters in length. The life cycle of parasite is completed with this mode of infection and cysticercosis does not develop. Adult tapeworms shed eggs in the feces of human beings.
- **Ingestion of eggs:** When intermediate hosts (pigs or humans) ingest eggs in food or water contaminated with human feces, the eggs develop into larvae, penetrate the gut wall and enter the blood circulation. They spread to various sites like muscle, brain and form **cystic larvae termed cysticercus cellulosae**.

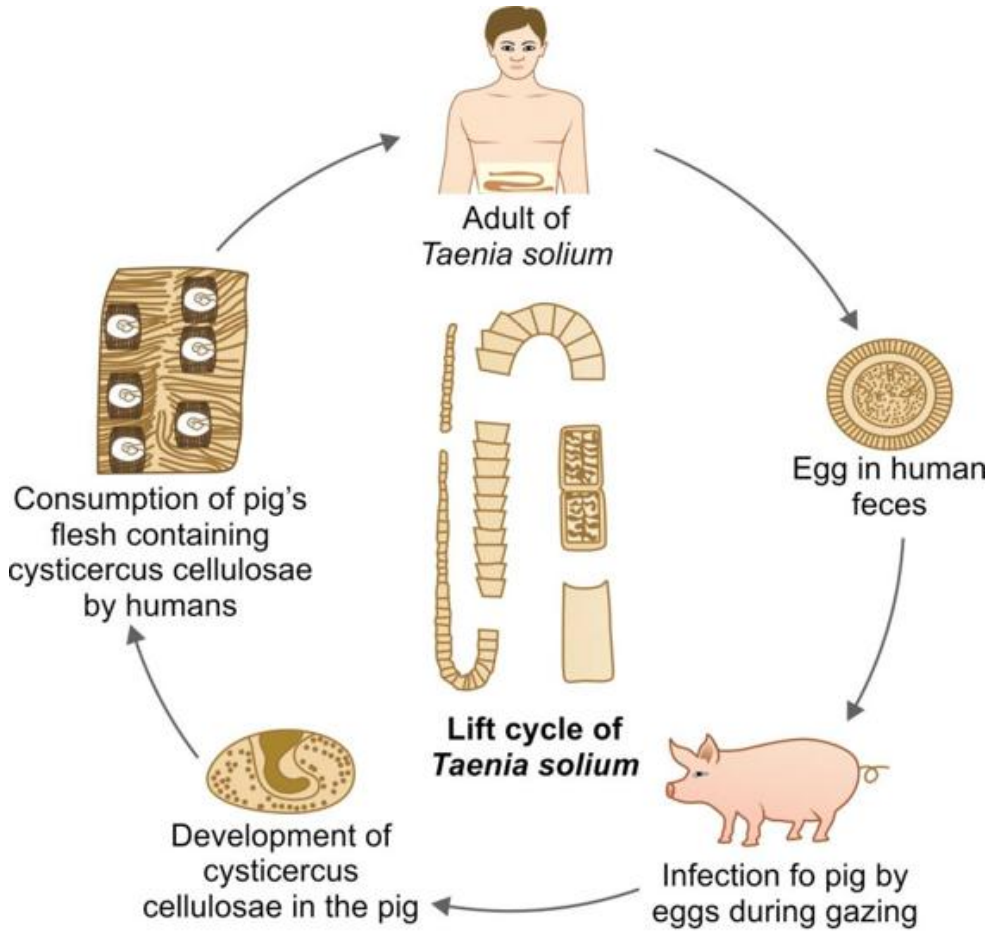


Fig. 4.13: Life cycle of *Taenia solium*.

MORPHOLOGY

- **Organs affected:** Cysticerci may be found in any organ, but the more common locations include the **brain, skeletal muscles, skin, and heart**.
- **Number:** The cysticercus can be single or multiple.
- **Appearance:** Cysticercus consists of round to oval white to opalescent cyst, 0.5–1 cm (often grape-sized) in diameter containing little, clear cyst fluid. It contains an invaginated scolex (which has four suckers) and circle of birefringent hooks on its wall. They remain viable for long time. Once the embryo dies, it induces granulomatous reaction with eosinophils and may later show scarring and calcification, which may be visible by radiography.

Clinical features

- **Adult tape worm** in the intestine can produce abdominal pain, diarrhea and loss of appetite.

- **Cysticercus cellulosae**
  - **Neurocysticercosis:** The most serious manifestations of cysticercosis are due to involvement of the brain (neurocysticercosis). Cerebral symptoms depend on the location of the cysts. It can produce epilepsy (convulsions), hydrocephalus, increased intracranial pressure, blurred vision and other neurologic disturbances.

Echinococcosis/Hydatid Disease

- Hydatid disease is a parasitic infestation by cestode (tapeworms) ***Echinococcus granulosus***.
- **Life cycle:** ***Echinococcus granulosus*** requires two mammalian hosts to complete its life cycle.
  - **Definitive host** (in which the worm reaches sexual maturity)**:** Dog, jackal, and fox.
  - **Intermediate host** (in which the worm does not reach sexual maturity)**:** Sheep, pig, goat, cattle, man (dead end host).

Pathogenesis (Fig. 4.14)

- Adult *Echinococcus granulosus* worm is found in small intestines of dogs and other canines. The dog is infected by eating the viscera of sheep containing hydatid cysts.
- The adult worm in small intestines of dogs, discharges eggs in feces which are ingested by man, sheep, pigs and other mammals. Man acquires infection by:
  - Handling dogs.
  - Eating food/vegetables contaminated with eggs shed by dogs or foxes.
- The eggs hatch in the human duodenum and are carried to the liver by portal venous system and other sites through systemic circulation. Those which are trapped in the hepatic sinusoids develop into hydatid cyst.

MORPHOLOGY

- **Sites:** About 65% human *E. granulosus* cysts are found in the liver and 5 to 15% in the lung, and the remaining 10 to 20% in bones and brain or other organs.

- **Hydatid cysts** begin at microscopic levels and grow slowly. They may eventually attain a size over 10 cm in diameter in about 5 years. *E. granulosus* usually produces unilocular hydatid cyst while *E. multilocularis* produces multilocular hydatid disease in the liver.
- **Microscopy:** The cyst wall consists of 3 distinguishable zones enclosing an opalescent fluid:
  1. **Pericyst (outer, capsular layer):** It shows inflammatory reaction and consists of fibroblasts, giant cells, and mononuclear and eosinophils.
  2. **Ectocyst(intermediate opaque, non-nucleated layer):** It is distinctive and has innumerable delicate laminations.
  3. **Endocyst** (inner, nucleated, germinative layer with daughter cysts and scolices projecting into the lumen)**:** Daughter cysts can develop within the large mother cyst. They appear first as minute projections of the germinative layer that develop central vesicles and thus form tiny brood capsules. Degenerating scolices of the worm produce a fine, grain-like (sand-like) sediment within the hydatid fluid (hydatid sand).

Clinical Features

- Liver: Produces pressure effects due to cyst.
- Cerebral: Epilepsy

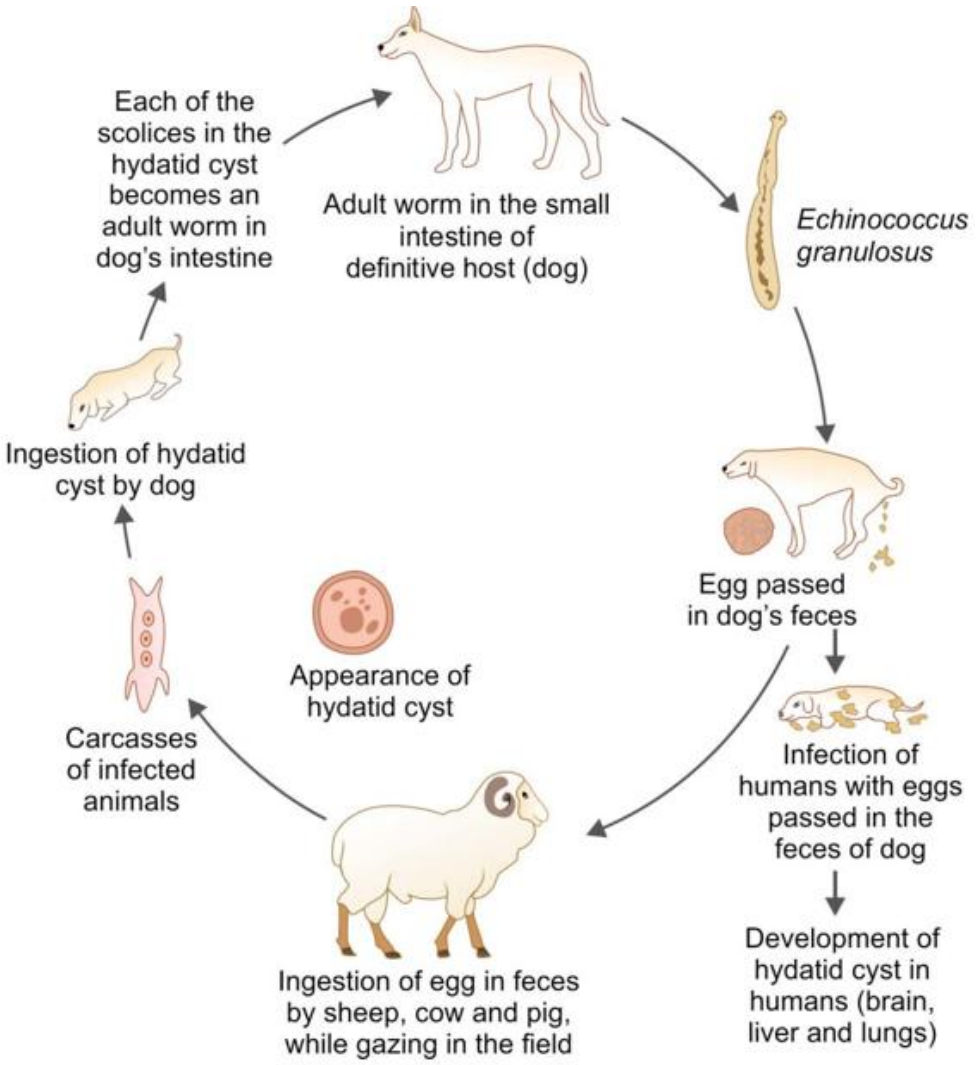


Fig. 4.14: Life cycle of *Echinococcus granulosus.*

- Renal: Hematuria
- Rupture of cyst: The liberation of antigenic proteins in the hydatid fluid into the circulation produces eosinophilia and may even cause anaphylactic reactions.

Laboratory diagnosis: Casoni test.

Filariasis

Filariasis is transmitted by mosquitoes and is caused by closely related nematodes, *Wuchereria bancrofti* and *Brugia* species (*B. malayi*).

Diseases

Filariasis causes a spectrum of diseases.

1. Asymptomatic
2. Recurrent lymphadenitis
3. Chronic lymphadenitis with elephantiasis
4. Tropical pulmonary eosinophilia.

Pathogenesis

- Definitive host is man and intermediate host is mosquito.
- During the mosquito bite to humans, the infective larvae are released by mosquitoes into the tissues. They develop within lymphatic channels into adult males and females which mate and release microfilariae into the bloodstream.
- During the mosquito bite of the infected persons, the mosquitoes can take up the microfilariae. These microfilariae undergo further development in the mosquito and they become infective and transmit the disease to humans.

MORPHOLOGY

- In chronic filariasis, the adult parasites lodge in the lymphatics and stimulate fibrosis and the formation of granulomas around the adult parasites. Thus, chronic filariasis is leads to **persistent lymphedema** of the extremities (*refer* **Fig. 5.6**), scrotum, penis, or vulva.
- In severe and long-standing swollen leg may develop tough subcutaneous fibrosis and epithelial hyperkeratosis, termed **elephantiasis (Fig. 4.15A).** Adult filarial worms (live, dead, or calcified) are observed in the draining lymphatics or nodes. Microfilaria **(Fig. 4.15B)** is the embryo of *Wuchereria bancrofti* and can be seen in blood and body fluids.
- Filariasis can also cause hydrocele.

OTHER INFECTIONS

Rhinosporidiosis

Q. Write short note on rhinosporidiosis.

Rhinosporidiosis is an inflammatory disease caused by *Rhinosporidium seeberi*. Usually, it occurs in nasopharynx as polyp but may also be observed

in larynx and conjunctiva. It is endemic in India and Sri Lanka and sporadic in other parts of the world.

Microscopy (Figs. 4.16A and B)

- Structure of nasal mucosa.
- Many spherical cysts called as sporangia measuring up to 200 nm in diameter having thick-wall (chitinous wall) are seen. Each of these cysts (i.e., sporangium) contain numerous small basophilic round spores of the size of erythrocytes. On rupture of a sporangium, the spores may be discharged into the submucosa or on to the surface of the mucosa.
- Chronic inflammatory (plasma cells, lymphocytes, histiocytes, neutrophils) infiltrate in the intervening and subepithelial layer.

Molluscum Contagiosum

Q. Write short note on molluscum contagiosum.

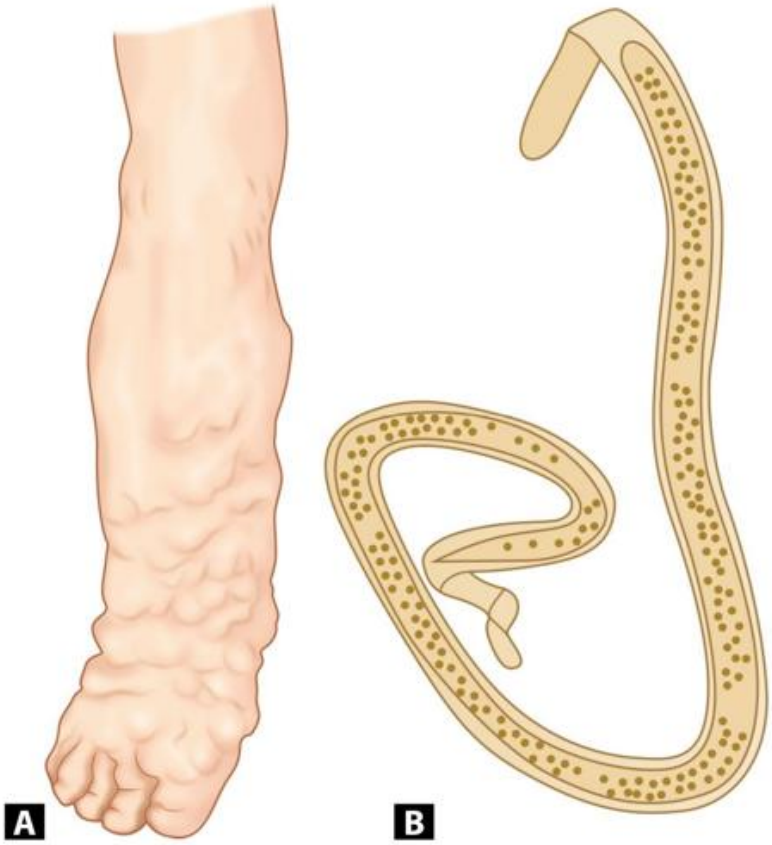
Molluscum contagiosum is a common, self-limited, highly contagious viral disease of the skin caused by a double-stranded DNA poxvirus.

Mode of infection: Usually spread by **direct contact**. Common among children and young adults.

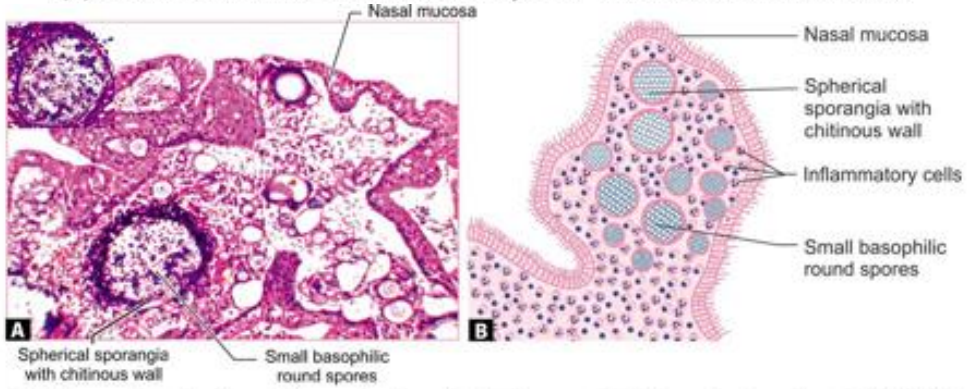
MORPHOLOGY

Lesions

Infection leads to multiple lesions on the skin and mucous membranes, with a predilection for face, trunk and anogenital areas. Individual lesions are small, firm, smooth, often pruritic, pink to skin-colored, **dome-shaped papules**, generally ranging in diameter from 2 mm to 4 mm. Fully developed lesions have a characteristic **central umbilication** and in a fully-developed lesion,



Figs. 4.15A and B: (A) Elephantiasis of leg; (B) Diagrammatic apperance of microfilaria (embryo of *Wuchereria bancrofti*).



Figs. 4.16A and B: Rhinosporidiosis of nasopharynx showing spherical sporangia (A) Hematoxylin and eosin (H & E); (B) Diagrammatic.

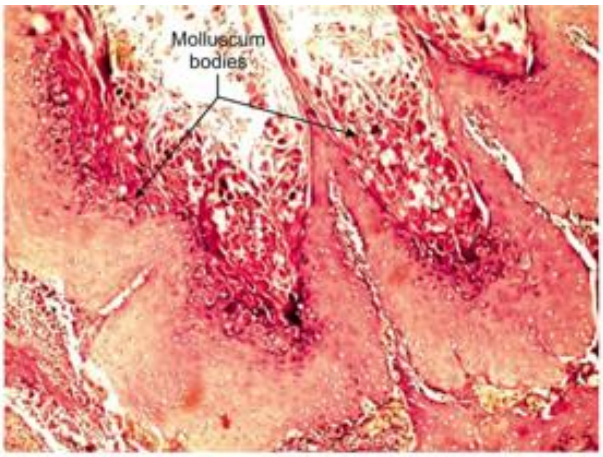


Fig. 4.17: Molluscum contagiosum. Epithelial cells of epidermis show ellipsoid cytoplasmic inclusions.

small amount of cheesy (curd/paste-like) keratinous material can be expressed on pressing from the central umbilication. This material if smeared onto a glass slide and stained with Giemsa may shows diagnostic molluscum bodies.

Microscopy (Fig. 4.17)

The microscopic picture is characteristic.

- **Infected epithelial cells:** Typical lesion consists of a sharply circumscribed (delimited) lobulated, cup-shaped mass of proliferating infected epithelial cells of epidermis growing down into the dermis.

- **Molluscum body:** As the infected epithelial cells differentiate within the mass, their cytoplasm is gradually filled by viral inclusion. These inclusions enlarge the epithelial cells and displace the nucleus. The viral inclusions are diagnostically specific structures (which appear ellipsoid) and are termed as **molluscum** bodies**.** The viral inclusions are found in cells of the stratum granulosum and the stratum corneum. Under hematoxylin and eosin stain, these inclusions appear **faintly granular eosinophilic in the blue-purple stratum granulosum** and **pale blue in the red stratum corneum**. These molluscum bodies contain numerous viral particles. Most lesions spontaneously regress.

CHAPTER 5

Hemodynamic Disorders, Thromboembolism, and Shock

DISORDERS OF PERFUSION

CC: Define and describe hyperemia, congestion, and hemorrhage.

Hyperemia

Q. Write short note on hyperemia.

Hyperemia and congestion are characterized by **locally increased blood volume.**

Definition: Hyperemia is an **active process** in which **arteriolar dilation** leads to increased **blood flow to a tissue/organ**.

Causes

- **Physiological:** Response to **increased functional demand (**e.g., heart and skeletal muscle during exercise).
- **Pathological:** Seen in **inflammation** and is responsible for the two cardinal signs of inflammation namely **heat** (calor) and **redness (**rubor/**erythema)**.

Congestion

Q. Write short note on chronic passive congestion.

Definition: Congestion is a **passive process** resulting from **reduced venous outflow of blood** from a tissue/organ.

Types and Causes

1. **Systemic** (e.g., congestive heart failure, congestion involves liver, spleen, and kidneys).
2. **Local:** Examples include:
   - Congestion of leg veins due to deep venous thrombosis → edema of the lower extremity.
   - Local congestion at various sites due to compression of veins, e.g., tight bandage, plasters, tumors, pregnancy, hernia, etc.

Onset

1. **Acute congestion:** It develops during shock, or sudden right-sided heart failure. It may occur in lung and liver.
2. **Chronic passive congestion:** It usually produces edema in the organ/tissue in which the venous outflow is reduced.
   - **Appearance:** Congested tissues have a **dusky reddish-blue color (cyanosis)** due to stasis of red blood cells (RBCs) and the accumulation of deoxygenated hemoglobin.

Chronic Venous Congestion of Lung

Q. Write short note on CVC lung/brown induration of lung.

Causes

- **Mitral stenosis** (e.g., rheumatic mitral stenosis).
- **Left-sided heart failure:** It develops secondary to coronary artery disease or hypertension.

Mechanism of of chronic venous congestion (CVC) of lung is presented in **Flowchart 5.1**.

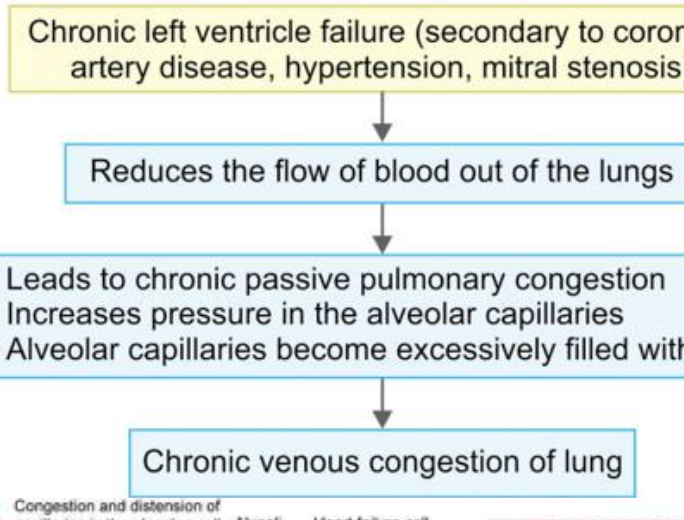
Consequences

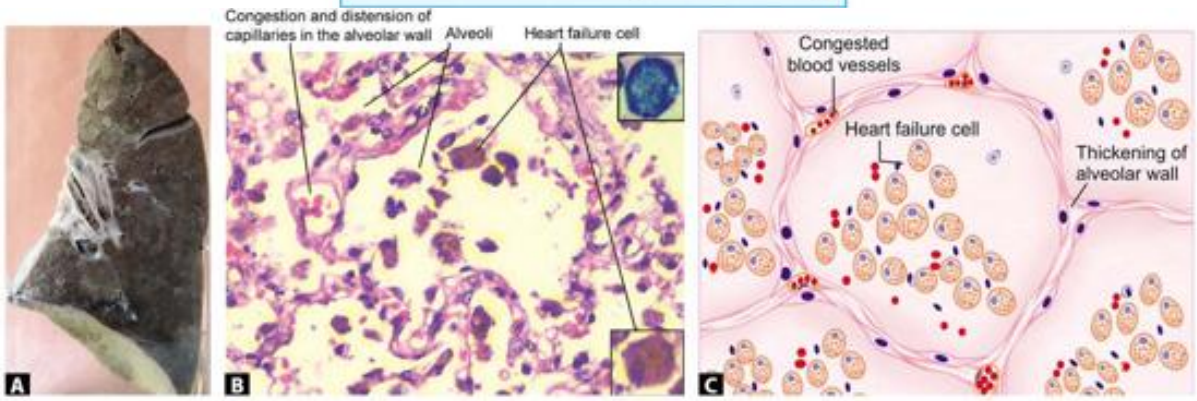
Q. Write short note on heart failure cells and the special stain used for its demonstration.

Four major consequences are:

1. **Microhemorrhages:** The wall of alveolar capillaries may rupture → minute hemorrhages into the alveolar space → release RBCs → hemoglobin breakdown → **liberation of iron containing hemosiderin pigment** (brown color) → alveolar macrophages phagocytose hemosiderin. **Hemosiderin-laden macrophages** are known as **heart failure cells**.
2. **Pulmonary edema:** It is due to forced movement of fluid from congested vessels into the alveolar spaces.
3. **Fibrosis:** It develops due to increased fibrous tissue in the interstitium of lung.
4. **Pulmonary hypertension**: It is due to transmission of pressure from the alveolar capillaries to the pulmonary arterial system.

Flowchart 5.1: Mechanism of chronic venous congestion of lung.





Figs. 5.1A to C: Chronic venous congestion lung. (A) Brown induration of lung; (B) Hematoxylin and eosin and (C) Diagrammatic section from lung shows thickened alveolar walls and hemosiderin-laden macrophages (heart failure cells) in the alveolar lumen Inset of B, lower right-hemosiderin-laden macrophage and upper left—Perl's stain imparting blue-black color to hemosiderin in the cytoplasm.

MORPHOLOGY

Gross

- Lung is **heavy**.
- Cut section (c/s) **rusty brown color** (due to hemosiderin pigment), **firm in consistency** (due to fibrosis) → known as **brown induration of lung (Fig. 5.1A).**

Microscopy (Figs. 5.1B and C)

- **Distension** and **congestion of capillaries** in the alveolar septa of lung.
- **Thickened alveolar septa** due to increase in the fibrous connective tissue → responsible for the firm consistency of the lung.
- **Heart failure cells** are seen in the alveoli.

Chronic Venous Congestion of Liver

Q. Write short note on causes, gross and microscopic features of chronic venous congestion of liver/CVC liver/nutmeg liver.

Causes

The hepatic veins empty into the inferior vena cava just inferior to the heart, so the liver is vulnerable to acute or chronic passive congestion.

- **Right-sided heart failure** is the most common cause.
- **Rare:** Constrictive pericarditis, tricuspid stenosis and obstruction of inferior vena cava and hepatic vein.

Mechanism of chronic venous congestion of liver is presented in **Flowchart 5.2**.

MORPHOLOGY

Gross

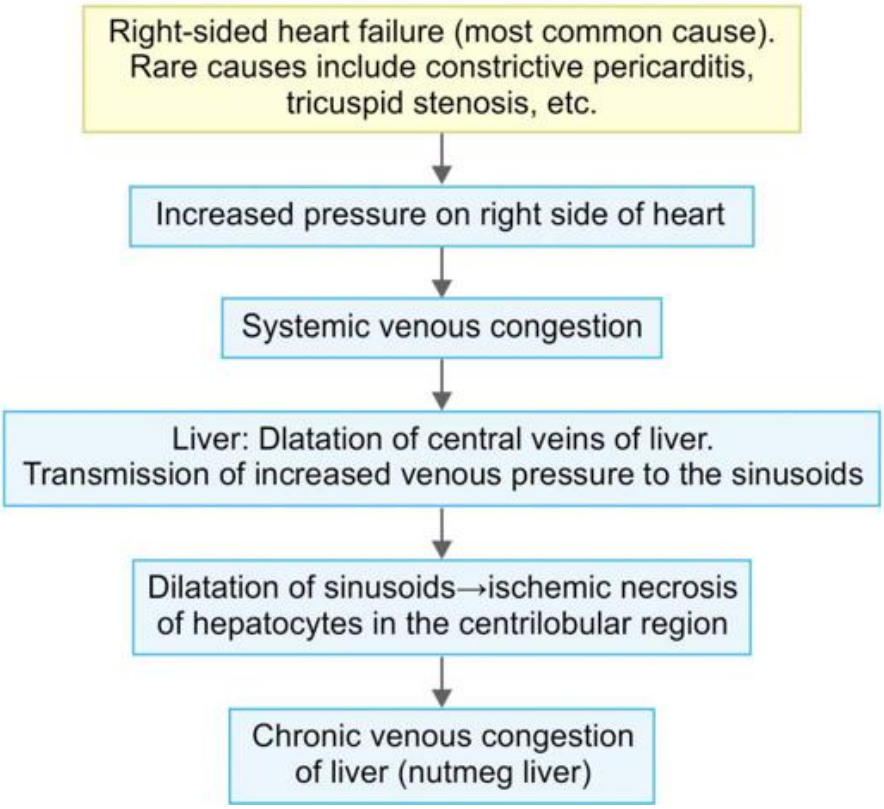
- **Liver increases in size** and weight and the **capsule appears tense.**
- **Cut section** shows **alternate (**combination of) **dark and light areas (Fig. 5.2A)** and resembles cross-section of a nutmeg (**nutmeg liver**).
  - **Congested centrilobular regions** (with hemorrhage and necrosis) appear **dark red-brown**. Congestion is most prominent around terminal hepatic venule (central veins) within hepatic lobules.

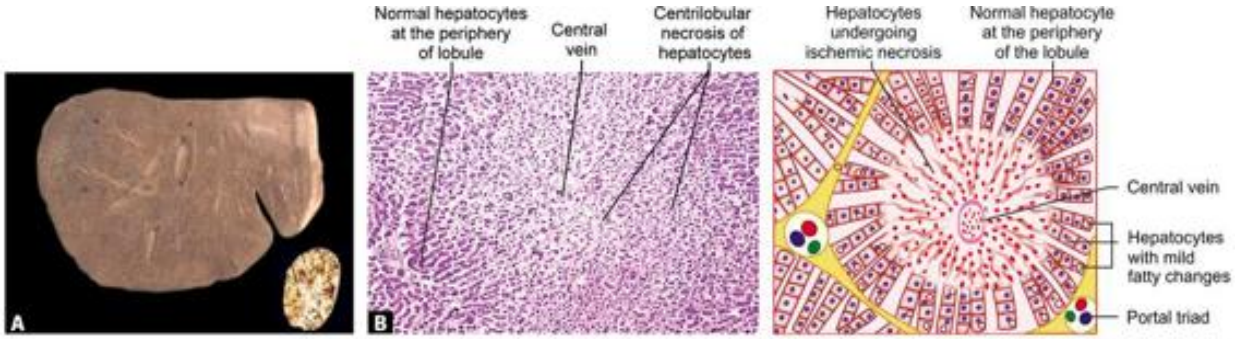
- **Periportal** (better oxygenated) **region** of the lobules appear **pale** and may show fatty change.

Microscopy (Fig. 5.2B)

- **Centrilobular region**:
  - **Congestion and hemorrhage** in the central veins (terminal hepatic venule) and adjacent sinusoids.
  - The **severe central hypoxia may produce centrilobular hepatocyte necrosis**.
  - **Thickening of central veins and fibrosis** in prolonged venous congestion.
  - **Cardiac sclerosis/cardiac cirrhosis** may occur with sustained chronic venous congestion (e.g., due to constrictive pericarditis or tricuspid stenosis).
- **Periportal region:** It shows **fatty change** in hepatocytes.

Flowchart 5.2: Mechanism of chronic venous congestion of liver.





Figs. 5.2A and B: Chronic venous congestion of liver. Liver shows alternate dark and light area and resembles the cut surface of a nutmeg (inset) (A); Microscopy shows centrilobular necrosis with degenerating hepatocytes surrounded by apparently normal hepatic parenchyma in the periportal region. (A) Photomicrograph; (B) Diagrammatic.

Congestive Splenomegaly (CVC Spleen)

Q. Write short note on CVC spleen/congestive splenomegaly.

Congestion and enlargement of spleen is called as congestive splenomegaly.

Causes

Chronic obstruction to the outflow of venous blood from spleen leads to higher pressure in the splenic vein.

- **Intrahepatic obstruction to blood flow: Cirrhosis of the liver** is the main cause (e.g., alcoholic cirrhosis, pigment cirrhosis).
- **Extrahepatic disorders:**
  - Systemic or central venous congestion (e.g., tricuspid or pulmonic valvular disease, chronic cor pulmonale, right heart failure or following left-sided heart failure). Splenomegaly is only moderate and rarely exceeds 500 g in weight.
  - Obstruction of the extrahepatic portal vein or splenic vein: Due to spontaneous portal vein thrombosis, which is usually caused by intrahepatic obstructive disease, or inflammation of the portal vein (pylephlebitis). Thrombosis of the splenic vein can also develop by infiltrating tumors arising in neighboring viscera, such as carcinomas of the stomach or pancreas.

MORPHOLOGY

Gross

Q. Write short note on Gamna-Gandy bodies.

- Spleen is **enlarged, firm and tense (*refer* Fig. 12.37)**. In long-standing chronic splenic congestion, spleen is markedly enlarged (1000–5000 g). **Capsule is thickened**.
- Cut section oozes dark blood.
- Enlarged spleen **may show excessive functional activity** termed as **hypersplenism** → leads to hematologic abnormalities (e.g., thrombocytopenia pancytopenia).
- CVC spleen: Hypersplenism.

Microscopy (Figs. 5.3A to C)

- **Red pulp**
  - **Dilatation and congestion** in the early stages.
  - Hemorrhage and fibrosis in later stages.
  - **Capillarization of sinusoids** may occur, in which **s**inusoids get converted into capillaries.
- **Thickened fibrous capsule and trabeculae**.
- May show **Gamna-Gandy bodies (Fig. 5.3C),** which consist of **iron-containing, fibrotic, and calcified foci of old hemorrhage**.**Slowing of blood flow** from the cords to the sinusoids → prolongs the exposure of the blood cells to macrophages in the spleen → leads to **excessive destruction of blood cells (hypersplenism)**.

Hyperemia: Active process whereas congestion is a passive process. Both are characterized by locally increased blood volume.

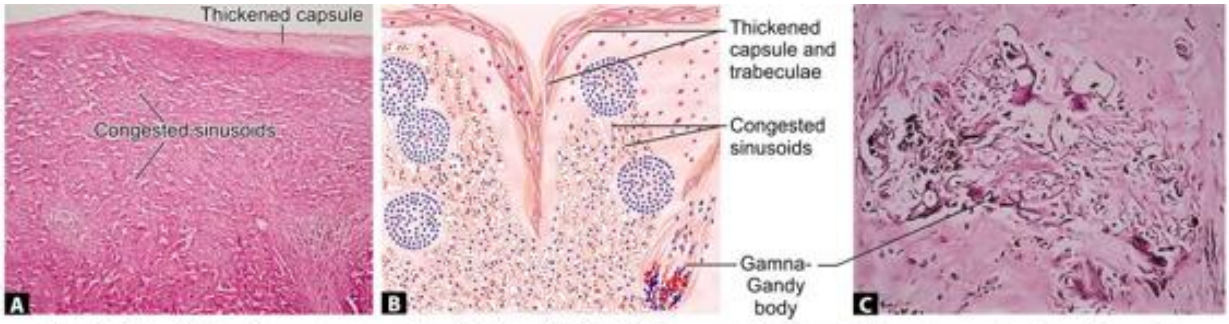
Heart failure cells:
1. Hemosiderin-laden macrophages. Hemosiderin pigment in these cells stain blue with Prussian blue stain (Perl's stain).
2. Found in lung affected by CVC lung and not in the heart.

Hemosiderin-laden macrophages are known as heart failure cells. The term heart failure does not mean that these are seen in heart failure.

CVC liver: Nutmeg liver.

CVC liver if sustained for long time: Cardiac sclerosis/cardiac cirrhosis develops.

Gamna-Gandy bodies: Iron-containing, fibrotic, and calcified foci of old hemorrhage. They contain:
- Hemosiderin (Perl's stain positive)
- Calcium (Von Kossa stain positive)



Figs. 5.3A to C: Chronic venous congestion of spleen (A) H&E; (B) diagrammatic. Shows thickened capsule, dilated sinusoids; (C) Gamna-Gandy body.

HEMORRHAGE

Definition: Hemorrhage (bleeding) is defined as the escape or extravasation of blood from vessels.

Etiology

The various causes of hemorrhage are:

1. **Trauma to the vessel wall:** The most common and obvious cause is trauma that **damages the blood vessels,** e.g., penetrating wound in the heart or great vessels, during labor, etc.
2. **Vascular diseases:**
   a. **Rupture of aneurysm:** Severe atherosclerosis may weaken the wall of the aorta (mainly abdominal aorta) and produce permanent abnormal dilatation of vessel called as aneurysm. These aneurysms may rupture and bleed into the retroperitoneal space. Aneurysm may congenital due to weak cerebral artery (berry aneurysm) and may rupture and cause subarachnoid hemorrhage.
   b. **Inflammatory lesions of the vessel wall:** Infections (e.g., blood vessels traversing a tuberculous cavity in pulmonary tuberculosis of the lung) bleeding from chronic peptic ulcer, typhoid ulcers, polyarteritis nodosa, septicemia.
3. **Neoplastic invasion of vessel:** Invasive neoplasms may erode blood vessels and result in hemorrhage (e.g., hemorrhage following vascular invasion in carcinoma of the tongue).

4. **Hemorrhagic (bleeding) diatheses:** The risk of hemorrhage is these clinical disorders/conditions is increased and usually occur spontaneously or follow insignificant injury. There is **defective clot formation.** They have diverse causes, including inherited or acquired defects in vessel walls, platelets, or coagulation factors. Examples include purpura, scurvy, acute leukemias. Scurvy is associated with capillary fragility and bleeding, due to the defective supporting connective tissue structures.
5. **Raised pressure within the vessels:** It may occur in systemic hypertension causing bleeding (e.g., cerebral and retinal hemorrhage), or high pressure in the veins (e.g., varicose veins legs or esophagus or pulmonary capillaries in chronic venous congestion of lung).

Manifestations

The bleeding may occur **externally** (exterior of the body), **or internally.** Internally blood may accumulate within a tissue (e.g., hematoma) or into the serous cavities/body spaces **(Box 5.1)** or into a hollow viscus. Hemorrhage may be manifested by different appearances and may have various clinical consequences. Terminology used in bleeding disorders is discussed on page 369.

BOX 5.1: Definition of hemorrhage into various body cavities.

- **Hemothorax:** Hemorrhage into the pleural cavity
- **Hemopericardium:** Hemorrhage into the pericardial space
- **Hemoperitoneum:** Bleeding into the peritoneal cavity
- **Hemarthrosis:** Bleeding into a joint space

Clinical Significance

The blood loss may be acute (large and sudden), or chronic (small repeated blood loss may occur over a period of time).

Effects

The effect of hemorrhage ranges from trivial (e.g., bruise) to fatal (e.g., a massive retroperitoneal hematoma resulting from rupture of a dissecting aortic aneurysm). The effects of hemorrhage depend upon (i) the amount

(volume) of blood loss, (ii) the speed/rate of blood loss and (iii) the site of hemorrhage.

- **Amount and the speed of hemorrhage**
  - Acute hemorrhage:
    - A sudden/rapid loss of up to 20% of the blood volume or slow loss of even larger amounts usually has little clinical effects in healthy adults because of compensatory mechanisms.
    - A sudden/acute loss of 33% of blood volume may lead to hypovolemic shock resulting in death.
    - Loss of up to 50% of blood volume gradually over a period of 24 hours may not be fatal.
  - Chronic or recurrent loss of blood:
    - External bleeding: Bleeding into an internal cavity, as in gastrointestinal hemorrhage from a peptic ulcer (arterial hemorrhage) or esophageal varices (venous hemorrhage) or menstrual bleeding frequently leads to iron deficiency anemia due to loss of iron in hemoglobin.
    - Internal bleeding: Iron is efficiently recycled from phagocytosed red cells when the bleeding is internal (e.g., a hematoma). Hence, it does not produce iron deficiency anemia.
  - Jaundice: Extensive hemorrhages can occasionally produce jaundice due to massive breakdown of red cells and hemoglobin.
- **Site of hemorrhage:** Bleeding may be trivial in the subcutaneous tissues. However, it can cause death if located in the brain.

EDEMA

CC: Define and describe edema, its types, pathogenesis and clinical correlations.

Q. Define edema.

Definition: An abnormal accumulation of fluid in the interstitial space within tissues is called edema.

Special forms of edema are listed in **Table 5.1**.

Types of Edema Fluid

Q. Tabulate the differences between transudate and exudate.

The edema fluid may be either transudate or exudate. The differences between transudate and exudate are presented in **Table 3.3.**

1. **Transudate:** It is protein-poor fluid caused by increased hydrostatic pressure or reduced plasma protein.
   - **Causes:** Transudate is observed in heart failure, renal failure, hepatic failure, and certain forms of malnutrition.
2. **Exudate:** It is **protein-rich fluid** produced due to increased vascular permeability and is seen in **inflammation**.

Pathophysiologic Categories of Edema (Table 5.2)

Q. Discuss the causes and pathogenesis of generalized edema.

Q. Classify and list the different pathophysiological categories of edema. Describe the pathogenesis of cardiac edema.

Edema may be localized or generalized in distribution.

Local/Localized Edema

Q. Discuss the causes and pathogenesis of generalized edema.

It is **limited to an organ or part** (e.g., arm, leg, epiglottis, larynx).

- **Obstruction of vein or lymphatic** [e.g., edema of limb (usually the leg) develops due to venous or lymphatic obstruction caused by **thrombophlebitis, chronic lymphangitis**, **resection of regional lymph nodes, filariasis,** etc.].
- **Inflammation:** It is the **most common cause** of local edema.
- **Immune reaction** [e.g., urticaria (hives), or edema of the epiglottis or larynx (angioneurotic edema)].

Generalized Edema

It is **systemic** in distribution and affects visceral organs and the skin of the trunk and lower extremities.

Causes: Disorder of fluid and electrolyte metabolism.

- Heart failure
- Nephrotic syndrome (renal diseases with massive loss of serum proteins into the urine)
- Cirrhosis of the liver.

TABLE 5.1: Special forms of edema.

Terminology	*Body cavity involved*
Hydrothorax	Pleural cavity
Hydropericardium	Pericardial cavity
Hydroperitoneum (ascites)	Peritoneal cavity

TABLE 5.2: Pathophysiologic categories of edema.

Mechanism	*Causes*
Increased hydrostatic pressure	Impaired venous return • Generalized – Congestive heart failure – Constrictive pericarditis • Regional – Ascites in cirrhosis – Obstruction (e.g., thrombosis) or compression of veins (e.g., external mass) – Arteriolar dilation: Heat
Decreased plasma osmotic pressure (hypoproteinemia)	• Nephrotic syndrome • Ascites in cirrhosis of liver • Malnutrition • Protein-losing gastroenteropathy
Lymphatic obstruction	• Inflammatory • Neoplastic • Postirradiation • Postsurgical
Inflammation	Acute and chronic inflammation, angiogenesis

TABLE 5.2: Pathophysiologic categories of edema.	
Mechanism	***Causes***
Sodium retention	• Excessive salt intake with renal insufficiency • Increased tubular reabsorption of sodium, e.g., increased renin-angiotensin-aldosterone secretion

Mechanism/Pathogenesis of Edema

Q. Discuss the pathogenesis of edema in cirrhosis.

Normal Fluid Balance

- The smallest vessels of the vascular system and the lymphatic vessels are termed as the *microcirculation*. Normally, the fluid flows out from the arteriolar end of the microcirculation into the interstitium. This is balanced by flowing in of the fluid at the venular end.
- As fluid moves through the interstitial space, most of it returns to the capillary bed. Normally, a small amount of fluid (approximately 10% of the fluid) is left in the interstitium and is drained (absorbed) by the adjacent lymphatic vessels, and it reaches the general bloodstream via the thoracic duct.
- The movement of water and salts between the intravascular and interstitial spaces is controlled by four distinct forces or pressures. These include: (1) vascular hydrostatic pressure, (2) interstitial fluid colloid osmotic pressure, (3) plasma colloid osmotic pressure, and (4) interstitial fluid pressure. This delicate balance of forces is summarized by **Starling's hypothesis(Fig. 5.4)**. According to this, the net filtration is equal to the combined forces that favor filtration minus the combined forces that oppose filtration.
- Clinically, **vascular hydrostatic pressure** (the force that drives the fluid out of circulation) and plasma **colloid osmotic pressure** (force which drives the fluid into circulation) are the **most important.**

Mechanism of Edema (Fig. 5.5)

Any mechanism, which interferes with the normal fluid balance, may produce edema.

- **Increased capillary hydrostatic pressure or decreased colloid osmotic pressure** produces increased interstitial fluid.
- If the movement of fluid into tissues (or body cavities) exceeds lymphatic drainage, the fluid accumulates in the interstitial tissue. Edema may also develop when the permeability of capillaries is increased and they "leak" fluid (e.g., inflammation).
- These pathophysiologic mechanisms may operate singly or in combinations.

Increased Hydrostatic Pressure

Hydrostatic pressure at the capillary end of microcirculation drives the fluid out of the capillary into the interstitial tissue space. Any conditions, which increase the hydrostatic pressure, can produce edema. The increased hydrostatic pressure **may be regional or generalized**.

- **Local increase in hydrostatic pressure:** It can be due to **local impairment in venous return**. Examples,
  - **Deep venous thrombosis** in a lower extremity may produce localized edema in the affected leg.
  - **Postural edema** may be seen in the feet and ankle of individuals who stand in erect position for long duration.
- **Generalized increase in hydrostatic pressure:** It produces generalized edema. Most common cause is **congestive heart failure (CHF)**.
  - Congestive heart failure may be failure of the left ventricle, right ventricle or both.
  - **Right-sided heart failure** results in pooling of blood on the venous side of the circulation → increases the hydrostatic pressure in the venous circulation → increases movement of fluid into the interstitial tissue spaces → shows characteristic **peripheral pitting edema**.
  - **Left-sided heart failure** results in increased hydrostatic pressure in the pulmonary circulation → produces **pulmonary edema**.

Decreased Plasma Osmotic Pressure

Q. Discuss the pathogenesis of renal edema.

Plasma osmotic pressure normally tends to draw the fluid into the vessels. The **plasma osmotic pressure is dependent on plasma proteins, mainly on albumin** (major plasma protein). Decreased plasma osmotic pressure may be due to:

- **Reduced albumin synthesis:** Occurs in **severe liver diseases** (e.g., cirrhosis) or **protein malnutrition** (due to decreased intake of proteins).

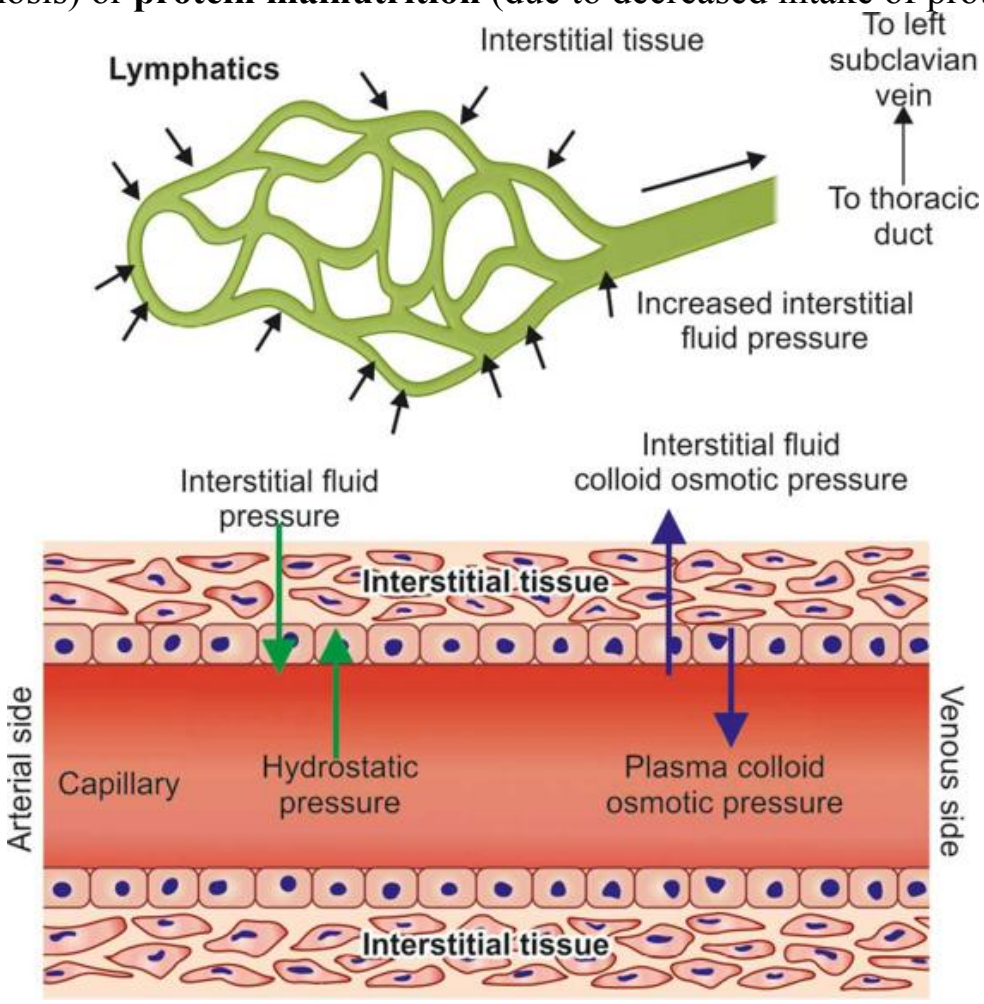


Fig. 5.4: Factors influencing the movement of fluid across capillary walls. Normally there is balance between hydrostatic and osmotic pressure so that there is only little net movement of fluid into the interstitium. Mechanism that can produce edema include increased hydrostatic pressure or diminished plasma osmotic pressure. Normally lymphatics in the interstitial tissue return much of the excess fluid into the circulation by way of the thoracic duct. If the fluid accumulated exceeds the capacity of lymphatic drainage also can produce local edema.

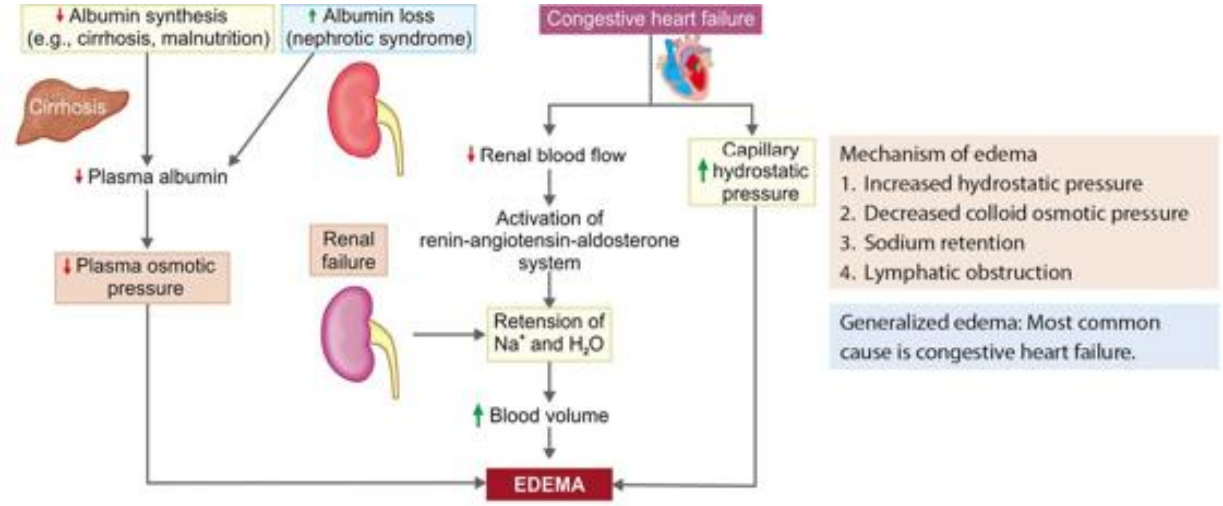


Fig. 5.5: Pathogenesis of systemic edema from congestive heart failure, renal failure, or reduced plasma osmotic pressure.

- **Loss of albumin:** May occur in the **urine or stool**. **Nephrotic syndrome** is an important cause of loss of albumin in urine. **Malabsorption and protein losing enteropathy** are characterized by loss of protein in the stool.

Consequences of decreased plasma osmotic pressure are shown in **Flowchart 5.3.**

Sodium and Water Retention

Increased **retention of sodium salt is invariably associated with retention of water**. Sodium and water retention may be a primary cause of edema.

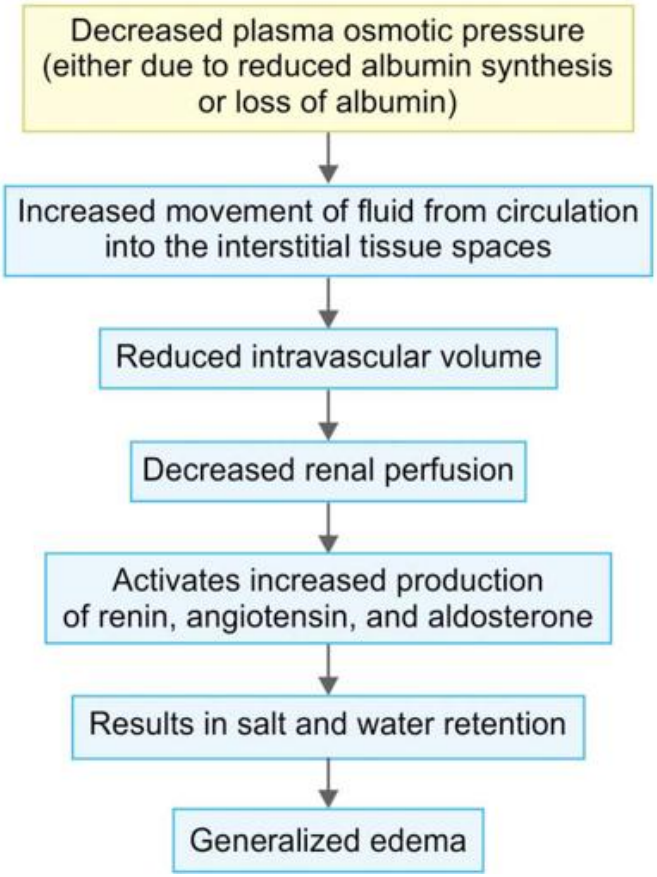
- **Mechanism**:
  - **Increased hydrostatic pressure** due to increased plasma volume
  - **Decreased plasma colloid osmotic pressure** due to dilution effect on albumin.
- **Causes:** May be primary or secondary
  - **Primary:** It is associated with disorders of kidney such as renal failure, glomerulonephritis.
  - **Secondary:** It develops in disorders that decrease renal perfusion, most important cause being congestive heart failure.

Q. Mention the pathogenesis/mechanism of cardiac edema.

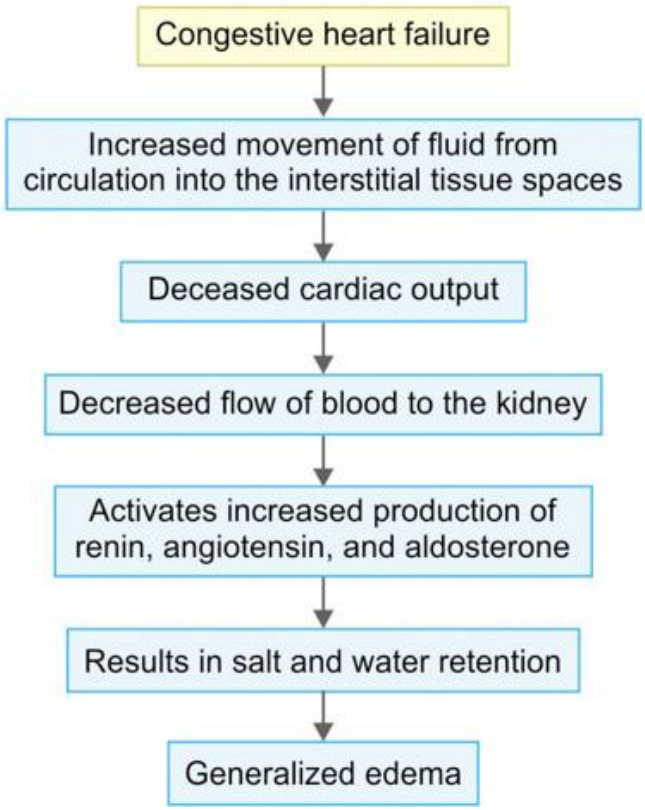
- **Mechanism of edema in congestive heart failure (Flowchart 5.4)**

Other adaptations also occur, which includes increased vascular tone and elevated levels of antidiuretic hormone (ADH).

Flowchart 5.3: Mechanism of generalized edema due to reduced plasma osmotic pressure.



Flowchart 5.4: Mechanism of generalized edema in congestive heart failure.



- **Water retention by ADH mechanism**
  - ADH is released from the posterior pituitary, when there is reduced plasma volume or increased plasma osmolarity.
  - Primary retention of water can occur due to the increased release of ADH.
  - **Increased secretion of ADH** is seen in association with lung cancer and **pituitary disorders**.
  - This can lead to hyponatremia and cerebral edema.

Lymphatic Obstruction

Q. Write short note on localized edema.

Lymphatic obstruction causes impaired drainage of lymph and produces localized edema, called as **lymphedema**.

Causes of lymphatic obstruction

- **Chronic inflammation of lymphatics** associated with fibrosis (e.g., lymphedema occurring at scrotal and vulvar region due to **lymphogranuloma venereum**).
- **Invasive malignant tumors** [e.g., **lymphedema of breast** due to blockage of subcutaneous lymphatics **by malignant cells** gives rise to **orange skin (peau d' orange) appearance** to the involved region of skin in the breast].
- **Pressure over lymphatic drainage** from outside (e.g., tumors obstructing thoracic ducts).
- **Damage by surgery/radiation:** Patients with breast cancer may develop severe edema of the upper arm as a complication of **surgical removal and/or irradiation of the breast** and **associated axillary lymph nodes**.
- **Parasitic infestations:** In **filariasis** (caused by *Wuchereria bancrofti)*, the parasite (**Fig. 5.6**B—microfilaria) may cause extensive obstruction of lymphatics and lymph node fibrosis. If the block is in the inguinal region, it can produce edema of the external genitalia and lower limbs (upper arm if axillary region is involved) which may be massive and resemble the leg of an elephant and is known as **elephantiasis (Fig. 5.6A and** *refer* **Fig. 4.15)**.
- **Hereditary disorder: Milroy's disease** is a hereditary disorder characterized by abnormal development of lymphatics. The edema may be seen in one or both lower limbs.

Through lymph, proteins in the interstitial space are returned to the circulation. So, **edema fluid** produced due to lymphatic obstruction has a **high protein** concentration. The increased protein content may **stimulate fibrosis** in the dermis of the skin and is responsible for the induration found in lymphedema.

MORPHOLOGY

Edema can be **easily detected on gross examination**. It may involve any organ or tissue, but is **most common in subcutaneous tissues, the lungs,** and the **brain**. Microscopically, edema appears as a clear space, which separates the extracellular matrix.

Generalized Edema

It is seen mainly in the subcutaneous tissues.

- **Subcutaneous edema:** It may be diffuse or more easily noticed in regions with high hydrostatic pressures. In most cases, the distribution of **edema is dependent on gravity** and is termed **dependent edema**. Thus, it is prominent in the legs when standing, and in

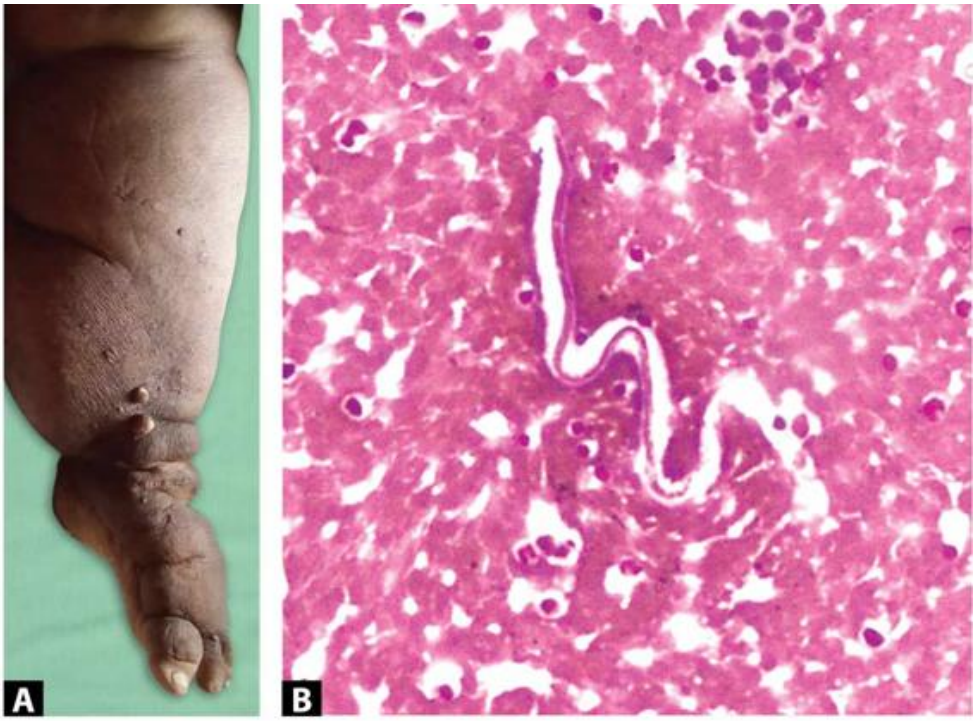
the sacrum when recumbent. **If pressure is applied** by a finger over substantially edematous subcutaneous tissue, it **displaces the interstitial fluid and leaves a depression**. This sign is called as **pitting edema (Fig. 5.7).**

- **Edema of renal origin:** It can **affect all parts of the body**. Initially, it is observed **in tissues with loose connective tissue matrix**, such as the **eyelids** (*refer* **Fig. 20.14**) **and scrotal region**. Edema in the eyelids is called **periorbital edema** and is a characteristic of severe renal disease.

Q. Write briefly on pulmonary edema.

Pulmonary Edema

- **Gross:** The **weight of lungs is increased** 2 to 3 times of normal weight. **Cut section** shows frothy, **blood-tinged fluid** (due to mixture of air, edema, and extravasated red cells) **oozing from the lung**.
- **Microscopy:** The edema fluid is seen in the alveolar septa around capillaries and reduces the diffusion of oxygen. Edema fluid present in the alveolar spaces favors bacterial infection.



Figs. 5.6A and B: Elephantiasis due to obstruction of lymphatics (A) by *Wuchereria bancrofti* (microfilaria—B).



Fig. 5.7: Pitting edema in which when pressure by finger is applied, it displaces interstitial fluid and leaves a depression at that site.

Q. Write briefly on brain edema.

Clinical Consequences

They range from minimal effects to rapidly fatal effect.

- **Generalized subcutaneous tissue edema:** It indicates the presence of an underlying **cardiac or renal disease**. Severe subcutaneous edema may delay wound healing or the clearance of infection.
- **Pulmonary edema:** It is common and most commonly **caused by left ventricular failure**. Other causes include renal failure, acute respiratory distress syndrome, and pulmonary inflammation or infection.
- **Cerebral/brain parenchymal edema:** It is **life-threatening**. In severe brain edema, the brain substance may herniate (extrude) through the foramen magnum, or occlude the blood supply to the brainstem. Both conditions may damage the medullary centers and lead to death.
- **Myxedema:** It is a form of **non-pitting** edema involving skin of face and visceral organs **observed in hypothyroidism (*refer* page 797)**. The edema is due to excessive deposition of glycosaminoglycans and hyaluronic acid, in skin, subcutaneous tissue, and visceral organs.
- **Papilledema: Swelling of the optic nerve head** is called as papilledema. The concentric increase in pressure encircling the optic nerve produces stasis of venous outflow which leads to swelling of the optic nerve head. The causes are:
  - Compression of the nerve (e.g., primary neoplasm of the optic nerve)
  - Raised cerebrospinal fluid pressure surrounding the nerve.

Edema: Excess fluid in the interstitial spaces within tissues.

Transudate is a protein-poor and cell-poor fluid. Exudate is protein-rich and cell-rich fluid.

Anasarca: Severe form of generalized edema.

Inflammatory carcinoma of breast: Local lymphedema due to invasion and obstruction of subcutaneous lymphatics by tumor cells.

Nephrotic syndrome: Massive loss of albumin urine → decreased serum albumin → decreased plasma osmotic pressure → generalized edema.

Angioneurotic edema:
- Autosomal dominant
- Mediated by vasoactive peptides such as bradykinin
- Low levels or abnormal function of a regulatory complement protein in the plasma, C1 inhibitor (C1 INH deficiency).

- Lymphatic edema: Fluid in edema has high protein content.
- Lymphedema: Lymphatic obstruction after modified radical mastectomy, radiation and filariasis.
- Peau d 'orange appearance of skin in the carcinoma breast: Lymphedema of breast due to blockage of subcutaneous lymphatics by malignant cells.

- Pitting edema in right heart failure is due to increased hydrostatic pressure.
- Pitting edema in cirrhosis is due to reduced osmotic pressure.

Cerebral edema:
- It may be **localized or generalized**. In generalized edema, the brain is grossly swollen with **distended gyri and narrowed sulci**. The ventricular cavities are compressed and the brain expands, it may herniate.

NORMAL HEMOSTASIS

CC: Define and describe normal hemostasis.

Hemostasis is a well-regulated physiological process by which blood is maintained in liquid state inside the vessels, yet arrests bleeding at the site of vascular injury by forming a hemostatic clot.

Steps in Hemostasis

Hemostasis is initiated to arrest hemorrhage in response to vascular damage (injury) by forming a blood clot. It includes several sequences of events at a

site of vascular injury**(Figs. 5.8A to D)**.

Normally, blood vessels are lined by a smooth, nonthrombogenic endothelium.

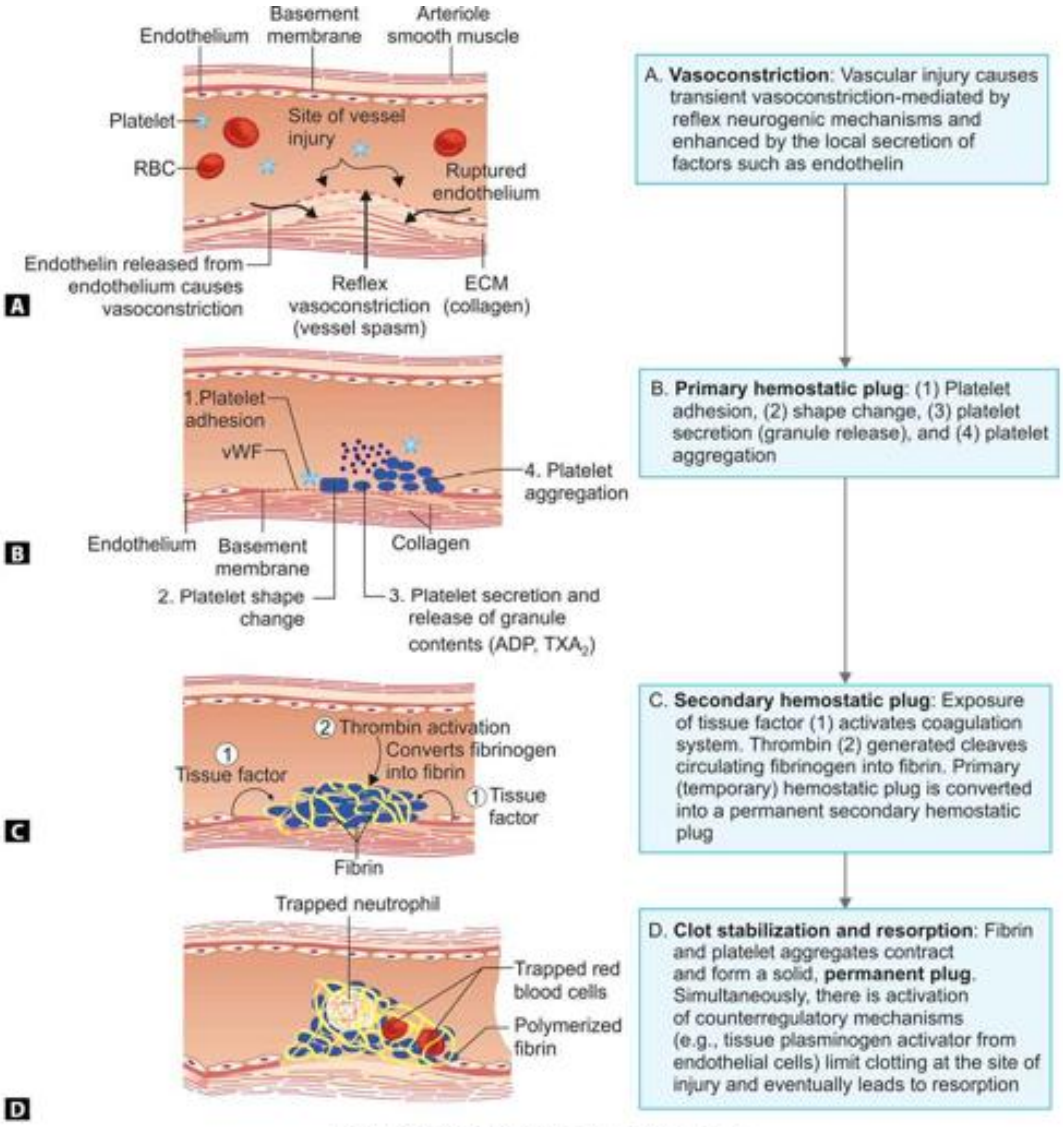
Injury to the endothelium causes transient vasoconstriction and **exposes highly thrombogenic subendothelial extracellular matrix** (ECM), **initiating the platelet events.** These events forms hemostatic plug and prevents or limit any further hemorrhage at the site of vascular injury. Hemostasis is a precisely orchestrated process and involves platelets, clotting (coagulation) factors, endothelium of blood vessels and the fibrinolytic system**.** The general sequence of events of hemostasis at a site of vascular injury is as follows:

Arteriolar Vasoconstriction

It occurs immediately after vascular injury and markedly reduces flow of blood to the injured site. It is mediated by reflex neurogenic mechanisms and enhanced by the local secretion of factors such as **endothelin**(a powerful endothelium-derived vasoconstrictor). This vasoconstriction is transient and bleeding may resume if there is no activation of platelets and coagulation factors.

Primary Hemostatic Plug

Platelets play an important role in hemostasis. Primary hemostasis is the term is used for platelet plug formation at the site of injury. It occurs immediately within seconds of injury and is responsible for cessation of bleeding from microvasculature. It is characterized by the formation of the primary platelet plug that initially seals vascular defects. Platelet plug provides a surface that binds and concentrates activated coagulation factors. Damage or disruption of the endothelium lining the blood vessels after a traumatic vascular injury, exposes subendothelial connective tissue, such as von Willebrand factor (vWF) and collagen. Both vWF and collagen produce sequence of reactions that promote platelet adherence and activation. These lead to the formation of a platelet plug. Primary hemostasis involves three steps: Platelet adhesion and shape change, platelet secretion (granule release) and platelet aggregation.



Figs. 5.8A to D: Different steps in hemostasis.

Platelet Adhesion and Shape Change

- **Platelet adhesion at the site of vascular injury:** Initial step is **adhesion of platelets** to subendothelial structures at the site of injury. The link between the receptor sites (GpIb-IX which is an integrin) on the surface of platelet with exposed subendothelial collagen is brought out by an adhesion glycoprotein called **von Willebrand factor.** vWF is synthesized by both endothelial cells and megakaryocytes. Genetic deficiencies of vWF (von Willebrand disease) or GpIb (Bernard–Soulier syndrome) can produce bleeding disorders.

- **Platelets shape change**: Following adhesion of platelets to the subendothelial region, there is a dramatic change in the shape of platelets. Platelets change their shape from round to spherical smooth discs to spiky "sea urchins" (urchins = young child who is poorly or raggedly dressed) with protrusions, thereby markedly increasing the surface area. This change in shape of platelet is accompanied by:
  - Changes in glycoprotein IIb/IIIa that **increase its affinity for fibrinogen**.
  - Translocation of negatively charged phospholipids (especially phosphatidylserine) to the surface of platelets. These negatively charged phospholipids on the surface bind calcium and serve as nucleation sites for the **formation of coagulation factor complexes**.

Platelet Secretion (Release Reaction), Activation and Recruitment

Soon after platelets shape change, platelets **release granule contents.** These processes of shape change along with release of granule content are together known as **platelet activation.** Platelet activation is brought out by a number of factors, including the coagulation factor thrombin and ADP.

- **Thrombin** activates platelets by switching on a special type of G-protein–coupled receptor [protease-activated receptor (PAR)].
- **ADP** is a present in dense body granules of platelets. Platelet activation and release of ADP leads to additional rounds of platelet activation and this phenomenon is called as **recruitment** which contain pro aggregatory substances (that recruit additional platelets) like ADP, serotonin (5-hydroxytryptamine), fibrinogen and vWF. Calcium is also released and is required for the coagulation.

Platelet Aggregation

- Platelet aggregation (clumping together) follows platelet activation. The secreted products of platelets recruit additional platelets and cause platelet aggregation (i.e., bridging/adhesion of adjacent platelets) through the receptor sites (Gp IIb-IIIa). This process is mediated by fibrinogen which acts as an intercellular bridge between adjacent platelets. Inherited deficiency of GpIIb-IIIa produces a bleeding disorder called Glanzmann

thrombasthenia. Activated platelets also produce the prostaglandin called thromboxane A_2 (TxA_2) which induces strong aggregation of platelets.

- These clumps of platelets so formed quickly (within 3 to 7 minutes) stop bleeding from the site of injury and are known as **primary hemostatic plug.** The process of primary hemostatic plug formation is termed as **primary hemostasis.**

Secondary Hemostatic Plug

It is characterized by deposition of fibrin at the site of vascular injury. This process takes an average of 3 to 10 minutes.

- **Exposure of tissue factor** at the site of injury. Tissue factor is a membrane-bound procoagulant glycoprotein. It is normally expressed by subendothelial cells in the vessel wall namely smooth muscle cells and fibroblasts. Tissue factor binds and activates factor VII. This in turn activates cascade of reactions that generates thrombin. Thrombin is a potent activator of platelets and causes additional platelet aggregation (of primary hemostatic plug) at the site of injury. The initial platelet aggregation is reversible. However, concurrent activation of thrombin stabilizes the platelet plug by causing further activation and aggregation which in turn promotes irreversible contraction of platelets. Platelet contraction is dependent on the cytoskeleton of platelets.
- Thrombin cleaves circulating fibrinogen into insoluble fibrin, thereby forming a fibrin meshwork at the site of vascular injury. The platelets contract, form an irreversibly fused mass. The fibrin formed from fibrinogen cement the platelets and produces a definitive secondary hemostatic plug. The red cells and leukocytes also get trapped in the fibrin meshwork and are found in hemostatic plugs. The process of conversion of the initial temporary primary hemostatic (platelet) plug into a permanent secondary hemostatic plug is known as **secondary hemostasis.** Secondary hemostasis consolidates the initial platelet plug.
- The solid permanent plug so formed prevents any further hemorrhage. The hemostatic plugs/clots thus formed are confined to the site of injury by the fibrinolytic system. Subsequently, vascular repair is accomplished by thrombolysis and recanalization of the occluded site.

Clot Stabilization and Resorption

Polymerized [large number of units (monomers) bonded together] fibrin and platelet aggregates contract and form a solid, **permanent plug.** This prevents further hemorrhage. Simultaneously, there is activation of counter-regulatory mechanisms [e.g., tissue plasminogen activator (t-PA) released from endothelial cells] which limit clotting at the site of injury and eventually leads to resorption and tissue repair.

Failure of the hemostatic system to restore the integrity of an injured vessel causes bleeding. Inability to maintain the fluidity of blood results in thrombosis.

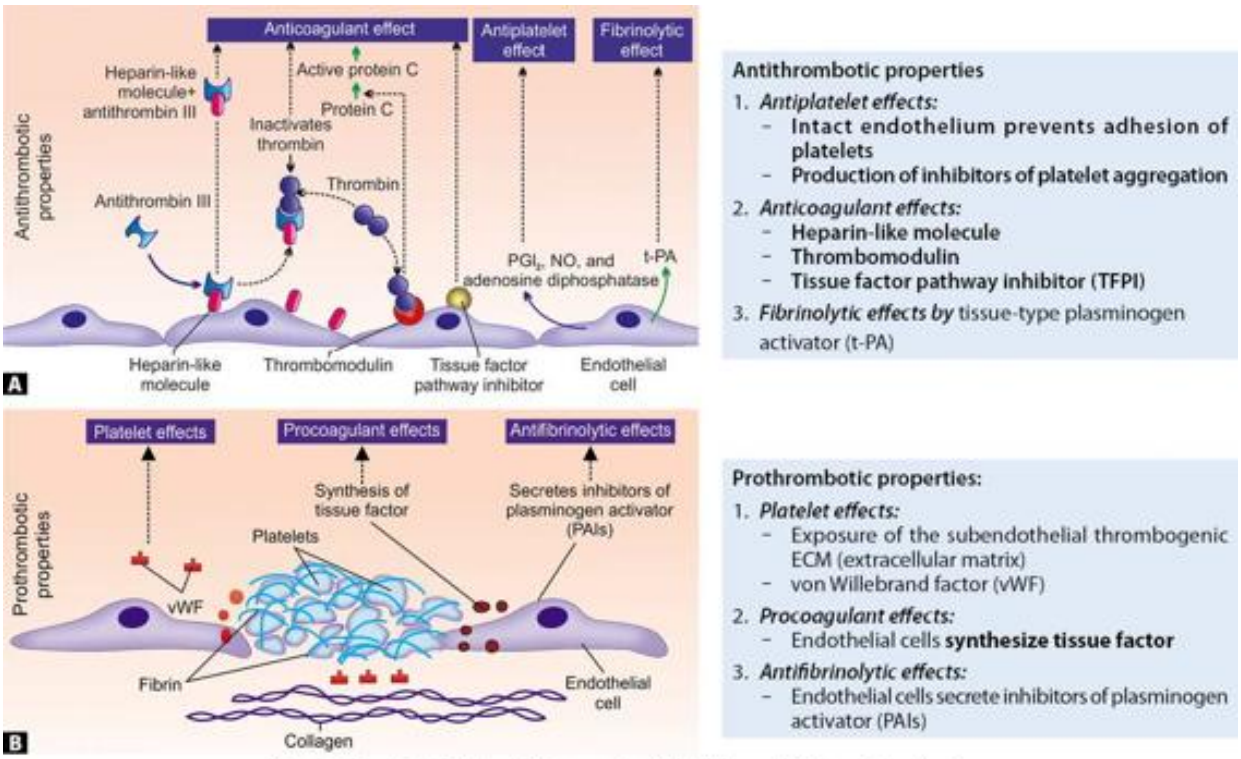
FUNCTIONS OF NORMAL ENDOTHELIUM

Endothelial cells play an important role in both homeostasis and thrombus formation. They have both antithrombotic and prothrombotic (procoagulant) properties. The balance between these two opposing endothelial properties determines the thrombus formation.

Antithrombotic Properties

Q. Write a short note on antithrombotic properties of endothelium.

Normally, the endothelial cells have antiplatelet, anticoagulant and fibrinolytic properties which prevent thrombosis (and also coagulation) **(Figs. 5.9A and B)**.



Figs. 5.9A and B: Endothelial factors that (A) inhibit and (B) favor thrombosis.
(PGI_2: prostacyclin; NO: nitric oxide; t-PA: tissue plasminogen activator; vWF: von Willebrand factor)

Antiplatelet Effects

They prevent platelet adhesion and aggregation following mechanism:

- **Intact endothelium prevents adhesion of platelets** (and plasma coagulation factors) to the highly thrombogenic subendothelial extracellular matrix (ECM).
- **Production of inhibitors of platelet aggregation** by endothelial cells: These include: **prostacyclin** (PGI_2), **nitric oxide (NO)** and **adenosine diphosphatase** (which degrades adenosine diphosphate—ADP).

Anticoagulant Effects

The endothelium inhibits coagulation by following molecules:

- **Heparin-like molecules:** Found in the endothelium and exert their anticoagulant effect indirectly through **antithrombin III.** They inactivate thrombin and coagulation factors (Xa and IXa).
- **Thrombomodulin:** Present on the endothelial cells and binds to thrombin and activates protein C, which **inhibits clotting** by proteolysis of factor Va and VIIIa.

- **Tissue factor pathway inhibitor** (TFPI): Inhibits tissue factor/factor VIIa complexes.

Fibrinolytic Effects

Endothelial cells synthesize tissue-type plasminogen activator (*t-PA*) which degrades a thrombi whenever it is formed.

Prothrombotic Properties

Q. Write a short note on prothrombotic properties of endothelium.

Endothelial cells may be damaged or activated by several ways. These include trauma, inflammation, infectious agents, hemodynamic forces, plasma mediators, and cytokines. The damaged or activated endothelial cells promote prothrombotic state by its platelet, procoagulant and antifibrinolytic effects.

Platelet Effects

- Endothelial damage **exposes the subendothelial thrombogenic ECM** and allows adhesion of platelets from circulation to ECM.
- *von Willebrand factor (vWF)* produced by normal endothelial cells is essential cofactor that helps platelet binding to matrix elements.

Procoagulant Effects

- Endothelial cells **synthesize tissue factor** in response to cytokines [e.g., tumor necrosis factor (TNF) or interleukin-1 (IL-1)] or bacterial endotoxin. Tissue factor activates the extrinsic coagulation cascade.
- Activated endothelial cells increases the catalytic function of activated coagulation factors IXa and Xa.

Antifibrinolytic Effects

Endothelial cells secrete inhibitors of plasminogen activator (PAIs). They reduce fibrinolysis and tend to favor thrombosis.
Antithrombotic and prothrombotic properties of endothelium are listed in **Table 5.3**.

Intact, nonactivated endothelial cells inhibit thrombus whereas endothelial injury or activation promotes thrombus formation.

Ultrastructurally, endothelial cells contain Weibel Palade bodies.

THROMBOSIS

CC: Define and describe the etiopathogenesis and consequences of thrombosis.

Q. Define thrombus and describe the pathogenesis of thrombus. Add a note on fate of thrombus.

Q. Define thrombus. Discuss the etiopathogenesis and pathology of thrombosis. Add a note on fate of thrombus.

Definition: Thrombosis is defined as the **process of formation of a solid mass** in the **circulating blood from the constituents of flowing blood**.
The **solid mass formed** is called as **thrombus** and it consists of an aggregate of coagulated blood containing platelets, fibrin, and entrapped cellular elements of blood.

Etiology

Q. What is Virchow's triad? List its components.

Q. Describe the etiopathogenesis of thrombus.

Three primary abnormalities can lead to formation of a thrombus and constitute Virchow's triad **(Fig. 5.10)**. These include:

1. **Injury to endothelium** (changes in the vessel wall).
2. **Stasis or turbulent blood flow** (changes in the blood flow).
3. **Hypercoagulability of the blood** (changes in the blood itself).

Injury to Endothelium (Changes in the Vessel Wall)

Endothelial injury may be either physical damage or endothelial dysfunction (or activation).

Physical Endothelial Injury

It is important for formation of thrombus in the **heart or the arterial circulation**. Normally, high flow rates in the heart and arterial circulation prevent adhesion of platelet to endocardium/endothelium and wash out any activated coagulation factors. The endothelial cell injury **promotes adhesion of platelets at the site of injury**.

TABLE 5.3: Antithrombotic and prothrombotic properties of endothelium.

Antithrombotic properties	***Prothrombotic properties***
Antiplatelet effects • Acts as a barrier between platelets and subendothelial thrombogenic ECM • Produce inhibitors of platelet aggregation (e.g., PGI_2, NO and adenosine diphosphatase	**Platelet effects** • Endothelial damage **exposes the subendothelial thrombogenic** ECM • *von Willebrand factor (vWF)* produced by normal endothelial cells helps platelet binding to ECM
Anticoagulant effects • Heparin-like molecules • Thrombomodulin • Tissue factor pathway inhibitor (TFPI)	**Procoagulant effects** • **Synthesis of tissue factor**→ activates the extrinsic coagulation cascade • Activated endothelial cells increase the catalytic function of factors IXa and Xa
Fibrinolytic effect through tissue-type plasminogen activator (t-PA)→conversion of plasminogen to plasmin→cleaves fibrin	**Antifibrinolytic effects** through secretion of inhibitors of plasminogen activator (PAIs)→reduce fibrinolysis

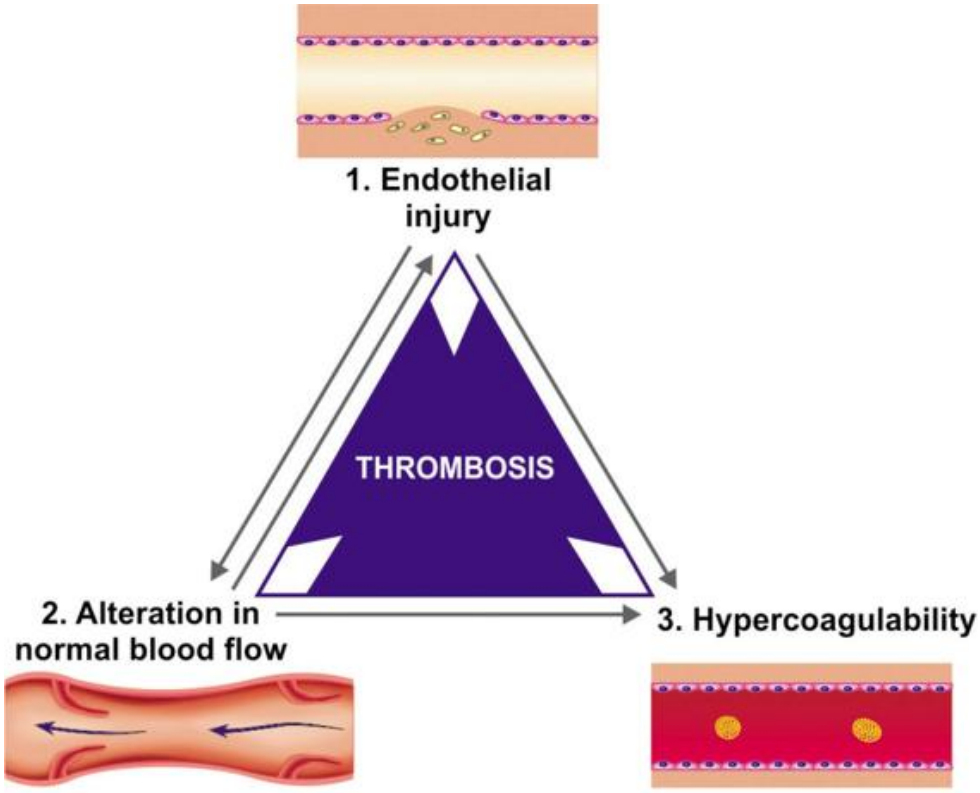


Fig. 5.10: Virchow's triad in thrombosis. (1) Endothelial injury is the most important factor, (2) Alteration in blood flow (stasis or turbulence) and (3) Hypercoagulability.

Causes:

- **Heart:**
  - Chambers of heart (e.g., endocardial injury due to myocardial infarction with damage to the adjacent endocardium, catheter trauma).
  - Valves: Small thrombi on the valves are called as vegetations.
    - Infective endocarditis: Thrombi on valves (e.g., mitral, aortic valve) damaged by a blood-borne bacteria or fungi
    - Damaged valves (e.g., due to rheumatic heart disease, congenital heart disease)
    - Libman–Sacks endocarditis in systemic lupus erythematosus
    - Nonbacterial thrombotic endocarditis: They are sterile vegetations on noninfected valves with hypercoagulable states.
- **Arteries** [e.g., ulcerated **atherosclerotic plaques**, traumatic or inflammatory vascular injury **(vasculitis)]**.

- **Capillaries:** Causes include **acute inflammatory lesions**, vasculitis and disseminated intravascular coagulation **(DIC)**.

Mechanism: Physical loss of endothelium exposes thrombogenic subendothelial ECM. Platelets adhere to the site of endothelial injury and release prothrombotic tissue factor. There is local depletion of antithrombotic factors like PGI_2.

Endothelial Dysfunction

Definition: Endothelial dysfunction is defined as an **altered state**, **which induces an endothelial surface that is thrombogenic or abnormally adhesive to inflammatory cells**. Thus, thrombus can develop without any denudation or physical disruption of endothelium.

Causes: Hypertension, turbulent blood flow, toxins (e.g., bacterial endotoxins, toxins from cigarette smoke), radiation injury, metabolic abnormalities (e.g., homocystinemia or hypercholesterolemia).

Mechanism: Endothelial dysfunction can **disturb the balance between prothombotic** and **antithrombotic activities of endothelium by:**

- **Producing more procoagulant factors**, e.g., platelet adhesion molecules, tissue factor, PAIs or
- **Synthesizing less anticoagulant effectors**, e.g., thrombomodulin, PGI_2, t-PA.

Alterations in Normal Blood Flow

Normally, blood flows through a blood vessel in layers and the velocity of the layers varies. This normal blood flow is termed as **laminar (Fig. 5.11)**. Blood in the center moves faster than blood in the outer layers. This is because, the blood in the center layer is in contact with blood only, whereas the outermost layer is also in contact with the intima of the blood vessel wall, which produces friction against the cellular components (**platelets** and other cellular elements) of the blood. Many blood cells stick or adhere to the intima from this outer layer because of minimal flow.

Causes

- **Turbulence** (disturbed movement of blood): The streamlined laminar flow may be disrupted by normal anatomy and by pathologic processes creating turbulent flow. Turbulent flow is an interruption in the forward current of blood flow by crosswise flow **(Fig. 5.12)**. Turbulence results if blood flows around an obstruction in the vessel or over a roughened intimal surface (e.g., atheromatous plaque). It can produce thrombus in the arteries and heart.
- **Stasis:** It is **major cause for venous thrombosis**.

Mechanism

Stasis and turbulence produce thrombus by the following mechanism:

- **Promote endothelial** injury/**activation** and increases the procoagulant activity.
- **Brings platelets into contact with the endothelium**.
- **Prevent cleansing and dilution of activated clotting factors** by fresh flowing blood.
- **Prevents flowing in of clotting factor inhibitors**.

Clinical disorder associated with turbulence and stasis:

- **Heart:**
  - Acute myocardial infarction
  - Arrhythmias/atrial fibrillation [e.g., rheumatic mitral stenosis in conjugation with disordered atrial rhythm (atrial fibrillation), it predisposes to mural thrombi in atria].

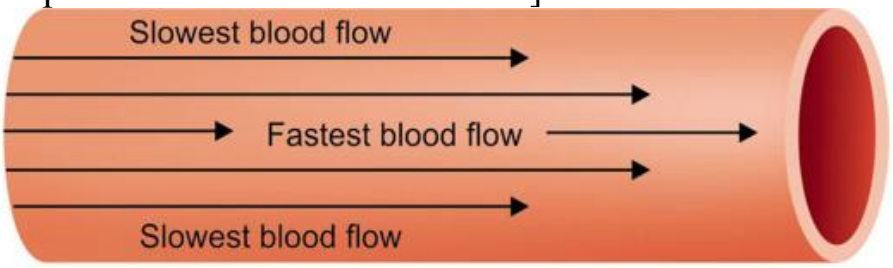


Fig. 5.11: Laminar blood flow in normal vessel.

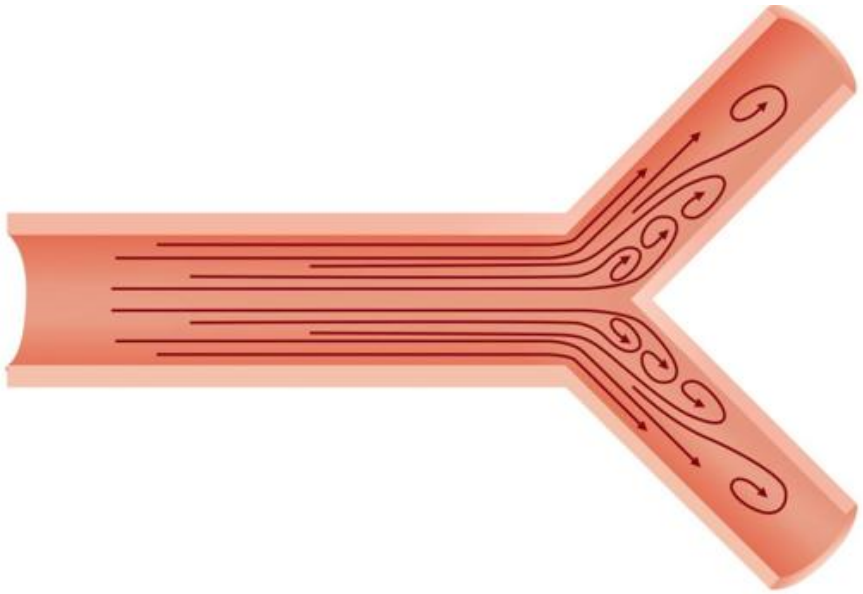


Fig. 5.12: Turbulent flow at the bifurcation of a blood vessel.

- Dilated cardiomyopathy

- **Arteries:**
  - Ulceration of atherosclerotic plaques
  - Aneurysms: They cause local stasis.
- **Veins:** Thrombi develop in the saphenous veins with varicosities or in deep veins.

Other causes

- **Hyperviscosity,** e.g., with **polycythemia vera**
- **RBC disorders,** e.g., **sickle cell anemia** can cause vascular occlusions and stasis.

Hypercoagulability (refer page 379)

Q. Write short note on causes of coagulable state.

Q. Write short note on primary conditions associated with increased risk of thrombosis.

Definition: Hypercoagulability state (also known as thrombophilia) is defined as a **systemic disorder associated with increased tendency to develop thromboembolism**.

Causes: It is a less frequent cause of thrombosis. Causes can be divided into primary (genetic) and secondary (acquired) disorders **(Box 5.2)**.

Secondary/acquired disorders (Box 5.2): The pathogenesis of acquired thrombophilia is usually multifactorial.

Terminology

Q. Write short note on mural thrombi.

Mural thrombus: It is **attached to the wall** and projects into the lumen, **without complete occlusion of the lumen (Figs. 5.13B and 5.14)**. It occurs in heart chambers or in the aortic lumen.

Occlusive thrombus: It **occludes the lumen of the blood vessel (Fig. 5.14)** and prevents the flow of blood. It usually occurs in veins or smaller or medium sized arteries.

Q. Write short note on vegetations.

Vegetation: It is a **thrombus on heart valve** (*refer* **Fig. 16.24**) and appears as polypoid mass projecting into the lumen (e.g., infective endocarditis).

Types of thrombi: Thrombi may be **arterial or venous type**. Differences between arterial and venous thrombus are shown in **Table 5.4**.

MORPHOLOGY OF THROMBI

Layers in Thrombus

- **First** layer of the thrombus on the endothelium/endocardium is a **platelet layer**.
- **On top of the platelet layer, fibrin** is precipitated to **form upstanding laminae** which anastomose to form an intricate structure which **resembles coral** (coralline thrombus). In **between the upstanding laminae** and anastomosing fibrin

Site and Types

Thrombi: Its size and shape depends on the site of origin and its cause. Thrombi can develop anywhere in the cardiovascular system.

meshwork, the **red blood cells get trapped**. **Retraction of fibrin produces a ribbed appearance** on the surface of thrombus.

Q. Write briefly on lines of Zahn.

Lines of Zahn

Both gross and microscopy of thrombus show **alternating light** (pale or white) area of platelets held together by fibrin, **and dark retracted area** of fibrin meshwork with trapped RBCs. These alternating laminations of light and dark are known as **lines of Zahn (Figs. 5.13A and B).**

BOX 5.2: Major causes of hypercoagulable state.

A. Primary (genetic)

Deficiency of antithrombotic (anticoagulant) factors

- Antithrombin III deficiency
- Protein C deficiency
- Protein S deficiency
- MTHFR gene mutation

Increased prothrombotic factors

- Activated protein C (APC) resistance (factor V mutation/ factor Va/ factor V Leiden)
- Excessive levels of prothrombin (prothrombin G20210A mutation)
- High levels of factors VII, XI, IX, VIII; von Willebrand factor; fibrinogen
- Homocystinuria

B. Secondary (acquired)

High-risk for thrombosis

- Prolonged bed rest or immobilization
- Myocardial infarction, atrial fibrillation
- Tissue injury (e.g., surgery, fracture, burn)
- Disseminated intravascular coagulation
- Cancer, prosthetic cardiac valves, heparin-induced thrombocytopenia
- Antiphospholipid antibody syndrome

Lower risk for thrombosis

- Nephrotic syndrome
- Hyperestrogenic states (pregnancy and postpartum), oral contraceptive use
- Cardiomyopathy, smoking, sickle cell anemia

Arterial thrombi: Seen in

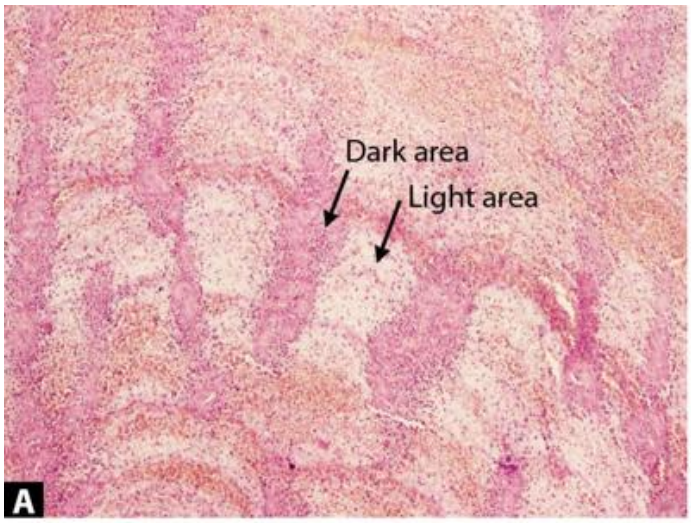
Homocysteinemia: Inherited or acquire disorder associated with both arterial and venous thrombosis.

When thrombosis develops in patient below the age of 50 years, genetic causes of hypercoagulability must be considered, even if there are acquired risk factors.

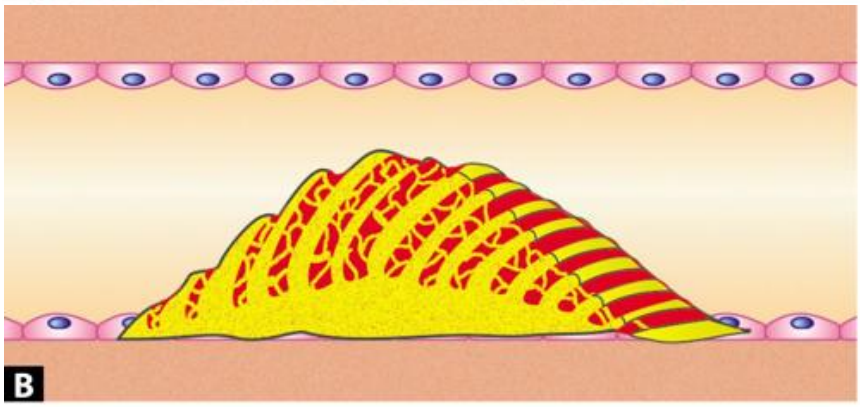
Hypercoagulability due to defective factor V gene is called Leiden mutation. It is the common inherited cause of hypercoagulability.

TABLE 5.4: Differences between arterial and venous thrombus.

Characteristics	*Arterial thrombus*	*Venous thrombus*
Main cause	Injury to endothelium	Stasis
Rate of blood flow	Rapid	Slow
Usual type of thrombus	Mural	Occlusive
Common sites	Aorta, coronary, cerebral and femoral arteries	Superficial varicose veins and deep veins of leg
Gross		
Color	Gray-white	Red-blue
Lines of Zahn	More prominent	Less prominent
Composition	Friable meshwork of platelets, fibrin, RBCs and degenerating leukocytes	More trapped RBCs and relatively few platelets
Propagation	Retrograde manner from point of attachment of thrombus (i.e., towards heart)	In antegrade manner from point of attachment towards the direction of blood flow (i.e., towards the heart)
Effects	Ischemia causing infarction of area supplied by the artery containing thrombus	Thromboembolism, edema and ulceration
Aspirin: Prevents arterial thrombosis.		
Heparin and warfarin: Prevents venous thrombosis.		



Thrombus: Lines of Zahn.



Figs. 5.13A and B: Appearance of thrombus: (A) Microscopic and (B) diagrammatic showing alternating dark and light areas (lines of Zahn).

Heart

- **Cardiac thrombi:** Usually develops at sites of turbulence or endocardial injury.
  - More common in the atrial appendages.
  - Can also develop on the endocardial surface over the site of acute myocardial infarction (*refer* **Fig. 16.22B**).
- **Valves:** Thrombi on heart valves are called **vegetations** (*refer* **Fig. 16.24**). They are more common on **mitral or aortic valves**.

Blood Vessels

- **Arteries:** Arterial thrombi tend to be **white**.
  - Aorta or larger arteries usually develop mural thrombi.

- Thrombi developing in the medium or smaller arteries are frequently occlusive. They develop (in decreasing order of frequency) in the coronary, cerebral and femoral arteries.

Veins

Venous thrombosis (phlebothrombosis) are usually occlusive, and form a long cast of the lumen. They occur usually at sites of stasis, and contain more trapped RBCs (and relatively few platelets). They are therefore known as red, or stasis thrombi.

Postmortem Clots

Q. Describe the appearance of postmortem clot.

Determination of whether a clot is formed during life (antemortem thrombi) or after death (postmortem clot) is important in a medical autopsy and in forensic pathology. Differences between antemortem venous thrombi and postmortem clots are listed in **Table 5.5**. After death, the red blood cells settle and produce two layers.

- **Lower layer:** It contains **many RBCs**, which have settled by gravity → forms a dark red lower portion. This has a reddish and gelatinous appearance which resembles **currant jelly**.
- **Upper layer:** It is **poor in cells and is yellow-white**. It is firm representing coagulated plasma without red blood cells. It is called **chicken fat** because of its color and consistency.

Fate of Thrombus (Fig. 5.14)

Q. Describe fate of thrombus.

1. Dissolution/lysis of thrombi without any consequences.
   - **Recent thrombi may totally disappear** due to activation of fibrinolysis.
   - **Old thrombi** are **more resistant to lysis**.

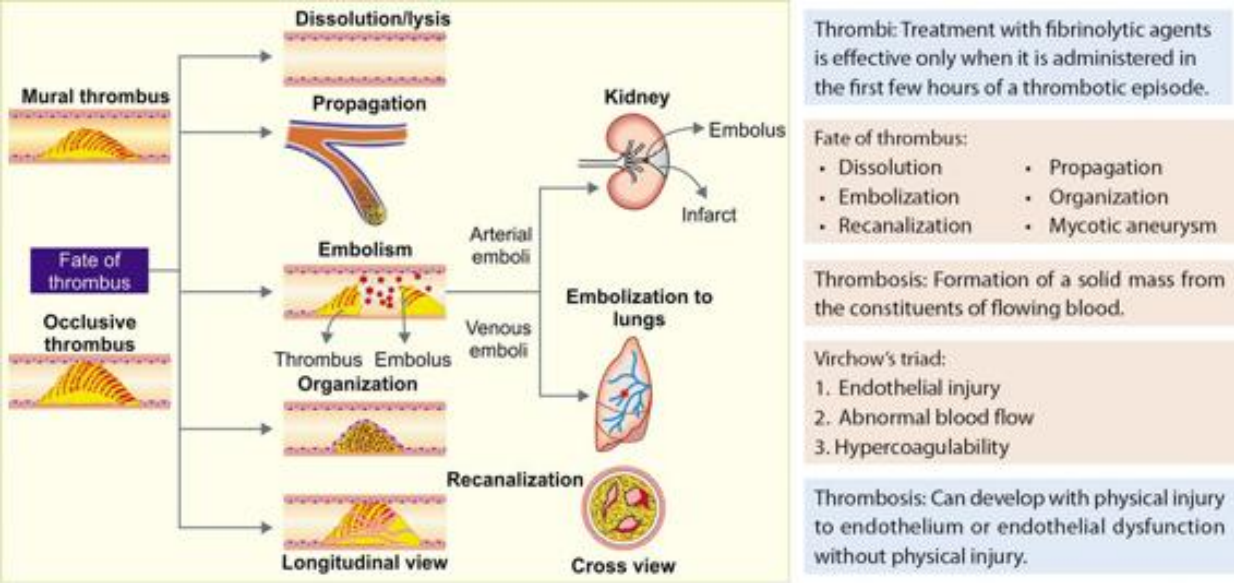


Fig. 5.14: Fate of thrombus.

TABLE 5.5: Differences between antemortem venous thrombi and postmortem clots.

Characteristics	*Antemortem venous thrombi*	*Postmortem clots*
Attachment to vessel wall	Focally and firmly attached	Not attached
Consistency	Dry, granular, firm and friable	Gelatinous, soft and rubbery
Shape	May or may not fit the vascular contours	Have the shape of the vessel in which it is found
Appearance	Alternate dark and white areas	Currant jelly and chicken fat appearance
Lines of Zahn	Present	Absent
Mechanism	Changes in blood flow (stasis) and hypercoagulability	Occurs in stagnant blood in which gravity fractionates the blood

2. **Propagation of thrombi:** It is the **process in which thrombi grow and increase in size**. The thrombus which was initially **mural, may become occlusive** thrombus. The propagating portion of a thrombus is poorly attached to the wall and therefore, **prone to fragmentation and embolization**.
   - **Arterial thrombi grow retrograde** from the point of attachment
   - **Venous thrombi extend in the direction of blood flow.**
3. **Embolization:** Thrombi may get detached from its site of origin and form emboli. These emboli can travel to other sites through the

circulation and lodge in a blood vessel away from the site of thrombus formation. The **consequences depend on the site of lodgment**. Large venous thrombi may get detached and travel to the pulmonary circulation to the lungs as pulmonary emboli.

4. **Organization:** If thrombi are not dissolved (either spontaneously or by therapy), these older thrombi become organized **by the ingrowth of endothelial cells, smooth muscle cells, and fibroblasts**. Small, organized thrombi may be incorporated into the vessel wall.
5. **Canalization/recanalization:** New lumen/channels lined by endothelial cells may form in an organized thrombus. These capillary channels may form thoroughfare channels and can re-establish the continuity of the original lumen.
6. **Mycotic aneurysm** (*refer* page 132)**:** Rarely, the central region of the thrombi may undergo enzymatic digestion due to lysosomal enzymes released from trapped leukocytes and platelets. If bacteremia develops, these thrombi may become infected and produce an inflammatory mass. This region of the vessel becomes weak and can produce mycotic aneurysm.

Venous thrombosis: Stasis is the major cause.

Antiphospholipid syndrome: Associated with
- Venous thrombosis
- Recurrent abortion
- Antibody to lupus

Mural thrombus: Occurs in heart chambers or in the aortic lumen.

Lines of Zahn: They help to distinguish antemortem thrombus from postmortem clot.

Rarely, a large round thrombus may form on the mitral valve and obstruct the lumen of the valve.

Agonal thrombi: Thrombi developing on one or both ventricles shortly before death.

Venous thrombus: Deep vein of the lower extremity (90% of cases) is the most common site.

Attachment: Thrombi are focally attached to the underlying surface.

Postmortem clot: Currant jelly and chicken fat appearance.

VENOUS THROMBOSIS (PHLEBOTHROMBOSIS)

Q. Write short note on phlebothrombosis and discuss the causes and pathogenesis of venous thrombosis.

Veins Involved

Most commonly superficial or deep veins of the leg are involved.

Superficial Venous Thrombi

- **Site:** They develop in the **varicosities** involving **saphenous veins**.
- **Effects:** It can cause **local congestion, swelling (edema), pain, and tenderness**. The local edema and impaired venous drainage **predispose** the overlying **skin to infections** from slight trauma and to the development of **varicose ulcers**. Embolization is very rare.

Deep Venous Thrombosis (DVT)

- Lower extremity DVTs are found in **association with venous stasis and hypercoagulable states**.
- **Sites:** Larger veins in the leg at or above the knee (e.g., popliteal, femoral, and iliac veins).
- **Effects:**
  - Even though DVTs can cause local pain and edema, the venous block produced by them is usually rapidly balanced by the development of collateral channels.
  - More prone to embolization into the lungs and produce pulmonary infarction. About 50% of DVTs are asymptomatic and are detected after embolization.

Pathogenesis of DVT (Phlebothrombosis)

Q. Discuss the causes and pathogenesis of venous thrombosis/phlebothrombosis.

Deep venous thrombosis is caused by the same etiological factors that favor arterial and cardiac thrombosis. These include endothelial injury, stasis, and a hypercoagulable state.

Different stages in the development of DVTs **(Fig. 5.15)** are:

- **Primary platelet thrombus:**
  - Damage to the intima of the vein causes adhesion of platelets at damaged site → platelets aggregate to form **pale platelet thrombus**.
  - Venous stasis favors accumulation of coagulation factors, which is activated to form fibrin.
- **Coralline thrombus:** The fibrin and thrombin formed encourages further accumulation of platelets. The **platelets along with fibrin form upright laminae** growing across the stream. Between the laminae, stasis promotes further deposition of fibrin with trapped RBC and WBCs. This **produces alternate layers of fused platelets and fibrin with trapped blood cells**. The contraction of fibrin produces a **characteristic ribbed (ripple) appearance** on the surface of thrombus. These **raised platelet ridges are known as lines of Zahn**.
- **Occluding thrombus: Further growth of thrombus** progressively **occludes the lumen** of the vein and forms occluding thrombus.
- **Consecutive clot:** Occlusive thrombus **stops the blood flow**. Since, thrombi can develop only in the streaming blood, the **blood column beyond the occluding thrombus clots to form a consecutive clot**. Thereafter, the consecutive clot may be halted and endothelialized or it can spread (**propagate**).
- **Propagated clot:** There are **two methods** of propagation **(Figs. 5.16A and B):**
  - **Thrombus formation in each tributary:** The consecutive clot when reaches the entrance of venous tributary may form another coralline thrombus over the clot. This causes occlusion of opening of tributary. A consecutive clot will again form up to the opening of next venous tributary. Thus, **several thrombi with associated consecutive clot** may be formed.

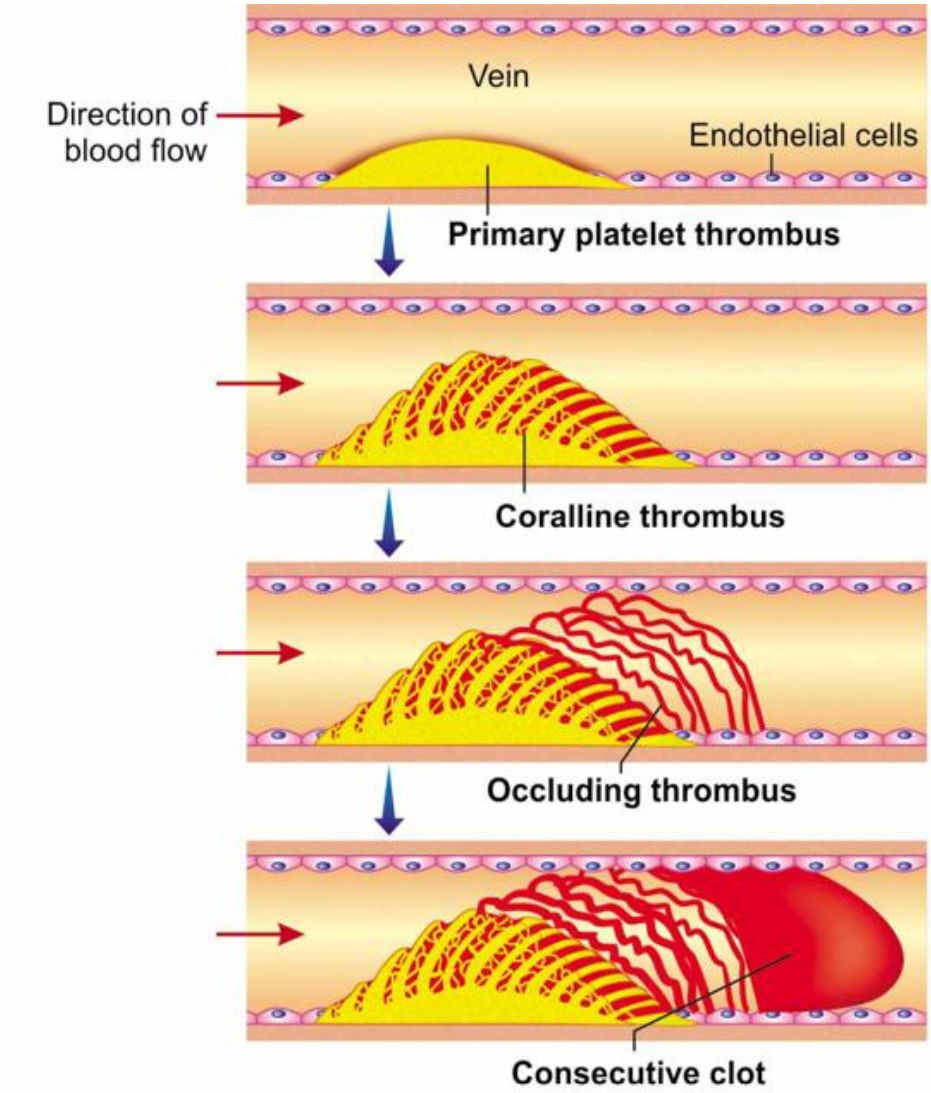
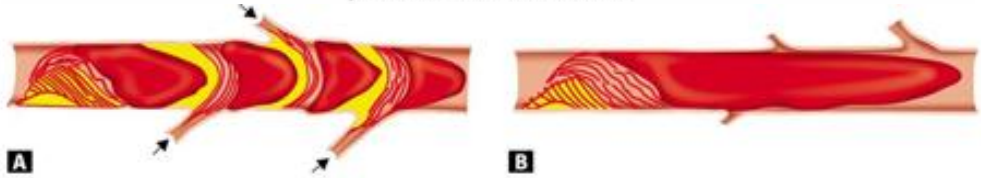


Fig. 5.15: Various stages in the pathogenesis of phlebothrombosis.



Figs. 5.16A and B: Propagation of venous thrombi. (A) Thrombus formation in each tributary; (B) Clotting en mass beyond the thrombus.

- **Clotting en mass beyond the thrombus:** Another method of propagation is **formation of long column of consecutive clot** attached to only one thrombus. These consecutive **clots may break and produce fatal massive pulmonary embolism**.

Thrombophlebitis

Inflammation of the wall of vein causes damage to the endothelium and **may lead to thrombus formation.** The thrombus formed is firmly attached to the wall of the vein and do not embolize. Sterile inflammation may be produced by direct trauma, chemicals or ionizing radiation. Bacterial inflammation of veins may be produced in the veins near the infected areas.

Thrombophlebitis migrans (migratory thrombophlebitis or Trousseau syndrome)

- **Characterized by recurrent thrombotic episodes** involving the superficial and deep veins, especially of the extremities.
- May develop as a **complication of deep-seated cancers** such as cancer of pancreas (tail and body), lung, stomach, and female genital tract.
- First described by Trousseau who had pancreatic cancer, when he noticed it on himself and suggested that it is a **sign of visceral cancer**. It is known as Trousseau's syndrome.

Consequences of Thrombi

It depends on the site of the thrombosis.

- **Obstruction of involved vessel:** Thrombi can cause obstruction of involved arteries and veins.
  - **Arterial thrombi:** They may cause **infarctions** in the region supplied by the involved vessel. Occlusion at certain locations (e.g., a coronary artery) can be life-threatening.
  - **Venous thrombi:** Small venous thrombi may cause no symptoms. Larger thrombi can cause **congestion and edema in region distal to obstruction** by thrombus. Forced dorsiflexion of the foot produces tenderness in the calf associated with DVT and is known as **Homan sign**.
- **Embolization:** Arterial, cardiac and venous thrombi can undergo fragmentation and detach to form emboli. It is the major complication and these are thromboemboli. The consequences of embolism depends on: (1) site of lodgement of emboli, (2) tissue affected and (3) source of thromboemboli.

- **Arterial and cardiac thromboemboli:** The most common sites of lodgment of emboli are the brain, kidneys, and spleen because of their rich blood supply. The various effects are mentioned in page 129.
- **Venous emboli:** They may lodge in the lungs causing various consequences of **pulmonary embolism** (*refer* page 127-8).

Conditions associated with both arterial and venous thrombi are listed in **Box 5.3.**

Superficial venous thrombi:
- Varicose ulcers
- Predisposition to infection of the overlying skin
- Embolization very rare

Deep vein thrombosis: More prone to embolization to lungs.

Primary platelet thrombus: Adhesion and aggregation of platelets at the site of intimal injury is similar to snowdrift during a snowstorm or flies sticking to an oily paper.

Coralline thrombus: Laminae anastomose to form a structure which resembles coral (sea weed).

Homan sign: Forced dorsiflexion of the foot produces tenderness in the calf when there is DVT.

BOX 5.3: Conditions associated with both arterial and venous thrombi.

- Homocysteinuria
- Disseminated intravascular coagulation (DIC)
- Cancer
- Antiphospholipid antibody
- Heparin-induced thrombocytopenia
- Paroxysmal nocturnal hemoglobinuria (PNH)
- Hyperhomocysteinemia
- Essential thrombocythemia
- Polycythemia vera
- Dysfibrinogenemia

Migratory thrombophlebitis:
- Known as Trousseau's syndrome
- Recurrent venous thrombosis
- Complicates deep-seated cancers (e.g., pancreas, lung)

Complications of arterial and cardiac thrombi:
- Fragmentation and embolization
- Common sites of embolization: Organs with rich blood supply, i.e., brain, kidneys, and spleen

EMBOLISM

CC: Define and describe embolism and its causes and common types.

Q. Define embolism. Mention the different types of embolism /embolus with examples. Add a note on air embolism and fat embolism.

Definition: An embolus is a **detached intravascular** solid, liquid, or gaseous **mass** that is **transported in the blood to a site distant from its point of origin**.

Types of Emboli

Q. Mention different types of emboli.

Classification: Depending on:

1. **Physical nature of the emboli:**
   - **Solid: Thromboemboli**, atheromatous material, **tumor emboli**, tissue fragments, bacterial clumps or parasites, foreign bodies.
   - **Liquid: Fat**, bone marrow and amniotic fluid.
   - **Gaseous: Air** or other gases.
2. **Whether infected or not**
   - **Bland:** Sterile.
   - **Septic:** Infected.
3. **Source (Fig. 5.15):** The emboli may be **endogenous** (form within the body) or **exogenous** (introduced from outside).
   - **Cardiac emboli:** Usually they arise from left side of the heart. Example, emboli from: (1) atrial appendage, (2) left ventricle in **myocardial infarction**, (3) vegetations on the valves in **infective endocarditis**.
   - **Vascular emboli:**
     - **Arterial** emboli (e.g., atheromatous plaque, aneurysms).
     - **Venous** emboli (e.g., deep vein thrombus, tumor emboli).
     - **Lymphatic** emboli (e.g., tumor emboli).
4. **Flow of emboli.**

Q. Write short note on paradoxical embolism.

Paradoxical emboli: They are rare and the **emboli originate** in the **venous circulation** and **bypass the lungs** by traveling **through a right-to-left shunt** such as an atrial septal defect (incompletely closed/patent foramen ovale) or interventricular defect. Then, they **enter the left side of the heart** and **block the blood flow to the systemic arteries** (*refer* page 481).

Q. Write short note on retrograde embolism.

Retrograde emboli: Emboli, which **travel against the flow of blood** are known as retrograde emboli. Example, **prostatic carcinoma metastasis to the spine**. It occurs through retrograde spread via intraspinal veins which carry the emboli from large thoracic ducts and abdominal veins due to increased pressure in the body cavities (e.g., during coughing or straining).

Embolus: Detached intravascular mass transported to a site distant from its point of origin.

Unless otherwise specified, emboli should be considered thrombotic in origin and the process is known as thromboembolism.

PULMONARY EMBOLISM

Q. Write short note on pulmonary embolism.

Definition: Pulmonary embolism (PE) is defined as an **embolism in which emboli occlude pulmonary arterial tree**.

Site of Origin of Emboli (Fig. 5.17)

- **Deep leg veins:** DVTs are the source in more than 95% of cases of pulmonary emboli. Deep leg veins include **popliteal, femoral or iliac veins**.
- **Other sites:** Pelvic veins, vena cava.

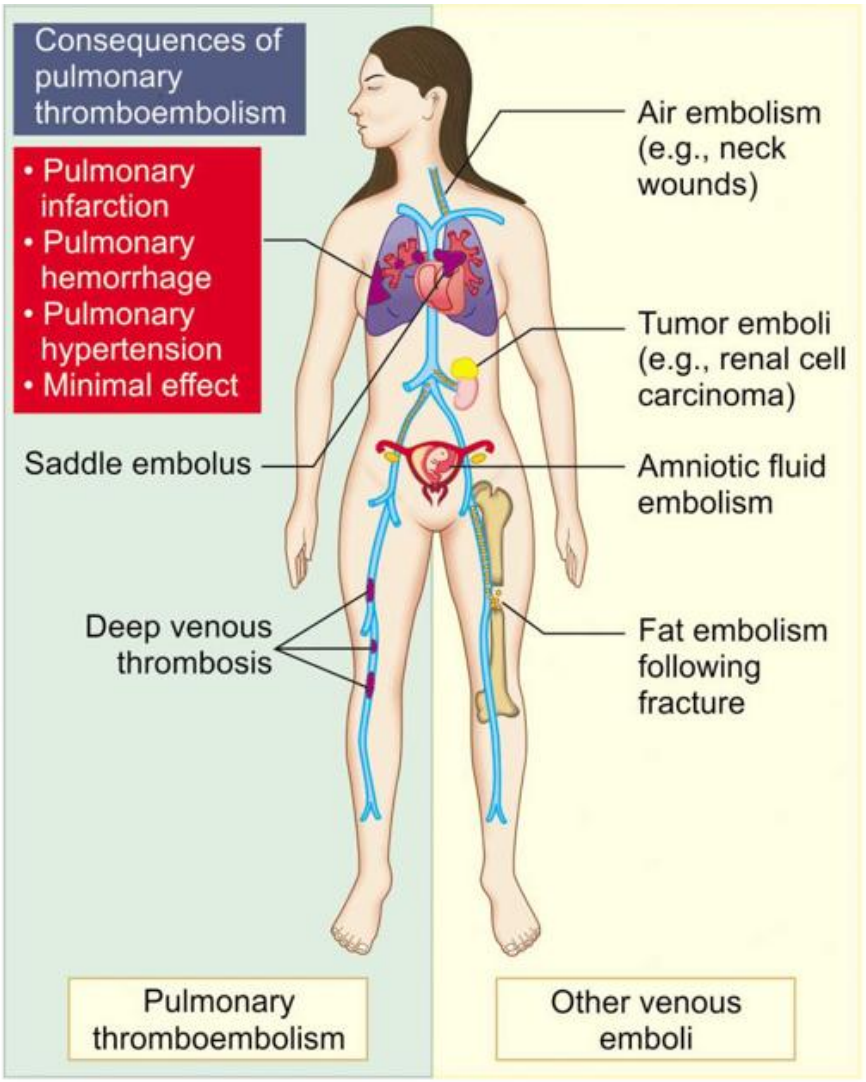


Fig. 5.17: Sources and effects of venous emboli.

Risk of pulmonary embolism: Major risk factor is after surgery. The risk increases with advancing age, obesity, prolonged operative procedure, postoperative infection, cancer, and pre-existing venous disease.

Mechanism: DVTs undergo fragmentation → these thromboemboli are carried through progressively larger vascular channels → into the right side of the heart → right ventricle → they enter into the pulmonary arterial vasculature.

Fate of Pulmonary Embolism

Fate depends on the size of the embolus.

1. **Resolution or organization: Small** pulmonary **emboli** may travel into the smaller branches of pulmonary arteries and may **resolve completely**. Most (60–80%) of them are clinically **silent**. With passage of time they become **organized** and are incorporated into the wall of pulmonary vessel.
2. **Massive pulmonary embolism:** When emboli **obstruct 60% or more of the pulmonary circulation**, it is known as massive pulmonary embolism.

Q. Write short note on saddle embolism.

- **Saddle embolus:** It is a **large pulmonary embolus** which **lodges at the bifurcation of the main pulmonary artery**. It produces acute massive obstruction of the blood flow to both lungs.
- **Effects:**
  - **Acute right ventricular failure.**
  - **Shock:** Right ventricular failure → reduction in left ventricular cardiac output → sudden severe hypotension (or shock) → may result in sudden death.

3. **Multiple recurrent pulmonary emboli:** These **may fuse to form** a **single large mass.** Usually, the patient who has had one PE is likely to have recurrent emboli.
4. **Paradoxical embolism** (*refer* page 127).

Consequences (Fig. 5.17)

1. **Pulmonary infarction**:
   - Most (about 75%) small pulmonary emboli do not produce infarcts. However, an embolus can **produce infarction in the patients with congestive heart failure or chronic lung disease**.
   - **Gross (Fig. 5.20B):**
     - **Type:** Usually **hemorrhagic type, because of blood supply to the infarcted (necrotic) area by the bronchial artery**.
     - **Shape: Pyramidal** in shape with the base of the pyramid on the pleural surface. When the blood in the infarcted area is resorbed, the center of the infarct becomes pale.

- **Fate:** Granulation tissue grows from the edges of the infarct and results in organization of infarct and forms a **fibrous scar**.
- **Clinical features:** Cough, stabbing pleuritic pain, shortness of breath, and occasional hemoptysis. Pleural effusion is a common complication and pleural fluid is often blood stained.

2. **Pulmonary hemorrhage: Obstruction of medium-sized pulmonary arteries** by emboli and subsequent rupture of these vessels can **result in pulmonary hemorrhage.**
3. **Pulmonary hypertension: Multiple recurrent pulmonary emboli → may cause mechanical blockage of the arterial bed → result in pulmonary hypertension → right ventricular failure**.
4. **Minimal effect:** Obstruction of **small end-arteriolar branches** of pulmonary artery by emboli usually **neither produces hemorrhage nor infarction**.

Pulmonary thromboembolism: Majority of the cases the source is femoral veins.

Paradoxical embolism: Embolus passes through an interatrial/interventricular defect and gains access to the systemic circulation.

Pulmonary infarction: It is rare, because lung has a dual blood supply by the bronchial arteries and the pulmonary artery.

Pulmonary embolism: Only 10% of emboli cause pulmonary infarction.

Pulmonary infarct:
- Uncommon in the young
- About 3/4 affect lower lobes
- Pyramidal in shape with apex pointing toward the hilus of the lung.

Pulmonary embolism: Patient who has had one PE is at a high-risk of developing another one.

SYSTEMIC THROMBOEMBOLISM

Definition: It is defined as an embolism in which **emboli occlude systemic arterial circulation**. Systemic arterial embolism **usually produces infarcts** in the region supplied by the involved vessel.

Sources of Systemic Emboli (Fig. 5.17)

- **Heart:** Most common source of thromboemboli.
  - **Intracardiac mural thrombi:** Most common source. Examples:
    - Myocardial infarct of left ventricular wall
    - In mitral stenosis, dilatation of left atrium and atrial fibrillation predisposes to thrombus and embolization.
  - **Paradoxical emboli:** Rare source
  - **Valvular source:** Examples, **bacterial endocarditis** (valvular vegetation from aortic or mitral valves) or prosthetic valves
- **Blood vessels:** Thrombi on **ulcerated atherosclerotic plaques** or from aortic **aneurysms**
- **Unknown origin**.

Consequences

- The arterial emboli can travel to a wide variety of sites. This is in contrast to venous emboli, which lodge mainly in one vascular bed namely the lung.
- The arterial emboli tend to pass through the progressively narrow arterial lumen and lodge at points where the vessel lumen narrows abruptly (e.g., at bifurcations or in the area of an atherosclerotic plaque).
- **Fate of thromboembolus at the site of arrest:**
  - **Propagation and obstruction:** Thromboemboli may grow (propagate) locally at the site of arrest and produce severe obstruction leading to **infarction of the affected** tissues **(Fig. 5.18)**.
  - **Fragmentation and lysis**.

Major Sites Affected by Arterial Thromboemboli (Fig. 5.18)

1. **Lower extremity** (75%): Embolism to an artery of the leg may produce **gangrene**.
2. **Brain:** Arterial emboli to the brain may produce **ischemic necrosis** in the brain (strokes).
3. **Intestine:** Emboli in the mesenteric vessels may produce **infarction of the bowel**.
4. **Kidney:** Renal artery embolism may cause small peripheral **infarcts** in the kidney.

5. **Blood vessels:** Emboli originating from bacterial vegetation may cause inflammation of arteries and produce **mycotic aneurysm**.
6. **Other sites:** Spleen and upper extremities are less commonly affected.

Systemic thromboembolism: Majority of the cases the source is left side of the heart.

Source of cardiac mural thrombi:
1. Myocardial infarction of left ventricle (2/3)
2. Left atrial dilation and fibrillation (1/4)

FAT AND MARROW EMBOLISM

Q. Describe fat embolism.

Fat and marrow embolus **consists of microscopic globules of fat with or without bone marrow** elements. Release of these elements into the circulation produces fat embolism.

Causes

- **Trauma to adipose tissue with fracture:** Severe trauma to adipose tissue, particularly accompanied by **fractures of bone** release fat globules or fatty marrow (with or without associated hematopoietic marrow cells) into ruptured blood vessels. Fat embolism occurs in about 90% of individuals with **severe skeletal injuries**, but less than 10% of them have clinical findings.
- **Soft tissue trauma and burns**.
- **During vigorous cardiopulmonary resuscitation**.

Manifestation

In **most of the cases** it is **asymptomatic**. **Sometimes**, it may manifest as potentially **fatal fat embolism syndrome**.

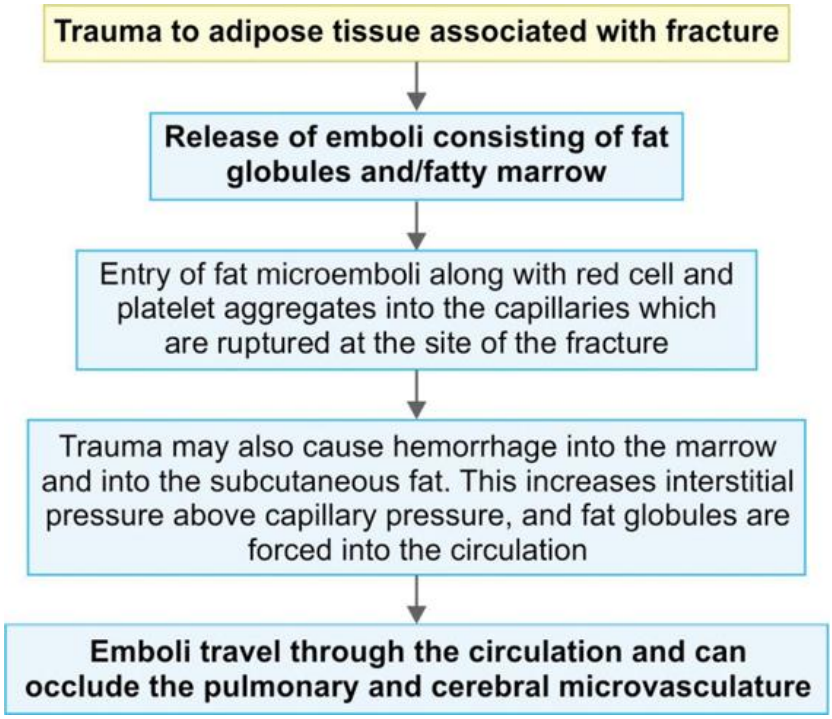
Fat embolism syndrome: It is the term applied when the patients develops **symptoms due to severe fat embolism**. It develops in only minority of patients.

Pathogenesis

Fat embolism syndrome involves both mechanical obstruction and biochemical injury.

Mechanical Obstruction (Flowchart 5.5)

Flowchart 5.5: Pathogenesis of fat embolism by mechanical obstruction.



Biochemical Injury (Flowchart 5.6)

- The chemical composition of the fat present in the lung in fat embolism is different from that in adipose tissue. The mechanical obstruction alone cannot explain this difference. So, pathogenesis probably involves mechanical obstruction associated with biochemical injury.

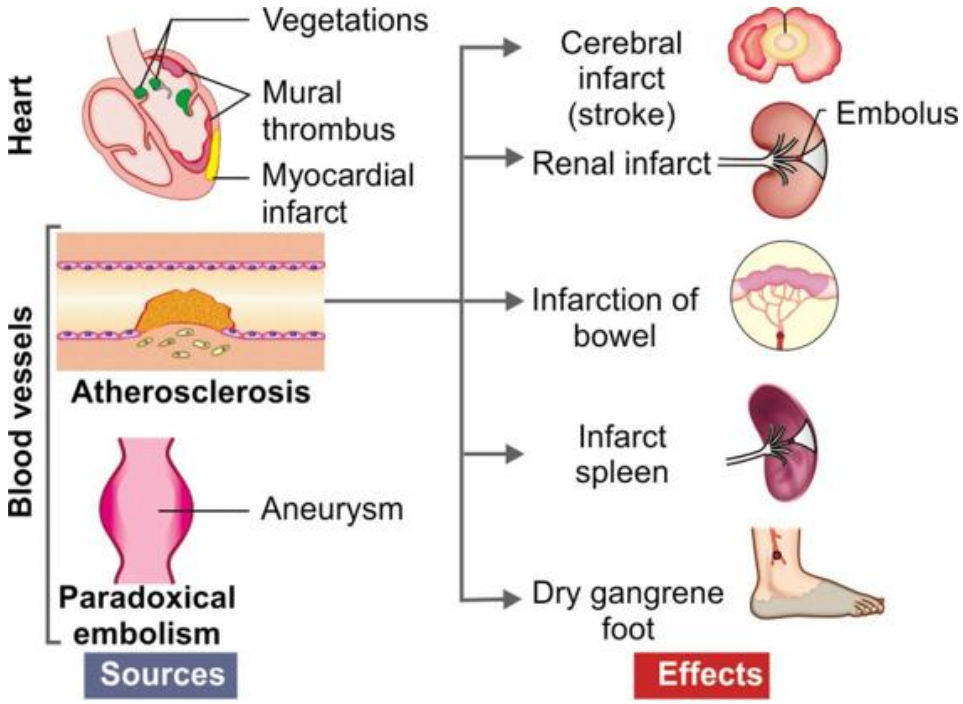
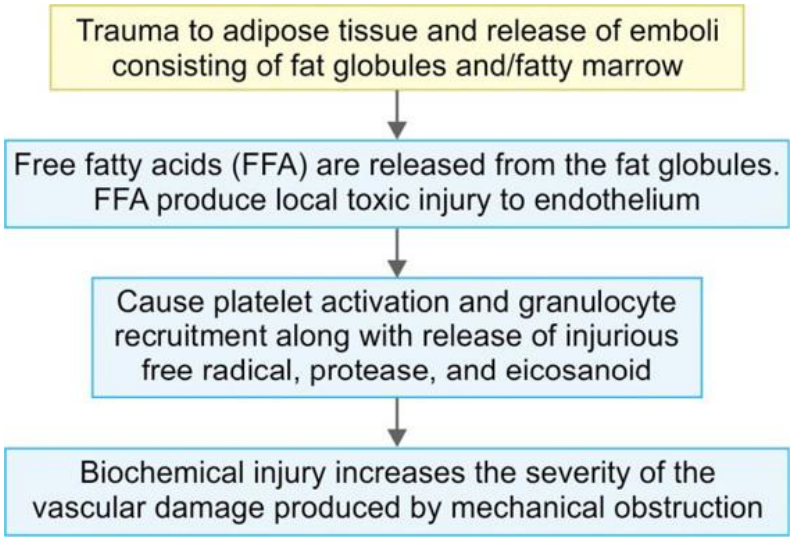


Fig. 5.18: Common sources and effects of systemic arterial emboli. It usually arises from the left side of the heart or from major arteries. Usual consequence is either infarction or gangrene at the site of lodgment.

Flowchart 5.6: Pathogenesis of fat embolism by biochemical injury.



Consequences of Fat Embolism

It depends on the size and quantity of fat globules and whether the emboli are arrested in the pulmonary or systemic circulation. The paradoxical fat

emboli may reach systemic circulation (e.g., through patent foramen ovale) and gets deposited in brain, kidney, etc.

- **Sites of arrest of fat emboli:**
  - Emboli in the venous side lodge **in the lungs**.
  - **If emboli pass into systemic circulation**, they may be arrested in **brain**, **kidneys** and other organs.
- **Autopsy findings: Numerous fat globules** can be found impacted **in the microvasculature** of the lungs (in pulmonary emboli) and brain and sometimes other organs (in systemic emboli).
  - **Lung:** The lungs typically show the changes of **acute respiratory distress syndrome**.
  - **Brain:** The lesions include **cerebral edema, small hemorrhages**, and occasionally microinfarcts.
- **Demonstration of fat embolism:** Fat is dissolved during routine tissue preparations by the solvents (xylene/xylol) used in paraffin embedding. The microscopic demonstration of fat microglobules requires **frozen sections and special stains for fat** (e.g., Sudan III and IV, Oil Red O, and osmic acid).

Clinical Presentation

The most severe form of fat embolism syndrome may be fatal.

- **Time of development:** It develops **1 to 3 days after the traumatic injury**.
- **Respiratory symptoms:** These include sudden onset of **tachypnea, dyspnea and tachycardia** which may lead to **respiratory failure**.
- **Neurologic symptoms:** These include **irritability, restlessness,** delirium and coma.
- **Hematological findings:**
  - **Thrombocytopenia:** Rapid onset of thrombocytopenia produces **diffuse petechial rash** (found in 20–50% of cases) and may be a useful **diagnostic** feature.
  - **Anemia**: It is due to aggregation of red cells and/or due to hemolysis.
- **Chest radiography:** It shows diffuse opacity of the lungs → may progress to an **opacification of lungs** (whiteout)—characteristic of **acute respiratory distress syndrome**.

Fat embolism: Commonly develop following fracture of long bones.

Fat embolism: Fatty acids from fat globules produce local toxic injury to endothelium.

Special stains for fat: Sudan III, Sudan IV, Oil Red O, and osmic acid.

Anemia in fat embolism: Due to aggregation of RBCs and hemolysis.

Fat embolism syndrome—clinical features: Dyspnea, petechial rash, irritability and restlessness.

Fat embolism: Fatal only in 10% of cases.

Thrombocytopenia in fat embolism: Due to platelet adhesion by fat globules.

AIR EMBOLISM

Q. Write short note on air/gas embolism/Caissons disease/decompression sickness.

Air embolism occurs when **air is introduced into venous or arterial circulation**.

Causes

- **Trauma/injury:** Air may enter the venous circulation through **neck wounds and chest wall injury**.
- **Surgery/invasive procedures:** These include invasive surgical procedures such as **thoracocentesis**, punctures of the great veins during **obstetric or laparoscopic procedures**, into the **coronary artery during bypass surgery**, cerebral circulation by neurosurgery in the "sitting position", or hemodialysis.
- **Criminal abortion**.

Amount of air required: It is usually **more than 100 cc** to have a clinical effect of air embolism.

Mechanism: In the circulation, air/gas bubbles tend to coalesce to form frothy masses → which physically obstruct vascular blood flow in the right side of the heart.

Microscopy: Air bubbles are seen as empty spaces in capillaries and small vessels of the lung/brain.

Decompression Sickness

It is a form of gas embolism and may be acute or chronic.

Acute Decompression Sickness

Cause: It develops when individuals are **exposed to sudden decrease in atmospheric pressure**. Risk factors include:

- Individuals when exposed to high atmospheric pressure, such as **scuba and deep-sea divers and underwater construction workers** (e.g., tunnels, drilling platform construction), during rapid ascent to low pressure.
- Individuals in **unpressurized aircraft** during rapid ascent.
- **Sport diving**.

Mechanism of acute decompression sickness is shown **Flowchart 5.7.**

Chronic Decompression Sickness

Caisson Disease

- A chronic form of decompression sickness is known as **Caisson disease** (named for the pressurized vessels/diving bells used in the bridge construction).
- Workers in these pressurized vessels may develop both acute and chronic forms of decompression sickness.
- **Characteristic features:** Avascular necrosis—gas embolus in vessel produces obstruction to blood flow → causes multiple foci of **ischemic (avascular) necrosis of bone.** The more commonly involved bone includes the head of the femur, tibia, and humerus.

Air embolism: More than 100 cc of air is required to have clinical effect.

Nitrogen has an affinity for adipose tissue. Hence, obese individuals are at increased risk of developing decompression sickness.

Treatment of acute decompression sickness is by placing the individual in a high pressure chamber. This will force the gas bubbles back into solution.

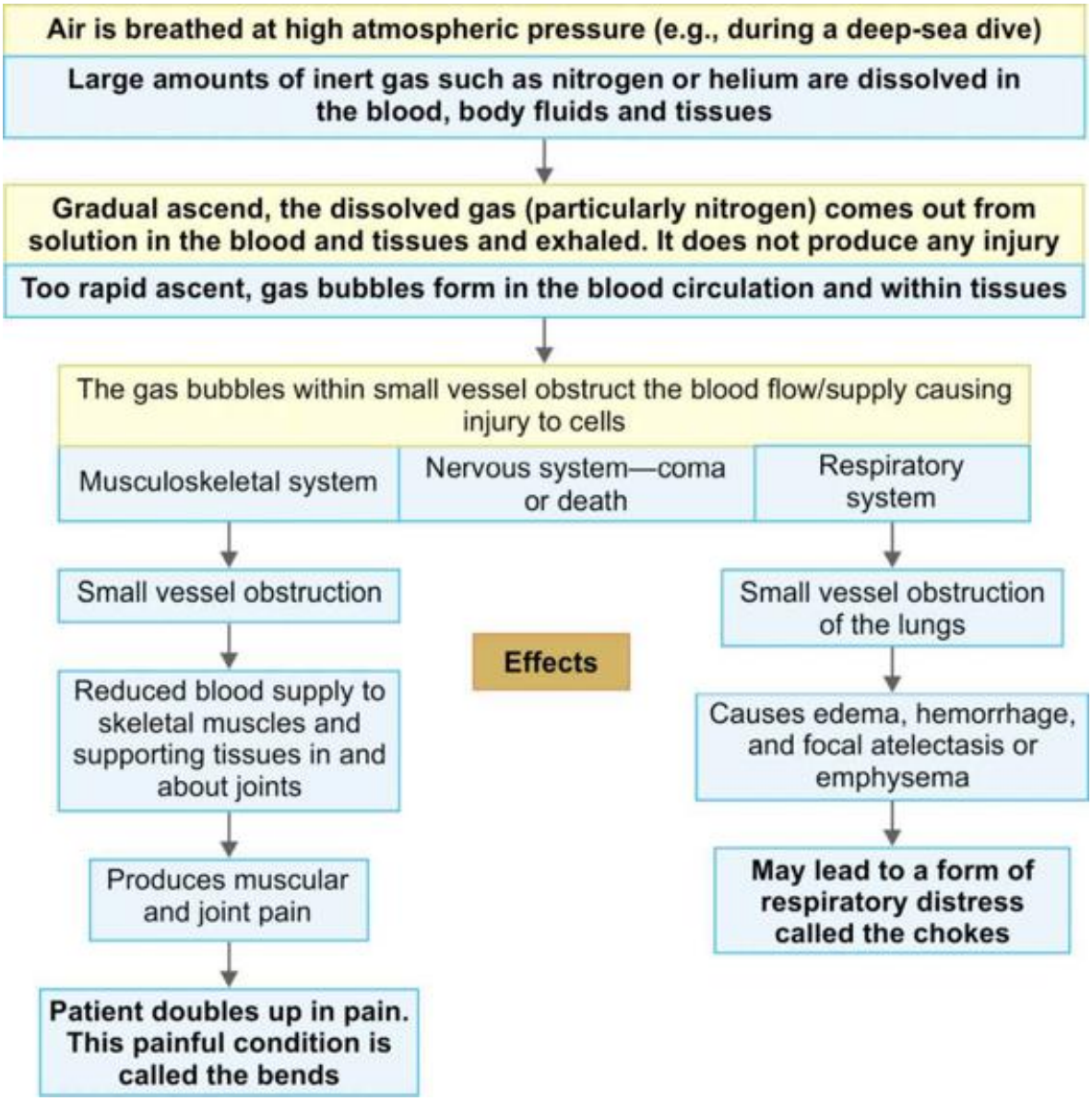
Decompression sickness: Bends and chokes-nitrogen gas bubbles occlude lumen of blood vessels.

AMNIOTIC FLUID EMBOLISM

Q. Write short note on amniotic fluid embolism.

Amniotic fluid embolism **develops when amniotic fluid along with fetal cells and debris enter the maternal circulation**. The entry occurs through open (ruptured) uterine and cervical veins or a tear in the placental membranes **(Fig. 5.19)**.

Flowchart 5.7: Mechanism of acute decompression sickness.



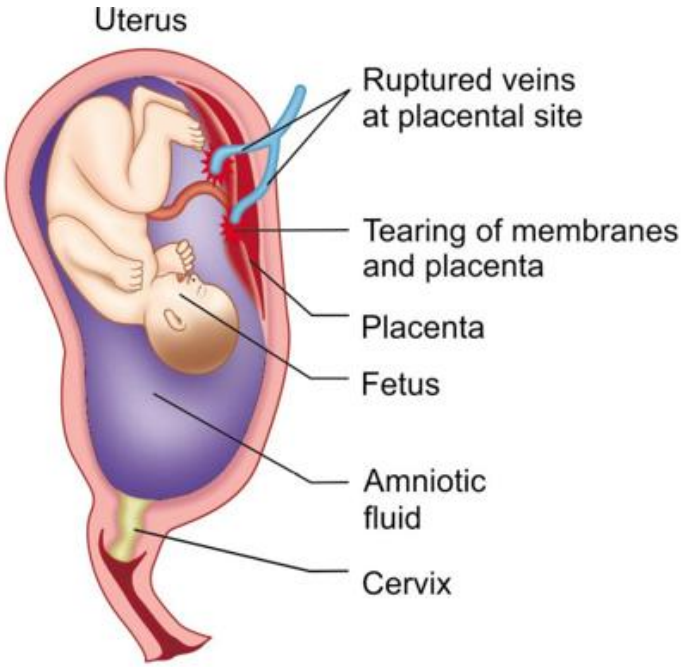


Fig. 5.19: Amniotic fluid embolism.

Time of occurrence: It is a rare maternal threatening complication, which occurs **at the end of labor and the immediate postpartum period**.

Consequences: From the venous circulation, amniotic fluid emboli enter the right-side of the heart and finally rest in pulmonary circulation. Amniotic fluid has a high thromboplastin activity and initiates a potentially **fatal disseminated intravascular coagulation (DIC).**

MORPHOLOGY

- **Amniotic fluid contents within pulmonary vasculature**: Amniotic fluid emboli are composed of squamous cells shed from fetal skin, lanugo hair, fat from vernix caseosa, and mucin derived from the fetal respiratory or gastrointestinal tract.
- **Other findings:** These include marked pulmonary edema, **diffuse alveolar damage**, and features of DIC.

Clinical Features

- **Abrupt onset:** It develops during immediate postpartum period, and is characterized by sudden onset of severe dyspnea, cyanosis, and neurologic impairment ranging from headache to seizures. Patient develops shock, coma and death.

- **Bleeding:** If the patient survives the initial acute crisis, patient develops bleeding due to disseminated intravascular coagulation (DIC).
- **Acute respiratory distress syndrome.**

Amniotic fluid embolism: Abrupt onset of dyspnea, hypotension and bleeding due to DIC—at the end of labor or immediate postpartum period.

MISCELLANEOUS PULMONARY EMBOLI

- **Foreign bodies:**
  - **Talc emboli**: It may occur in intravenous drug abusers who use talc as a carrier for illicit drugs.
  - **Cotton emboli:** It may occur due to cleansing of the skin by cotton prior to venipuncture.
- **Schistosomiasis:** The ova of schistosoma may gain entry into the circulation from bladder or gut and lodge in the lungs.
- **Tumor emboli:** It may occur during hematogenous dissemination of cancer.
- **Atheromatous emboli** (cholesterol emboli): Fragments of atheromatous plaque may embolize.
- **Platelet emboli:** During early stages of atherosclerosis, there is platelet deposition in the intimal surface of blood vessels. They may form platelet emboli.
- **Infective emboli:** In infective endocarditis, the vegetations seen on the diseased heart valves may become infected. These infected vegetations may break off and form infective emboli. Their effects are due to both emboli and infective agent that may weaken the wall of the vessel → may lead to the formation of a **'mycotic' aneurysm** (*refer* pages 124, 132 and 443). **Mycotic is a misnomer because the infective agent is usually bacterial, not fungal**.

Mycotic aneurysm: Mycotic is a misnomer because the infective agent is usually bacterial, not fungal.

INFARCTION

CC: Define and describe ischemia/infarction, its types, etiology, morphologic changes and clinical effects.

Q. Define Infarct.

Definition: An infarct is a **localized area of ischemic necrosis** caused by occlusion of either the arterial blood supply or the venous drainage. The process of **producing** infarct is known as **infarction**.

Mostly infarct is coagulative type of necrosis due to sudden occlusion of arterial blood supply. If the patient survives, the infarct heals with a scar. Common and important infarcts are shown in **Table 5.6.**

Causes of Infarction

Q. What are the causes of red and pale infarct?/Types of infarcts with examples.

- **Arterial causes:** Most important
  - **Occlusions of lumen:** It is the most common cause and may be due (1) **thrombus** or (2) **embolus(Fig. 5.18).**

TABLE 5.6: Common and important infarcts.

Organ/tissue affected	*Infarction*
Heart	Myocardial infarction
Brain	Cerebral infarction
Lung	Pulmonary infarction
Bowel/intestine	Intestinal infarct
Extremities	Gangrene

  - **Causes in the wall** (e.g., local vasospasm, **hemorrhage into an atheromatous plaque** or thromboangiitis obliterans).
  - **External compression of vessel:** Tumor.
- **Venous causes:**
  - **Occlusions of lumen** may be due (1) thrombus or (2) embolus
  - **Extrinsic vessel compression:** Tumor, torsion of a vessel (e.g., in testicular torsion or bowel volvulus), **strangulated hernia**.

Factors that Determine the Outcome of an Infarct

Q. Mention the factors that influence the development of an infarct.

The outcome of vascular occlusion may range from noor minimal effect to the death of a tissue or individual.The **major factors** that determine the outcome of infarct are:

1. **Nature of the vascular supply:**
   - **Dual/parallel blood supply:** Organs or tissues with double or parallel blood supply are **less likely to develop infarction**, e.g., lung, liver, hand and forearm.
   - **End-arterial blood supply:** Kidney and spleen has blood supply, which are end-arteries with little or no collaterals. Obstruction of vessels in these organs usually causes tissue death and **infarction**.
2. **Rate of occlusion: Slow occlusion** is **less likely to produce infarction than rapid occlusion**. This is because it provides time to develop alternate perfusion pathways.
3. **Vulnerability of tissue to hypoxia:**
   - **Neurons are highly sensitive to hypoxia**. They undergo necrosis even if the blood supply is occluded for 3 to 4 minutes.
   - In heart, myocardial cells are also quite sensitive to hypoxia, but less sensitive than neurons. Myocardial cells die after only 20 to 30 minutes of ischemia.
4. **Oxygen content of blood:** In a normal individual, partial obstruction of a small vessel may not produce any effect, but in a patient with anemia or cyanosis same may produce infarction.

Classification (Table 5.7)

Q. Write short note on different types of infracts, their causes and common sites of occurrence.

Q. Mention the organs involved in red and pale infract.

White/Pale Infarcts

They occur:

- With **arterial occlusions**
- In **solid organs**
- With **end-arterial circulation without a dual blood supply** (e.g., heart, spleen, and kidney)
- **Tissue with increased density** which prevents the diffusion of RBCs from adjoining capillary beds into the necrotic area.

Red/Hemorrhagic Infarcts

They occur:

- With **venous occlusions**, e.g., ovary.
- In **loose textured tissues**, e.g., lung: They allow red cells to diffuse through and collect in the necrotic zone.
- In **tissues with dual (double) blood supply**, e.g., lung, liver and small intestine: It allows blood flow from an unobstructed parallel blood supply into a necrotic zone.
- In **tissues previously congested** due to decreased venous drainage.
- Organs with extensive collateral circulation, e.g., **small intestine and brain**.
- **Reperfusion of infarcted area:** When **blood flow is re-established to a site of previous arterial occlusion** and necrosis, e.g., may occur in heart when the infarcted area is reperfused following coronary angioplasty of an obstructed coronary artery.

MORPHOLOGY

Q. Write short note on organs involved in pale and red infracts.

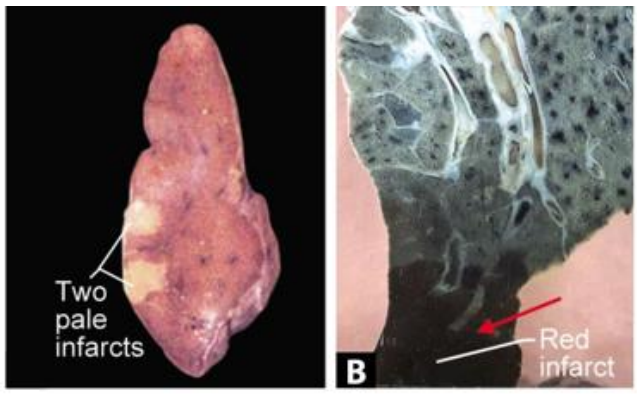
White/pale Infarcts

Organs involved includes **heart, kidneys, spleen,** and dry gangrene of the extremities.

Gross:

- Usually **wedge-shaped (Fig. 5.20A)**
- **Occluded blood vessel** is seen **at the apex** and the **periphery/surface** of the organ **forms the wide base**
- **Acute infarcts** are **poorly defined** and **slightly hemorrhagic**

- After 1 to 2 days, the infarct becomes soft, sharply demarcated, and light yellow in color



Figs. 5.20A and B: (A) Infarct spleen showing two wedge shaped pale/white infarct; (B) Lung showing sharply circumscribed, dark red to purple hemorrhagic infarct.

TABLE 5.7: Classification of infarct.

According to color	*Presence or absence of infection*	*According to the age of infarct*
White/pale (anemic)	Septic, when it is infected	Recent or fresh
Red (hemorrhagic)	Bland, when it is free of infection	Old or healed

- **Margins of infarct appear well-defined** because of narrow rim of congestion caused by inflammation
- **As time passes**, infarcts progressively become **paler** and more **sharply defined**.

Red/Hemorrhagic Infarcts

Gross (Fig. 5.20B): Appear as **sharply circumscribed** area of necrosis, **firm** in consistency and **dark red to purple** in color.

Microscopy of Infarct

- **Both pale and red infarct** characteristically shows **ischemic coagulative necrosis.**
- Microscopic changes of **frank necrosis** appear after about **4 to 12 hours**.
- **Acute inflammation cells** infiltrate the necrotic area from the viable margins all-round the infarcts within a few hours. It becomes **prominent within 1 to 2 days.**
- Followed by a **reparative process,** which begins **at the preserved margins**. The **necrotic cells** in infarcts and extravasated red cells are **phagocytosed by macrophages**.
- In tissues composed of stable or labile cells, **parenchymal regeneration** can occur at the periphery where stromal architecture is preserved.
- **Granulation tissue** may **replace the infarcted area** which **matures to** form **scar tissue.**
- If the infarct is large (e.g., in heart or kidney), the necrotic center may persist for months.

Septic infarctions: They may occur in two situations:

1. **Infection:** Infarct may get infected when it is **seeded by pyogenic bacteria**, e.g., infection of pulmonary infarct.
2. **Septic emboli:** They contain organisms and can produce septic infarct, e.g., vegetations of bacterial endocarditis may cause septic infarct of spleen.

The organisms present in a **septic infarct** convert infarct into a frank **abscess.**

Infarct: Localized area of ischemic necrosis caused by occlusion of either the arterial blood supply or the venous drainage.

Venous thrombosis: Infarcts caused by venous thrombosis usually occur in organs with a single efferent vein (e.g., testis and ovary).

Although venous thrombosis can cause infarction, mostly it produces congestion.

White/pale infarct: Seen in heart, kidney, spleen.

Hemorrhagic infarct: Seen in ovary, lung, small intestine.

In red/hemorrhagic infarcts there is bleeding into the necrotic area from adjacent arteries and veins which is not observed in pale infarct.

White infarct: Wedge-shaped with occluded vessel at the apex and periphery of the organ forms the base.

In contrast to other organs, the central nervous system infarction shows **liquefactive necrosis.** The necrotic focus may become cystic and filled with fluid and is referred to as a cystic infarct.

SHOCK

CC: Define and describe shock, its pathogenesis and its stages.

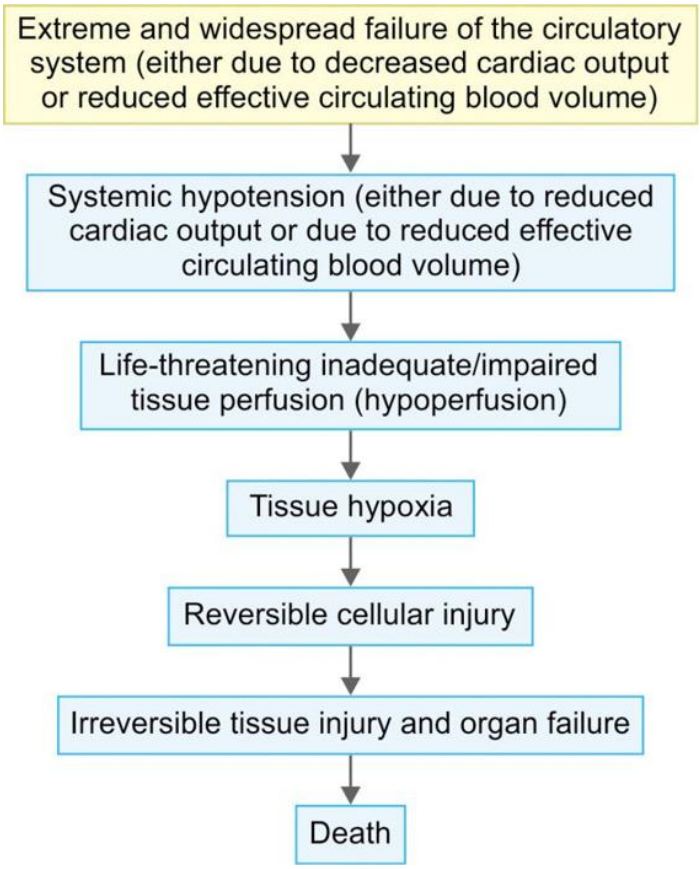
Q. Define shock. Enumerate the types of shock and discuss the pathogenesis and morphological changes in various organs in shock.

Q. Define, classify and enlist the various stages in the evolution of shock. Describe the pathogenesis of septic shock.

Introduction: Shock is the most common, important, and very serious medical condition. It is the final common pathway for several clinical events, which are capable of causing death. These events include severe hemorrhage, extensive trauma or burns, large myocardial infarction, massive pulmonary embolism, and severe microbial sepsis. Characteristic features of shock are presented in **Flowchart 5.8.**

Definition: Shock is a **pathological process** that results from inadequate tissue perfusion, **leading to cellular dysfunction and organ failure**.

Flowchart 5.8: Characteristic features of shock.



Classification

Q. Classify shock.

According to etiology (cause) shock can be classified into three major general categories **(Table 5.8)**.

Etiology and Pathogenesis

Q. Describe the etiology and pathogenesis of shock.

Q. Write short note on hypovolemic shock.

Hypovolemic Shock

Hypovolemic shock results from low cardiac output due to:

- **Loss of blood** (e.g., **massive hemorrhage**).
- **Loss of plasma** (e.g., **severe burns**).

Q. List the main types of shock with suitable examples.

TABLE 5.8: Major types of shock.

Types of shock	*Principal mechanisms*	*Clinical example*
Hypovolemic shock		
Loss of blood/plasma/fluid → decreased circulating blood volume → low cardiac output → hypotension, and shock	Loss of blood volume	Massive hemorrhage, trauma
	Loss of plasma volume	Massive burns
	Loss of fluid	Vomiting, diarrhea, severe gastroenteritis
Cardiogenic shock		
Direct myocardial damage or a mechanical abnormality of the heart → low cardiac output → reduced cardiac output and blood pressure	Myocardial damage	Myocardial infarction, ventricular rupture, arrhythmia, cardiac tamponade, pulmonary embolism
	Mechanical	• Valvular failure (stenosis or incompetence) • Hypertrophic cardiomyopathy • Ventricular septal defect
	Arsythmic	Ventricular arrhythmias

TABLE 5.8: Major types of shock.

Types of shock	*Principal mechanisms*	*Clinical example*
Septic shock	Peripheral vasodilation and pooling of blood. Endothelial activation/injury; leukocyte-induced endothelial damage, activation of cytokines, and disseminated intravascular coagulation	Overwhelming microbial infections [bacterial, (gram-negative sepsis, gram-positive septicemia) fungal, viral, rickettsial. Superantigens (e.g., toxic shock syndrome)]
Others		
Neurogenic shock	Result of loss of vascular tone and peripheral pooling of blood	Anesthetic accident or a spinal cord injury
Anaphylactic shock	Acute widespread systemic vasodilation and increased vascular permeability results in tissue hypoperfusion and hypoxia	IgE–mediated hypersensitivity reaction

Common sources of infection associated with septic shock: Pneumonia, peritonitis, pyelonephritis, abscess (especially intra-abdominal), primary bacteremia, etc.

- **Loss of fluid: Vomiting, diarrhea, severe gastroenteritis**, e.g., cholera.

The pathogenesis of hypovolemic shock is presented in **Flowchart 5.9**.

Cardiogenic Shock

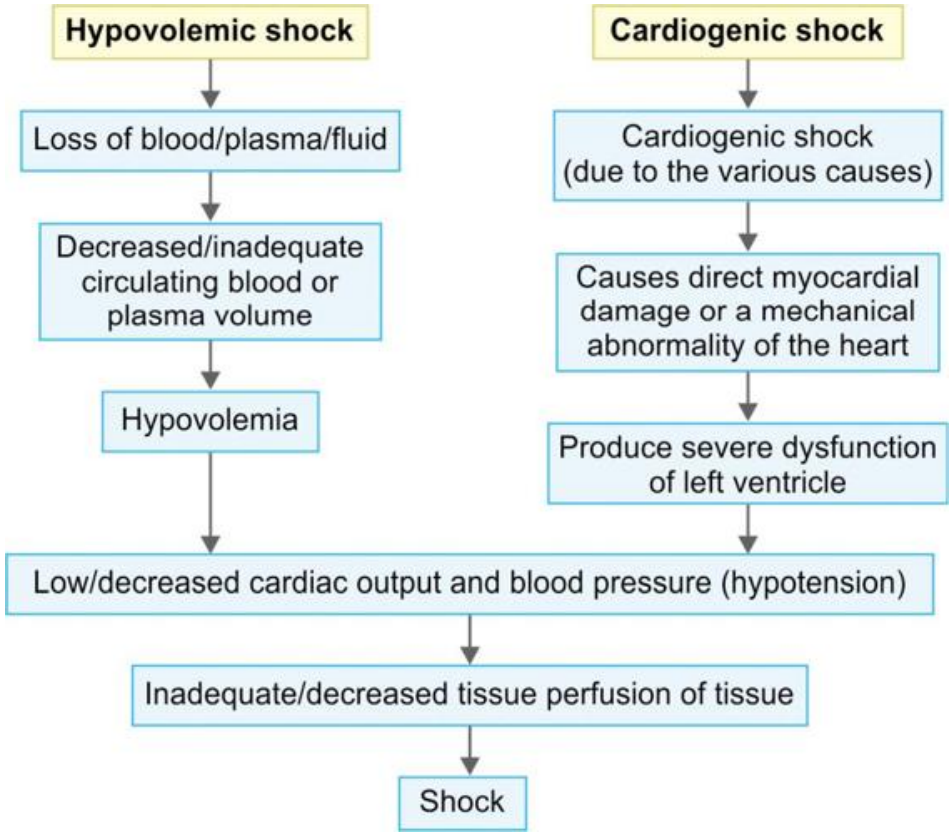
Q. Write short note on cardiogenic shock.

Cardiogenic shock results from low cardiac output due to:

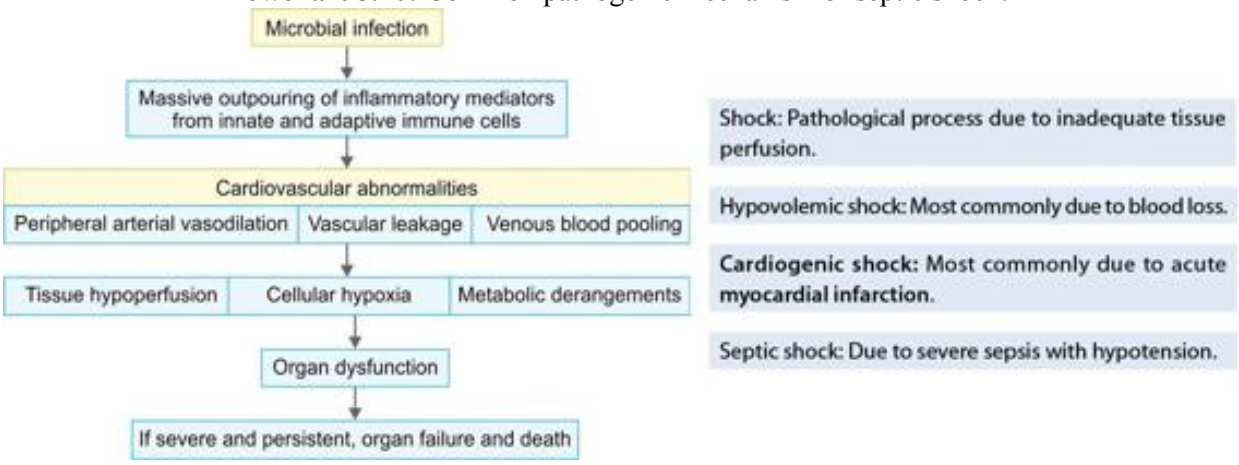
- **Intrinsic myocardial damage** (e.g., **massive myocardial infarction**, ventricular arrhythmias).
- **Extrinsic pressure or compression of heart** (e.g., **cardiac tamponade**).
- **Obstruction to the outflow blood from ventricles** (e.g., pulmonary embolism).

The pathogenesis of cardiogenic shock is presented in **Flowchart 5.9.** The left-sided heart failure also reduces the entry of blood from pulmonary vein into the left atrium. This leads to movement of fluid from pulmonary vasculature into the pulmonary interstitial space and into the alveoli resulting in **pulmonary edema**.

Flowchart 5.9: Pathogenesis of hypovolemic and cardiogenic shock.



Flowchart 5.10: Common pathogenic mechanism of septic shock.



Septic Shock

Q. Describe the pathogenesis of septic shock.

Definition: Septic shock is defined as **shock due to severe sepsis with hypotension**, which **cannot be corrected by infusing fluids**.

Septic shock results from vasodilation and peripheral pooling of blood and is associated with dysfunction of multiple organs distant from the site of

infection.

Causative Organisms

- Septic shock may be caused by **gram-positive** (most common) **or gram-negative bacteria, fungi**, and, very rarely, protozoa or rickettsiae. Hence, the older term "endotoxic shock", is not appropriate.
- The common gram-positive bacteria include ***Staphylococcus aureus,*** **enterococci,** ***Streptococcus pneumoniae,*** and gram-negative bacilli which are resistant to usual antibiotics.

Major Pathogenic Pathways in Septic Shock

- **Trigger:** Septic shock is **triggered by microbial infections and is associated with severe systemic inflammatory response syndrome (SIRS)**. Most of septic shocks are triggered by **bacteria or fungi** that normally do not produce systemic disease in immunocompetent hosts. Apart from microbes, SIRS may be triggered by other conditions such as burns, trauma, and/or pancreatitis.
- **Hallmark of septic shock:** The common pathogenic mechanism is a massive outpouring of inflammatory mediators from innate and adaptive immune cells that produce arterial vasodilation, vascular leakage, and venous blood pooling. These cardiovascular abnormalities result in tissue hypoperfusion, cellular hypoxia, and metabolic derangements that lead to organ dysfunction and, if severe and persistent, organ failure and death. It is **tissue hypoperfusion** due to **decrease in peripheral vascular resistance** as a result of **systemic arteriolar vasodilation vascular leakage,** and venous **pooling of blood in the periphery**. Cardiac output may be normal or even increased in early stages.
- **Initiation of shock:** Several **microbial constituents** can initiate the pathogenesis of septic shock. These constituents **and mediators produced by host** act in an incompletely known way to produce septic shock.

Pathogenesis of Septic Shock

Common pathogenic mechanism of septic shock is presented in **Flowchart 5.10**.

Major factors contributing to the pathogenesis of septic shock are listed in **Box 5.4**.

BOX 5.4: Major factors contributing to the pathogenesis of septic shock.

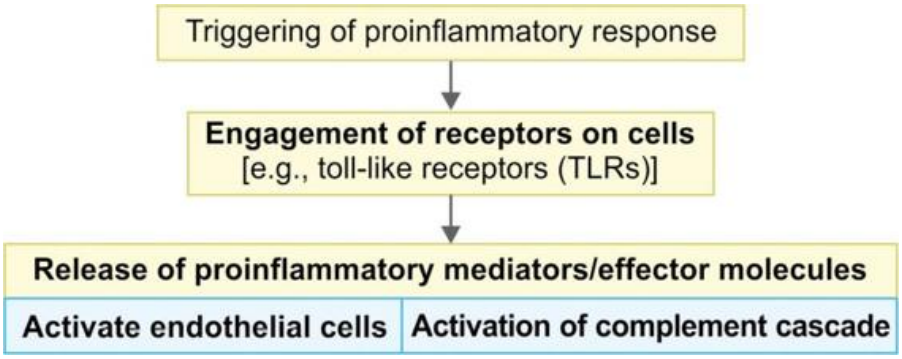
- Inflammatory and counter-inflammatory responses
- Endothelial cell activation and injury
- Induction of a procoagulant state
- Metabolic abnormalities
- Organ dysfunction
- Immune suppression

Inflammatory and Counter-inflammatory Responses (Flowcharts 5.11 and 5.12)

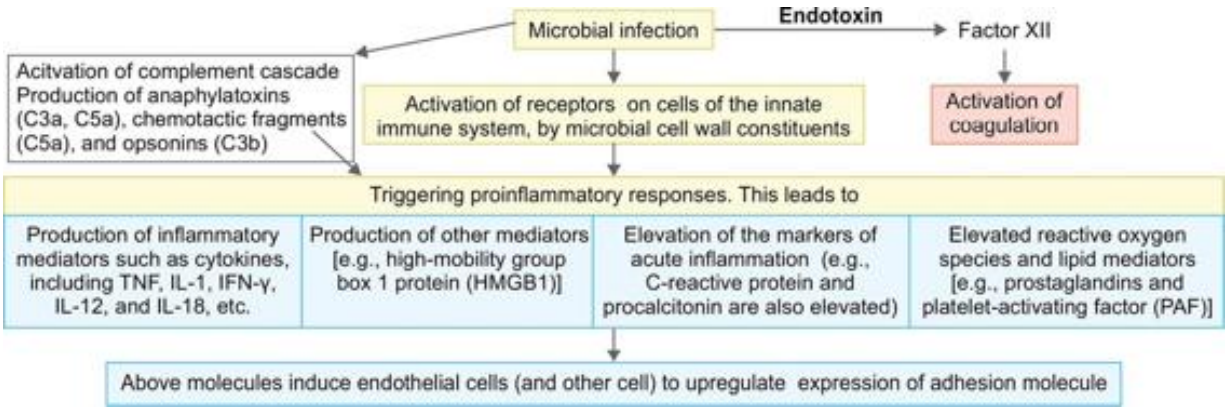
1. Triggering of proinflammatory response:

The proinflammatory/inflammatory responses/reactions are directed to eliminate the causative pathogen and are responsible for the organ/tissue damage. The causative pathogens may also cause direct damage to organs. In contrast, counter/anti-inflammatory responses are responsible for increased susceptibility to secondary infections that occurs later in the course. Proinflammatory response is triggered through activation of receptors on cells of the innate immune system by microbial cell well constituents.

Flowchart 5.11: Effects of proinflammatory response.



Flowchart 5.12: Proinflammatory responses in septic shock.



- **Activation of receptors on cells by microbial cell wall constituents:** In sepsis, various microbial components of cell wall (e.g., bacterial peptides) engage receptors [e.g., Toll-like receptors (TLRs) which recognize "pathogen-associated molecular patterns" (PAMPs) of microbes] present on cells **of the innate immune system**.
- **Release of proinflammatory mediators:** The activated receptors on cells of the immune system trigger **production of proinflammatory mediators.** These include:
  - Production of inflammatory mediators such as cytolines including **TNF, IL-1, IFN-γ, IL-12,** and **IL-18**.
    - **Cytokine-like** mediators such as **high mobility group box 1 protein (HMGB1)**.
  - Markers of acute inflammation (e.g., C-reactive protein, procalcitonin) are also elevated and are useful indicator of septic shock.
  - They also produce reactive oxygen species and lipid mediators such as prostaglandins and platelet activating factor (PAF).
- **Effect of inflammatory mediators:** These proinflammatory effector molecules **activate endothelial cells** (and other cell types) to **upregulate expression of adhesion molecule**. This in turn **stimulates production of cytokine and chemokine**.
- **Activation of complement cascade:** It occurs due to microbial components, resulting in the **production of anaphylatoxins (C3a, C5a), chemotactic fragments (C5a), and opsonins (C3b)**. All these complement products contribute to the proinflammatory state.
- **Activation of coagulation:** Microbial components can also activate coagulation directly through factor XII and indirectly through altered endothelial function.

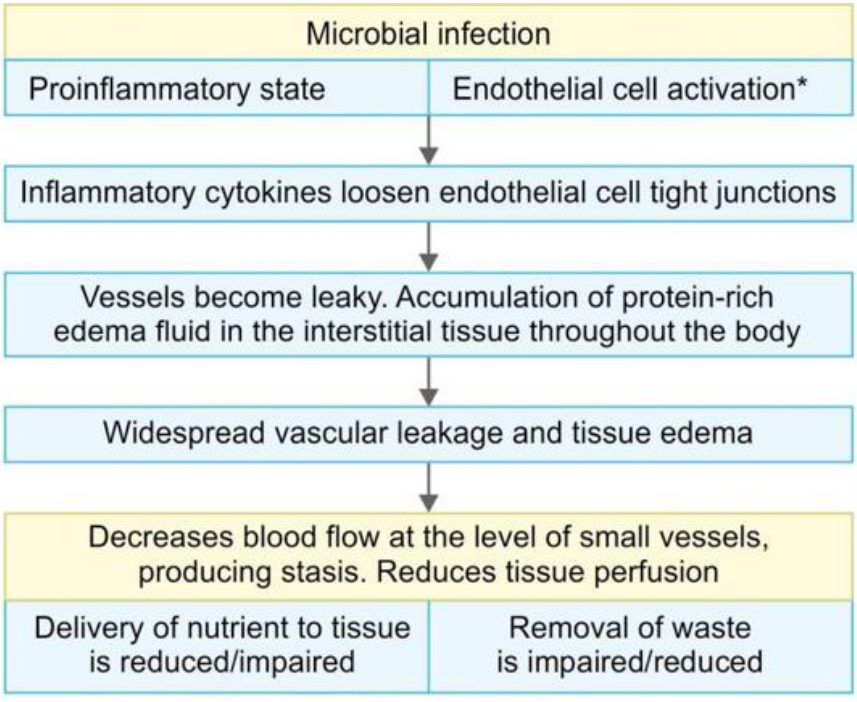
2. Activation of counterregulatory immunosuppressive mechanisms:

- The hyperinflammatory state produced by sepsis **also activates counter-regulatory immunosuppressive mechanisms**. This involves both innate and adaptive immune cells. Thus, in a patient with sepsis, there may be **oscillation between hyperinflammatory and immunosuppressed states**.
- **Mechanisms for the immune suppression:** These include a shift from proinflammatory (T_H1) to anti-inflammatory (T_H2) cytokines, production of anti-inflammatory mediators (e.g., soluble TNF receptor, IL-1 receptor antagonist, and IL-10) and lymphocyte apoptosis.

Endothelial Cell Activation and Injury(Flowchart 5.13)

- Endothelial cell activation/injury is caused **by either microbial constituents or proinflammatory state** (leukocyte-derived inflammatory mediators).

Flowchart 5.13: Endothelial cell activation and injury in septic shock.



*Endothelium cell activation also upregulates production of nitric oxide (NO) and other vasoactive inflammatory mediators (e.g., C3a, C5a, and PAF)—causes relaxation of vascular smooth muscle and systemic hypotension.

- Inflammatory cytokines cause **loosening of endothelial cell tight junctions**. This causes **widespread vascular leakage of protein-rich fluid from vessels into the interstitial tissue** resulting in the accumulation of **edema** fluid in the interstitial tissue throughout the body.
- **Edema** impairs/reduces both supply of nutrient and removal of waste. This **impairs tissue perfusion** and may be exacerbated by attempts to support the patient with intravenous fluids.
- Endothelial activation also **upregulates production of nitric oxide (NO) and other vasoactive inflammatory mediators** (e.g., C3a, C5a, and PAF). These may cause **relaxation of vascular smooth muscle and systemic hypotension**.

Induction of a Procoagulant State (Flowchart 5.14)

Sepsis causes expression of many factors that favor coagulation or procoagulant state. Factors activating coagulation system in sepsis:

- **Activation of factor XII** by microbial components such as **endotoxin**.
- **Proinflammatory cytokines (e.g., IL-6):**
  - They **increase the production of tissue factor** by monocytes and possibly endothelial cells.
  - **Reduce the production of endothelial anticoagulant factors**, such as tissue factor pathway inhibitor, thrombomodulin, and protein C.
  - **Reduce fibrinolysis** by increasing plasminogen activator inhibitor-1 expression.

Consequences of activation of coagulation system:

- This leads to **systemic activation of thrombin and the deposition of fibrin-rich thrombi in small vessels**, often throughout the body. This **produces dangerous complication DIC** in about 50% of septic patients. This compromises tissue perfusion formation. The consumption of coagulation factors and platelets leads to deficiencies of these factors and cause **bleeding and hemorrhage.**
- The vascular leak and tissue edema reduces the flow of blood flow in the small vessels, produces stasis and diminishes the clearing of activated coagulation factors.

Metabolic Abnormalities

1. **Insulin resistance and hyperglycemia:** It is **due to the action of proinflammatory cytokines** such as TNF and IL-1, stress-induced hormones (e.g., glucagon, growth hormone, and glucocorticoids), and **catecholamines.** The proinflammatory cytokines suppress the release of insulin, promote insulin resistance in the liver and other tissues, and impair the surface expression of GLUT-4 (glucose transporter). **Hyperglycemia decreases neutrophil function**, suppresses its bactericidal activity and causes increased expression of adhesion molecule on endothelial cells.
2. **Decreased glucocorticoid production:** Initially, there is increased glucocorticoid production, and is later followed by decreased production due to adrenal insufficiency. **Adrenal necrosis may also develop due to DIC (Waterhouse–Friderichsen syndrome).**
3. **Lactic acidosis:** Cellular hypoxia and diminished oxidative phosphorylation may produce increased lactate and lactic acidosis.

Organ Dysfunction

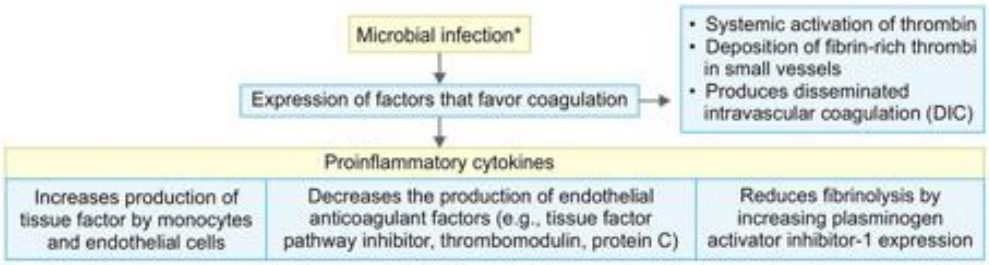
- **Decrease supply of oxygen and nutrients to the tissues:** Due to systemic hypotension, interstitial edema, and thrombi in the small vessels. Oxidative stress causes damage to the mitochondria and impairs oxygen use.
- **Decreased contractibility of myocardium and cardiac output:** It is **due to increased levels of cytokines and secondary mediators**. This along with increased vascular permeability and endothelial injury can lead to the **adult respiratory distress syndrome.**
- **Multiorgan failure: Finally, above factors lead to failure** of multiple organs, mainly the **kidneys, liver, lungs,** and **heart** resulting in death.

Immune Suppression

It occurs in patients with septic shock. It is probably **due to**:

- **Production of anti-inflammatory mediators** (e.g., soluble TNF receptor (STNFR), IL-1 receptor antagonist, and IL-10).

Flowchart 5.14: Activation of procoagulant state in septic shock.



*The vascular leak and tissue edema produced due to endothelial cell decreases blood flow in the small vessels, producing stasis. This diminishes the clearing/removal of activated coagulation factors.

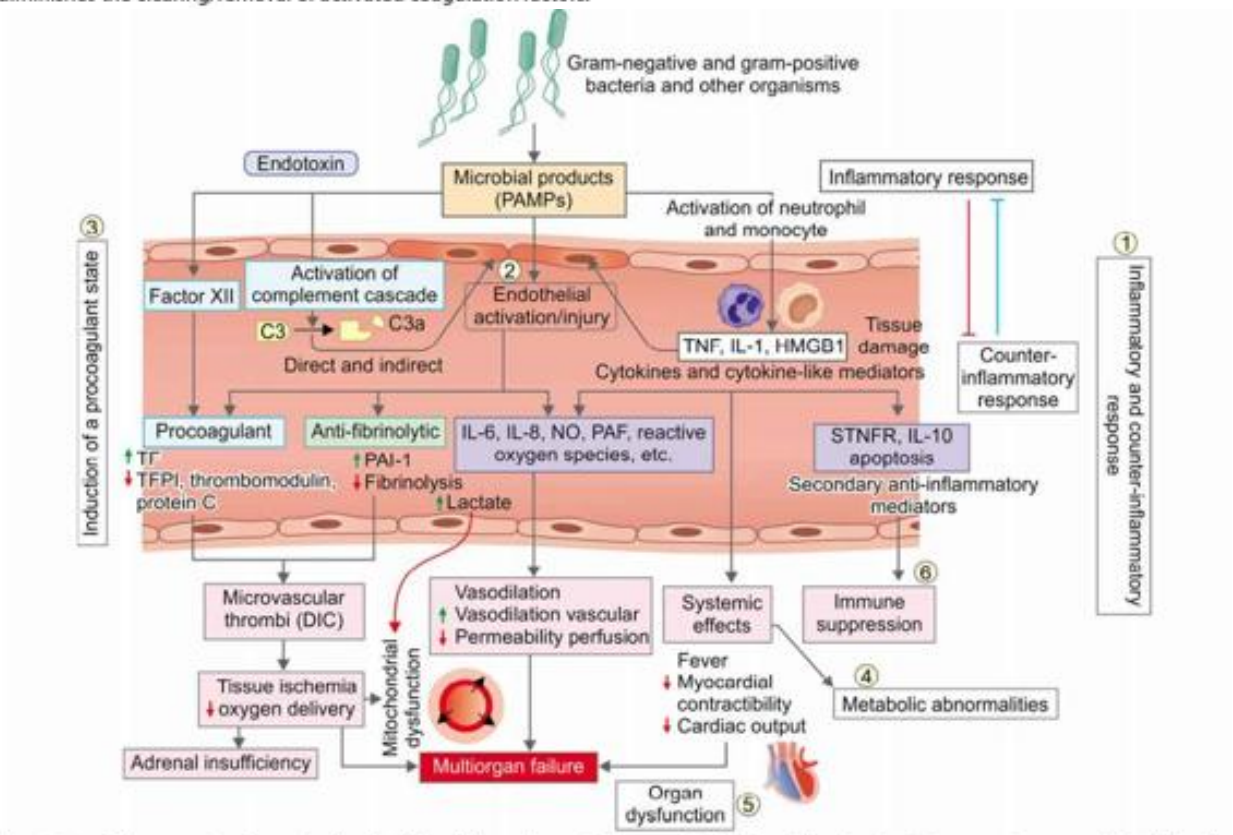


Fig. 5.21: Pathogenesis of septic shock. Microbial products initiate endothelial cell activation/injury activates endothelial cells, complement activation, activation of neutrophils and macrophages, factor XII. These initiating events lead to end-stage multiorgan failure. Major factors contributing to the pathogenesis of septic shock are: (1) Inflammatory and counter-inflammatory responses, (2) Endothelial cell activation and injury, (3) Induction of a procoagulant state, (4) Metabolic abnormalities, (5) Organ dysfunction and (6) Immune suppression.

(DIC: disseminated intravascular coagulation; HMGB1: high mobility group box 1 protein; NO: nitric oxide; PAF: platelet activating factor; TF: tissue factor; STNFR: soluble tumor necrosis factor receptor)

- **Widespread apoptosis of lymphocytes**.

Another group of proteins secreted by bacteria called **superantigens** also produce a syndrome similar to septic shock (e.g., *toxic shock syndrome*). Superantigens are polyclonal T-lymphocyte activators which induce the release of high levels of cytokines. These will produce a variety of clinical features, ranging from a diffuse rash to vasodilation, hypotension, shock, and death.

The summary of various mechanism involved in the pathogenesis of septic shock is presented in **Figure 5.21**.

Stages of Shock

Q. Describe three different /various stages of shock.

Shock is a progressive disorder, which if not treated, leads to death. It can be divided into three phases.

1. **Nonprogressive (compensated/reversible) phase:** During the initial phase, homeostatic compensatory mechanisms **redistribute the blood supply** in such a way that the effective **blood supply to the vital organs is maintained**. This is achieved by neurohumoral mechanisms, which try to maintain cardiac output and blood pressure.

Compensatory changes: The neurohumoral mechanism produces the following compensatory changes:

- **Widespread vasoconstriction** except vital organs. **Coronary and cerebral vessels** usually **maintain relatively normal blood flow, and oxygen delivery**. Cutaneous vasoconstriction → produces the coolness and pallor of the skin.
- **Fluid conservation by kidney**.
- **Tachycardia**.

2. **Progressive phase:**
   - **If the underlying causes are not corrected**, shock **passes to the progressive phase**.
   - Characterized by **widespread tissue hypoperfusion** and **hypoxia** → intracellular aerobic respiration replaced by **anaerobic glycolysis** → **increased** production of **lactic acid** → metabolic **lactic acidosis** → **decreases** the tissue **pH** → dilatation of arterioles → peripheral **pooling of blood into the microcirculation** → **decreases** the **cardiac output** → produces **anoxic injury** to endothelial cell → favors development of **DIC** → widespread tissue hypoxia and **damage of vital organs**.

TABLE 5.9: Summary of main morphological features of shock.

Organ	*Changes*
Adrenal	Lipid depletion in the cortical cells

TABLE 5.9: Summary of main morphological features of shock.

Organ	*Changes*
Kidney	Acute tubular necrosis (ATN)/acute renal failure
Lungs	Relatively resistant to hypoxic injury. However, in septic shock shows diffuse alveolar damage (shock lung) with hyaline membrane
Heart	Coagulative necrosis and contraction band necrosis
Liver	Congestion and necrosis of centrilobular region of the liver
Brain	Encephalopathy (ischemic or septic) and cortical necrosis
Gastrointestinal tract	• Diffuse gastrointestinal hemorrhage • Erosions of the gastric mucosa and superficial ischemic necrosis in the intestine

3. **Irreversible phase:**
   - **Without intervention, the shock eventually** enters an **irreversible stage**.
   - At this phase, **cellular and tissue injury is so severe** that even if the hemodynamic defects are corrected, **survival is not possible**.
   - Widespread cell injury results in leakage of lysosomal enzymes, which aggravate the shock state.
   - **Myocardial contractile function worsens** partly due to nitric oxide synthesis.
   - If ischemic intestine allows microbes from the intestinal flora to enter into the circulation, it may lead to **superimposed bacteremic shock.**
   - The patient develops **acute tubular necrosis** and results in death.

MORPHOLOGY (TABLE 5.9)

Q. Describe the morphological changes in various organs in shock.

Changes in Cardiogenic or Hypovolemic Shock

These are mainly due to hypoxic injury. Morphological changes are particularly evident in adrenals, kidneys, lungs, brain, heart, and gastrointestinal tract.

Adrenal

- **Lipid depletion in cortical cell:** It is due to conversion of the relatively inactive vacuolated cells to metabolically active cells. The active cells utilize stored lipids for the synthesis of steroids.
- **Focal hemorrhage:** It occurs in the inner cortex of adrenal in **severe** shock.
- **Massive hemorrhagic necrosis** of the entire adrenal gland is found in the Waterhouse–Friderichsen **syndrome**, which is **associated with severe meningococcal septicemia**.

Q. Write a short note on renal changes in shock

Kidney

Acute tubular necrosis (acute renal failure) is a major complication of shock.

- **Gross:** Kidney is **enlarged, swollen**, **congested**, and the **cortex may appear pale**. Cut section shows blood pooling in the outer region of the medulla.
- **Microscopy:**
  - **Tubules:** Dilation of the proximal tubules and **focal necrosis of tubular epithelial cells**. Frequently, the tubular lumen may show **pigmented casts** formed due to leakage of hemoglobin or myoglobin.
  - **Interstitium**: It shows **edema** and **mononuclear cells** in the interstitium and within tubules.

Q. Write short note and lung changes in shock/diffuse alveolar damage.

Lungs

- Lungs are relatively resistant to hypoxic injury and are usually not affected in pure hypovolemic shock.
- However, when shock is due to bacterial sepsis or trauma, it shows **diffuse alveolar damage** which can leads to acute respiratory distress syndrome (ARDS), also known as shock lung.
- **Gross:** The lung is **firm and congested. Cut surface** shows **oozing out** of **frothy fluid**.
- **Microscopy:**
  - **Edema:** It first develops around peribronchial **interstitial** connective tissue and later in the **alveoli**.
  - **Necrosis**: Endothelial and alveolar epithelial cells undergo necrosis and leads to formation of **intravascular microthrombi**.
  - **Hyaline membrane**: It is usually seen lining the alveolar surface. It may also line alveolar ducts and terminal bronchioles.

Heart

- **Gross:** It shows **petechial hemorrhages** in the epicardium and endocardium.

- **Microscopy**: **Necrosis** of the myocardium is seen which may range from minute focus to large areas of necrosis. Prominent **contraction bands** are seen by light microscopy.

Liver

- **Gross:** Liver is enlarged. Cut section shows a **mottled** (blotched) appearance due to marked pooling of blood in the centrilobular region.
- **Microscopy**: The centrilobular region of the liver shows **congestion and necrosis.**

Brain

Encephalopathy (ischemic or septic) and cortical necrosis.

Gastrointestinal Tract

Shock produces **diffuse gastrointestinal hemorrhage**. **Erosions of the gastric mucosa** and superficial **ischemic necrosis** in the intestine lead to gastrointestinal bleeding.

Changes in Septic Shock

- Septic shock can lead to **DIC** which is characterized by **widespread** formation of fibrin-rich **microthrombi**, particularly in the brain, heart, lungs, kidney, adrenal glands, and gastrointestinal tract.
- The utilization of platelets and coagulation factors in DIC produces **bleeding manifestations**. It may show **petechial hemorrhages** on serosal surface and the skin.

Clinical Consequences

The clinical features of shock depend on the cause.

- **Hypovolemic and cardiogenic shock:** Usually present with features of hypotension and hypoperfusion. The features include **altered sensorium, cyanosis, oliguria, weak rapid pulse, tachypnea**, and **cool, clammy extremities**.
- **Septic shock:** The **skin initially may be warm and flushed** because of peripheral vasodilation.

The initial underlying cause that precipitated the shock may be life-threatening (e.g., myocardial infarct, severe hemorrhage, or sepsis). Later, the organ dysfunction involving **cardiac, cerebral, and pulmonary** function worsen the situation. The **electrolyte disturbances and metabolic acidosis** may further exacerbate the situation.

Patients who survive the initial complications may develop **renal insufficiency** characterized by a progressive decrease in urine output and severe fluid and electrolyte imbalances.

Prognosis

The prognosis depends on the cause and duration of shock.

- Patients with hypovolemic shock may survive with appropriate management.

Septic shock, or cardiogenic shock associated with massive myocardial infarction, usually have high mortality rate.

Organisms causing septic shock:
- Gram-positive: *Staphylococcus aureus*, enterococci, *Streptococccus pneumoniae*
- Gram-negative resistant to usual antibiotics

Septic shock: Microbial components activate both innate and adaptive immunity. The activated inflammatory cells produce inflammatory mediators.

Septic shock: Microbial constituents or inflammatory mediators cause endothelial cell activation.

Septic/endotoxic shock: Initiating mechanism is endothelial injury/activation.

Septic shock—consequences of endothelial activation:
- Activation of thrombosis
- Increased vascular permeability
- Vasodilation

Septic shock: Multiorgan failure such as kidneys, liver, lungs, and heart.

Toxic shock syndrome is similar to septic shock and is produced by a group of microbial exotoxins called superantigens.

Metabolic abnormalities in septic shock:
- Insulin resistance
- Hyperglycemia
- Decreased glucocorticoid production

Stages of shock:
(i) Nonprogressive (ii) Progressive (iii) Irreversible

Septic shock can initially cause cutaneous vasodilation, which produces warm skin.

Shock lung: Diffuse alveolar damage.

Histopathological features of shock:

- Acute tubular necrosis
- Depletion of lipids in adrenal cortex
- Pulmonary congestion
- Hepatic necrosis

Shock: Morphological changes mainly observed in adrenals, kidneys, lungs, brain, heart, and gastrointestinal tract.

Clinical triad of septic shock: (i) DIC, (ii) hypotensive shock and (iii) metabolic disturbances (e.g., insulin resistance and hypoglycemia).

Procalcitonin is an acute phase reactant which is now useful marker for sepsis.

Cause of death in shock: Most commonly due to multiorgan failure.

CC (Show how): Identify and describe various forms of cell injuries, their manifestations and consequences in gross and microscopic specimens.

(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Gross and microscopic specimens to be shown are myocardial infarction, infarct spleen, chronic venous congestion (lung, liver and spleen).

CHAPTER 6

Immunopathology, AIDS, and Amyloidosis

The normal immune system is essential for protection against infection. Immune system is like a double-edged sword. Though it is protective in most of the situations, sometimes a hyperactive immune system may cause fatal diseases.

IMMUNITY

CC: Describe the principles and mechanisms involved in immunity.

Definition: Immunity is **resistance** (defense mechanism) exhibited **by host against invasion by any foreign antigen**, including microorganisms.

Types: There are two types namely innate and adaptive immunity.

Innate (Natural/Native) Immunity

General Features

- **First line of defense present by birth**.
- Provides **immediate initial protection** against an invading pathogen.
- **Does not depend on the prior contact** with foreign antigen or microbes.
- **Lacks specificity**, but **highly effective**. No memory, and no self/non-self recognition.
- **Triggers the adaptive immune response**.
- **No memory** is seen.

Major Components

Physical/Anatomical Barriers

It includes epithelium lining of skin, gastrointestinal and respiratory tracts which act as **mechanical barriers**, produce antimicrobial molecules such as defensins.

Cells

- **Phagocytic cells:** It consists of mainly **monocytes (macrophages in tissue) and neutrophils** in the blood. Phagocytic cells use several receptors to sense microbes and are called as "microbial sensors" (pattern recognition receptors) (*refer* page 50).
  - **Pathogen associated molecular patterns (PAMPs):** Microbes have few highly conserved common molecular structures shared by entire classes of pathogens. These structures are called **pathogen associated molecular patterns** and are essential for the infectivity of these pathogens.
  - **Pattern recognition receptors (PRRs)** (*refer* **Fig. 3.1):** Phagocytic cells involved in innate immunity recognize PAMP using a group of cellular receptors (microbial sensors) called pattern recognition receptors. Examples for PRRs:
    - **Toll-like receptors (TLRs):** These are transmembrane receptors and about 10 types of human TLRs have been identified. Each receptor recognize a unique set of microbial patterns. For example, TLR2 recognizes various ligands (e.g., lipoteichoic acid) expressed by gram-positive bacteria, TLR4 recognize lipopolysaccharides (LPS) of gram-negative bacteria.
    - **Receptors for mannose residues.**
    - **NOD (nucleotide-oligomerization domain protein)-like receptors:** They are located in the cytoplasm and serve as intracellular sensors for microbial products.
    - **Receptors for opsonins**.
- **Dendritic cells:** These cells **function as antigen presenting cells** to T-cells. They produce type I interferons (IFN) (e.g., IFN-α), which inhibit viral infection and replication.
- **Natural killer (NK) cells:** They provide defense against many viral infections and other intracellular pathogens (*refer* pages 145-6).

Soluble Molecules in the Blood and Tissues

- **Complement system**

- Proteins that coat microbes and aid in phagocytosis, e.g., mannose-binding lectin and C-reactive protein.

Functions of Innate Immune Response

- **Inflammation** and destruction of invading microbe
- **Antiviral defense** is mediated by **dendritic cells and NK cells**.

Adaptive Immunity

If the innate immune system fails to provide effective protection against invading microbes, the adaptive immune system is activated.

General Features

Q. Write short note on humoral immunity.
Q. Write short note on cellular immunity.

- **Second line of defense** acquired during life
- **Capable of recognizing both microbial and nonmicrobial substances**
- **Takes more time to develop** and is **more powerful than innate immunity**
- **Long-lasting protection**
- Prior exposure to antigen is present
- Three **characteristic features** are: (1) **specificity**,(2) **diversity** and (3) **memory**.

Components

1. **Humoral immunity**: It is mediated by soluble protein products called antibodies produced by B lymphocytes. Antibody is capable of reacting with the specific antigen responsible for its production.
2. **Cellular immunity (cell-mediated immunity):** It is localized reaction to organism, usually intracellular pathogens. It is mediated by T lymphocytes, macrophages and their soluble products called cytokines. Cell-mediated cytotoxicity is important in lysis of virus infected cells and rejection of allograft and tumor cells. Other cytotoxic cells involved in cell mediated immune response are null cells (NK). These NK cells are able to attack the target cells and kill them.

Functions of Adaptive Immune Response

- **Antibodies:** Protection against extracellular microbes in the blood, mucosal secretions and tissues.
- **T lymphocytes**:
  - Defense against viruses, fungi and intracellular bacteria either by direct killing of infected cells by cytotoxic T lymphocytes or by activation of phagocytes to kill the ingested microbes.
  - Important immunoregulatory role, orchestrating and regulating the responses of other components of the immune system.

Different types of adaptive immunity and their differences are shown in **Table 6.1**.

Both B and T lymphocytes express highly specific receptors for a wide variety of substances, called **antigens**.

Main physiological function of immune system is protection against infectious microbes.

Immunity types: (1) Innate and (2) Adaptive.

Innate immunity: Early and first line response to microbes.

Innate immunity components:
- Physical barriers
- Phagocytic cells, NK cells
- Soluble plasma proteins (complements)

Innate immunity: One of the manifestations is inflammatory response.

Toll-like receptor causes activation of NF-kB and AP-1.

TLR-4: *Chlamydia* and lipopolysaccharide of gram-negative bacteria (except *Leptospira*) recognizes toll-like receptor-4 (TLR-4).

TLR-2: Peptidoglycan of gram +ve bacteria and lipopolysaccharide of *Leptospira* recognizes toll-like receptor-2 (TLR-2).

TLR-3: Recognizes dsRNA viruses.

Natural killer cells: Attack cells which are not able to express class I MHC molecules **(Fig. 6.1B)**

Adaptive immunity: Develops slowly but is more powerful and specialized than innate immunity.

Humoral immunity: Mediated by antibodies secreted byB lymphocytes and are effective against extracellular microbes and their toxins.

Terms "immune system" and "immune response" *refer* to adaptive immunity.

CELLS OF THE IMMUNE SYSTEM

Cells of immune responses (lymphocytes and other cells) migrate among lymphoid and other tissues and the vascular and lymphatic circulations.

Naïve Lymphocytes

These are mature lymphocytes which have not encountered the antigen (immunologically inexperienced). After the lymphocytes are activated by recognition of antigens, they differentiate into:

- **Effector cells:** They perform the function of eliminating microbes.
- **Memory cells:** They live in a state of heightened awareness and are better able to combat the microbe in case it infects again.

T Lymphocytes

- **Development:** T (thymus-derived) lymphocytes develop from precursors in the thymus.
- **Distribution:** Mature T-cells are found in:
  - **Peripheral blood** where it constitute 60–70% of lymphocytes
  - **T-cell zones of peripheral lymphoid organs** namely paracortical region of lymph node and periarteriolar sheaths of spleen.
- **T-cell receptor:** T-cell recognizes a specific cell-bound antigen by means of an antigen-specific T-cell receptor (TCR).

TABLE 6.1: Differences between two types of adaptive immunity.

Type	*Mediator*	*Protection against*
Humoral immunity	B lymphocytes which secrete antibodies	Extracellular microbes and their toxins
Cell-mediated (or cellular) immunity	T (thymus-derived) lymphocytes	Intracellular microbes

- **Markers:** Leukocyte cell surface molecules are named systematically by assigning them a **'cluster of differentiation' (CD) antigen number** that helps in their identification.
  - **Primary T-cell associated** CD molecules: **CD1, CD3, CD4, CD5 and CD8**.
  - **CD3** is involved in signal transduction and is also known as **pan T-cell marker**. It is involved in T-cell activation.

- **Subsets of T lymphocytes:** Naïve T-cells can differentiate into two subtypes, namely **CD4 and CD8.** Both subtypes serve as "coreceptors" in T-cell activation. They are called as coreceptors because they work with the antigen receptor in responses to antigen.

Q. Write short note on T helper cell.

- **$CD4^+$ T-cell:** These subset of T-cells have CD4 molecule and are called as **helper T-cells**. They **constitute about 60% of mature T-cells**. The CD4 cells function as cytokine-secreting helper cells that help macrophages and B lymphocytes to combat infections. They are **subcategorized as T_H1, T_H2 and T_H17 $CD4^+$ T-cells**.
- **$CD8^+$ T-cell:** These subset of T-cells have CD8 molecule and are called as **cytotoxic/killer T-cells.** They **constitute about 30% of T-cells**. $CD8^+$ T-cells function as cytotoxic (killer) T lymphocytes (CTLs) to destroy host cells harboring microbes and tumor cells.

When the antigen presenting cells (APCs) present antigen to T-cells, $CD4^+$ T-cells recognize and bind only to class II MHC molecules and $CD8^+$ T-cells bind only to class I MHC molecules.

B Lymphocytes

- **Development:** B (bone marrow-derived) lymphocytes develop from precursors in the bone marrow.
- **Distribution:**
  - **Peripheral blood:** Mature B-cells constitute 10–20% of the circulating peripheral lymphocyte population.
  - **Peripheral lymphoid tissues:** Lymph nodes (cortex), spleen (white pulp), and mucosa-associated lymphoid tissues (pharyngeal tonsils and Peyer's patches of GIT).
- **B-cell receptor (BCR):** B-cells have receptors composed of IgM and IgD on their surface and has unique antigen specificity.
- **Functions of B-cells:** All the mature, naïve B-cells express membrane-bound immunoglobulins (Ig) on their surface that functions as B-cell receptors (BCRs) for antigen. B-cells recognize antigen via these BCRs.
  - **Production of antibodies:** The primary function of B-cells is to **produce antibodies**. After stimulation by antigen and other signals, B-cells develop into **plasma cells.** These cells secrete antibodies which are the mediators of humoral immunity. Salient features of various antibodies are presented in **Table 6.2**.

- **Antigen presenting cell:** B-cells also serve as APCs and are very efficient at antigen processing.

- **Markers:** B-cell markers include: CD10 (CALLA), CD19, CD20, CD21 (also called EBV receptor), CD23, CD79a.

Dendritic Cells

As the name suggests these cells have numerous fine cytoplasmic processes that resemble dendrites. These are important antigen presenting cells in the body and can be functionally of the following types:

TABLE 6.2: Salient features of antibodies (immunoglobulins).

Features	***IgM (millionaire's antibody)***	***IgG (subtypes: IgG1, IgG2, IgG3, IgG4)***	***IgA***	***IgE (reaginic/homocytotropic antibody)***	***IgD***
Approximately % of total Ig	5%	80% (maximum)	15%	Trace	Trace
Molecular weight	900,000 (maximum)	150,000	150,000–300,000	190,000	180,000
Type of heavy chain	μ	γ	α	ε	d
Structure	Pentamer (maximum size)	Monomer	Dimer (in glandular secretions), monomer (in serum)	Monomer	Monomer
Complement activation	Yes (classical pathway)	Yes (classical pathway)	Activates alternate complement pathway	No	No
Transport across placenta	No	Yes	No	No	No
Half-life (days)	5	21	6	2	3
Main function	Primary immune response	• Secondary immune response • Functions as B-cell receptor	• Mucosal immunity • Highly effective at neutralizing toxins	Allergic diseases, defense against parasite infection and anaphylactic reaction	Unknown

- **Interdigitating dendritic cells (IDC):** They are the **most important APCs** for initiating primary T-cell responses against protein antigens.
  - **Location: (1) Common location is below the epithelial lining: Immature dendritic cells within the epidermis are known as Langerhans cells; (2) Interstitia of all tissues.**
- **Follicular dendritic cell:**
  - **Location: It is present in the germinal centers of lymphoid follicles in the spleen and lymph nodes (hence named as follicular dendritic cell).**

Macrophages

Q. Write short note on macrophage and its function.

Macrophages are a part of the mononuclear phagocyte system.

Role in Adaptive Immune Responses

Processing of antigen: Macrophages process the antigens present in the phagocytosed microbes and protein antigens. After processing, the antigen is presented to T-cells and thus, they function as APCs in T-cell activation.

Effector Cell in Immunity

- **Cell-mediated immunity:** Macrophages are main effector cells in certain types of cell-mediated immunity, the reaction that serves to eliminate intracellular microbes. In this type of response, T-cells activate macrophages and increase their capability to kill ingested microbes.
- **Humoral immunity:** Macrophages also participate in the effector phase of humoral immunity. Macrophages get activated by IFN-γ.
- **Phagocytosis:** Macrophages efficiently phagocytose and destroy microbes which are opsonized (coated) by IgG or C3b through their respective receptors.

Natural Killer Cells

Q. Write short note on natural killer cell.

- Non-phagocytic **large** (little larger than small lymphocytes) **granular** (numerous cytoplasmic **azurophilic granules**) **lymphocytes**.

- **Markers:** They **do not bear the markers for T- or B-cells**. Two cell surface molecules, **CD16 and CD56**, are commonly used to identify them.
- Comprise **about 5–15% of human peripheral lymphoid cells**.

Function

- Natural killer (NK) cells provide **defense against many viral infections** and other **intracellular pathogens** and also **has antitumor activity**, causing lysis of cells with which they react. Killing of the cells is performed without prior exposure to or activation by the microbes or tumors. Because of this ability, NK cells acts an early line of defense against viral infections and few tumors.
- They recognize abnormal cells in two ways:
  - **Antibody-dependent cellular cytotoxicity** (ADCC): NK cells bear (CD16) immunoglobulin receptors (F_cR) and bind antibody-coated targets leading to lysis of these cells. This phenomenon is called as antibody-dependent cell-mediated cytotoxicity (type IV).
  - **Perforin-granzymes system (Figs. 6.1 and 6.12):** NK cells have a variety of **surface receptors for MHC** (major histocompatibility complex) **class I**. These receptors can either be having **inhibitory or activating functions**. The function of NK cells is regulated by a balance between signals from these activating and inhibitory receptors **(Figs. 6.1A and B)**.
    - **Inhibitory receptors:** MHC class I molecules are normally expressed on healthy/normal host cells. NK cell inhibitory receptors recognize self-class IMHC molecules, which are expressed on all normal healthy host cells (MHC class I positive). They prevent NK cells from killing normal host cells by inhibiting the death pathway.
    - **Activating receptors:** If the target cell with which NK cells interact, do not have MHC molecules on their surface, there is no binding of MHC receptor of NK cells. The downregulation of class I MHC molecules (leading to absence of MHC molecules) may occur in cells due to various kinds of stress such as infection by viruses and DNA damage as in tumor. These activating receptors make holes in the target cell membrane by secreting perforins. Granzymes secreted by NK cells are injected through these pores and cause apoptosis of target cell. NK cells kill cells that are infected by some microbes or cells that are damaged beyond repair.

CD45: Present in all leukocytes. Also known as leukocyte common antigen (LCA).

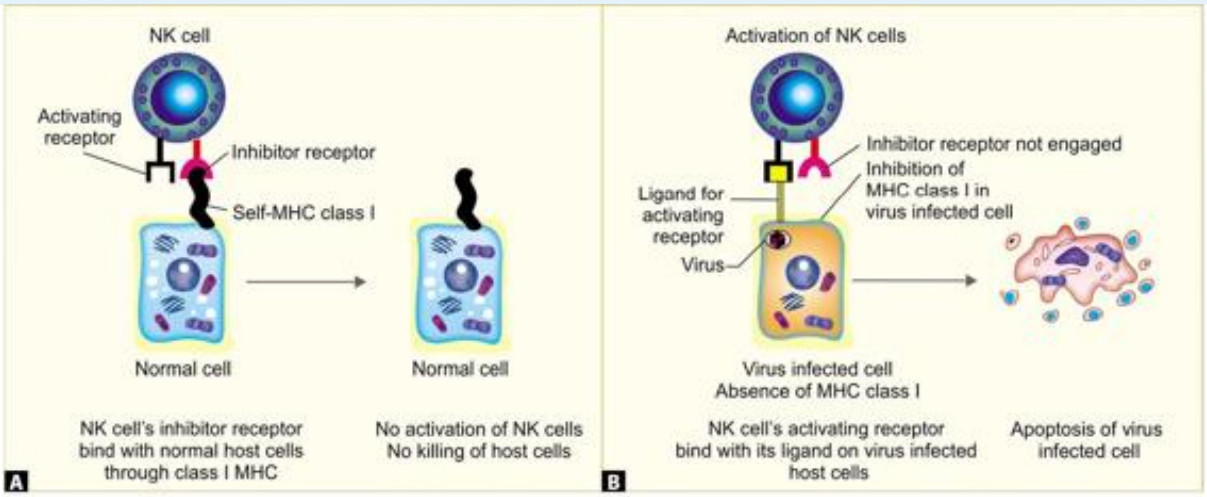
Lymphocytes: Activated to proliferate and differentiate into (1) effector and (2) memory cells.

Memory T-cells can be identified by using the marker CD45RO.

T lymphocytes which function to suppress immune responses are called as *regulatory T lymphocytes*.

$CD4^+$ helper T-cells:
- Help B-cells to produce antibodies/immunoglobulin production
- Activate macrophages to destroy ingested microbes
- Stimulate leukocyte recruitment
- Regulate all immune responses to protein antigens

Functions of $CD4^+$ helper T-cell is mediated by cytokines.



Figs. 6.1A and B: Function of natural killer (NK) cells: (A) Normal host cells express self-class I MHC molecules, which are recognized by inhibitory receptors of NK cells that binds them and prevent from killing normal cells; (B) In infected and stressed cells, class I MHC expression is reduced so that the inhibitory receptors of NK cells are not engaged. This results in activation of NK cells and killing of infected cells/stressed cells.

$CD4^+$ (helper) T-cells: Recognize and bind only to class II MHC molecules present on the antigen presenting cells (MHC-II restricted). They recognize peptides presented on HLA class II molecules (HLA-DR, HLA-DP and HLA-DQ). They are the master regulator of immune system.

$CD8^+$ ('cytotoxic') T-cells: Recognize and bind only to class I MHC molecules present on the antigen presenting cells (MHC-I restricted). They recognize antigenic peptides in association with HLA class I molecules (HLA-A, HLA-B, HLA-C). They kill infected cells directly through the production of pore-forming molecules such as perforin, or by triggering apoptosis of the target cell.

Normal ratio between $CD4^+$ T-cell and $CD8^+$ T-cell is 2:1.

Cytotoxic T-cells produce cytotoxic granules perforins and granzymes.

Naïve cells: Immunologically inexperienced mature lymphocytes that have not encountered the antigen for which they are specific.

Memory cells: Lymphocytes with heightened awareness and better ability to combat the microbe (in

case it returns).

B-cells also express several receptors. Type 2 complement receptor (CR2, or CD21) is the receptor for the Epstein-Barr virus (EBV), and hence EBV infects B-cells.

CD19 is a pan B-cell marker and involved in signal transduction.

CD3 is a pan T-cell marker and involved in T-cell activation.

Dendritic cells: Most efficient APCs which are located in epithelia and most tissues.

Langerhans cells are dendritic cells in the epidermis.

Follicular dendritic cell acts as reservoir for HIV in AIDS.

Macrophage associated markers: CD13, CD14, CD15 and CD33.

Antigen-presenting cells:
1. Macrophages (wide distribution)
2. Langerhans cells (in skin)
3. Dendritic cells (in the mucosa, lymph and blood)

Effector cells of immune system:
- NK cells
- $CD4^+$ T helper cells
- Plasma cells
- $CD8^+$ CTLs

Cell lysis by NK cells is unique:
1. Not mediated by immune response
2. MHC unrestricted
3. Does not involve an antigen-antibody interaction

Ability of NK cells to kill target cells is inversely related to target cell expression of MHC class I molecules.

Hyporesponsiveness of NK cells found in Chediak-Higashi syndrome.

Markers for NK cell: CD16 (surface receptor for Fc portion of IgG) and CD56 (surface receptor for NCAM-1).

Cell-mediated immunity: Mediated by T lymphocytes and protects against intracellular microbes.

CYTOKINES

- Immune responses involve multiple interactions among many cells. These include lymphocytes, dendritic cells, macrophages, other inflammatory cells (e.g., neutrophils), and endothelial cells.

- Some of these interactions are cell-to-cell contact. However, many interactions and effector functions of leukocytes are mediated by **short-acting soluble small proteins** called **cytokines**. These cytokines represent the **messenger molecules of the immune system** and are involved in cell signaling. **Interleukins (ILs)** are a group of cytokines that regulate the immune and inflammatory responses.

Classification

Most of the cytokines have many effects and can be classified depending on their functions.

Cytokines of Innate Immunity

- These cytokines are produced rapidly in response to microbes and other stimuli
- Mainly secreted by macrophages, dendritic cells and NK cells
- Mediate inflammation and antiviral defense
- These cytokines include TNF, IL-1, IL-12, type I IFNs, IFN-γ and chemokines.

Cytokines of Adaptive Immune Responses

- These cytokines are produced mainly by $CD4^+$ T lymphocytes in response to antigen and other signals
- They promote lymphocyte proliferation and differentiation and activate effector cells
- This category include IL-2, IL-4, IL-5, IL-17, and IFN-γ.

Colony-stimulating Factors

- These cytokines stimulate hematopoiesis and are assayed by their ability to stimulate formation of blood cell colonies from bone marrow progenitors.
- They increase leukocyte numbers during immune and inflammatory responses.

Cytokines: Messenger molecules of the immune system.

HYPERSENSITIVITY REACTIONS

CC: Describe the mechanism of hypersensitivity reactions.

Q. Classify and briefly describe hypersensitivity reactions.

Immune response is usually a protective process but sometimes it may be injurious. Hypersensitivity means that the body responds to a particular antigens in an exaggerated fashion, where it does not happen in normal circumstances. Thus, hypersensitivity is considered as a immune response that is inappropriately triggered, excessive, or produces undesirable effects on the body.

Definition: Hypersensitivity reaction is a **pathological, excessive and injurious immune response to antigen**, **leading to tissue injury, disease** or sometimes death **in a sensitized individual**. The resulting diseases are named as hypersensitivity diseases.

General Features of Hypersensitivity Disorders

The basic mechanism that triggers hypersensitivity is a specific antigen–antibody reaction or a specific antigen–lymphocyte interaction.

- **Priming or sensitization:** It occurs in **individuals who had previous contact with the antigen** (allergen).
- **Nature of antigens:** It may be **exogenous or endogenous** origin.
  - **Exogenous antigens** (e.g., antigens in dust, pollen, food, drugs, microbes, chemicals and few blood products).
  - **Endogenous antigens:** Self or autologous antigens → cause **autoimmune diseases**.
- **Genetic susceptibility:** Hypersensitivity diseases are usually associated with the inheritance of particular susceptibility genes (e.g., HLA genes).
- **Imbalance between control and effector of immune mechanisms:** It produces damage to host tissues.
- **Mechanism of tissue injury:** Same as the effector mechanisms of defense against infectious pathogens.
  - However, these reactions are poorly controlled, excessive, or misdirected (e.g., against normally harmless environmental and self-antigens).

Classification of Hypersensitivity Reactions (Table 6.3)

Hypersensitivity reaction: Pathological, excessive and injurious immune response to antigen leading to

tissue injury.

TYPE I (IMMEDIATE) HYPERSENSITIVITY REACTIONS

Q. Write short note on type I hypersensitivity reactions and its pathogenesis.

Type I hypersensitivity is an immediate or IgE-mediated reaction, such as anaphylaxis. Usually known as **allergic or atopic disorders** and the environmental antigens that elicit these reactions are known as **allergens**.

Definition: Type I hypersensitivity reaction is a type of immunological tissue reaction. It **occurs rapidly (within 5–10 minutes) after the interaction of antigen** (allergen) **with IgE antibodies bound to the mast cells in a sensitized person**.

Characteristics

- **Immediate** reaction occurring within minutes (5–10 minutes). Hence, type I hypersensitivity is also known as ***immediate hypersensitivity***. Most are caused by excessive T_H2 responses.
- **Antibodies:** Mediated by **IgE antibody**.
- **Develops after the interaction of an environmental antigens (allergens) with IgE antibodies** bound to **mast cells**.
- **Genetic susceptibility:** Occurs in genetically susceptible individuals **previously sensitized** to the antigen. There is a strong genetic or hereditary linkage regarding the IgE response to antigens (allergens).

TABLE 6.3: Classification of hypersensitivity reaction according to the effector immune mechanism.

Types	***Cause of injury***
Antibody mediated	
1. Immediate hypersensitivity reaction (type I hypersensitivity)	T_H2 cells, IgE antibodies, mast cells and other leukocytes
2. Antibody-mediated disorders (type II hypersensitivity)	IgG and IgM antibodies that bind to fixed tissue or cell surface antigens

TABLE 6.3: Classification of hypersensitivity reaction according to the effector immune mechanism.

Types	***Cause of injury***
Antibody mediated	
3. Immune complex–mediated disorders (type III hypersensitivity)	IgG and IgM antibodies bind antigens, usually in the circulation, and form antigen-antibody complexes. They deposit in vascular beds and induce inflammation
Cell mediated	
4. Cell-mediated immune disorders (type IV hypersensitivity)	T lymphocytes of the T_H1 and T_H17 subsets produce cytokines and induce inflammation

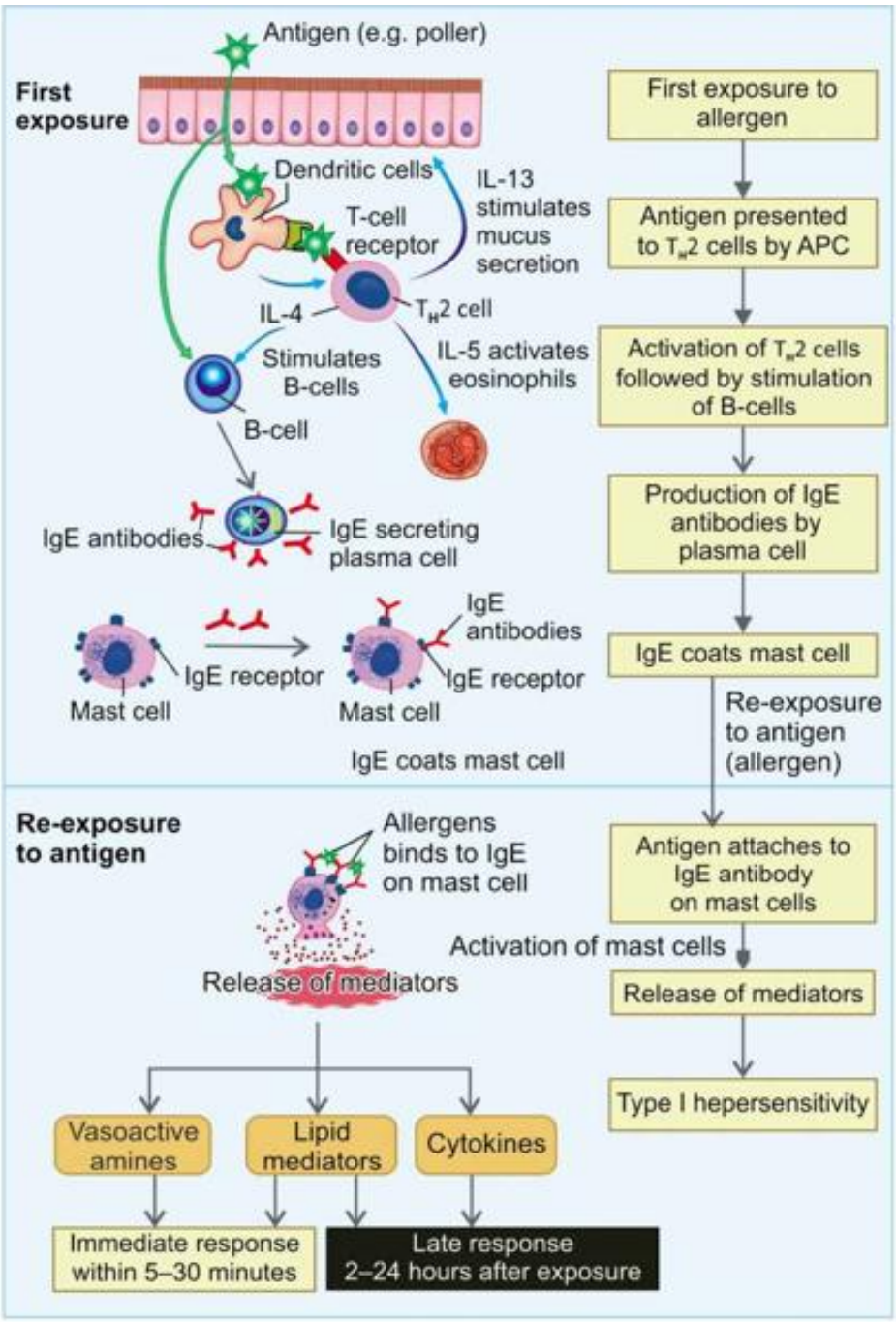


Fig. 6.2: Sequence of events in type I hypersensitivity. It is initiated by the exposure to an allergen, which stimulates T_H2 responses and IgE production, in genetically susceptible individuals. IgE binds to mast cells. On re-exposure to the allergen, antigen binds to IgE on the mast cells and activates it to secrete the mediators. These mediators produce the manifestations of type I hypersensitivity. (APC: antigen presenting cell)

This genetic component involves both the ability to respond to an allergen and the general ability to produce an IgE antibody response.

- **Antigens (allergens):** Many allergens (e.g., house-dust mite, pollens, animal danders or moulds) in the environment are **harmless for majority of individuals**. Allergens elicit significant IgE reactions only in **genetically predisposed individuals**, who are said to be **atopic.**

Pathogenesis

Sequence of Events (Fig. 6.2)

During Initial Exposure to Antigen (Sensitization)

In a genetically susceptible individual, the following events occur**:**

1. **Exposure to sensitizing antigen:** Individuals are exposed to environmental allergens. They may be introduced by: **(1) inhalation, (2) ingestion or (3) injection**.
2. **Activation of T_H2 cells:** In genetically susceptible individual, antigens (allergens) activate **T_H2 subset of $CD4^+$ helper T-cells** (through dendritic cells) → **secretes cytokines (e.g., IL-4**, **IL-5 and IL-13)**.
3. **Production of IgE antibody: IL-4** secreted by T_H2 cells **stimulates B-cells to secrete** cytotropic **IgE antibodies**. IL-5 activates eosinophils and IL-13 stimulates epithelial cells to secrete mucus.
4. **Sensitization of mast cells by IgE antibody:**
   - **Mast cells** are mainly concentrated near blood vessels and nerves and in subepithelial tissues (common sites of type I hypersensitivity).
   - Mast cells possess F_c-epsilon ($F_c\varepsilon R1$) receptor, which have **high affinity for IgE antibodies**.
   - **IgE antibodies** produced by B-cells **attach to** the $F_c\varepsilon R1$ on the **mast cells**. These **IgE antibody bearing mast cells are sensitized to react if antigens binds to these antibodies**.
   - **Eosinophils** also express $F_c\varepsilon R1$ and are involved in IgE mediated defense against helminth infections.

During Subsequent Exposure to Antigen (Activation Phase)

In sensitized individual (the mast cell has attached IgE antibodies), during subsequent re-exposure to the specific allergen, following events occur:

Mast cell activation: The **antigen (allergen) binds** to more than one **IgE antibody molecules on mast cells** → generate signals → **causes mast cell degranulation → secretion of preformed (primary) mediators** that are stored in the granules.

Two phases: IgE triggered reactions can be divided into two phases:

1. **Immediate response:**
   - Develops within 5–30 minutes after exposure to an allergen and subside in 60 minutes.
   - Characterized by vasodilation, vascular leakage, and smooth muscle spasm or glandular secretions.
2. **Late-phase reaction:**
   - Develops in 2–24 hours after the exposure to antigen which may **last for several days**.
   - Characterized by **infiltration of tissues with eosinophils, neutrophils, basophils, monocytes, and T_H2 cells**. It also shows mucosal **epithelial cell damage**.

Mediators of Type I Hypersensitivity Reactions (Fig. 6.3)

1. Preformed Mediators (Primary Mediators)

They are stored in mast cell granules. The local mast cell degranulation releases preformed and newly formed proinflammatory mediators. These released mediators cause an inflammatory response. Their biological effects start immediately following their release. These include:

- **Vasoactive amines:** Most important being **histamine,** which causes:
  - Vasodilatation
  - **Increased vascular permeability**
  - **Smooth muscle contraction**
  - **Increased secretion of mucus** by nasal, bronchial and gastric glands.
- **Enzymes:** It includes neutral proteases (chymase, tryptase) and several acid hydrolases. These enzymes **cause tissue damage** and generate kinins and activates components of complement (e.g., C3a) by acting on their precursor proteins.
- **Proteoglycans:** It includes heparin (anticoagulant), and chondroitin sulfate.

2. Secondary (Newly Synthesized) Mediators

- **Lipid mediators:** They are synthesized and secreted by mast cells, includes leukotrienes and prostaglandins.
  - **Leukotrienes C_4 and D_4:** These are the most powerful (several thousand times than histamine) and cause **increased vascular permeability** and **bronchial smooth muscle contraction**.
  - **Leukotriene B_4** is **chemotactic** for neutrophils, eosinophils and monocytes.
  - **Prostaglandin D_2:** It causes bronchospasm and increased mucus secretion.
- **Cytokines:** Mast cells can produce many cytokines, which may be involved in immediate hypersensitivity reactions. The cytokines include:
  - TNF, IL-1 and chemokines promote leukocyte recruitment (during the late-phase reaction).
  - IL-4 and IL-5 amplifies the T_H2 response and IL-13 stimulates mucus secretion by epithelial cells.

Clinical Manifestations

Clinical manifestations of type I hypersensitivity reaction vary in severity and intensity. In most of the individuals, it produces annoying reactions, such as hives (urticaria), seasonal allergic rhinitis, eczema, or mild bronchoconstriction. In few individuals, the symptoms are more problematic, such as tightening of the throat, localized edema, wheezing, and tachycardia as is associated with localized angioedema reactions or severe airway reactions. Very rarely in few allergic individuals, it can be expressed as a life-threatening allergic reaction known as ***anaphylaxis***.

Examples of type I hypersensitivity reactions are listed in **Table 6.4**.

Diagnosis of Type I Hypersensitivity

- Typical clinical history and examination
- Skin-prick testing
- Measuring specific IgE in the serum.

Allergen: Antigen that evoke allergic response.

Immunoglobulin involved in type I hypersensitivity reaction: IgE.

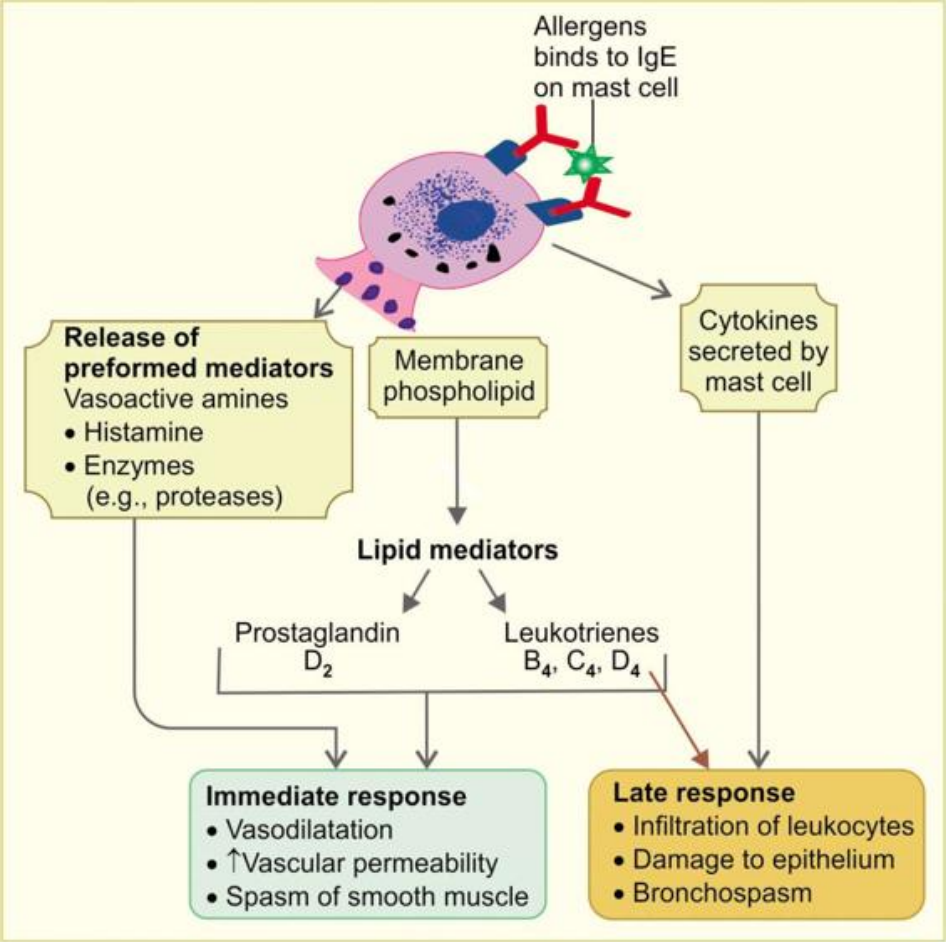


Fig. 6.3: Mast cell mediators involved in type I hypersensitivity.

TABLE 6.4: Examples of type I hypersensitivity reactions.

Localized type I hypersensitivity	***Systemic type I hypersensitivity***
• Bronchial asthma (extrinsic) • Hay fever/allergic rhinitis • Allergic conjunctivitis • Urticaria • Atopic dermatitis/eczema • Angioedema • Allergic gastroenteritis (food allergy)	Anaphylaxis due to: • Antibiotics: Most commonly penicillin • Bee stings • Insect bite • Foreign proteins (e.g., antisera) • Foods (peanuts, fish and shellfish) • Food additives

Type I hypersensitivity: Produced by environmental antigens (allergens) in a genetically susceptible individuals.

T_H2 cells: Play a central role in immediate hypersensitivity reactions.

Type I hypersensitivity reaction commonly referred as allergy.

Type I hypersensitivity: Principal mediators involved are:

- Histamine
- Enzymes (e.g., proteases)
- Prostaglandins
- Leukotrienes
- Cytokines

- IL-4: Responsible for secretion of IgE from the B-cells.
- IL-5: Most potent eosinophil-activating cytokine.

Type I hypersensitivity: First exposure to allergens elicit a strong T_H2 response which stimulates production of IgE by B-cells → IgE attaches to mast cells.
On re-exposure antigens cross-link IgE and stimulate mast cell to secrete mediators.

Type I hypersensitivity reaction: Release of mediators occur in two phases:

1. Immediate response
2. Late-phase reaction.

Type I hypersensitivity: It may manifest as systemic fatal anaphylaxis or more commonly as local allergic reactions.

Bee sting reaction is mediated by IgE (type I hypersensitivity).

ANTIBODY-MEDIATED (TYPE II) HYPERSENSITIVITY REACTIONS

Q. Write short note on type II hypersensitivity reactions.

Definition: Type II hypersensitivity disorders are **caused by antibodies (IgG/IgM)**, which react with **target antigens on the surface of specific cells or fixed in the extracellular matrix (tissues)**.

Characteristics

Antibodies: IgG (usually) and IgM (rarely) type of antibodies mediate type II reactions.
Antigen: It may be endogenous or exogenous:

- **Endogenous antigens:** It may be normal molecules **intrinsic to the cell membrane or extracellular matrix** (e.g., autoimmune diseases).
- **Exogenous antigens:** These antigens may get **adsorbed on a cell surface or extracellular matrix** → may cause altered surface antigen (e.g., drug metabolite).

Mechanism of Injury

Three mechanism of type II hypersensitivity reactions are as follows:

1. Opsonization and Phagocytosis (Fig. 6.4A)

Complement injure the target cells by promoting their phagocytosis.

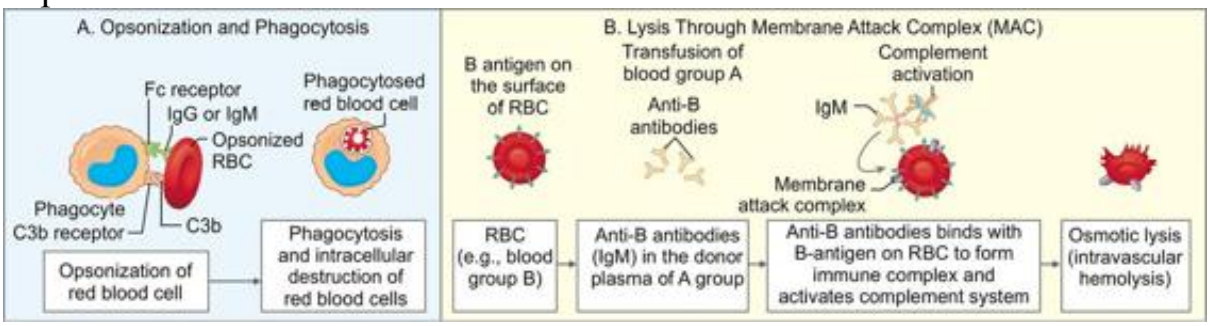
- **Production of antibodies: Antigen may be intrinsic to target cells** (e.g., RBC or platelets) **or exogenous antigen adsorbed to its cell surface** (e.g., drug metabolites). **B-cells produce IgG antibodies** (e.g., autoantibodies) against target antigens.
- **Activation of complement:** Antigen antibody complexes are formed on the surfaces of the target cells → may activate the complement system by the classical pathway.
- **Opsonization: Complement components such as C3b**, **acts as opsonins** and gets deposited on the surfaces of the target cells.
- **Phagocytosis:** Opsonized cells are recognized by phagocytes through Fc and C3b receptors on its surface → results in **phagocytosis of the opsonized cells → destruction of cells by phagocytes** (e.g., macrophages in spleen).

  Examples:
  - **Autoimmune hemolytic anemia: Target antigen is RBC membrane protein (Rh or I antigen)** and antibodies against their own red blood cells.
  - **Autoimmune thrombocytopenia purpura: Target antigen is GpIIb/IIIa** or Ib-IX of platelets.
  - **Drug-induced hemolytic anemia.**

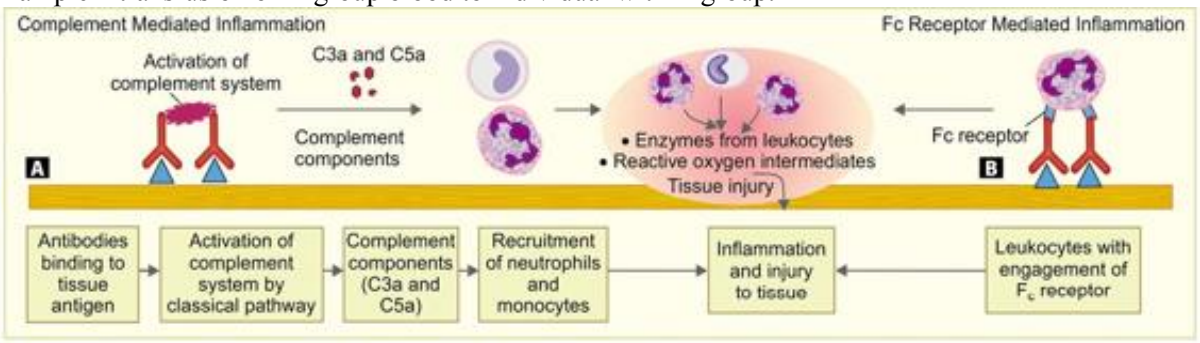
Lysis of Target Cells Through Membrane Attack Complex (Fig. 6.4B)

- Complement **activation on cells can generate membrane attack complex** [MAC (C5–9)]. MAC disrupts membrane integrity and causes **lysis of the cells (Fig. 6.4B)**.

- **Example: (1) Transfusion reactions** in which the cells from an incompatible donor react with and are opsonized by preformed antibody in the recipient. (2) **Hemolytic disease of newborn** (erythroblastosis fetalis), in which IgG anti–red blood cell antibodies from the mother cross the placenta and cause destruction of fetal RBCs.



Figs. 6.4A and B: Type II hypersensitivity reaction: (A) Opsonization and phagocytosis: The opsonized red cells coated by antibodies and complement components are phagocytosed followed by lysis of red cells inside the phagocytes (e.g., macrophage in the spleen). (B) Cell lysis through membrane attak complex (MAC). Binding of IgG or IgM antibody to an antigen promotes complement fixation. Activation of complement leads to formation of membrane attack complex which causes cell lysis. Example—transfusion of A group blood to individual with B group.



Figs. 6.5A and B: Type II-hypersensitivity reaction—complement and Fc receptor mediated inflammation: (A) Antibody binds to a surface antigen, activates the complement system and leads to the recruitment of tissue-damaging inflammatory cells. Several complement-derived peptides (e.g., C5a) are potent chemotactic factors; (B) Inflammation may also be induced by antibody binding to Fc receptors of leukocytes.

2. Tissue Injury by Complement and Fc Receptor Mediated Inflammation (Figs. 6.5A and B)

Complement induces inflammation and causes injury to target cells in two ways: (a) through activation of classic complement pathway and (b).

a. **Antibodies against matrix components in fixed tissue antigens (Fig. 6.5A)**, such as basement membranes and extracellular matrix may **activate**

complement system by classical pathway.

- Complement components may **cause injury due to inflammation**. This may be due to (1) chemotactic agents (mainly C5a) produced at the site of deposition of antibody and (2) anaphylatoxins (C3a and C5a), which increase vascular permeability.
- Activated **inflammatory cells** (leukocyte) **release lysosomal enzymes and reactive oxygen species** which **damage tissues**.

b. Inflammation may also be induced by antibody binding to Fc receptors of leukocytes **(Fig. 6.5B)**.

- **Example: Goodpasture syndrome** in which anti-glomerular basement membrane antibody binds to a glomerular basement membrane antigen and activates the complement system. The recruitment of inflammatory cells damages the basement membrane.

3. Antibody-mediated Cellular Dysfunction

It is characterized by deposition of antibodies against target cell surface receptors, which may impair or dysregulate function of the target cell without causing cell injury or inflammation. Examples:

- **Antibody-mediated stimulation of cell function: In Graves' disease**, antibodies against the thyroid-stimulating hormone receptor on thyroid epithelial cells stimulate the cells. This results in hyperthyroidism **(Fig. 6.6)**.
- **Antibody-mediated inhibition of cell function:** In **myasthenia gravis (Fig. 6.7)**, antibodies directed against acetylcholine receptors in the motor end plates of skeletal muscles block neuromuscular transmission. This causes muscle weakness.

Mechanism of type II hypersensitivity reactions are summarized in **Flowchart 6.1**.

Examples of type II hypersensitivity diseases are presented in **Table 6.5**.

Type II hypersensitivity:

- Antibodies can coat (opsonize) cells with or without complement and target these cells for phagocytosis by macrophages.
- Macrophages express Fc receptor and receptor for complement.

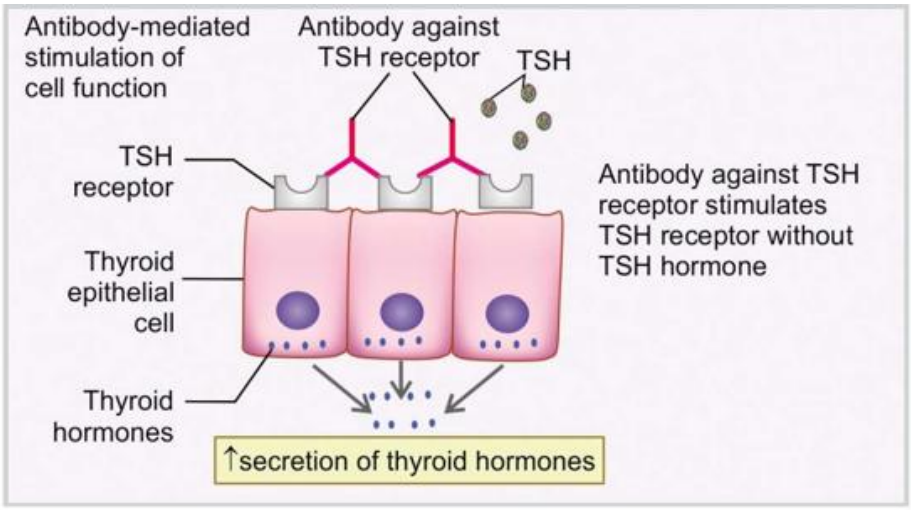


Fig. 6.6: Type II hypersensitivity reaction: Antibody-mediated stimulation of cell function. Autoantibodies bind against the thyroid-stimulating hormone (TSH) receptor and activate thyroid cells to produce excessive production of hormones and causing hyperthyroidism in Graves' disease.

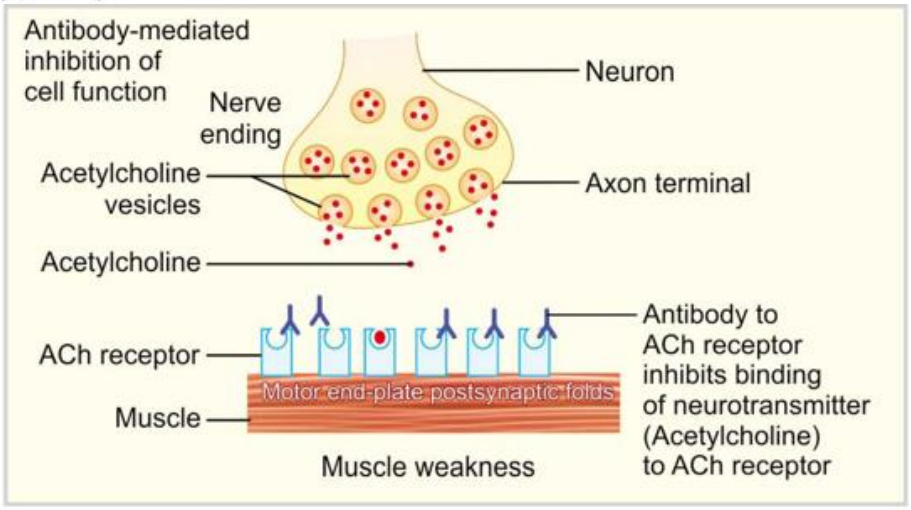
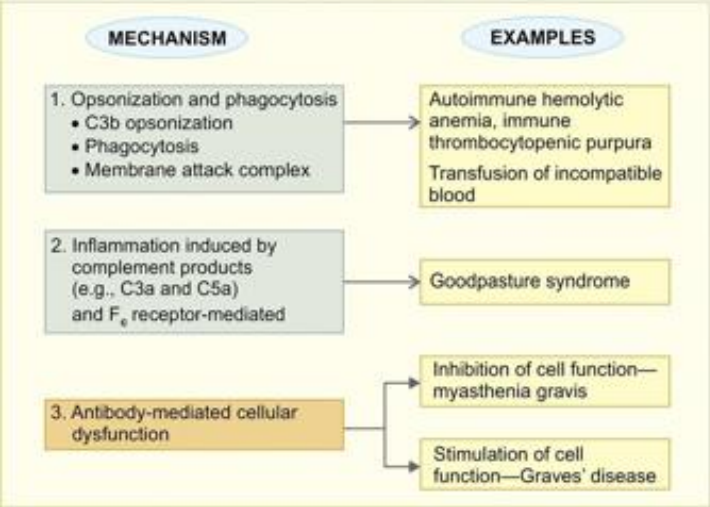


Fig. 6.7: Type II hypersensitivity reaction: Antibody-mediated inhibition of cell function. Anti-receptor antibodies may inhibit/ disturb the normal function of receptors. Example—autoantibodies to the acetylcholine (ACh) receptor on skeletal muscle cells in myasthenia gravis produce disease by blocking neuromuscular transmission and causing progressive muscle weakness.

Flowchart 6.1: Summary of mechanism of type II hypersensitivity reactions.



Type II hypersensitivity: Caused by antibodies (IgG/IgM), that react with antigens on the surface of cells or fixed in the extracellular matrix.

Antibody-mediated (type II) hypersensitivity reaction:
- Transfusion reactions
- Hemolytic disease of newborn
- Autoimmune hemolytic anemia

Mechanism of type II hypersensitivity:
- Complement dependent
- Complement independent (antibody-dependent)

Complement-dependent reactions:
- Opsonization and phagocytosis
- Lysis by MAC
- Complement and Fc receptor mediated inflammation

TABLE 6.5: Examples of type II hypersensitivity (antibody-mediated) diseases.

Mechanism and disease	*Target antigen*
1. Opsonization and phagocytosis (IgG-mediated)	
Autoimmune hemolytic anemia	Cell-surface antigens (Rh blood group antigens, I antigen)
Autoimmune thrombocytopenic purpura	Platelet membrane glycoprotein IIb: IIIa integrin
Transfusion reactions	The cells from an incompatible donor react with and are opsonized by preformed antibody in the recipient
2. Complement and Fc receptor-mediated inflammation (IgG-mediated)	
Goodpasture syndrome	Antibody against matrix antigens (basement membrane noncollagenous protein of kidney glomeruli and lung alveoli)
3. Antibody-mediated (Complement independent) cellular dysfunction	
a. Graves' disease (hyperthyroidism)	Antibody against receptors: Thyroid-stimulating hormone (TSH) receptor (agonistic antibodies)
b. Myasthenia gravis	Antibody against receptors: Acetylcholine receptor (antagonistic antibodies)

Type II hypersensitivity: Antibody-dependent cellular dysfunction to:
- Stimulation of cell function (Graves' disease) or
- Inhibition of cell function (myasthenia gravis)

IMMUNE COMPLEX-MEDIATED (TYPE III) HYPERSENSITIVITY REACTIONS

Q. Write short note on type III hypersensitivity reactions.

Type III hypersensitivity reaction is IgG/IgM immune complex mediated reaction (e.g., immune complex glomerulonephritis and SLE). It results from failure of the immune and phagocytic systems to effectively remove antigen–antibody immune complexes and is not tissue specific. It is not an immediate reaction. It occurs over a period of several hours or longer (i.e., glomerulonephritis) and is usually ongoing process.

Definition: Type III hypersensitivity reactions are **characterized by formation of immune** (antigen and antibody) **complexes in the circulation**. They may **get deposited in blood vessels, leading to complement activation and a subsequent self-sustaining acute inflammation.** The inflammatory cells recruited (neutrophils and monocytes) **release lysosomal enzymes → generate toxic free radicals → cause tissue damage**.

Less commonly, the immune complexes may be formed at sites where antigen has been "planted" previously (in situ immune complexes).

Characteristics

Antibodies: Complement-fixing antibodies namely IgG, IgM and occasionally IgA.

Antigen: It may be exogenous or endogenous.

- **Exogenous:** Various **foreign proteins** such as foreign serum protein injected (e.g., diphtheria antitoxin, horse anti-thymocyte globulin) or produced by an infectious microbe.
- **Endogenous: Antibody against self-components** (autoimmunity), e.g., nucleoproteins.

Sites of Antigen–Antibody Formation

- **Circulating** immune complexes**:** They are **formed within the circulation**.
- **In situ immune complex:** They formed at **extravascular sites** where antigen might have been previously planted.

Sites of Immune Complex Deposition

- **Systemic:** Circulating immune complexes may be **deposited in many organs**.
- **Localized:** Immune complexes may be **deposited or formed in particular organs/tissues such as kidney** (glomerulonephritis), **joints** (arthritis), **small blood vessels of the skin**.

Cause of Tissue Damage

- **Activation of complement**
- **Inflammation at the sites of deposition.**

Examples of immune complex disorders are listed in **Table 6.6**.

Systemic Immune Complex Disease—Acute Serum Sickness

Q. Write short note on serum sickness.

This was a frequent sequelae to the administration of large amounts of foreign serum (e.g., serum from immunized horses used for protection against diphtheria). Nowadays, foreign serums are not used. Hence, it is infrequent.

Pathogenesis (Fig. 6.8)

Divided into three phases:

1. **Formation of immune complexes:**
   - **Introduction of protein antigen:** It initiates an immune response.
   - **Formation of antibody:** It usually forms a week (7–12 days) after the injection of the foreign protein and are secreted into the blood.
   - **Formation of immune complexes:** They are formed in the circulation when antibodies react with the antigen. Most immune complexes are removed effectively before they can cause tissue injury. In type III hypersensitivity, the immune complexes are not removed.

TABLE 6.6: Examples of immune complex-mediated diseases.

Disease	*Antigen*	*Manifestations*
Exogenous antigen		

TABLE 6.6: Examples of immune complex-mediated diseases.		
Disease	***Antigen***	***Manifestations***
Poststreptococcal glomerulonephritis	Streptococcal cell wall antigen(s)	Glomerulonephritis
Serum sickness	Various proteins, e.g., foreign serum protein (horse anti-thymocyte globulin)	Arthritis, vasculitis, nephritis
Arthus reaction	Various foreign proteins	Cutaneous vasculitis
Endogenous antigen		
Systemic lupus erythematosus (SLE)	Nuclear antigens	Glomerulonephritis, skin lesions, arthritis, others

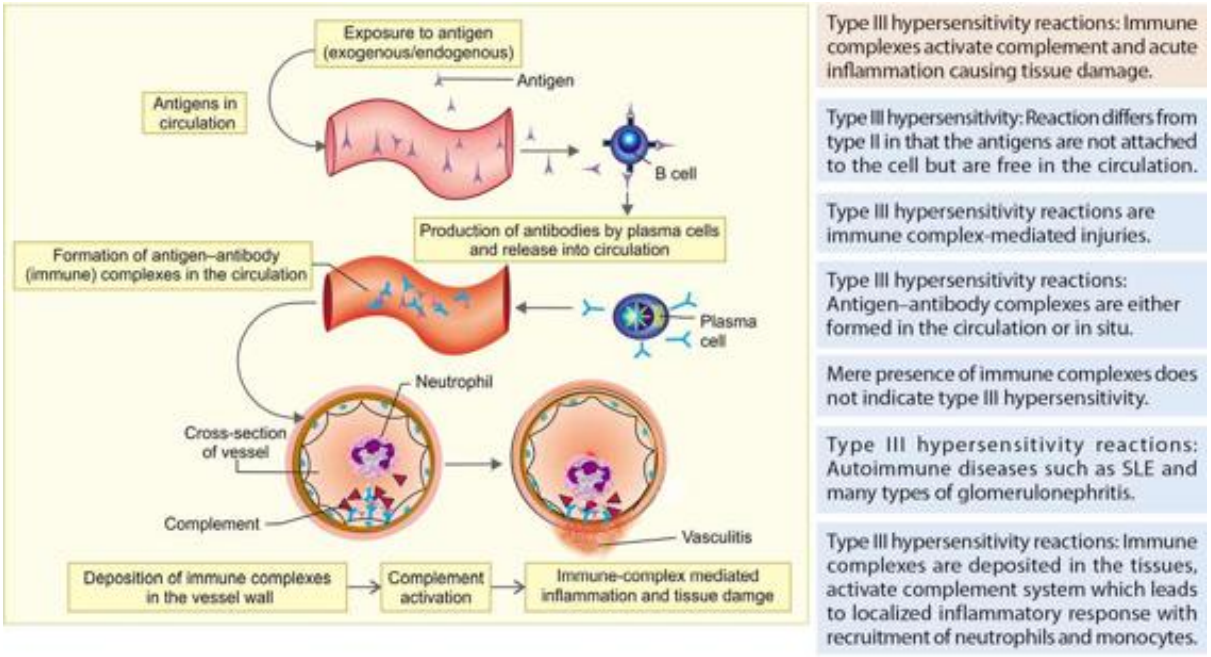


Fig. 6.8: Pathogenesis of systemic immune complex-mediated disease (type III hypersensitivity). Immune complexes are deposited in tissue activate complement system and recruit tissue-damaging inflammatory cells. The pathogenic ability of immune complexes to mediate tissue injury depends on size, solubility, net charge and ability to fix complement.

2. **Deposition of immune complexes:**
   - **Immune complexes** of **medium size** and with **slight antigen excess** are the **most pathogenic**. They cause an inflammatory process leading to tissue injury.
   - **Sites of deposition:**
     - **Blood vessels:** It causes vasculitis.
     - **Renal glomeruli:** It causes glomerulonephritis.
     - **Joints:** It causes arthritis.

3. **Inflammatory reaction and tissue injury:** Mechanism of tissue injury include:
   - **Inflammatory reaction: Immune complexes** in the tissue **activates complement**, the products (e.g., chemotactic C5a) of which causes chemotactic **recruitment of acute inflammatory cells** (neutrophils and monocytes) to the site.
   - **Tissue damage:** Activated inflammatory cells (leukocyte) **release lysosomal enzymes, arachidonic acid products and reactive oxygen species** → which produce **tissue damage**.

Clinical features: Fever, urticaria, joint pains (arthralgias), lymph node enlargement and proteinuria appear during this phase.

MORPHOLOGY

General Features

- **Acute necrotizing vasculitis:** It is the **main feature** and is characterized by **necrosis of the vessel wall and intense neutrophilic infiltration**.
- **Fibrinoid necrosis:** It consists of necrotic tissue, immune complexes deposits, complement and plasma protein. It produces a **smudgy eosinophilic appearance** (*refer* **Fig. 2.19**) at the site of deposit and obscures the cellular detail.

Kidney

- **Immunofluorescence microscopy:** It appears as **granular lumpy deposits** (*refer* **Fig. 20.8**) of immunoglobulin and complement.
- **Electron microscopy:** It appears as **electron-dense deposits** along the glomerular basement membrane (*refer* **Fig. 20.9**).

Fate of the Lesion

- **Single dose of antigen:** If the disease is due to a single large dose of antigen, the lesions tend to be **self-limiting and lesions resolve**. This is because continued rise in antibody produces larger immune complexes, which are catabolized by phagocytosis, e.g., acute serum sickness, perhaps **acute poststreptococcal glomerulonephritis**.
- **Repeated dose of antigen:** A chronic form of serum sickness results from repeated or prolonged exposure to an antigen. Examples:
  - **Systemic lupus erythematosus** (SLE), which is associated with persistent antibody responses to autoantigens.

- **Membranous glomerulonephritis, polyarteritis nodosa** and several other vasculitides.

Local Immune Complex Disease—Arthus Reaction

Q. Write short note on Arthus reaction.

- Arthus reaction is a **local area of tissue necrosis usually in the skin**, resulting from acute immune complex vasculitis.
- Arthus reaction can be experimentally produced by intracutaneous injection of an antigen to a previously immunized animal (with circulating antibodies against the antigen). As the antigen diffuses into the vascular wall, it locally binds to the antibody and form large immune complexes at the site of injection.
- **Immune complexes deposited in the vessel walls, cause fibrinoid necrosis and thrombosis leading to ischemic injury**.

Type III hypersensitivity: Immune complexes of **medium size** and with **slight antigen excess** are pathogenic.

Type III hypersensitivity: Small quantity of immune complexes is formed during normal immune responses and is usually destroyed by phagocytosis.

Very large immune complexes (with antibody excess) are cleared from circulation by macrophages in the spleen and liver. They are usually harmless.

Type III hypersensitivity: During the active phase, activation of complement system leads to a decrease level of C3 in the serum and can be used to monitor disease activity.

Raji cell assay are used to quantitate immune complexes.

Fibrinoid necrosis: Seen in

- Polyarteritis nodosa
- Malignant hypertension
- Aschoff bodies
- Arthus reaction
- SLE
- Rheumatoid nodule

Type III hypersensitivity reactions: Inflammatory cells, complement, and accompanying release of potent inflammatory mediators is responsible for injury.

T-CELL MEDIATED (TYPE IV) HYPERSENSITIVITY REACTIONS

Q. Write short note on type IV hypersensitivity reactions/delayed hypersensitivity reactions.

- Type IV hypersensitivity reaction is **mediated by T lymphocytes** (T cell-mediated diseases) including **$CD4^+$ and $CD8^+$ T-cells**.
- It develops in response to antigenic exposure in a previously sensitized individual.
- **Reaction is delayed by 48–72 hours after exposure to antigen.** Hence also called as delayed-type hypersensitivity (DTH).
- This hypersensitivity reaction is involved in several **autoimmune diseases** (e.g., rheumatoid arthritis, Hashimoto's thyroiditis), pathological **reactions to environmental chemicals** (e.g., poison ivy, nickel) and **persistent microbes (e.g., tuberculosis, leprosy)**.

Types: Two types, namely (1) **Cytokine-mediated** inflammation in which **$CD4^+$ T-cells produce cytokines** and (2) **Direct cell toxicity** mediated by **$CD8^+$ T-cells**.

$CD4^+$ T-Cell-mediated Inflammation (Fig. 6.9)

$CD4^+$ T cytokines produced by the $CD4^+$ T is responsible for $CD4^+$ T cell-mediated hypersensitivity reactions. The cytokines can induce chronic and destructive inflammation. The classical example for T cell–mediated inflammation is ***delayed-type hypersensitivity (DTH)***. In DTH reaction, an antigen is administered into the skin of a previously immunized individual. This leads to cutaneous reaction within 24–48 hours. Since the reaction is delayed by 24–48 hours, it is termed delayed, in contrast to immediate hypersensitivity (which occurs immediately). The mechanism is as follows:

- **Type of antigen:** Antigen may be either **exogenous** environmental antigens or **endogenous** (**self-antigens** causing autoimmune disease).
- **Recognition of tissue antigen by naïve $CD4^+$ T-cells** presented by antigen presenting cell (APC).
- **Differentiation of $CD4^+$ T-cells into effector cells of T_H1 type or T_H17 type depending on the cytokine.**
- **T_H1 cells → production of cytokines** (e.g., **IFN-γ and TNF)**. IFN-γ (**most powerful macrophage activating cytokine) → activates macrophages**. The macrophages produce substances that destroy intracellular microbes and damage tissues.

- **T_H17 cells:** They **produce IL-17, IL-22, chemokines and other cytokines** that **promote inflammation** by recruiting more **neutrophils and monocytes** to the site of reaction.

Three examples of $CD4^+$ T cell-mediated inflammatory reactions are discussed below. These are: (i) tuberculin reaction, (ii) granulomatous inflammation and (iii) contact dermatitis.

Tuberculin reaction (Montoux test): Tuberculin reaction is a classical example for delayed-type hypersensitivity.

- It is produced by the **intracutaneous injection of purified protein derivative** (PPD, also called tuberculin), a protein-containing antigen of the tubercle bacillus.
- **In a previously sensitized individual, the injection site becomes red and indurated** in 8–12 hours, reaches a peak (usually 1–2 cm in diameter) in 24–72 hours, and thereafter slowly subsides.
- Microscopically, the injected site shows perivascular accumulation ("cuffing") of $CD4^+$ T-cells and macrophages.

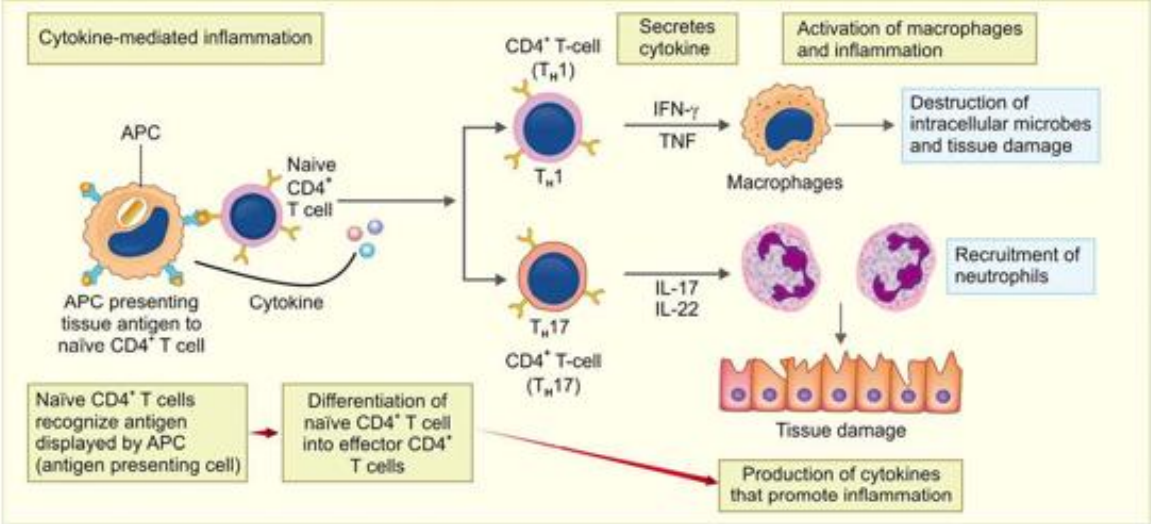


Fig. 6.9: Mechanisms of $CD4^+$ T-cell mediated (type IV) hypersensitivity reactions. In delayed-type hypersensitivity reactions, antigens are presented to naïve T-cells by antigen presenting cell (APC). Depending on the cytokines produced by APC, naïve T-cells may differentiate into $CD4^+$ T_H1 or $CD4^+$ T_H17. $CD4^+$ T_H1 cells secrete cytokines that activate macrophage leading to tissue injury. $CD4^+$ T_H17 cells produce cytokines that produce inflammation by recruiting neutrophils. Both mechanisms produce tissue damage.

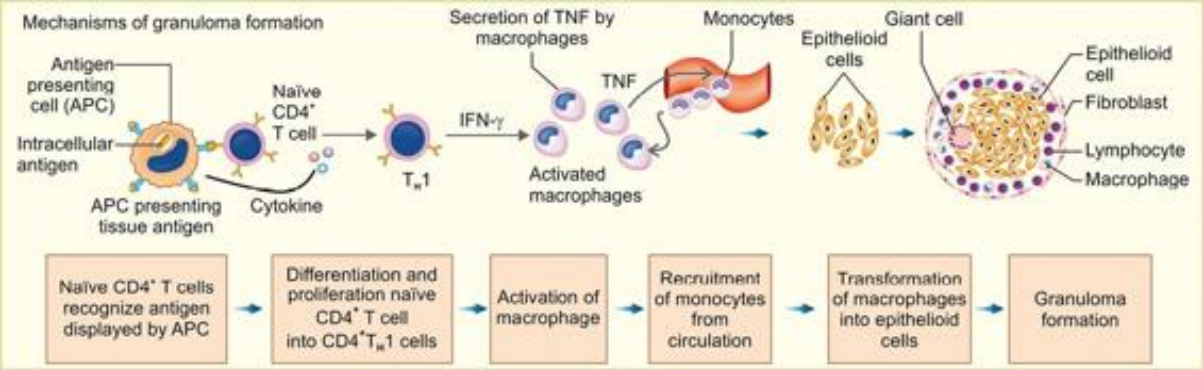


Fig. 6.10: Mechanisms of granuloma formation in cell-mediated (type IV) hypersensitivity reactions.

Granulomatous inflammation: Prolonged DTH reaction against persistent microbes (e.g., tubercle bacilli) **or other nondegradable** (foreign bodies) **injurious agent** may produce a special type of microscopic reaction known as **granulomatous inflammation**.

Mechanisms of granuloma formation in cell-mediated (type IV) hypersensitivity reactions (Fig. 6.10): Different steps involved are:

1. **Exposure to antigen.**
2. **Antigen presenting cells (APCs) present the antigen to naïve $CD4^+$ T-cells.**
3. **Differentiation and proliferation of $CD4^+$ T-cells into $CD4^+T_H1$ cells.**
4. **Replacement of $CD4^+$ T_H1-cells by activated macrophages over a period of 2 or 3 weeks**.
5. **TNF secreted by activated macrophages causes recruitment of monocytes from circulation.**
6. The activated macrophages undergo a morphologic evidence of activation. These include—transformation into large, flat, eosinophilic and epithelium-like cells referred to as **epithelioid cells**. The cytokines (e.g., IFN-γ) may cause fusion of epithelioid cells to form multinucleated **giant cells**.
7. **Granuloma** is a **microscopic aggregate of epithelioid cells (Fig. 6.11)**, **surrounded by a rim of lymphocytes**. Older granulomas are enclosed by rim of fibroblasts and connective tissue.

Contact dermatitis: Contact with various environmental antigens (e.g., poison ivy, metals such as nikel and chromium, chemicals like hair dyes, cosmetics, soaps) may evoke inflammation with blisters in the skin at the site of contact known as contact dermatitis.

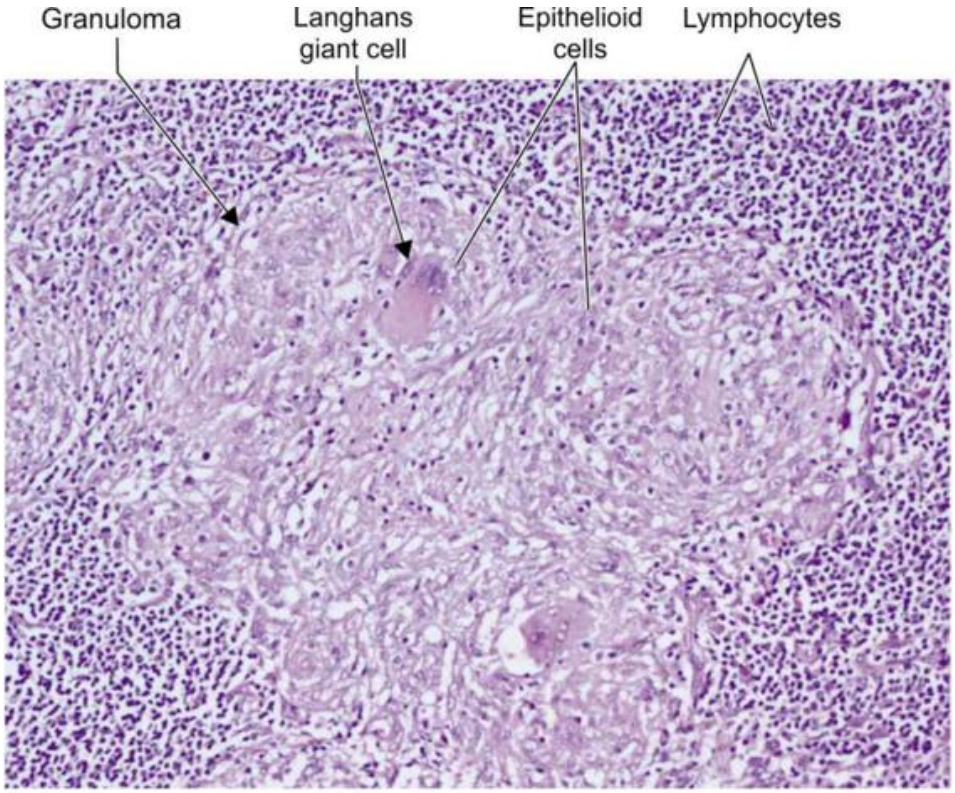


Fig. 6.11: Granulomatous inflammation. Section of a lymph node with granuloma. It consists of an aggregate of epithelioid cells surrounded by lymphocytes. The granuloma shows several multinucleate giant cells.

$CD8^+$ T-Cell-mediated Cytotoxicity (Fig. 6.12)

It is a type of T-cell mediated tissue injury due to $CD8^+$ T lymphocytes (also called as cytotoxic T lymphocytes or CTLs), which kill antigen-bearing target cells. Destruction of tissues by CTLs may play important role in some T cell-mediated diseases. Examples include type 1 diabetes, graft rejection, killing of virus infected cells (e.g., in viral hepatitis) and some tumor cells. By killing the infected cells, it eliminates the infection. However, in few cases, it is responsible for cell damage that accompanies the infection (e.g., in viral hepatitis).

Examples of T-cell mediated (type IV) hypersensitivity are shown in **Table 6.7**.

Salient features and differences between hypersensitivity reactions are presented in **Table 6.8**.

CD3 is known as pan T-cell marker and is involved in T-cell activation.

IFN-γ activated macrophages:

- Increased phagocytic and microbicidal power
- Express more class II MHC molecules on the surface→ facilitate further antigen presentation
- Secrete TNF, IL-1 and chemokines→ promote inflammation
- Produce more IL-12→ amplify T_H1 response

IL-2 is characteristic product in T_H1 response.

Positive tuberculin test indicates that the individual is previously exposed to tuberculosis. However, immunosuppression (e.g., HIV) may be associated with negative tuberculin test despite the presence of severe infection.

Type IV hypersensitivity: $CD8^+$ cytotoxic T-cells (CTLS) kill cells (by apoptosis) that express antigens in the cytoplasm that are seen as foreign. Example—virus infected cells, tumor cells and donor graft cells.

Feature common to both cytotoxic T-cells and NK cells is that they are effective against virus infected cells.

AUTOIMMUNE DISEASES

CC: Define autoimmunity. Enumerate autoimmune disorders.

Definition: Autoimmunity is defined as **immune reactions** in which **body produces autoantibodies** and **immunologically competent T lymphocytes against self-antigens**.

Autoimmunity is an important cause of certain diseases in humans **(Table 6.9)**.

- **Organ-specific disease:** It may be restricted to a single organ or tissue (e.g., type 1 diabetes).
- **Systemic or generalized disease** [e.g., systemic lupus erythematosus (SLE)].

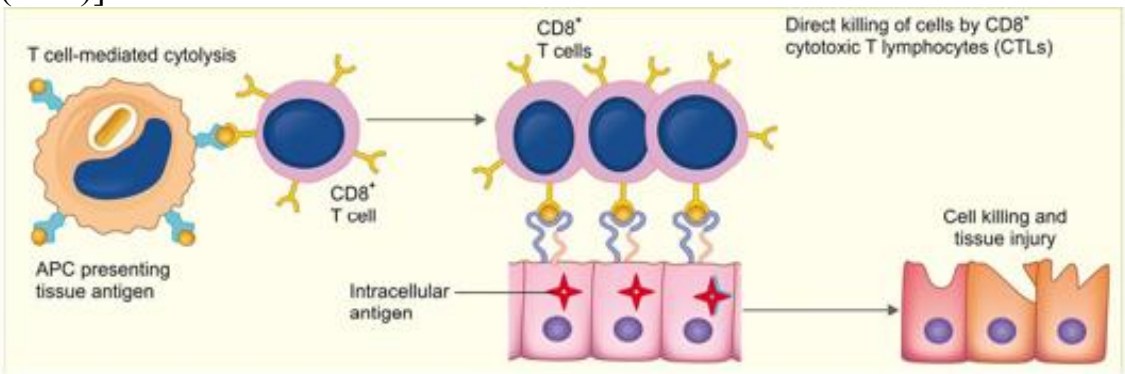


Fig. 6.12: Mechanisms of T-cell mediated (type IV) hypersensitivity reactions by direct cell toxicity mediated by $CD8^+$ cytotoxic T lymphocytes.

TABLE 6.7: Examples of T-cell mediated (type IV) hypersensitivity.

Disease	*Antigen*	*Manifestations*
Type 1 diabetes mellitus	Antigens of pancreatic islet β cells (insulin, glutamic acid decarboxylase, others)	Insulitis (chronic inflammation in islets), destruction of β cells; diabetes mellitus
Rheumatoid arthritis	Collagen; citrullinated self-protein	Chronic arthritis, inflammatory destruction of articular cartilage and bone
Inflammatory bowel disease	Enteric bacteria, self-antigen	Chronic inflammation of intestine, ulceration
Hashimoto thyroiditis	Thyroglobulin and other thyroid proteins	Hypothyroidism
Contact sensitivity (dermatitis)	Environmental chemicals (e.g., poison ivy)	Inflammation of skin and blisters

Most potent stimulator of naïve T-cell is mature dendritic cell.

TABLE 6.8: Salient features and differences between hypersensitivity reactions.

Features	***Type I***	***Type II***	***Type III***	***Type IV***
Antigens	Exogenous allergens include: Pollen, moulds, mites, drugs, food, etc.	Cell surface or tissue bound	Soluble exogenous (viruses, bacteria, fungi, parasites) or endogenous autoantigens	Cell/tissue bound
Antibody involved	IgE	IgG and IgM	IgG, IgM, IgA	None
Mediators	From mast cells	Complement and lymphokines	Complement	T lymphocytes, activated macrophages
Time taken for reaction to develop	5–10 min	6–36 hours	4–12 hours	48–72 hours
Immunopathology	Edema, vasodilatation, mast cell degranulation, eosinophils	Antibody-mediated damage to target cells/tissue	Acute inflammatory reaction, neutrophils, vasculitis	Perivascular inflammation, mononuclear cells, fibrin, granulomas, caseation and necrosis in TB

TABLE 6.8: Salient features and differences between hypersensitivity reactions.

Features	*Type I*	*Type II*	*Type III*	*Type IV*
Examples of diseases and conditions produced	• Asthma (extrinsic) • Urticaria/edema • Allergic rhinitis • Food allergies • Anaphylaxis	• Autoimmune hemolytic anemia • Transfusion reactions • Hemolytic disease of newborn • Goodpasture syndrome • Acute rheumatic fever • Pernicious anemia • Myasthenia gravis	• Autoimmune, e.g., SLE • Glomerulonephritis • Rheumatoid arthritis • Farmer's lung disease • Hypersensitivity pneumonitis • Arthus reaction (localized)	• Pulmonary TB • Contact dermatitis • Tuberculin test • Leprosy • Graft-versus-host

Q. List autoimmune diseases.

TABLE 6.9: Examples of autoimmune diseases.

Diseases mediated by antibodies	*Diseases mediated by T-cells*
Organ-specific	**Organ-specific**
Autoimmune hemolytic anemia	Type 1 diabetes mellitus
Autoimmune thrombocytopenia	Hashimoto thyroiditis
Goodpasture syndrome	Crohn's disease
Myasthenia gravis	Multiple sclerosis
Graves' disease	
Systemic	**Systemic**
Systemic lupus erythematosus	Rheumatoid arthritis

- **Involving more than one organ** (e.g., Goodpasture syndrome, in which lung and kidney are involved).

Normal individuals are unresponsive (tolerant) to their own (self) antigens and autoimmune disorders results from the loss of self-tolerance.

Autoimmune diseases: May be mediated by:
- Autoantibodies or

- T-cells against self-antigens

Autoimmunity: Presence of immune responses against self tissue. Autoimmune diseases occur if these immune responses cause significant tissue/organ damage.

Immunological Tolerance

Immunological tolerance is the phenomenon in which there is **no immune response to specific (usually self) antigens**. It is the result of exposure of lymphocytes to that specific antigen.

Self-tolerance: It is absence of immune response to an individual's own antigens.

Mechanisms of Self-tolerance

- Numerous different antigen receptors are produced in the developing T and B lymphocytes.
- These receptors are capable of recognizing self-antigens and these lymphocytes have to be eliminated or inactivated as soon as they recognize the antigens, to prevent immune reaction against own antigens.
- The mechanism by which this is achieved can be broadly classified into two groups: (1) central tolerance and (2) peripheral tolerance **(Figs. 6.13A and B)**.

Central Tolerance (Fig. 6.13A)

Q. Write short note on central immune tolerance.

It is the **process by which self-reactive T and B lymphocytes** (which recognize self-antigens) are **deleted** (killed) during their maturation **within the central (or generative) lymphoid organs**. These **organs are thymus for T-cells and the bone marrow for B-cells**.

Mechanisms of central tolerance

T-cells:

- **Negative selection or deletion:** It is a process by which immature **self-reactive T lymphocytes** that encountered antigens are eliminated by **apoptosis**. It occurs **in the thymus**. **AIRE (autoimmune regulator)**, a protein product of AIRE gene is **critical for deletion of immature self-**

reactive T-cells. Mutations in AIRE gene are the cause of an autoimmune polyendocrinopathy.

- **Regulatory T-cells:** Some T-cells may differentiate into regulatory T-cells.

B-cells:

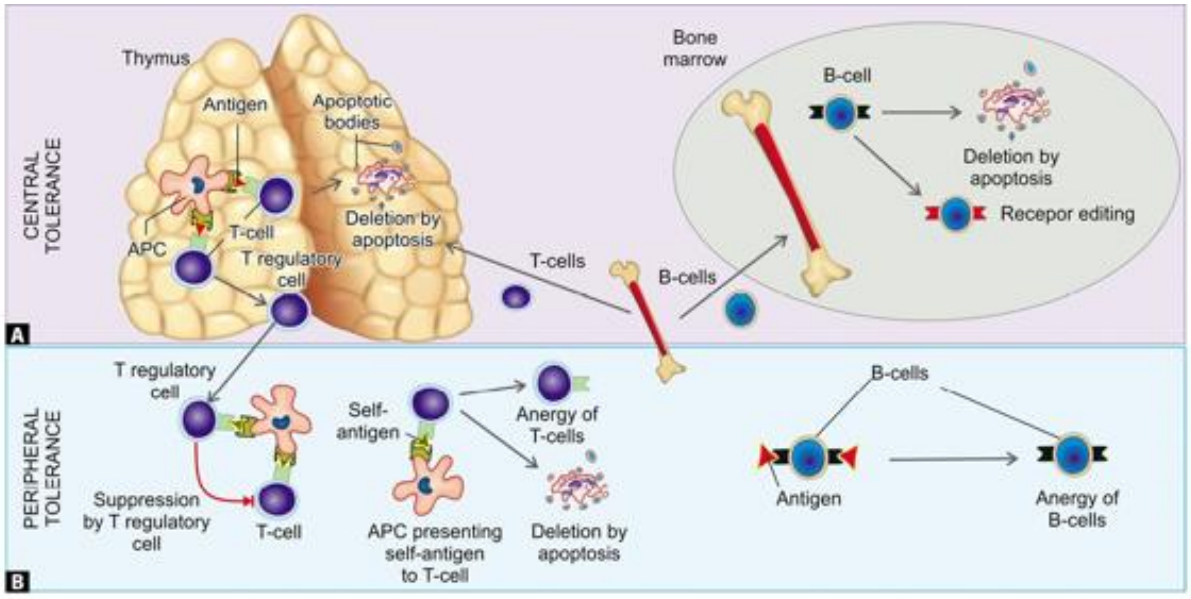
- **Apoptosis:** Immature **B-cells** that recognize self-antigens may also undergo **apoptosis in the bone marrow**.
- **Receptor editing:** It is a **process by which some self-reactive B-cells undergo rearrangement of antigen receptor genes and express new receptors**. These receptors are no longer self-reactive.

Peripheral Tolerance (Fig. 6.13B)

Silencing of potentially autoreactive T- and B-cells in peripheral tissues is called as peripheral tolerance.

Mechanisms of peripheral tolerance:

1. **Anergy:** It refers to functional **inactivation of autoreactive lymphocytes** in the peripheral tissues.
   - **Anergy of T-cells:** Normally, activation of T-cells require two signals from antigen presenting cells (APCs): (1) **peptide antigen** on the surface of APCs and (2) **co-stimulatory signals** ("second signals").
     - If **the antigen is presented by APCs without co-stimulatory signals**, a negative signal is delivered by APCs to the antigen-specific T-cells and the **T-cell becomes inactive** (i.e., anergic).



Figs. 6.13A and B: Main mechanisms of central and peripheral immunological self-tolerance. (APC: antigen-presenting cell).

- **Anergy of B-cells:** It may develop, if **B-cells encounter self-antigen in the absence of specific helper T-cells**.

2. **Suppression by regulatory T-cells:** It plays a major role in **preventing immune reactions against self-antigens**.
3. **Activation-induced cell death:** It is a mechanism in which **apoptosis of mature activated self-reactive lymphocytes** is produced. Apoptosis may be by **intrinsic (mitochondrial) pathway or by extrinsic pathway** (*refer* pages 35-6).

Immunological tolerance: Unresponsiveness to self-antigen is of two types:
1. Central tolerance
2. Peripheral tolerance

Central tolerance: Self-reactive lymphocytes that recognize self antigens are killed by apoptosis in the central lymphoid organs.

Peripheral tolerance: Autoreactive lymphocytes that recognize self-antigens in peripheral tissues are inactivated (anergy) or suppressed by regulatory T-cells or undergo apoptosis.

Mechanisms of Autoimmunity (Fig. 6.14)

Q. Mechanism of autoimmune disorders.

- Breakdown of self-tolerance may lead to autoimmunity.
- The mechanism of autoimmunity may be the result of combination of the two main factors, namely (1) **genetic** and (2) **environmental factors**.

Genetic Factors

Role of susceptibility genes: Most autoimmune diseases are complex multigenic disorders and genetic factors have an important role.

- **Runs in families:** The incidence is greater in monozygotic than in dizygotic twins.
- **Association with HLA genes:** It is most significant.

Environmental Factors

A. **Role of infections:** A variety of **microbes may trigger autoimmunity by several mechanisms**.
   - **Molecular mimicry: Few viruses and microbes may express antigens that have the same amino acid sequences as self-antigens**. Immune responses against them may attack self-tissue and this phenomenon is known as molecular mimicry. For example, **rheumatic heart disease** in which antibodies formed against streptococcal bacterial proteins cross-react with myocardial proteins and cause myocarditis.
   - **Breakdown of anergy: Tissue necrosis and inflammation** produced **by microbial infections can cause upregulation of costimulatory molecules** on APCs. This may **favor breakdown of energy and activation of T-cells**.

B. **Other environmental factors:**
   - Ultraviolet radiation
   - Cigarette smoking
   - Local tissue injury
   - Hormones

Autoimmunity: Due to breakdown of tolerance.

HLA class III region genes: Important elements in governing susceptibility to autoimmunity.

HLA typing is useful in:
- Organ transplant
- Disputed paternity

SYSTEMIC LUPUS ERYTHEMATOSUS

CC: Define and describe the pathogenesis of systemic lupus erythematosus.

Systemic lupus erythematosus (SLE) is a **chronic autoimmune disease** having following characteristics:

- **Protean manifestation and variable behavior**.
- **Remission and relapses**.
- **Multisystemic involvement:** Mainly affects **skin, kidneys, joints, serous membranes and heart**.
- **Broad spectrum of autoantibodies**, most important is antinuclear antibodies (ANAs).

Etiology

Q. Discuss the etiology and pathogenesis of systemic lupus erythematosus (SLE).

Systemic lupus erythematosus is an **autoimmune disease** in which fundamental defect is **failure of self-tolerance**. It leads to production of **many autoantibodies** that **damage the tissue either directly or indirectly by depositing immune complex deposits**. A combination of genetic and environmental factors plays a role in the pathogenesis of SLE.

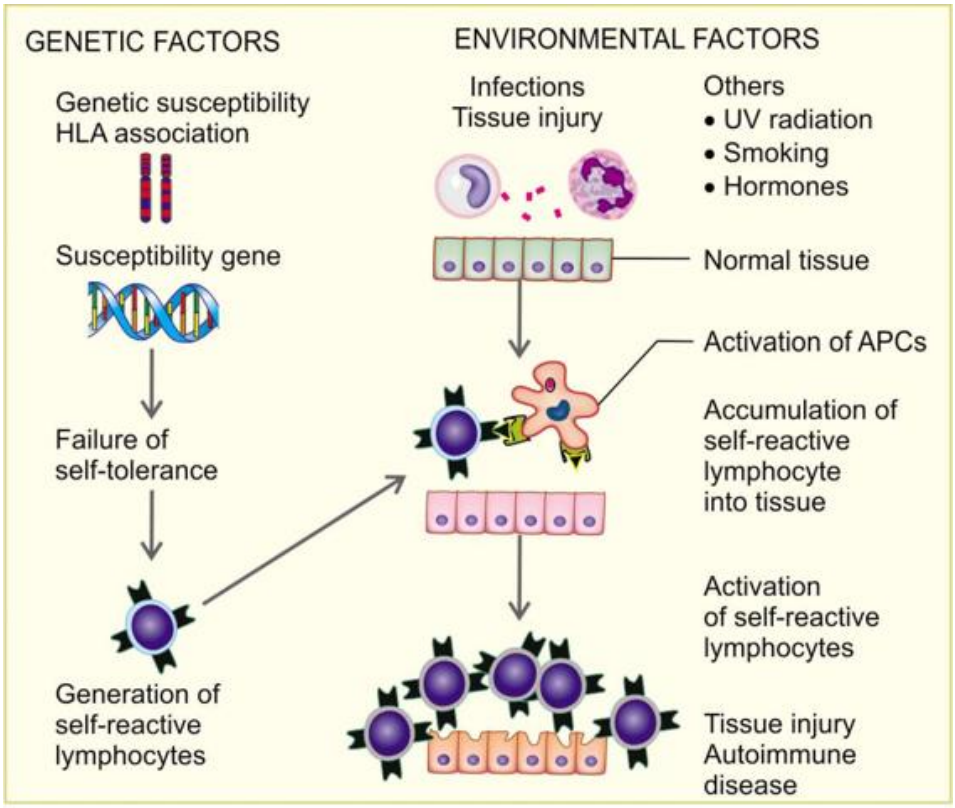


Fig. 6.14: Pathogenesis of autoimmunity.

Genetic Factors

Evidence to support genetic predisposition are:

- **Familial association:**
  - **Family members** of SLE patients have an **increased risk** of SLE. About 20% of unaffected first-degree relatives may show autoantibodies.
  - **High rate** of concordance (>25%) in **monozygotic twins** when compared with dizygotic twins (1–3%).
- **HLA association:** Risk is more with HLA-DR2 or HLA-DR3.
- **Other genetic factors:**
  - Genetic deficiencies of early complement components (such as C2, C4 or C1q): It may result in—(1) impaired removal of circulating immune complexes by the mononuclear phagocyte system, (2) defective phagocytic clearance of apoptotic cells and (3) failure of B-cell tolerance. If apoptotic cells are not cleared, their nuclear components may elicit immune responses.

- Polymorphism in the inhibitory Fc receptor → inadequate control of B-cell activation.

Environmental Factors

- **Ultraviolet (UV) radiation:** Exposure to sunlight exacerbates the lesions of the disease.
  - **Mechanism:** UV irradiation → causes **apoptosis** of host cells → **increases burden of nuclear antigens** and **promote inflammation**.
- **Cigarette smoking:** It is associated with development of SLE.
- **Sex hormones:** SLE is 10 times greater during the reproductive period (17 through 55 years) **in women** than in men. SLE shows exacerbation during normal menses and pregnancy.
- **Drugs:** Examples include **hydralazine, procainamide, isoniazid and D-penicillamine** can produce SLE–like disease and disease remits after withdrawal of the drug.

Immunological Abnormalities

Several immunological abnormalities of both innate and **adaptive immune** system have been observed in SLE.

- **Type I interferons:**
  - These are antiviral cytokines normally produced by B-cells during innate immune responses to nucleic acid of viruses.
  - **IFN-α** is a type I interferon produced by plasmacytoid dendritic cells and **large amounts is produced** in SLE. It may indirectly produce autoantibodies.
- **Toll-like receptor (TLR) signals** (*refer* **Fig. 3.1**)**:**
  - TLRs present in B lymphocytes normally sense microbial products, including nucleic acids.
  - In SLE, nuclear DNA and RNA within the **immune complexes may activate B lymphocytes by engaging with TLRs**. These **activated B-cells** specific for nuclear antigens may **produce antinuclear autoantibodies**.
- **Failure of B-cell tolerance:** Occurs due to **defects in both central** (i.e., bone marrow) and **peripheral tolerance → higher autoreactive B-cells**.
- $CD4^+$ helper T-cells specific for nucleosomal antigens: These escape tolerance and produce high-affinity pathogenic autoantibodies.

Pathogenesis of SLE (Fig. 6.15)

Different steps are:

1. **Increased apoptosis triggered by environmental agents:** UV irradiation and other environmental agents may cause death of cells by **apoptosis**.
2. **Inadequate clearance of apoptotic bodies:** It results in accumulation of large amount of nuclear antigens. It is partly due to defect in complement proteins.
3. **Susceptibility genes with failure of self-tolerance:** Genetic abnormality in B and T lymphocytes is responsible for failure of self-tolerance.
4. **Stimulation of self-reactive B-cells:** It produces antibodies against the self-nuclear antigens.
5. **Formation of antigen–antibody (immune) complexes** in the circulation.
6. **Endocytosis of immune complexes:** The antibody portion of immune complexes bind to Fc receptors on B-cells and dendritic cells (DCs) and the immune complexes may be internalized by endocytosis.
7. **TLR engagement by nuclear antigens:** Nucleic acid components of immune complexes bind to TLRs of B-cells and DCs.
8. **TLR stimulation of B-cells and DCs:** Binding to TLR—
   - Stimulate B-cells to produce autoantibodies.
   - Activate dendritic cells (mainly plasmacytoid DCs) to produce IFN-α → stimulate B- and T-cells to further amplify immune response → cause more apoptosis.
9. **Persistent production of autoantibodies:** Thus, a cycle of antigen release and immune activation → results in the persistent production of IgG autoantibodies.

Autoantibodies in SLE

Q. Write short essay on antibodies in systemic lupus erythematosus.

Systemic lupus erythematosus (SLE) is characterized by the production of **several diverse autoantibodies**. Some antibodies are against different nuclear and cytoplasmic components of the cell that are not organ specific. Other antibodies are directed against specific cell surface antigens of blood cells.

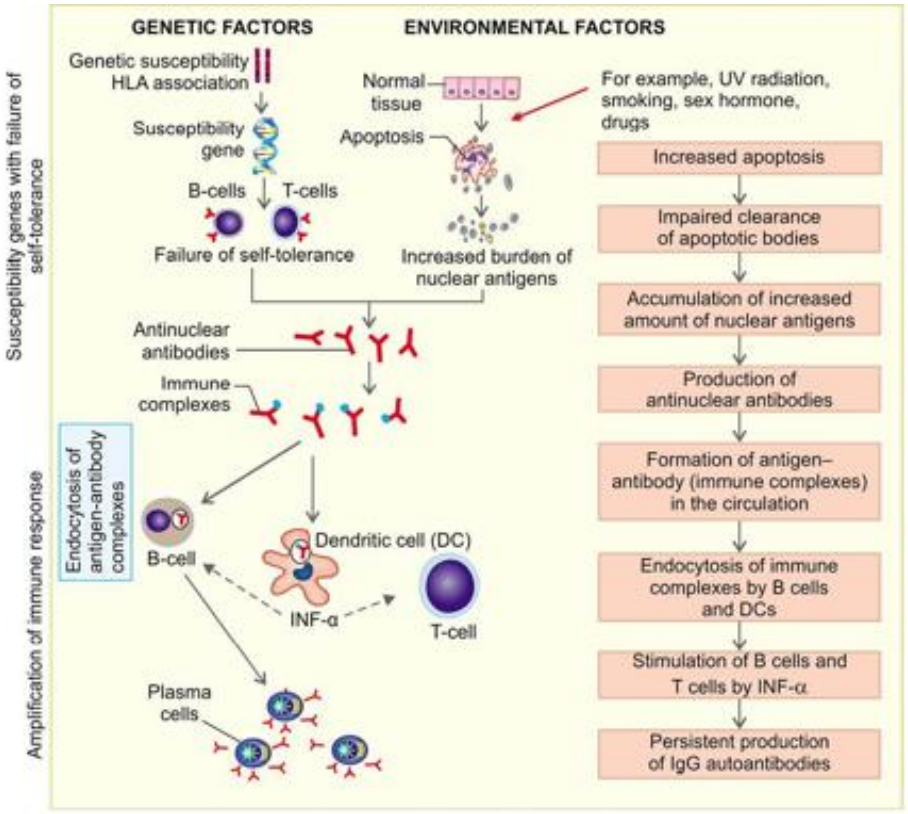


Fig. 6.15: Pathogenesis of systemic lupus erythematosus.

TABLE 6.10: Important antinuclear antibodies and their clinical utility.

Type of antinuclear antibodies	*Antigen recognized*	*Clinical utility*
Anti-dsDNA*	DNA (double-stranded)	High titers of IgG antibodies are SLE-specific (but not to single-stranded DNA)
Anti-Sm*	Nonhistone proteins bound to RNA	Specific for SLE; do not usually correlate with disease activity or clinical manifestations
Antihistone antibodies	Histones associated with DNA	More frequent in drug-induced lupus than in SLE
Antibodies to DNA	Multiple nuclear	Best screening test; if repeated test are negative SLE unlikely
Anti-Ro (SS-A)	RNP (ribonucleoprotein)	Not specific for SLE; predictive value indicates increased risk for neonatal lupus and sicca syndrome

*Antibodies specific to SLE

Females with child-bearing potential and SLE should be screened for aPL and anti-Ro.

Importance of autoantibodies: (1) **diagnosis and management** of patients with SLE and (2) responsible for **pathogenesis of tissue damage**.

Types of Antibodies

Antinuclear Antibodies (ANAs)

They are directed against various nuclear antigens including DNA, RNA and proteins (all together called generic ANAs) and can be grouped into different

categories **(Table 6.10)**. These ANAs are usually detected by indirect immunofluorescence.

Other Autoantibodies

- **Autoantibodies against blood cells**, namely (1) red cells, (2) platelets, (3) neutrophils and (4) lymphocytes.

Q. Write short note on antiphospholipid antibody/syndrome.

- **Antiphospholipid antibodies aPL** are detected in 40–50% of SLE patients but they are not specific for SLE.

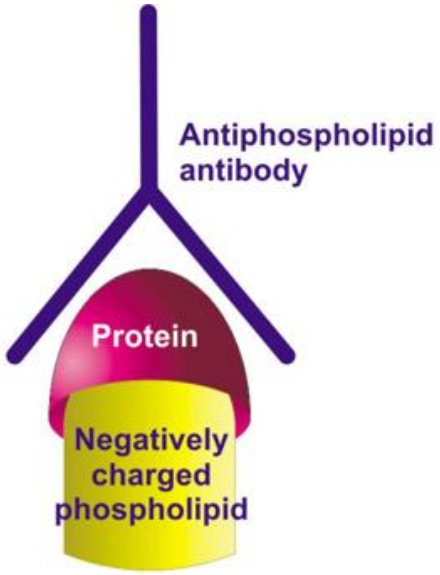
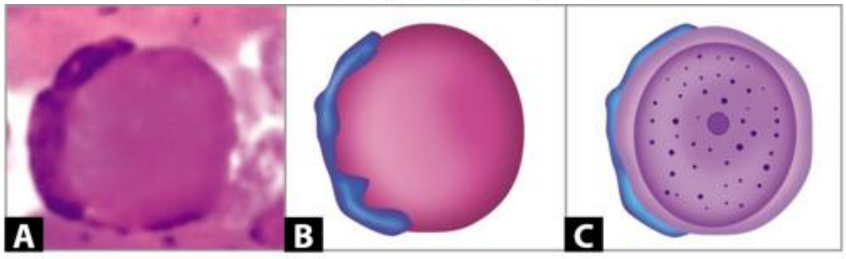


Fig. 6.16: Antiphospholipid antibody against plasma proteins bound to phospholipids.



SLE: Subendothelial immune complex deposits give rise to "wire-loop" lesions on light microscopy.

Figs. 6.17A to C: Appearance of LE cell; (A) Leishman stain, (B) diagrammatic and (C) tart cell.

- The term **antiphospholipid antibody** is misleading, because these antibodies react with plasma proteins of complexes rather than directly with phospholipids **(Fig. 6.16)**.
- Antiphospholipid antibody includes **lupus anticoagulant antibody**, **anticardiolipin antibody and anti-β_2 glycoprotein antibody**.
- **Complications:** These autoantibodies can lead to → **increased venous and arterial thrombosis and thrombocytopenia → recurrent spontaneous miscarriages** and **focal cerebral or ocular ischemia**.
- **Antibodies against phospholipid–β_2-glycoprotein complex** also bind to cardiolipin antigen. Since cardiolipin antigen is used in the serological test for syphilis, SLE patients **may give a false-positive serological reaction for syphilis**.

- **Two tests that measure different antibodies (anticardiolipin and the lupus anticoagulant):** (1) ELISA for anticardiolipin and (2) a sensitive phospholipid-based activated prothrombin time, such as the dilute Russell viper venom test.

Mechanisms of Tissue Injury

Autoantibodies mediate tissue injury.

- **Type III hypersensitivity:** It occurs with deposition of **immune complexes**. It is the most **common cause of tissue injury and visceral lesions**.
- **Type II hypersensitivity: Autoantibodies against cell surface antigens** specific for **RBCs**, **white cells and platelets** → opsonize these cells → promote their phagocytosis and lysis → **cytopenias**.
- **Antiphospholipid antibody syndrome:** Patients with antiphospholipid antibodies are prone to venous and arterial thromboses. This syndrome is may be associated with recurrent spontaneous miscarriages and focal cerebral or ocular ischemia.

LE Bodies or Hematoxylin Bodies

Q. Write short note on LE cell and its associated conditions.

LE Bodies

- ANAs cannot penetrate intact cells, but if nuclei of the cell are exposed, they can bind to them.
- In tissues, nuclei of damaged cells react with ANAs, lose their chromatin pattern, and appear homogeneous, to produce **LE bodies or hematoxylin bodies**.

LE Cell (Figs. 6.17A to C and refer page 389)

- It is related to LE bodies and can be demonstrated in vitro.
- The blood sample is agitated to damage the nucleated cells and it releases the nuclei.
- The nuclei of damaged cells react with ANAs to form a homogenous denatured nuclear material.
- The LE cell is any phagocytic leukocyte (blood neutrophil or macrophage) that has engulfed this denatured nucleus of an injured cell.
- The demonstration of LE cells in vitro was used as a test for SLE.
- With the advent of new techniques for detection of ANAs, this test is of only historical interest.
- Sometimes, LE cells can be found in body fluid such as pericardial or pleural effusions.
- **Interpretation:** LE cell is positive in about 70% of SLE. It may also be positive in conditions such as rheumatoid arthritis, lupoid hepatitis, penicillin sensitivity, etc.

MORPHOLOGY

SLE is a systemic autoimmune disease and morphologic changes in SLE are **extremely variable**.

The most **characteristic lesions** of SLE are **due to deposition of immune complexes** in **blood vessels, kidneys, connective tissue, and skin**.

Kidney

Kidney may be **involved in about 50%** of SLE patients and is one of the most important organs involved.

Pathogenesis of glomerulonephritis: Immune complexes composed of **DNA and anti-DNA antibodies** get deposited in the glomeruli → inflammation → proliferation of cells (endothelial, mesangial and/or epithelial).

Morphologic classification of lupus nephritis: Six patterns are recognized but none of these are specific for SLE.

1. **Minimal mesangial** lupus nephritis (class I): It is characterized by **immune complex deposition in the mesangium**, granular deposits of immunoglobulin and complement and no recognizable structural changes by light microscopy.

2. **Mesangial proliferative** lupus nephritis (class II): It is characterized by **immune complex deposition in the mesangium** and **mild-to-moderate increase in mesangial cells and mesangial matrix**.
3. **Focal proliferative** lupus nephritis (class III): It is seen in 20–35% of patients. The lesions are **focal and may be segmental** (affecting only a portion of the glomerulus) or **global** (involving the entire glomerulus). Affected glomeruli may show **proliferation of endothelial and mesangial cells, or parietal epithelial cells** (crescent formation), **fibrinoid necrosis, leukocyte infiltration, and eosinophilic deposits or intracapillary thrombi.**
4. **Diffuse proliferative** lupus nephritis (class IV): It is severe form and occurs in 35–60% of patients. Lesions are diffused (>50% of glomeruli) and most of involved glomeruli may show **proliferation of endothelial, mesangial and epithelial cells**.

The proliferation of parietal epithelial cells may produce cellular crescents. **Prominent, subendothelial deposits cause homogeneous thickening of the capillary wall**, which on light microscopy appear as a "**wire-loop**" lesion **(Fig. 6.18)**. These wire loops **may be seen in both focal and diffuse proliferative (class III or IV) lupus nephritis**.

5. **Membranous** lupus nephritis (class V): It is seen in 10–15% of patients and is characterized by **diffuse thickening of the capillary walls** similar to idiopathic membranous glomerulonephritis → nephrotic syndrome.
6. **Advanced sclerosing** lupus nephritis (class VI): It shows sclerosis of more than 90% glomeruli.

Interstitium and tubules: They may show changes, but are usually not dominant abnormality.

Immunofluorescence: It shows granular deposits of antibody and complement.

Electron microscopy: It shows electron-dense deposits (immune complexes) in mesangial, intramembranous, subepithelial, or subendothelial locations.

Blood Vessels

An **acute necrotizing vasculitis** (involving small arteries and arterioles) may be seen in any involved tissue. The arteritis is characterized by **fibrinoid necrosis** in the vessel walls. In **chronic stages**, vessels undergo fibrous thickening of wall and **narrowing of the lumen**.

Heart (*refer* page 474)

Any layer of heart may be involved. **Valvular endocarditis** (Libman–Sacks/**nonbacterial verrucous endocarditis)** appear as **single or multiple 1–3 mm warty** deposits **on either surface of the leaflets of any heart valves** (*refer* **Fig. 16.27B**).

Clinical Features

- SLE is a **multisystem** disease with **variable clinical presentation**.
- **Age:** It usually occurs in young woman between **20 and 30 years**, but may manifest at any age.
- **Sex:** It predominantly affects **women**, with female-to-male ratio of 9:1.
- **Onset:** Acute or insidious with fever.
- **Typical presentation: Butterfly rash over the face, fever, pain** without deformity in one or more **peripheral joints, pleuritic chest pain and**

photosensitivity. SLE patients are **susceptible to infections**, because of immune dysfunction and treatment with immunosuppressive drugs.

Diagnostic Criteria for Systemic Lupus Erythematosus (Table 6.11)

Q. Write short essay/note on diagnostic clinical criteria for systemic lupus erythematosus.

Laboratory Findings

Q. Write short essay/note on laboratory diagnosis of systemic lupus erythematosus (SLE).

- **Purpose:** (1) To establish or rule out the diagnosis, (2) follow the course of disease, and (3) to identify adverse effects of therapies.
- **Tests for autoantibodies** (*refer* pages 162-3 and **Table 6.10**)**:** ANAs are found in almost all patients, but it is not specific. Various methods of detecting antibodies include:
  - Indirect immunofluorescence assay (IFA): They can identify ANAs. Significance of IFA assay are:
    - Extremely sensitive (positive in more than 95%)

- Limited specificity because it is positive in patients with other autoimmune diseases, chronic infections and cancer.
- Multiplex flow cytometry immunoassay.
- ELISA (for smith antigen).

- **Standard tests for diagnosis:** Includes complete blood count, platelet count, ESR (raised) and urinalysis.
- **Tests for following disease course:** These tests indicate the status of organ involvement known to be present during SLE flares.

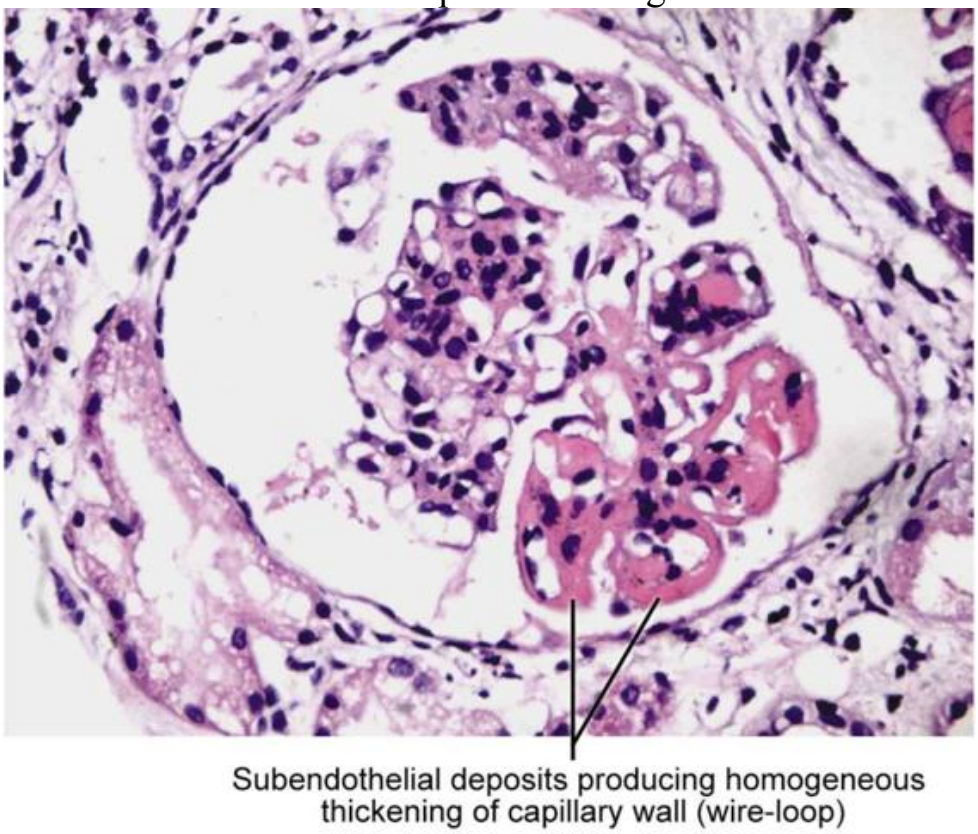


Fig. 6.18: Lupus nephritis showing glomerulus with "wire-loop" lesions due to extensive subendothelial deposition of immune complexes.

TABLE 6.11: Revised criteria for classification of systemic lupus erythematosus (American College of Rheumatology Criteria), 1997.

Criteria	*Description*
1. Malar rash	Fixed erythema, flat or raised, over the malar eminences tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging

3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by physician
5. Arthritis	Non-erosive arthritis involving two or more peripheral joints
6. Serositis	Pleuritis or pleural effusion or pericarditis or pericardial effusion
7. Renal disorder	Persistent proteinuria >0.5 g/day or >3 + if quantitation not performed or cellular casts (red blood cell, hemoglobin, granular, tubular, or mixed)
8. Neurologic disorder	Seizures: In the absence of offending drugs or known metabolic derangements (e.g., uremia, ketoacidosis, or electrolyte imbalance) or psychosis: In the absence of offending drugs or known metabolic derangements
9. Hematologic disorder	Hemolytic anemia with reticulocytosis or leucopenia (<4000/mm^3) total on two or more occasions or lymphopenia (<1500/mm^3) on two or more occasions or thrombocytopenia (<1,00,000/mm^3) in the absence of offending drugs
10. Immunologic disorder	Anti-DNA antibody to native DNA in abnormal titer, or Anti-Sm—presence of antibody to Sm nuclear antigen, or positive finding of antiphospholipid antibodies based on: (1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, (2) a positive test for lupus anticoagulant using a standard test, or (3) a false-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by negative *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test
11. Antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with drug-induced lupus syndrome
Presence of any four or more criteria of the 11 (serially or simultaneously, during any period of observation) is diagnostic of SLE.	

- **Renal involvement:** Urinalysis may show **hematuria**, **red cell casts**, **proteinuria**, or nephrotic syndrome.
- **Hematologic changes:** Hemoglobin levels (anemia) or platelet counts (thrombocytopenia) and ESR.
- Serum levels of creatinine or albumin.
- Decreased complement component levels in serum such as C3 and C4 are often indicators of enhanced consumption and increased disease activity.

Course: It is variable and unpredictable. It shows remissions and exacerbations.

Cause of death: Renal failure and intercurrent infections.

SLE: Systemic autoimmune disease caused by autoantibodies against numerous self-antigens and forms immune complexes.

SLE: Term lupus is derived from Latin for wolf, because of the skin lesion on the face looked as though eaten by a wolf.

SLE: Complex disorder of multifactorial origin which results from interactions of genetic, immunological and environmental factors.

SLE: (1) Genetic factors; (2) Environmental factors (3) Immunological abnormalities.

SLE: Caused by autoantibodies against numerous self-antigens, major being antinuclear antibodies (ANAs).

Anticardiolipin antibodies in SLE may produce false +ve VDRL test for syphilis.

SLE: Shows features of both type II (hematological abnormalities) and type III (visceral lesions) hypersensitivity reactions.

LE cell: Phagocytic leukocyte (neutrophil or macrophage) that has engulfed the denatured nucleus of an injured cell.

Tart cell: Usually monocyte that has ingested another cell or nucleus of another cell.

Wire loop lesions: Seen in diffuse proliferative glomerulonephritis (class IV) in SLE. It may also be seen in focal lupus nephritis (class III).

Vegetations:
- Larger in infective endocarditis.
- Smaller (verrucae), seen at the lines of closure of the valve leaflet in rheumatic heart disease.
- Single or multiple warty deposits on either surface of the leaflets of any heart valves in SLE.

Libman–Sacks endocarditis is seen in SLE.

SLE: Antigen and antibodies form immune complexes which are responsible for the tissue damage.

Antibodies specific to SLE (confirmatory tests): Antibody to—
- Double-stranded DNA (dsDNA)
- Spliceosomal proteins Smith (Sm) antigen

Serum ANA: Screening test for SLE.

Antiphospholipid syndrome: Increased risk for venous or arterial clotting and fetal loss.

OTHER AUTOIMMUNE DISORDERS

NC: Define and describe the pathogenesis of other common autoimmune diseases.

Rheumatoid Arthritis

Rheumatoid arthritis is an autoimmune disease that mainly affects the joints but also may involve extra-articular tissues (e.g., skin, blood vessels, lungs, and heart). It is discussed on pages 851-4.

Sjögren Syndrome

Definition: Sjögren syndrome is a chronic disease characterized bymarked lymphocytic infiltration of exocrine glands, primarily salivary and lacrimal glands leading to **dry eyes (*keratoconjunctivitis sicca* or *xerophthalmia*) and dry mouth (xerostomia). It is due to immunologically mediated destruction of the lacrimal and salivary glands.**

Types: It may present as an isolated primary disorder [as a single entity called as primary Sjögren syndrome also known as the ***sicca syndrome*** (SS)] or more commonly in association with another autoimmune diseases (e.g., rheumatoid arthritis, SLE, polymyositis, scleroderma, vasculitis, mixed connective tissue disease) and is called as secondary form of Sjögren syndrome.

Pathogenesis

The lacrimal and salivary glands characteristically show dense lymphocytic infiltration (mainly of activated $CD4^+$ helper T cells and some B cells) along with plasma cells. Though the exact basis of lymphocyte accumulation and the pathogenesis of Sjögren syndrome is not clear, the pathology and serology (frequently reveal autoantibodies), its association (albeit weak) with HLA alleles, suggests autoimmune disorder with activation of autoreactive T cells and B cells. The initial trigger may be a viral infection [EBV and human T-cell leukemia virus-1 (HTLV-1)] of the salivary glands causing local death of salivary epithelial cells and release of tissue self-antigens. In a genetically susceptible individuals, $CD4^+$ T cells and B cells specific for these self-antigens may escape tolerance and cause immune reactions. This produces damage to the tissues followed by fibrosis.

Autoantibodies: Most patients (about 90%) with Sjögren syndrome reveal antibodies against two cytoplasmic ribonucleoprotein (RNA) associated antigens, SS-A (Ro) and SS-B (La).

MORPHOLOGY

Exocrine glands: Lacrimal and salivary glands are the major sites of the disease.

Microscopy: The involved glands show dense infiltration by lymphocytes ($CD4^+$ helper T cells and some B cells) which may destroy acini and ducts. As the disease progresses, the affected glands shows atrophy and may be replaced by hyalinized fibrotic tissue.

Clinical Features

Gender and age: Sjögren syndrome are most common in females between 50 and 60 years of age.

Symptoms: These are the consequences of lack of tears and saliva.

- **Eye:** Keratoconjunctivitis produces eye discomfort, blurred vision, burning, and itching. May develop infections and ulcer of the cornea and conjunctivae.
- **Salivary gland:** Xerostomia produces dry mouth (dryness of the buccal mucosa), difficulty in swallowing solid foods, decrease in taste, cracks and fissures in the mouth.
- **Extraglandular disease:** May develop in one-third of patients. These include synovitis, pulmonary fibrosis, and peripheral neuropathy.

Systemic Sclerosis

Q. Write a short note on scleroderma.

Definition: Systemic sclerosis or *scleroderma* is a autoimmune disorder (with multiple autoantibodies) of unknown origin characterized by progressive fibrosis (excessive deposition of collagen) involving multiple tissues such as the skin, gastrointestinal tract, skeletal muscles , lungs, kidneys, heart, and other tissues. The fibrosis often produces **obliterative vascular disease.**

The term ***systemic sclerosis*** is preferred because excessive fibrosis is seen in multiple organs. Involvement of skin is the usual presenting manifestation and found in about 95% of patients. However, the visceral involvement (e.g.,

gastrointestinal tract and skeletal muscles) is responsible for morbidity and mortality. Disease restricted to the skin is called ***localized scleroderma.***

Classification: On the basis of its course, systemic sclerosis is classified into two groups:

1. **Diffuse systemic sclerosis:** It is characterized by initial involvement of skin, with rapid progression and early visceral involvement.
2. **Limited systemic sclerosis:** It is associated with relatively mild involvement of skin (usually the fingers and face).
   - Involvement of the viscera occurs late and it usually follows a benign course. This presentation also is called **CREST (Calcinosis, Raynaud phenomenon, Esophageal dysmotility, sclerodactyly, and telangiectasia) syndrome.**

Pathogenesis

The cause and the pathogenesis of systemic sclerosis is not well understood. The clinical and pathologic manifestations of systemic sclerosis **results from three interrelated processes namely (i) autoimmune responses** (humoral and cellular immunity)**, (ii) vascular damage** (fibroproliferative vascular lesions of small arteries and arterioles)**, and (iii)** excessive and often progressive **deposition of collagen**.

- **Autoimmunity:** Probably, $CD4^+$ T cells respond to an as yet-unidentified antigen and accumulate in the skin. They release cytokines (e.g., IL-13) and growth factor (e.g., TGF-β), that activate inflammatory cells and fibroblasts. They stimulate synthesis of collagen and extracellular matrix proteins (e.g., fibronectin) in fibroblasts. The cytokines also recruit leukocytes and propagate the chronic inflammation. The production of numerous disease-specific various autoantibodies such as ANAs provides diagnostic and prognostic information.Probably these antibodies stimulate fibrosis.
- **Vascular damage:** Microvascular disease is observed in the early stage of systemic sclerosis. The microvascular injury is characterized by structural and functional abnormalities in the endothelial cell (endothelial activation). Repeated cycles of endothelial injury followed by platelet aggregation releases platelet and endothelial factors (e.g.,PDGF, TGF-β). They cause proliferation of endothelial cells and fibrosis in the intima and perivascular region. This in turn produces narrowing of the microvasculature and leads to ischemic injury and scarring.

- **Fibrosis:** It is the characteristic feature of the disease. The fibroblasts of these patients are capable of producing excessive amounts of collagen. Progressive fibrosis and the vascular lesions may lead to ischemic damage.

MORPHOLOGY

Sites involved: Most prominently the skin, alimentary tract, musculoskeletal system, and kidney.

Skin: It is characterized by diffuse fibrosis of the skin and associated atrophy. It usually starts in the fingers and distal regions of the upper extremities. Then it extends proximally to involve the upper arms, shoulders, neck, and face.

- **Microscopy:** Skin shows **edema and perivascular infiltrates by $CD4^+$ T cells** accompanied by swelling and degeneration of collagen fibers (appear eosinophilic). **Capillaries and small arteries** may **show hyaline thickening of the walls** and **partial occlusion**. As the disease progresses **increased fibrosis occurs in the dermis** along with thinning of the epidermis and atrophy of dermal appendages. Subcutaneous calcifications may be seen in patients with CREST syndrome. In advanced stages the **fingers may produce claw-like appearance** with limited joint mobility, and the face appears like a drawn mask. Loss of blood supply may produce ulcerations and atrophic changes or autoamputation of the terminal phalanges.

Alimentary tract: It is the most commonly (in about 90% of patients) involved internal organ system. It is characterized by progressive atrophy and fibrous replacement of the muscularis at any level of the gut. The mucosa becomes thinned and may ulcerate. The lamina propria and submucosa shows excessive collagenization.

- **Esophagus:** Esophageal involvement is almost universal and most severe. The lower two-thirds of the esophagus may develop a rubber-hose–like inflexibility giving rise to symptoms of gastroesophageal reflux and its complications (e.g., strictures).
- **Small intestine:** It may show loss of villi and bacterial overgrowth may lead to secondary malabsorption syndrome.

Musculoskeletal system: Musculoskeletal symptoms may be the first manifestations of the disease. Severity may vary from mild polyarthralgias to more severe arthralgias. Inflammation of the synovium (synovitis) is common in the early stages and fibrosis later stages.

Kidneys: Renal involvement may be found in two-thirds of patients. The most prominent are the vascular lesions characterized by **thickening of intima of interlobular arteries and afferent arterioles** with luminal narrowing. There are no specific changes in the glomerulus.

Lungs: It may be affected in more than 50% of patients and may manifest **as pulmonary hypertension and interstitial fibrosis.**

Heart: It may be involved in one-third of patients. It may manifest as pericarditis with effusion, patchy myocardial fibrosis, and thickening of intramyocardial arterioles. The changes in the lung may lead to right ventricular hypertrophy and failure (cor pulmonale).

Clinical Features

Age and gender: Peak age of presentation is between 50 and 60 years of age. More frequent in females with a female-to-male ratio of 3:1.

Scleroderma can manifests as two distinct clinical syndromes either as a generalized or diffuse **(progressive systemic)** form and a **limited variant.**

Systemic sclerosis: The most distinguishing clinical features of systemic sclerosis are due to the progressive thickening and fibrosis of the skin and later involvement of specific internal organs.

- **Skin:** Symptoms usually begin with **Raynaud phenomenon**, characterized by intermittent episodes of ischemia of the fingers (due to vasoconstriction of the arteries and arterioles), marked by triphasic color changes, paresthesias and pain. Progressive collagen deposition in the skin produces **increasing stiffness** (especially of the hands) and followed by complete **immobilization of the joints**. "Stone facies" due to tightening of facial skin and restricted motion of the mouth may be seen. Progression of vascular lesions in the fingers can lead to ischemic ulceration of the fingertips.
- **Internal organs:**
  - **GIT:** Dysphagia due to esophageal fibrosis may be observed in more than 50% of patients. Abdominal pain, intestinal obstruction, or malabsorption syndrome can occur due to involvement of the small intestine.
  - **Respiratory system:** The pulmonary fibrosis may produce severe respiratory disability and is the most common cause of death. It may lead to right-sided cardiac dysfunction.
  - **Heart:** Myocardial fibrosis may produce chest pain, arrhythmias and cardiac failure.
  - **Renal system:** Mild proteinuria may be found in about 30% of patients. "Scleroderma renal crisis" typically begins abruptly, with malignant hypertension, rapidly progressive renal insufficiency and fatal renal failure.

Autoantibodies: Almost all patients have ANAs that react with a variety of nuclear antigens. Two ANAs strongly associated with systemic sclerosis are: (i) against DNA topoisomerase I (anti-Scl 70) and (ii) anticentromere antibody.

- **Anti-Scl 70:** It is highly specific and is associated with pulmonary fibrosis and peripheral vascular disease.
- **Anticentromere antibody:** It is associated with CREST syndrome.

Prognosis: Most of the patients,it steadily follows downhill course over the span of many years.

Inflammatory Myopathies

Definition: Inflammatory myopathies are uncommon, probably immunologically mediated. Heterogeneous group of disorders. They are characterized by injury and inflammation of mainly the skeletal muscles. Depending on clinical, morphologic, and immunologic features, three disorders have been described namely: (i) polymyositis, (iii) dermatomyositis, and (iii) inclusion body myositis.

Mixed Connective Tissue Disease

Definition: As the name suggests, mixed connective tissue disease (MCTD) is a disorder with clinical features of several different disorders such as SLE, systemic sclerosis, and polymyositis. It is more common in female and most are adults (mean age, 37 years).

Laboratory findings: The disease is characterized serologically by a most distinctive ANA directed against an extractable nuclear antigen. These patients have high titers of antibodies to U1 (uridine-rich) ribonucleoprotein (anti-U1-RNP antibody) and is used as a most diagnostic criteria for MCTD. However, their role in the development of any of the characteristic lesions of MCTD is not clear.

Clinical features: Clinical features of MCTD are shared with other diseases. Usually, it presents with synovitis of the fingers, Raynaud phenomenon, and mild myositis. They respond favorably to corticosteroids, at least in the short term.

Complications: These include pulmonary hypertension, interstitial lung disease, and renal disease.

Polyarteritis Nodosa and Other Vasculitides

Definition: Polyarteritis nodosa is an immunologic disease that belongs to a group of disorders characterized by necrotizing inflammation of the walls of blood vessels. It can involve any type of

vessel (arteries, arterioles,veins, or capillaries). These vasculitides are discussed in pages 448-51 of Chapter 14.

IgG4-related Disease

Definition: IgG4-related disease (IgG4-RD) is a newly identified fibroinflammatory disorder characterized rich infiltration by IgG4 antibody-producing plasma cells and lymphocytes (mainly T cells) in the tissues, associated with fibrosis and obliterative phlebitis.

Diagnostic feature: Increased numbers of IgG4-producing plasma cells in tissue are required for the diagnosis of this disorder. Though this disorder is often associated with elevated serum IgG4 concentrations, it need not be present in all cases.

Organs involved: IgG4-related disease can occur in any organ system. These include the biliary tree, salivary glands, periorbital tissues, lymph nodes, kidneys, lungs, meninges, aorta, prostate, thyroid, breast, pericardium, and skin.

IgG4-RD spectrum: Examples include Mikulicz syndrome (enlargement and fibrosis of salivary and lacrimal glands), Riedel thyroiditis, idiopathic retroperitoneal fibrosis, autoimmune pancreatitis, and inflammatory pseudotumors of the orbit, lungs, and kidneys.

Age group and gender: Usually affects middle-aged and older men.

Pathogenesis: Not known. Though IgG4 production in lesions is a characteristic of the disease, it is not known whether it contributes to the pathology of the disease.

Anti-topoisomerase I is marker of: Systemic sclerosis.

MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULES

CC: Describe the HLA system and the immune principles involved in transplant and mechanism of transplant rejection.

- All human cells have a series of molecules on their surfaces that are recognized by other individuals as foreign antigens. **Major histocompatibility complex (MHC) molecules** were discovered as **products of genes that evoke rejection of transplanted organs** and responsible for tissue compatibility between individuals.

- The **human** MHC, commonly called the **human leukocyte antigen (HLA) complex** is the name of the **loci of genes** densely packed (clustered) on a **small segment on chromosome 6 (**6p21.3). They were named HLA because in humans, MHC-encoded proteins were initially detected on leukocytes by the binding of antibodies.
- **Physiologic function of MHC molecules:** To **display peptide fragments of proteins for recognition by antigen-specific T-cells**.
- The MHC molecules are products of MHC gene. The best known of these genes are the HLA class I and class II genes. Their products are important for immunologic specificity and transplantation histocompatibility, and they play a major role in susceptibility to a number of autoimmune diseases.
- **Polymorphism of MHC gene:**
  - **MHC gene is highly polymorphic**. Polymorphism means that there are many alleles of each MHC gene resulting in extreme (high degree) variation in the MHC in human population (genetic diversity). Each person inherits one set of these alleles that is different from the alleles in most other persons. The possibility of two different individuals having the same combination of MHC molecules is very remote. Therefore, grafts exchanged between individuals are recognized as foreign and attacked by the immune system. **Polymorphism is an important barrier in organ transplantation.**
  - **HLA haplotype:** It is the combination of HLA alleles in each individual. Each individual inherits one set of HLA genes from each parent and thus typically expresses two different molecules for every locus.
- **Importance of MHC:** (1) In organ/tissue transplantation and (2) HLA is linked to many autoimmune diseases.

Classification

MHC gene product is classified based on their structure, cellular distribution, and function into **three groups (Fig. 6.19)**. MHC class I and class II gene products are critical for immunologic specificity and transplantation histocompatibility, and they play a major role in susceptibility to a number of autoimmune diseases.

Class I MHC Molecules

- They are the products of MHC class I genes and are **expressed on all nucleated cells and platelets** (except erythrocytes and trophoblasts).
- They are **encoded by three closely linked loci, designated *HLA-A*, *HLA-B* and *HLA-C***.
- **Highly polymorphic** in the population and most highly polymorphic segment known within the human genome.
- **Functions:** Products of MHC class I gene are integral participants in the **immune response to intracellular infections, tumors and allografts**.
- Class I molecules **interact with $CD8^+$ T lymphocytes during antigen presentation** and are **involved in cytotoxic reactions**. **$CD8^+$ T lymphocytes** recognize antigens only in the context of self-class I molecules, they are referred to as **class I MHC-restricted**.

Class II MHC Molecules

- They are encoded in a region called ***HLA-D***, which has three sub-regions: ***HLA-DP*, *HLA-DQ*, and *HLA-DR***.
- Class II antigens (HLA-D and -DR, D-related) **are expressed only on professional antigen-presenting cells (B lymphocytes, monocytes/macrophages, Langerhans cells, dendritic cells)**.
- **Function:** This locus contains genes that **encode many proteins involved in antigen processing and presentation**. The **class II-peptide complex is recognized by $CD4^+$ T-cells** (function as helper cells) and these CD4 molecule acts as the co-receptor. Because **$CD4^+$ T-cells can recognize antigens only in the context of self-class II molecules** → they are referred to as **class II MHC-restricted**.

Class III MHC Molecules

Their gene **encode** components of the **complement system, cytokines, tumor necrosis factor (TNF), lymphotoxin** and some proteins without apparent role in the immune system.

HLA and Disease Association (Table 6.12)

Q. Write short note on diseases associated with HLA.

Some diseases are associated with the inheritance of certain HLA alleles and these diseases can be broadly grouped into:

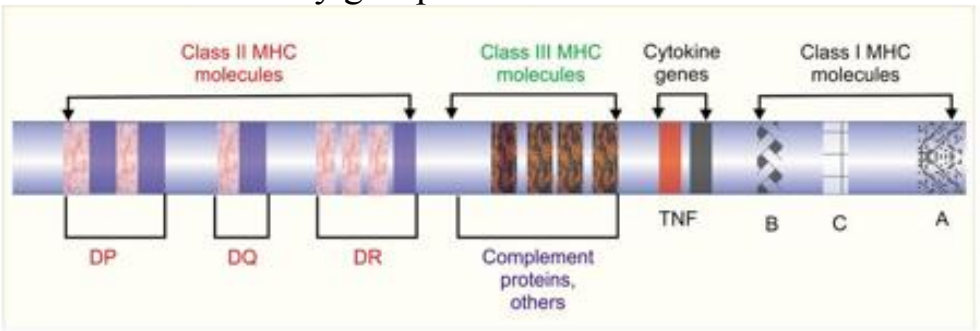


Fig. 6.19: Major histocompatibility complex (human leukocyte antigen complex) showing location of genes.

TABLE 6.12: Association with HLA alleles with diseases.

Disease	*HLA Allele*
Class I MHC molecules	
• Ankylosing spondylitis	B27
• Postgonococcal arthritis	
• Acute anterior uveitis	
• Behçet's syndrome	B51
• 21-hydroxylase deficiency	HLA-BW47
• Hereditary hemochromatosis	HLA-A
Class II MHC molecules	
• Chronic active hepatitis	DR3
• Primary Sjögren syndrome	
• Rheumatoid arthritis	DR4
• Ulcerative colitis	DR103
• Type 1 diabetes	DR3/DR4
• Primary biliary cirrhosis	DR8
• Graves' disease and myasthenia gravis	DR3

- **Inflammatory diseases (e.g., ankylosing spondylitis most strikingly associated with HLA-B27**).
- **Autoimmune diseases** (e.g., autoimmune endocrinopathies associated with alleles at the DR locus).

- **Inherited errors of metabolism** [e.g., 21-hydroxylase deficiency (HLA-BW47) and hereditary hemochromatosis (HLA-A)].

MHC is a cluster of genes located on short arm of chromosome 6 (6p21.3).

Tests for detection of HLA:
- Lymphocytotoxicity test (MHC class I)
- Mixed lymphocyte culture/reaction (MHC class II)
- Primed lymphocyte typing
- DNA analysis

Class I MHC molecules:
- Present in all nucleated cells and platelets
- Not present in mature RBCs and trophoblasts

Class II molecules are expressed only on professional antigen presenting cells.

Class III MHC gene code for:
- Complement proteins (except C3)
- Properdin B of alternate complement pathway
- Tumor necrosis factor α and β

HLA B27 is positive in ankylosing spondylitis.

Significance of HLA antigens:
- Organ transplantation
- Play major role in recognition of foreign antigen and immunity
- Transfusion medicine
- Its association with diseases

A super-antigen is a bacterial product that binds to beta chain of TCR and MHC class II molecules of APC simulating T-cell activation.

Type I MHC presents peptide antigen to T-cell, so that peptide binding site is formed by distal domain α 1 and 2.

Mixed lymphocyte culture is used to identify: MHC class II antigen.

HLA class III region genes are important elements in governing susceptibility to autoimmune disease.

REJECTION OF TRANSPLANTS

CC: Describe the immune principles involved in transplant and mechanism of transplant rejection.

Q. Write short note on transplant rejection.

- **Transplantation** is a **procedure for replacement of irreparably damaged tissue or organ to restore their lost function**.
- **Tissue or organ transplanted** is called as **transplant or graft**.
- Individual from which transplant is obtained is known as **donor** and the individual who receives it is called **recipient**.
- **Allograft** is the term used for **a graft from individual of the same species**.
- A **major barrier for transplantation** is the process known as **rejection**, in which the recipient's immune system recognizes the graft as being foreign and mounts the immunological reactions against it.

Recognition and Rejection of Allografts

In transplantation, donor MHC-encoded antigens are immunogenic and can stimulate rejection of transplanted tissues. **Rejection is a complex process in which both T lymphocytes and antibodies produced against graft antigens and they react against and destroy the grafts.**

Optimal survival of graft occurs when recipient and donor are closely matched for histocompatibility antigens. But an exact HLA match is not possible, except between monozygotic twins. Because HLA genes are highly polymorphic, there will be always some differences between individuals. **The major antigenic differences between a donor and recipient that result in rejection of transplants are mainly due to differences in HLA alleles.** Thus, after organ transplantation, immunosuppressive therapy and vigilant monitoring of graft function are necessary. Present therapeutic advances have greatly improved transplant success rates, even when there is a degree of histoincompatibility. Both cell-mediated immunity and circulating antibodies play a role in transplant rejection.

Recognition of Graft Alloantigens

The antigens in the allograft are termed as allogeneic antigens or alloantigens.

Pathways of recognition of graft alloantigens: Following transplantation, the recipient's T cells recognize donor alloantigens from the graft by two pathways.

TABLE 6.13: Classification and characteristics of transplant rejection.

Type	*Time*	*Mechanism*	*Pathological findings*
Hyperacute rejection	Minutes to hours	Preformed antibody and complement activation (type II hypersensitivity)	Arteritis, thrombosis and necrosis
Acute rejection			
• **Acute cellular rejection**	5 days to weeks	Activated T lymphocytes: $CD4^+$ and $CD8^+$ T-cells (type IV hypersensitivity)	Extensive interstitial mononuclear cell infiltration ($CD4^+$ and $CD8^+$), edema and endothelitis
• **Acute humoral rejection**		Antibody and complement activation	Necrotizing vasculitis, neutrophilic infiltration and thrombosis
Chronic rejection	Months to years	Immune and non-immune mechanisms	Fibrosis, scarring

Hyperacute rejection: Type II hypersensitivity.

Acute cellular rejection: Between 5 to 30 days of transplantation.

Acute rejection:
- Type II hypersensitivity and
- Type IV hypersensitivity.

Initial target of the antibodies in graft rejection is graft vasculature.

1. **Direct pathway:** In this pathway, the **graft antigens are presented directly to recipient T cells by graft APCs**.
2. **Indirect pathways:** In this pathway, the graft antigens are taken up by host APCs, processed (similar to any other foreign antigen), and presented to host T cells.

Both pathways activate $CD8^+$ T cells, which develop into cytotoxic T-cells (CTLs) and $CD4^+$ T cells. $CD4^+$T cells become cytokine-producing effector cells, mainly TH1 cells.

Classification of Rejection Reaction (Table 6.13)

Q. Write short note on transplant rejection reactions.

Depending on time of occurrence, the rejection reactions are classified as: **(1) hyperacute**, **(2) acute** and **(3) chronic**.

Hyperacute Rejection

Q. Write short note on hyperacute rejection.

- **Occurs within minutes or hours after transplantation**
- It is a special type of rejection, **occurs if the host has preformed antidonor antibodies in the circulation before transplantation**.
- These **antibodies bind to endothelium of graft organ → activates complement → vascular thrombosis**.
- Results in rapid and irreversible destruction of the graft.
- **Causes of preformed antidonor antibodies:**
  - **Multiparous women**, who develop anti-HLA antibodies against paternal antigens that is shed from the fetus.

- **Prior blood transfusions**, because platelets and white blood cells are rich in HLA antigens.
- Host has **previously rejected a renal transplant**.

Acute Rejection

- Occurs **within days to weeks** after transplantation in the non-immnuosuppressed host.
- Types:
  - **Acute cellular rejection:** It is mediated by activated T ($CD4^+$ and $CD8^+$) lymphocytes and results in deterioration in graft function.
  - **Acute humoral rejection (rejection vasculitis):** It is mediated by antibody (anti-donor antibodies) formed de novo after transplantation. Its consequences depend on specificity and ability to trigger other immune components such as the complement cascade.

Chronic Rejection

- Also known as **chronic allograft failure.**
- It is a major cause of graft loss.
- **Occurs months to years after transplantation.**
- Pathogenesis is poorly understood and may be due to both immunological and non-immunological mechanism.

Q. Differences between hyperacute and acute transplant rejection (for answer *refer* Table 6.13).

MORPHOLOGY

Kidneys were the first solid organs to be transplanted and are most commonly transplanted organ. Hence, the morphologic changes are discussed mainly in relation to renal transplants.

1. Hyperacute Rejection

- **Gross:**
  - The renal **graft rapidly becomes cyanotic, mottled, and flaccid**, and may excrete a few drops of bloody urine.
  - Later, cortex undergoes necrosis (infarction), and kidney becomes nonfunctional.
- **Microscopy: Blood vessels** show **widespread acute arteritis**, **arteriolitis** with **fibrinoid necrosis** of their walls → thrombosis → **ischemic necrosis**.

2. Acute Rejection

- **Type:** Either cellular or humoral immune mechanisms may predominate.

  - **Acute cellular rejection:** It occurs within few months after transplantation and develops renal failure. It shows cellular infiltration of $CD4^+$ and $CD8^+$ T-cells and mononuclear cells. $CD8^+$ T-cells may injure vascular endothelial cells, causing **endothelitis**.
  - **Acute humoral rejection** (rejection vasculitis)**:** Main target of the antibodies is the graft vasculature → manifest as **vasculitis**, endothelial cell necrosis and neutrophilic infiltration. Caused by anti-donor antibodies.

3. Chronic Rejection

It is associated with proliferation of transplant vascular smooth muscle, interstitial fibrosis and scarring. It presents **progressive renal failure**. It is characterized by vascular changes, interstitial fibrosis and loss of kidney parenchyma.

C4d Staining

- It is a fragment of complement protein C4.
- Its deposition in the capillaries of the graft indicates local activation of classic pathway of complement system. Thus, C4d staining provides an evidence for antibody-mediated (humoral) graft rejection.
- This is useful for the early detection of vascular rejection.

Transplantation of Hematopoietic Cells

Q. Indications for bone marrow/hematopoietic stem cell (HSC) transplantation.

Bone marrow transplantation was the original term used to describe the collection and transplantation of hematopoietic stem cells. Hematopoietic stem cell (HSC) transplantation is a preferred term, because the peripheral blood and umbilical cord blood are also used as sources of stem cells.

Definition: Hematopoietic stem cell (HSC) transplantation is a procedure which involves eliminating an individual's hematopoietic and immune system by chemotherapy and/or radiotherapy and replacing with stem cells either from another individual or with individual's own hematopoietic stem cells.

Types of Hematopoietic Stem Cell Transplant

- **Autologous** ("from self"): Own HSCs removed, cryopreserved and re-infused.
- **Allogeneic** ("from different genes"): HSCs obtained from another individual.
- **Syngeneic** ("from same genes"): HSCs obtained from an identical twin.

Sources of Hematopoietic Stem Cells

- **Bone marrow:** Richest store.
- **Peripheral blood:** Very few HSCs but can be mobilized from bone marrow by administering G-CSF or GM-CSF.
- **Umbilical cord blood:** Easily available and is a rich source.

Indications for HSC Transplantation

Refer **Box 6.1**.

Complications of HSC Transplantation

Autologous HSC transplants have fewer immunologic complications but have higher rates of relapse of the disease after transplant. Allogeneic HSC transplants have lower rates of relapse but have more immunologic complications, and GVHD, which can be fatal.

Graft versus Host Disease

Q. Write short note on graft versus host disease (GVHD). List the organs involved in GVHD.

It is the major complication that follows allogeneic HSC transplant. This is due to infused donor T lymphocytes ($CD4^+$ and $CD8^+$ T-cells) reacting against the recipient's tissues/organs. **Three conditions are necessary** for the development of GVHD:

1. **An immunocompetent graft** (i.e., one containing T-cells).
2. **HLA mismatch** (minor or major) between donor and recipient.
3. **An immunosuppressed recipient** who cannot mount an immune response to the graft.

When immunosuppressed recipients receive normal bone marrow cells from allogeneic donors, the **immunocompetent T-cells present in the donor** HSCs recognize **the recipient's HLA antigens as foreign** and react against them.

- **Acute GVH disease:** It occurs before 100 days. It often affects three primary **target organs** simultaneously, namely **skin, gastrointestinal (GI) tract and liver**. Direct cytotoxicity by $CD8^+$ T-cells and cytokines released by the sensitized donor T-cells are responsible for the damage.

BOX 6.1: Indication for hematopoietic stem cell transplantation.

Red blood cell disorders
- Severe aplastic anemia
- Thalassemia major
- Fanconi anemia
- Sickle cell disease
- Pure red cell aplasia

WBC disorders
- Leukemia
  - Acute lymphoblastic leukemia—relapse after initial chemotherapy induced remission
  - Chronic myeloid leukemia, acute myeloid leukemia
- Myelodysplastic syndromes, myelofibrosis
- Lymphomas: Hodgkin lymphoma, non-Hodgkin lymphoma
- Multiple myeloma

Solid tumors: Germ cell tumors, neuroblastoma

Immunological disorders
- Severe autoimmune disorders: Scleroderma, lupus erythematosus
- Immune deficiency syndromes

- **Chronic GVH disease:** It occurs after day 100 and can **affect the skin, GI tract, liver, eyes, lungs and joints**. GVHD is difficult to treat and in severe cases it is usually fatal.

Other Complications

- **Infections:** Patients are susceptible to a variety of infections (bacterial, viral and fungal) due to lack of granulocytes, as well as lack of a functioning immune system.
- **Organ toxicity:** Damage to GI tract, liver and lungs.
- **Immunodeficiency**: It **is a frequent complication of bone marrow transplantation**. The immunodeficiency may be due to prior treatment, preparation for the graft, a delay in repopulation of the recipient's

immune system, and attack on the host's immune cells by grafted lymphocytes. Immunodeficiency predisposes to infections, particularly infection with cytomegalovirus which can cause fatal pneumonitis.

ABO blood group compatibility: Most essential requirement for successful transplantation.

Graft rejection: Initiated by host T lymphocytes that recognize HLA antigen of graft as foreign.

Recognition of allograft may be direct (on APCs in the graft) or indirect (by host APCs).

Direct recognition: Important for acute graft rejection.

Indirect recognition: Important for chronic graft rejection.

Hyperacute rejection: Caused by preformed antibodies.

Hyperacute rejection:
- Type II hypersensitivity reaction
- Irreversible

Acute rejection:
- Most common
- Type IV and type II hypersensitivity

Acute humoral rejection: Antibodies destroy graft vessels.

Acute cellular rejection: T-cells destroy graft parenchymal cells and blood vessels by CTLs and inflammatory reaction.

Chronic rejection: Irreversible.

Corneal transplantation: Graft survival rate is very high.

Chronic rejection: Arteriosclerosis due to hyperplasia of vascular smooth muscle cells probably due to T-cell reaction and secretion of cytokines.

Solid organs that are transplanted include liver, heart, lungs, and pancreas.

C4d staining: Useful in the early diagnosis of vascular rejection.

Risk of graft versus host disease can be reduced by depletion of T cells from the graft.

Target organs of GVH disease: Skin, gastrointestinal (GI) tract and liver.

IMMUNODEFICIENCY SYNDROMES

Immunodeficiency is defect in immunity.

Classification

- **Primary immunodeficiency (PID)** disorders due to an intrinsic defect in the immune system.
- **Secondary immunodeficiency** states which may arise as complications of an underlying condition. The underlying condition includes cancers, infections, malnutrition, or immunosuppression, irradiation, or chemotherapy for cancer and other diseases.

Primary Immunodeficiency

Classification of primary immune deficiency diseases is presented in **Box 6.2**. Most of them manifest themselves in infancy, between 6 months and 2 years of life. They come to clinical attention because they are susceptible to recurrent infections.

X-linked Agammaglobulinemia (Bruton's Agammaglobulinemia)

- One of the more common primary immunodeficiency disease.
- Characterized by **defect in B-cell development**.

Etiology

- Due to mutations in a cytoplasmic tyrosine kinase gene, called **Bruton tyrosine kinase (*Btk*) gene.** The gene is located on the long arm of the X chromosome at Xq21.22.

BOX 6.2: Classification of primary immune deficiency diseases.

Deficiencies of the Innate Immune System

- **Phagocytic cells**: Impaired adhesion (leukocyte adhesion deficiency/LAD), impaired killing (chronic granulomatous disease/CGD)
- **Innate immunity receptors and signal transduction**: Defects in toll-like receptor signaling
- **Complement deficiencies:** Classical, alternative and lectin pathways.

Deficiencies of the Adaptive Immune System

- **B lymphocytes**: XL and AR agammaglobulinemia, hyper-IgM syndrome
- **T lymphocytes:** DiGeorge syndrome, Wiskott–Aldrich syndrome.

Regulatory Defects

- **Innate immunity**: Severe colitis
- **Adaptive immunity**: Autoimmune lymphoproliferation syndrome (ALPS)

- ***Btk gene*** product is a kinase that is required for maturation of pre B-cell to B-cell stage.
- **Mutation of *Btk gene* blocks B-cell maturation at pre B-cell stage → no production of light chains** and reduced production of immunoglobulin. They have intact T-cell mediated immunity.

Clinical Manifestation

- Seen in **males** and does not manifest till about 6 months of age (till maternal immunoglobulins are depleted).
- **Susceptible to infections:**
  - Recurrent bacterial infections of the respiratory tract, such as acute and chronic pharyngitis, sinusitis, otitis media, bronchitis and pneumonia.
  - Viral infections (e.g., echovirus, poliovirus and coxsackievirus) and ***giardia lamblia***.
- Increased **susceptibility to autoimmune diseases** (e.g., arthritis and dermatomyositis).

Characteristic Findings

- **Absent or markedly decreased B lymphocytes** in the circulation.
- **Decreased serum** levels of all classes of **immunoglobulin.**
- **Underdeveloped germinal centers** in lymph nodes, Peyer's patches, the appendix and tonsils.
- **Absence of plasma cells throughout the body**.
- **Normal T-cell mediated immunity**.

DiGeorge Syndrome (Thymic Hypoplasia)

T-cell immunodeficiency disorder: Absence of cell-mediated immunity due to low numbers of T lymphocytes in the blood and lymphoid tissues.

Etiology

- **Defective embryologic development of the third and fourth pharyngeal pouches**, which normally give rise to the thymus, parathyroid glands, some of the clear cells of the thyroid, the ultimobranchial body and influence conotruncal cardiac development.
- Patients develop a **variable loss of T-cell mediated immunity** (due to hypoplasia or lack of the thymus), tetany (due to lack of the parathyroids) and congenital defects of the heart and great vessels (due to conotruncal cardiac development).
- In the absence of a thymus, T-cell maturation is interrupted at the pre T-cell stage.
- Most patients with DiGeorge syndrome have a point deletion (**22q11 deletion**) in the long arm of chromosome 22.

Clinical Manifestations

- Usually presents during infancy with conotruncal congenital heart defects and severe hypocalcemia (due to hypoparathyroidism).
- Infants are prone to recurrent or chronic viral, bacterial, fungal and protozoal infections.
- The T-cell zones of lymphoid organs (paracortical areas of the lymph nodes and the periarteriolar sheaths of the spleen) are depleted.

Immunodeficiency with Thrombocytopenia and Eczema (Wiskott–Aldrich Syndrome)

X-linked recessive disease characterized by thrombocytopenia, eczema and a marked susceptibility to recurrent infection, ending in early death.

Etiology

- Caused by **mutations in the WASP gene** encoding ***Wiskott–Aldrich syndrome protein (WASP)***, which is located at Xp11.23 → reduced levels of WASP.
- WASP link membrane receptors (e.g., antigen receptors) to cytoskeletal elements.
- WASP gene mutations affect not only T lymphocytes but also the other lymphocyte subsets, dendritic cells and platelets.

Clinical Manifestations

Typically present with **recurrent bacterial infections, eczema and bleeding** caused by thrombocytopenia.

Other Features

- Thymus is morphologically normal.

- Later stages → progressive secondary depletion of T lymphocytes in the peripheral blood and in the T-cell zones (paracortical areas) of the lymph nodes, with variable loss of cellular immunity.
- Increased risk of developing non-Hodgkin B-cell lymphomas.

Bruton disease: Usually does not manifest until about 6 months of age, when maternal immunoglobulin are depleted.
Bruton disease: Underdeveloped or rudimentary germinal centers in lymph nodes, Peyer's patches, the appendix and tonsils.

DiGeorge syndrome: Defective embryologic development of the third and fourth pharyngeal pouches.
DiGeorge syndrome: Absence of thymus and parathyroid glands.

Wiskott Aldrich syndrome
- X-linked recessive
- Thrombocytopenia
- Eczema/atopic dermatitis
- Recurrent infections.

Wiskott Aldrich syndrome—diagnosis:
- Mutations in WASP gene at Xp11.23
- Reduced levels of WASP.

ACQUIRED IMMUNODEFICIENCY SYNDROME

CC: Define and describe the pathogenesis and pathology of HIV and AIDS.

Acquired immunodeficiency syndrome (AIDS) is caused by the retrovirus **human immunodeficiency virus** (HIV).

Characteristic Features

- **Infection and depletion of $CD4^+$ T lymphocytes**.
- **Severe immunosuppression → leads to opportunistic infections, secondary neoplasms and neurologic manifestations**.

Route of Transmission

HIV-1 and HIV-2 are relatively weak viruses outside of the body. HIV is found in every bodily fluid and is broadly disseminated in the body. Since it

is present in very low titers in saliva, tears, cerebrospinal fluid, amniotic fluid, and feces; it is not transmitted through these body fluids. Transmission of HIV occurs when there is an exchange of blood or body fluids containing the virus or virus-infected cells. The **three major routes** of transmission are:

1. **Sexual transmission:** It is the **main route of infection** in more than 75% of cases of HIV.
   - **Homosexual or bisexual men** or **heterosexual contacts:** It may be male-to-male, or male-to-female or female-to-male transmission.
   - **HIV is present in genital fluids** such as vaginal secretions and cervical secretions/cells (in women) and semen (in men).
   - Risk of sexual transmission of HIV is increased when there is coexisting sexually transmitted diseases, especially those associated with genital ulceration (e.g., syphilis, chancroid and herpes).
   - **Viral transmission** can occur in two ways:
     - **Direct inoculation of virus or infected cells into the blood vessels** at the site of breach caused by trauma, and
     - **By uptake into the mucosal dendritic cells** (DCs).
2. **Parenteral transmission:** It occurs via blood, blood products, or blood-contaminated needles or syringes. Three groups of individuals are at risk.
   a. **Intravenous drug abusers:** Transmission occurs by **sharing of needles and syringes contaminated** with HIV-containing blood.
   b. **Hemophiliacs:** Mainly those who received large amounts factor VIII and factor IX concentrates before 1985. Now increasing use of recombinant clotting factors have eliminated this mode of transmission.
   c. **Transfusion of blood or blood components:** Recipients of blood transfusion of **HIV-infected whole blood or components** (e.g., platelets, plasma) was one of the modes of transmission. Screening of donor blood and plasma for antibody to HIV has reduced the risk of this mode of transmission. Because of recently infected individual may be antibody-negative (seronegative), there is a small risk of acquiring AIDS through transfusion of blood. Organs from HIV-infected donors can also transmit AIDS.
3. **Perinatal transmission (mother-to-infant transmission):**
   - **Major mode of transmission of AIDS in children**.
   - Transmission of infection can occur by **three routes**:

a. **In utero:** It is transmitted by transplacental spread.
b. **Perinatal spread:** During normal vaginal delivery or child birth (intrapartum) **through an infected birth canal** and in the immediate period (peripartum).
c. **After birth:** It is transmitted by ingestion of breast milk or from the genital secretions.

Transmission of HIV infection to health care workers: There is an extremely small risk of transmission to healthcare professional, after accidental needle-stick injury or exposure of nonintact skin to infected blood.

Etiology

Properties of HIV

AIDS is caused by HIV, which is a **nontransforming human retrovirus** belonging to the lentivirus family. This subfamily is so named from the Latin word lentus, meaning "slow," because infection develops gradually. Retroviruses are single-stranded **RNA viruses having an enzyme called reverse transcriptase**, which **prepares a DNA copy of the RNA genome of the virus in host cell**.

Genetic forms: HIV occurs in two genetically different but related main forms, **HIV-1 and HIV-2.** Both are similar in structure and function, but are differentiated from each other by their envelope glycoproteins, point of origin, and latency periods. Many subspecies or strains of HIV also exist because of the rapid rate of HIV virion mutation.

- **HIV-1** is most common in the United States, Europe and Central Africa. About 10 subtypes of HIV-1 have been identified: group N (YBF30), group O, and group M with 8 subtypes (A, B, C, D, E, F, G, and H). The most common cause of AIDS in India is HIV-1 group M subtype C.
- **HIV-2 is common** in West Africa and confined to western and southern India (found near Goa). HIV-2 is differentiated from HIV-1 by a longer clinical latency period from the onset of infection to the development of symptoms. It is possible to have coinfection with both HIV-1 and HIV-2.

Structure of HIV (Fig. 6.20)

Q. Write short note on structure of HIV.

- HIV-1 is **spherical enveloped** virus which is about 90–120 nm in diameter.
- It consists of electron-dense, cone-shaped core surrounded by nucleocapsid cell which is covered by lipoprotein envelope.

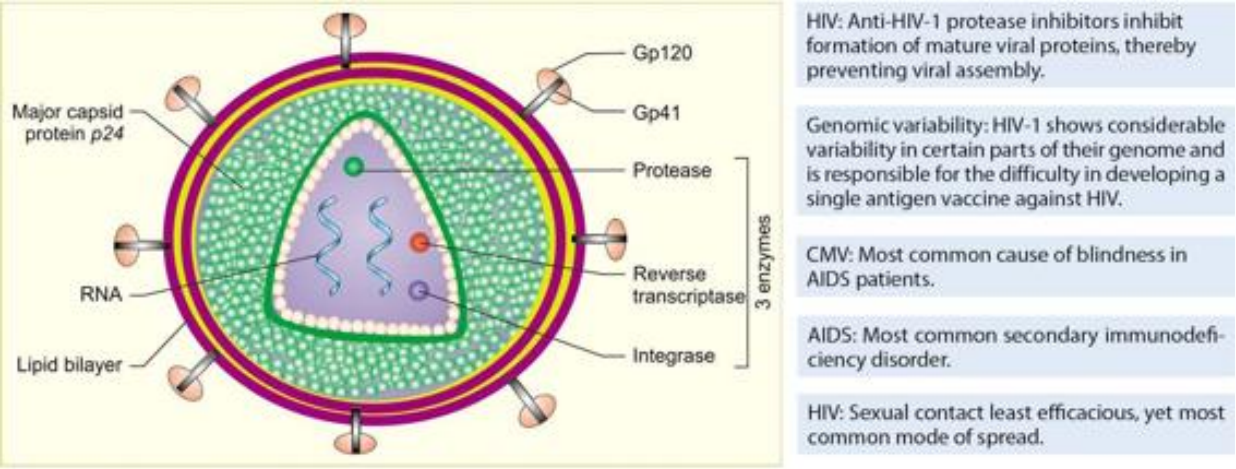


Fig. 6.20: Diagrammatic representation of structure of the human immune deficiency virus (HIV)-1 virion. The viral particle is covered by a lipid bilayer derived from the host cell and studded with viral glycoproteins gp41 and gp120.

A. Viral core: It contains:

1. **Major capsid protein *p24*:** This viral antigen and the antibodies against this are **used for the diagnosis of HIV infection** in enzyme-linked immunosorbent assay (**ELISA**).
2. **Nucleocapsid protein** ***p7/p9*** (not shown in**Fig. 6.20)**.
3. **Two identical copies of single stranded RNA genome**.
4. **Three viral enzymes:** These enzymes are very important because they facilitate the conversion of RNA to DNA. **(1) Protease** (works as a "molecular scissors" and splits the other viral components and activates the virions); **(2) reverse transcriptase** (RNA-dependent DNA polymerase which allows the virus to copy RNA into DNA), and **(3) integrase**. **When the virus infects a cell**, **viral RNA** is not translated, instead **transcribed by reverse transcriptase into DNA**. The **DNA form of the retroviral genome** is called a **provirus** which can be **integrated into the chromosome of host cell**.

B. Nucleocapsid: Between the lipid envelope and viral core, there is a protein layer called *p17*. The nucleocapsid or core is composed of a ***protein*** called ***p24***.

C. **Lipid envelope:** The virus contains a lipoprotein envelope, which **consists of lipid derived from the host cell and two viral glycoproteins**. These glycoproteins are: **(1) gp120,** project as a knob-like spikes (look like a studded ball) on the surface and **(2) gp41,** anchoring transmembrane pedicle. Gp120 and gp41 proteins cover the viral particle surface. Gp120 is the most external and distal part of each "stud/knob-like," whereas gp41 is the bridge that holds it onto the virion surface. **These glycoproteins are essential for HIV infection of cells**.

HIV Genome

It contains two main groups of genes and their products act as antigens. Its RNA encodes for nine genes, i.e., 3 standard genes and 6 accessory genes.

1. **Standard genes:** HIV-1 RNA genome **contains three standard retroviral genes**, which are **typical of retroviruses**. These include: *gag*, *pol*, and *env* genes. Initially, the protein products of the ***gag*** and ***pol*** genes are translated into **large precursor proteins** and are later cleaved by the viral enzyme protease to form → **mature proteins**. The *gag* gene encodes the core antigen proteins that form the virus particle. The *pol* gene encodes reverse transcriptase proteins. The *env* gene encodes the viral envelope protein glycoprotein gp160, which is split into two fragments, gp120 and gp41, by cellular protease.
2. **Accessory genes:** HIV contains 6 accessory genes namely *tat*, *rev*, *vif*, *nef*, *vpr*, and *vpu*. They **regulate the synthesis (regulatory genes) and assembly of infectious viral particles** and the **pathogenicity of the virus**.

Pathogenesis of HIV Infection and AIDS

Q. Write short note on pathogenesis of HIV infection and AIDS.

Infection is transmitted when the virus enters the blood or tissues of an individual.

Major targets: HIV can infect many tissues, but two major targets of HIV infection are:

- **Immune system:** HIV is an RNA retrovirus that causes a defect in cell-mediated immunity that may progress to AIDS.
- **Central nervous system (CNS)**.

Life Cycle of HIV

Q. Write note on life cycle of HIV.

Consists of four main steps, namely: (1) Infection of cells by HIV, (2) integration of the provirus into the host cell genome, (3) activation of viral replication, and (4) production and release of infectious virus **(Fig. 6.21)**.

1. **Infection of cells by HIV** (1 in **Fig. 6.21**): At the time of exposure to the HIV virus, infection occurs when the virus moves across the epithelium or mucosal membrane of the body.
   - **Cell tropism:** Once inside the body, HIV has **selective affinity** for host cells with surface **CD4 molecule receptor**. The cells with such receptors include **$CD4^+$ T-cells** and other $CD4^+$ cells such as **monocytes/macrophages** and **dendritic cells**. The HIV envelope contains two glycoproteins, surface gp120 (expressed in all HIV strains) noncovalently attached to a transmembrane protein and gp41.
   - **Binding of envelope protein gp120 to CD4 molecule receptor** on the host cell in the mucosal or other tissue at or near the site of exposure, is the first step in HIV infection. Binding alone is not enough for infection (for fusion of the virion and host cell) and **requires participation of a coreceptor molecule** namely chemokine receptor (CCR5 or CXCR4). Chemokines (chemoattractant cytokines that regulate leukocyte trafficking to sites of inflammation) play a significant role in the pathogenesis of HIV disease.
   - **Conformational change:** Binding to CD4 leads to a conformational change (change in shape by refolding) **in the HIV, that results in the formation of a new recognition site on gp120 for the coreceptors CCR5 or CXCR4**.
   - **Gp120 binding to chemokine receptor:** A number of important coreceptors on the target cells are necessary for the virus to gain entry into cells. These chemokine receptors include CCR5 (must be present for the HIV particles to bind to the $CD4^+$ cells, especially in early infection) and another CXCR4 often present in later infection. New

recognition site on gp120 of HIV bind to **chemokine receptors,** i.e., CCR5 and CXCR4. Chemokine receptors play a role in viral cell entry.

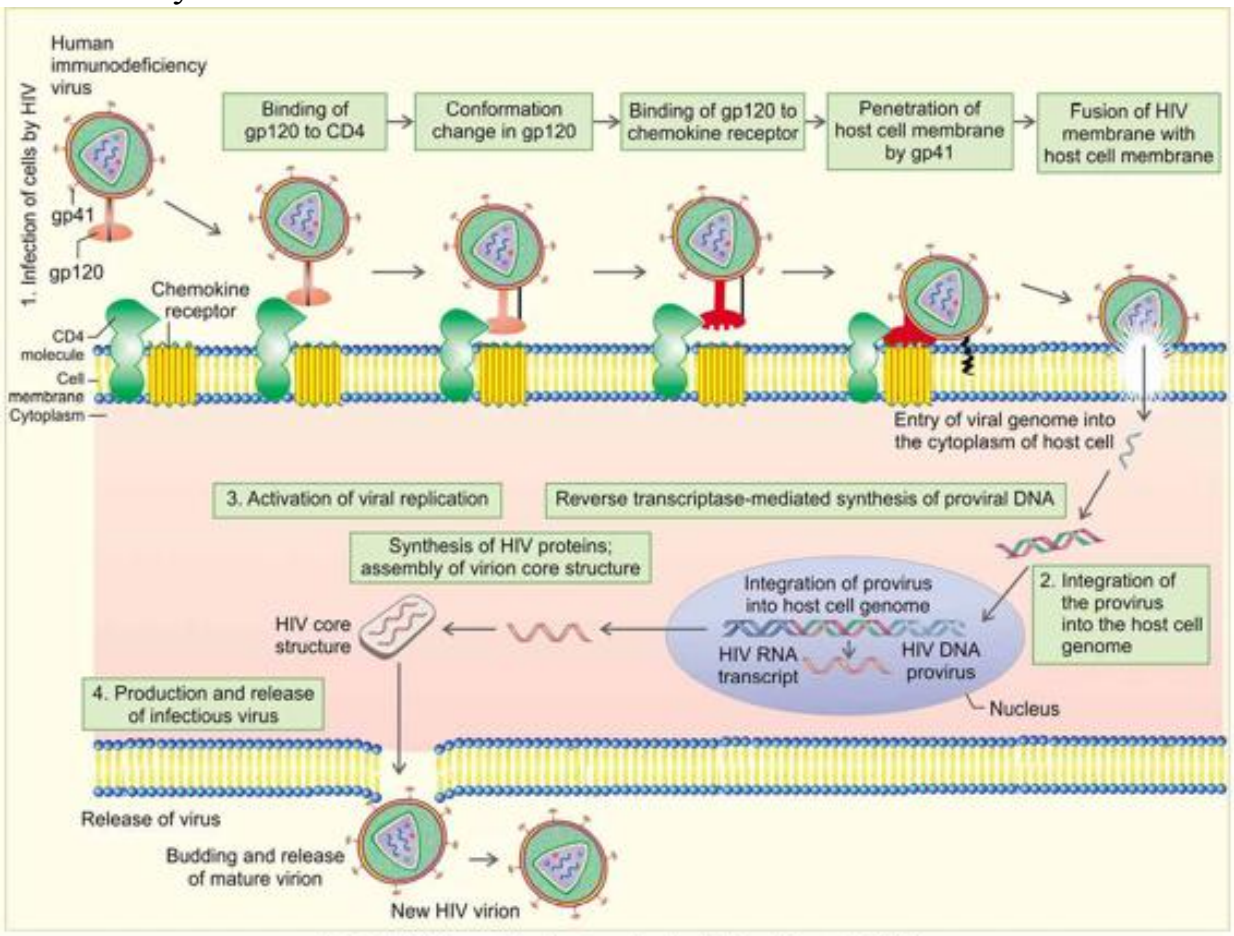


Fig. 6.21: Various molecular steps involved in the life cycle of HIV.

- **Penetration of host cell membrane by gp41:** Binding of gp120 to the chemokine coreceptors leads to **conformational changes in gp41**. After the binding of HIV particle to both the CD4 receptor and the chemokine receptor on the host cell, gp41 implants itself in the cell membrane.
- **Membrane fusion:** The conformational change in gp41 allows **HIV to penetrate the cell membrane of the target cells** (e.g., $CD4^+$ T-cells or macrophages), **leading to fusion of the virus with the host cell**.
- **Entry of viral genome into cytoplasm of host cell:** Once internalized, the virus core containing the HIV genome enters the cytoplasm of the host cell, and infection is produced.

2. **Integration of the proviral DNA into the genome of the host cell** (2 in **Fig. 6.21**)**:**
   - Once in the cytoplasm (after the internalization of the virus core), a **single-stranded DNA copy is made by reverse transcriptase** from the viral RNA. Using this single-stranded DNA as a template, DNA polymerase copies it to make **a second (complementary) DNA strand** and destroys the original RNA strands. Thus, it produces **double-stranded DNA**. This double-stranded complementary DNA is called as cDNA or proviral DNA. The accuracy of DNA transcription is poor, with mutations occurring frequently. This tendency to mutate makes HIV resistant to antiviral medications.
   - **Integration of the proviral DNA into the genome of the host cell.** Once formed, this new viral DNA is called provirus or preintegration complexes (PICs). This migrates to the nucleus of host cell and is actively transported in the nuclear compartment.
   - **Episomal form: In quiescent T-cells, HIV cDNA** may remain as a linear **episomal form in the cytoplasm of infected cell**.
   - **Integration of cDNA: In dividing T-cells**, HIV **cDNA enters the nucleus**, and **becomes integrated into the genome** of the host cell using several host proteins including a viral integrase protein.
3. **Viral replication** (3 in **Fig. 6.21**)**:** After the integration of proviral DNA into the host DNA, it can either be latent or productive infection.
   - **Latent infection:** During this, the **provirus remains silent** for months or years.
   - **Productive infection:** Once the proviral DNA is integrated into the host cell's DNA, the viral DNA is replicated together with the host cell's DNA during every cell division. Now the viral DNA is permanently part of the host cell's DNA.
4. **Production and release of infectious virus** (4 in **Fig. 6.21**)**:** The complete **virus particle** formed **buds from the cell membrane** and **releases new infectious virus**. This productive infection when extensive, leads to death of infected host cells.

The virus **infection remains latent for long periods in lymphoid tissues**. **Active viral replication is associated with more infection of cells and progression to AIDS**.

Dissemination: Virus disseminates to other target cells. This occurs either by fusion of an infected cell with an uninfected one or by the budding of

virions from the membrane of the infected cell.

Progression of HIV Infection

Acute Infection

HIV infection starts as an acute infection. It is only partially controlled by the host immune response and progresses to chronic infection of the peripheral lymphoid tissue.

- **Primary infection: HIV first infects memory CD4$^+$ T-cells** (express CCR5), which are present in **the mucosal lymphoid tissue** (largest reservoir of T-cells and where majority of memory cells are lodged). HIV **causes death of these cells** resulting in **significant depletion of T-cells**.
- **Spread to lymphoid tissue: Dendritic cells at the primary site of infection capture the virus** and **migrate to lymphoid tissue** such as lymph nodes and spleen. In the lymphoid tissues, **DCs are passed on to CD4$^+$ T-cells by direct cell-to-cell contact**.
- **Acute HIV (retroviral) syndrome: Virus replicates** and **causes viremia**, accompanied by **acute HIV syndrome** (nonspecific signs and symptoms similar to many viral diseases). The extent of viremia is measured as HIV-1 RNA levels in the blood. It is a useful marker of HIV disease progression and in the management of HIV infection.
- **Host immune response against HIV:** Virus spreads throughout the body and infects helper T-cells, macrophages and DCs in the peripheral lymphoid tissues. During this period, the host humoral and cell-mediated immune response develops against viral antigens. These include **anti-HIV antibodies** and **HIV-specific cytotoxic T-cells**. Immune responses partially control the infection and viral replication.

Chronic Infection: Clinical Latency Period

Following acute phase it progresses to chronic phase. This phase is **characterized by dissemination of virus, viremia,** and **development of immune response by host**.

- **Minimal/no symptoms:** In this phase, **virus continuously replicates in the lymph nodes and spleen**. The host immune response can handle most infections with opportunistic microbes with no or minimal clinical symptoms.

- **Progressive decrease of $CD4^+$ T-cells:** There is **continuous destruction of $CD4^+$ T-cells in the lymphoid tissue** accompanied by steady **decrease in their number in the peripheral blood**. During the early course of disease, the loss of $CD4^+$ T-cells can be replaced by new T-cells. However, over a period of years, the continuous cycle of viral infection and death of T-cells → leads to steady decrease in the number of $CD4^+$ T-cells both in the lymphoid tissue and in circulation.

Mechanism of T-cell depletion: Direct killing of T-cells by the virus is the major cause.

Inversion of $CD4^+/CD8^+$ ratio: Normal $CD4^+/CD8^+$ ratio is 2:1. Loss of $CD4^+$ cells in AIDS patient leads to inversion of ratio of 0.5 or less.

HIV infection of non T-cells: HIV can infect non T-cells such as macrophages and dendritic cells **(mucosal and follicular)**.

Abnormalities of B-cell Function

- **Polyclonal activation of B-cells → hypergammaglobulinemia → circulating immune complexes**.
- **Impaired humoral immunity → disseminated infections** caused by capsulated bacteria, such as ***S. pneumoniae*** and ***H. influenzae.***

Natural History of HIV Infection (Fig. 6.22)

Q. Write short note on natural history of HIV infection.

Virus usually enters the body through mucosal epithelia and clinical course can be divided into three main phases:

1. **Early acute phase:** It may present as an acute (*refer* above), usually **self-limited nonspecific illness**. **These symptoms include sore throat, myalgias, fever, weight loss and fatigue**. **Other features, such as rash, cervical adenopathy**, diarrhea and vomiting, may also occur.

Q. Write short note on CD count in HIV.

Centers for disease control (CDC) classification of HIV infection: It depends on blood $CD4^+$ T-cell count. This divides the patients into three categories with counts being:

a. **Greater than 500 cells/µL**

   b. **Between 200 and 500 cells/µL**
   c. **Less than 200 cells/µL**
2. **Middle chronic phase:** It may have **few or no clinical manifestations** and is called the clinical latency period (*refer* page 178). The **symptoms may be due to minor opportunistic infections**, such as oral candidiasis (thrush), vaginal candidiasis, herpes zoster, and perhaps mycobacterial tuberculosis.
3. **Final crisis phase:** It is **final phase of HIV with progression to AIDS**. It presents with **fever, weight loss, diarrhea, generalized lymphadenopathy, multiple opportunistic infections, neurologic disease and secondary neoplasms**. Most of untreated (but not all) patients with HIV infection **progress to AIDS after a chronic phase lasting from 7 to 10 years**.

Exceptions

- **Rapid progressors:** In these patients, the middle, **chronic phase is shortened to 2–3 years** after primary infection and they **rapidly progress to AIDS**.
- **Long-term nonprogressors:** It is defined as **untreated patients who are asymptomatic for 10 years or more, with stable $CD4^+$ T-cell counts and low levels of plasma viremia**.

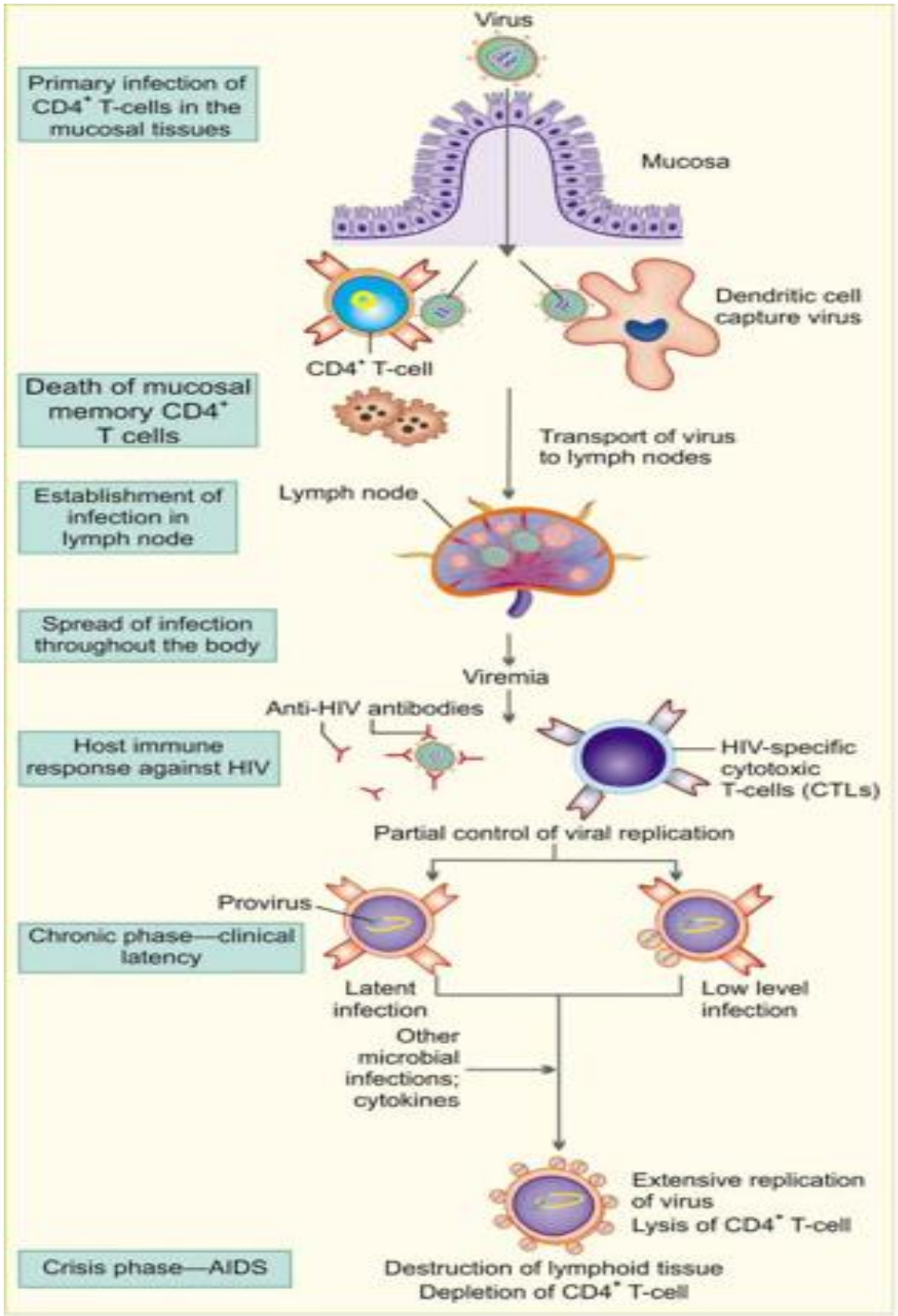


Fig. 6.22: Pathogenesis of HIV infection. HIV infects CD4+ T-cells and dendritic cells, and spreads to lymph nodes. Viral replication in lymph node leads to viremia and widespread seeding of lymphoid tissue. The viremia is controlled by the host immune response and the disease enters a phase of clinical latency. During this phase, viral replication in both T-cells and macrophages continues. Ultimately, there is progressive decrease of CD4+ cells and patient develops clinical symptoms of full-blown AIDS entering the crisis phase.

Natural history of HIV infection:
- Early acute phase
- Middle chronic phase
- Final crisis phase

Western blot: Confirmatory test for HIV.

The opportunistic infections and neoplasms found in patients with HIV infection are presented in **Table 6.14**.

CNS Lesions in AIDS

Q. List the CNS lesions found in AIDS.

- AIDS—dementia complex
- Non-Hodgkin B-cell lymphoma—primary lymphoma of the brain
- Progressive multifocal leukoencephalopathy
- Meningoencephalitis (tuberculous, cryptococcal)
- Aseptic meningitis
- Peripheral neuropathy
- Demyelinating lesions of the spinal cord

Diagnosis of HIV Infection or AIDS

Q. Write short note on laboratory diagnosis of AIDS.

1. **ELISA:** Detects antibodies against viral proteins. It is the **most sensitive** and best screening test for the diagnosis of AIDS.
2. **Western blot: Most specific** or the **confirmatory test** for HIV.
3. **Direct detection of viral infection:**
   - ***p24* antigen capture assay.**
   - **Reverse transcriptase polymerase chain reaction (RT-PCR).**
   - **DNA-PCR.**
   - **Culture of virus from the monocytes and $CD4^+$ T-cells.**

Prognosis: The prognosis of AIDS is poor.

HIV: Male-to-female transmission is more common compared to transmission from female-to-male.

Risk of transmission by needle prick injury is 0.3% for HIV whereas for hepatitis it is 30%.

HIV: Neither transmitted by casual personal contact (in the household, workplace or school) nor by insect bites.

Q. Write briefly on opportunistic/fungal infections in AIDS.

Q. Write briefly on common neoplasms in HIV patients.

TABLE 6.14: AIDS-defining opportunistic infections and neoplasms found in patients with HIV infection.

Opportunistic infections	
Protozoal and Helminthic Infections	**Organ or Site Involved or Type of Damage**
Cryptosporidiosis or isosporidiosis	Enteritis
Toxoplasmosis	Pneumonia or CNS infection
Fungal Infections	
Pneumocystosis	Pneumonia or disseminated infection
Candidiasis	Esophageal, tracheal, or pulmonary
Cryptococcosis	Infection of central nervous system
Coccidioidomycosis	Disseminated
Histoplasmosis	Disseminated
Bacterial Infections	
Mycobacteriosis	
• Atypical, e.g., *Mycobacterium avium-intracellulare*	Disseminated or extrapulmonary
• *M. tuberculosis*	Pulmonary or extrapulmonary
Nocardiosis	Pneumonia, meningitis, disseminated
Salmonella infections	Disseminated
Viral Infections	
Cytomegalovirus	Pulmonary, intestinal, retinitis, or CNS infections
Herpes simplex virus	Localized or disseminated
Varicella-zoster virus	Localized or disseminated
Progressive multifocal leukoencephalopathy	Central nervous system
Neoplasms	***Cause***
Kaposi sarcoma (KS)	Kaposi sarcoma herpes virus
Non-Hodgkin B-cell lymphoma—primary lymphoma of the brain	Epstein–Barr virus (EBV)
Cervical cancer in women	Human papillomavirus (HPV)
Anal carcinoma	HPV

HIV is cytotoxic to $CD4^+$ T-cells and leads to loss of cell-mediated immunity.

HIV affects most commonly: $CD\ 4^+$ T (helper) cells.

Normal ratio of CD4 to CD8 is 2:1.

Acute stage of HIV macrophages are affected and in chronic stage T_{H1} cells are affected.

Pneumocystis jiroveci: Most common fungal infection in AIDS in World.

Candidiasis: Most common fungal infection in AIDS in India.

Most common vascular tumor in AIDS patients is Kaposi's sarcoma.

M. tuberculosis: Most common infection with HIV in India.

Most common site for lymphoma in AIDS patients is CNS.

AIDS: Malignancy includes Kaposi sarcoma (most common), NHL cervical and anal cancer.

AIDS: Death is usually due to disseminated infection.

Vertical transmission: Commonest cause of AIDS in children.

Most common route for vertical transmission: Through infected birth canal during normal vaginal delivery.

Most common viral antigen used for diagnosis of HIV in blood before transfusion is *p24*.

p24 antigen is a product of gag gene of HIV.

HIV: Selective affinity for host cells with CD4 molecule receptor and includes:

- **$CD\ 4^+$ T-cells (worst affected)**
- **Monocytes/macrophages**
- **Dendritic cells**

Defective CCR5 receptors lead to protective effect of providing resistance to the development of AIDS.

Neutrophil is not a target for initiation and maintenance of HIV infection.

CNS in AIDS:

- Perivascular giant cell
- Vacuolization
- Microglial nodule

Weight loss in AIDS is so severe that it is also known as **"Slim's disease"**.

Anti-gp120: Detected by ELISA test.

AMYLOIDOSIS

CC: Describe the pathogenesis and pathology of amyloidosis.

Q. Define and classify amyloid. Describe the physical and chemical nature of amyloid. Enumerate the special stains for amyloid.

Q. Define and classify amyloid. Describe the gross and microscopic features of organs involved secondary amyloidosis.

Q. Describe the pathogenesis, morphology and staining characteristics of amyloid.

Q. Define amyloidosis.

Definition: Amyloid is a **pathologic fibrillar protein deposited in the extracellular space** in **various tissues and organs** of the body in variety of clinical condition.

General Features

- **Associated with** number of **inherited and inflammatory disorders**.
- **Extracellular deposits** cause structural and functional damage to involved tissue.

- **Basically a disorder of protein misfolding** and is produced by aggregation of misfolded proteins (normal folded proteins are soluble) or protein fragments.
- It also contains abundant charged sugar groups and has staining characteristics that were thought to resemble starch (amylose) and were called as amyloid. But these deposits are not related to starch.
- **Usually a systemic** (sometimes localized) disease.

Forms of Amyloid

All amyloid have same morphological and staining property but amyloidosis is **not a single disease**. It is a **group of diseases** having **in common the deposition of similar-appearing proteins** in which biochemical structure (more than 20 different proteins) and mechanism of formation are different.

Physical Nature of Amyloid

All types of amyloid are **composed of nonbranching fibrils of 7–10 nm diameter**.

- **Each fibril consists of β-pleated sheet polypeptide chains** and is wound around one another.
- **Congo-red dye** binds to these fibrils and produces **classic apple-green birefringence** (dichromism). Hence, Congo-red stain is used to identify amyloid deposits in tissues.

Chemical Nature of Amyloid

Q. Write short note on physical and chemical nature of amyloid.

Fibrillar proteins bind with variety of substances:

- **About 95%** of the amyloid material consists of **fibril proteins**.
- **Remaining 5%** consists of **proteoglycans**, **glycosaminoglycans**, **serum amyloid P, etc**.

Biochemical Forms of Amyloid

It consists of three major distinct proteins and more than 20 minor forms.

A. Major forms

These are **AL**, **AA** and **Aβ amyloid:**

1. **AL (amyloid light chain) protein:**
   - Consists of **complete immunoglobulin** (Ig) **light chains** or the **aminoterminal fragments of light chains**, **or both**.
   - **Produced by plasma cells** and associated with some monoclonal B-cell proliferation (e.g., plasma cell tumors).
2. **AA (amyloid-associated) protein:**
   - **Non-immunoglobulin**.
   - **Derived from** a larger precursor in the serum called **SAA (serum amyloid-associated)** protein **synthesized by the liver**. **Increased synthesis of SAA** protein occurs under the influence of cytokines (e.g., IL-6 and IL-1) **during inflammation**.
   - Associated with **chronic inflammation** (called as secondary amyloidosis).
3. **Aβ amyloid:**
   - **Derived from** transmembrane glycoprotein called **amyloid precursor protein (APP)**.
   - **Found in the cerebral lesions** of **Alzheimer disease**.

B. Minor types

1. **Transthyretin (TTR):**
   - **It is a normal serum protein** that transports thyroxine and retinol.
   - **Mutations** in gene encoding TTR → **alter its structure** → misfolds.
   - **Found in a familial amyloid polyneuropathies, heart of aged individuals** (senile systemic amyloidosis).
2. **β_2-microglobulin:**
   - It is a **normal serum protein**.
   - Amyloid fibril subunit namely **Aβ_2m is derived from β_2-microglobulin** and is found in amyloidosis of **patients on long-term hemodialysis**.
3. **Other minor types:** Serum amyloid P component, proteoglycans, and highly sulfated glycosaminoglycans.

Pathogenesis of Amyloidosis (Fig. 6.23)

Q. Write short note on pathogenesis of amyloidosis.

Misfolding of Proteins

Amyloidosis is a **disorder due to abnormal folding or misfolding of proteins**.

- **Normally, misfolded proteins are degraded either intracellularly** in proteasomes, or **extracellularly** by macrophages.
- **In amyloidosis**, there is **failure of control mechanism** → production of misfolded proteins, which exceeds the degradation → accumulation outside cells. These misfolded proteins are unstable and self-associated → deposited as fibrils in extracellular tissues.

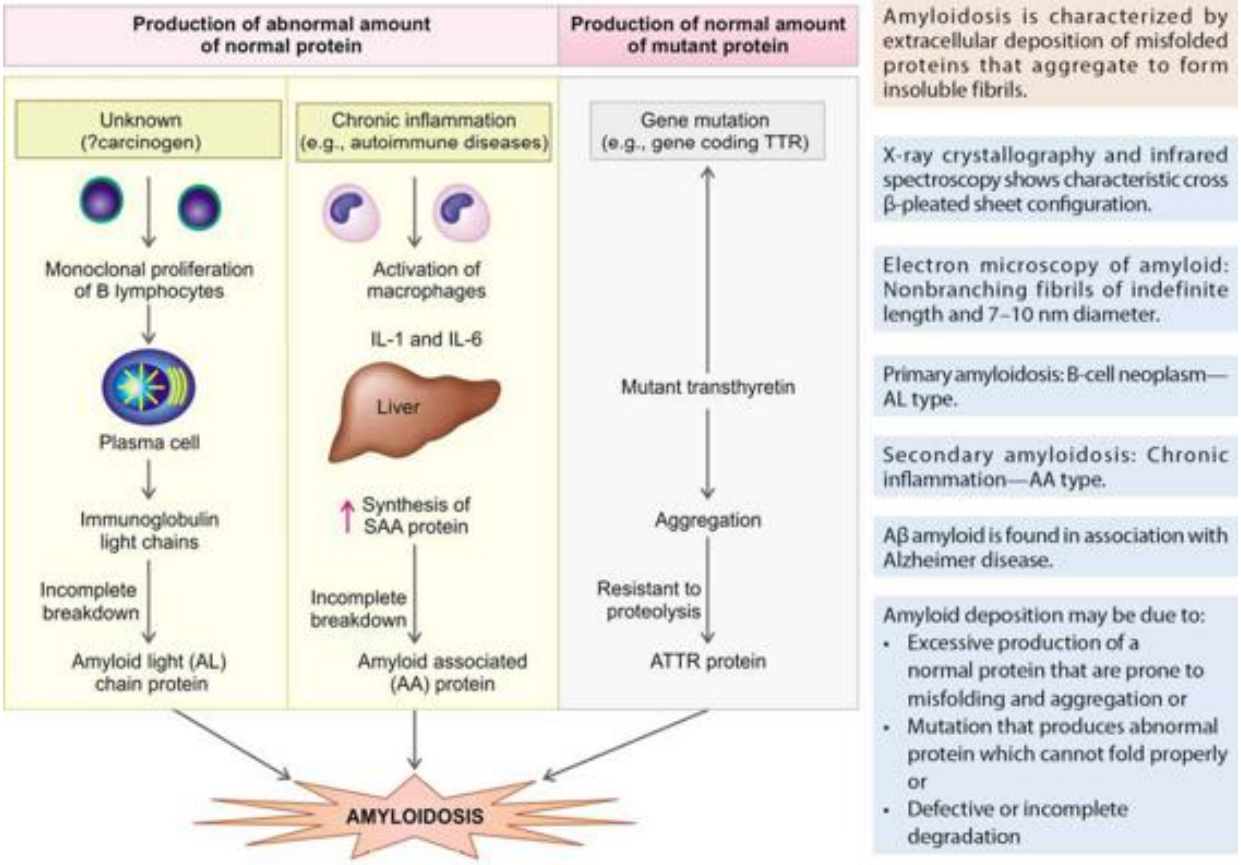


Fig. 6.23: Pathogenesis of amyloidosis. AL protein is seen in association with B lymphocyte and plasma cell proliferation which secrete immunoglobulin light chains that are amyloidogenic. AA protein is seen in variety of diseases associated with the activation of macrophages, which in turn, leads to the synthesis and release of SAA. The SAA is converted to AA protein. ATTR protein is due to mutant proteins which aggregate and deposit as amyloid.
(SAA: serum amyloid; ATTR: transthyretin).

Categories of Proteins

Misfolded proteins that form amyloid may be the result of:

Production of Abnormal Amounts of Normal Protein

- These proteins have an inherent tendency to fold improperly or undergo misfolding → associate and form fibrils. Example: During inflammation, SAA is synthesized by the liver cells under the influence of cytokines such as IL-6 and IL-1 secreted by activated macrophages, and is degraded by monocyte-derived enzymes.
- In individuals prone to amyloidosis, there may be defect in the monocyte-derived enzymes → incomplete breakdown of SAA → formation of insoluble AA molecules. Genetically defective SAA may also be responsible for resistant degradation by macrophages.

Production of Normal Amount of Mutant Protein

This is the protein that is prone to misfolding and subsequent aggregation to form amyloid.

Example: In familial amyloidosis, mutation of gene encoding TTR → alterations in structure of serum protein TTRs → proteins prone to misfolding → aggregate → are resistant to proteolysis.

Pathological Effects

- **Pressure on adjacent normal cells** → leads to **atrophy of cells**.
- **Deposition in the blood vessel wall** causes:
  - Narrowing of the lumen → lead to **ischemic damage**.
  - **Increased permeability** → escape of protein out of vessel.

Classification of Amyloidosis

Q. Describe the pathology of primary amyloidosis.

Q. Classify amyloidosis.

Amyloidosis is classified **depending on biochemical and clinical characteristics (Table 6.15)**.

Systemic (Generalized)

It involves several organ systems.

Q. Describe the pathology of primary amyloidosis.

Primary Amyloidosis

a. **Immunocyte dyscrasias with amyloidosis:** Usually **systemic** and is of **AL type**. **Many have underlying plasma cell dyscrasia**, e.g., **multiple myeloma**.
   - **Multiple myeloma:**
     - **5–15% of patients** develop amyloidosis.
     - **Tumor synthesize abnormal amounts of a single specific Ig** (monoclonal gammopathy) → appears as an **M (myeloma) protein spike on serum electrophoresis**.
     - Tumor **also synthesizes the light chains (known as Bence–Jones protein) of either the κ or the λ type** which are found in the serum.
     - **Bence–Jones protein** being of small molecular size **can be excreted in the urine**. The amyloid deposits in these patients contain the same light chain protein.
     - All the myeloma patients with amyloidosis invariably have Bence–Jones proteins in the serum or urine, or both. But majority of myeloma patients who have free light chains do not develop amyloidosis. This suggests that the presence of Bence–Jones proteins, though necessary, is by itself not enough for amyloidosis.

TABLE 6.15: Classification of amyloidosis.

Type	*Precursor protein*	*Fibril protein*	*Associated disease/s*
A. Systemic (generalized) amyloidosis			
1. Immunocyte dyscrasias with amyloidosis (primary amyloidosis)	Immunoglobulin light chains (mainly λ)	AL	Multiple myeloma, other plasma cell dyscrasias
2. Reactive systemic amyloidosis (secondary amyloidosis)	Serum amyloid associated (SAA)	AA	Chronic inflammatory process
3. Hemodialysis-associated amyloidosis	β_2-microglobulin	Aβ_2m	Chronic renal failure

TABLE 6.15: Classification of amyloidosis.			
Type	***Precursor protein***	***Fibril protein***	***Associated disease/s***
B. Hereditary or familial amyloidosis			
1. Familial Mediterranean fever	SAA	AA	
2. Familial amyloidotic neuropathies	Transthyretin	ATTR	
3. Systemic senile amyloidosis			
C. Localized amyloidosis			
1. Senile cerebral	Amyloid precursor protein (APP)	Aβ	Alzheimer disease
2. Endocrine			
◆ Thyroid	Calcitonin	A Cal	Medullary carcinoma
◆ Islets of Langerhans	Islet amyloid peptide	AIAPP	Type 2 diabetes

b. Primary amyloidosis without plasma cell dyscrasia:

- Majority of patients with AL amyloid do not have multiple myeloma or any other overt plasma cell neoplasm.
- But almost all these patients **have monoclonal immunoglobulins or free light chains, or both, in the serum or urine**.
- **Bone marrow** in most show **increase in the number of plasma cells**, which may secrete the precursors of AL protein. Thus, these may represent plasma cell dyscrasia characterized by production of an abnormal protein, instead of production of tumor masses.

Reactive Systemic (Secondary) Amyloidosis

Q. Write short note on reactive systemic amyloidosis.

- **Systemic in distribution** and is of **AA** type.
- Occurs as a **complication of** (secondary to) **chronic inflammatory** or **tissue-destructive process**. Hence, was known as **secondary amyloidosis**.
- **Complicates or occurs in association with diseases,** such as:

- **Chronic inflammatory conditions: Tuberculosis, bronchiectasis** and chronic osteomyelitis.
- **Autoimmune states: Rheumatoid arthritis**, ankylosing spondylitis, inflammatory bowel disease (Crohn's disease and ulcerative colitis).
- **Heroin abusers:** These patients develop chronic skin infections or abscesses due to subcutaneous self-administration of narcotics.
- **Nonimmunocyte-derived tumors:** Renal cell carcinoma and Hodgkin lymphoma.

Hemodialysis-associated Amyloidosis

Patients with **chronic renal failure on long-term hemodialysis** have high **levels of β_2-microglobulin** in the serum because it cannot be filtered through dialysis membranes → gets deposited as amyloid.

Hereditary or Familial Amyloidosis

Q. Write short note on heredofamilial amyloidosis.

It constitutes a heterogeneous group, are rare and occur in certain geographic areas.

Familial Mediterranean Fever

- **Autosomal recessive** disorder.
- Characterized by recurrent attacks of fever accompanied with inflammation of serosal surfaces (peritoneum, pleura and synovial membrane).
- The gene encodes a protein called **pyrin** (for its relation to fever), regulate inflammatory reactions by producing high levels of proinflammatory cytokines IL-1.
- The amyloid fibril proteins are of AA type probably produced due to recurrent bouts of inflammation.

Familial Amyloidotic Neuropathies

It is characterized by **deposition of amyloid in peripheral and autonomic nerves** and the **fibrils are made up of mutant TTRs**.

Localized Amyloidosis

Q. Write short note on localized amyloidosis.

- Amyloid deposits are **limited to a single organ** (e.g., **heart**) or tissue.
- Either grossly visible as nodular masses or detected only by microscopic examination.
- **Sites:** Lung, larynx, skin, urinary bladder and tongue.
- **Microscopy:** Amyloid deposits may be surrounded by lymphocytes and plasma cells.

Endocrine Amyloid

Q. Write short note on endocrine tumors showing microscopic deposits of amyloid.

- Endocrine tumors such as **medullary carcinoma of the thyroid** (*refer* pages 806-7 and **Fig. 24.14**), islet tumors of the pancreas, pheochromocytomas and undifferentiated carcinomas of the stomach.
- **Islets of Langerhans in type II diabetes mellitus**.

Amyloid of Aging

Q. Write briefly on amyloid of aging.

Senile systemic amyloidosis characterized by the systemic deposition of amyloid in elderly patients usually between 70 and 80 years. Also called senile cardiac amyloidosis because of the symptoms related to restrictive cardiomyopathy and arrhythmias. **The amyloid is derived from the normal TTR molecule**.

MORPHOLOGY

Main Organs Involved

- **Secondary amyloidosis:** Kidneys, liver, spleen, lymph nodes, adrenals and thyroid.
- **Primary amyloidosis:** Heart, GI tract, respiratory tract, peripheral nerves, skin and tongue.

Gross

- May or may not be apparent grossly.
- If large amount accumulates → affected organs are **enlarged, firm and have a waxy appearance (Fig. 6.24A).**

- **Cut surface:** If the amyloid deposits are large, **painting** the cut surface **with iodine** gives a **yellow color, which is transformed to blue violet after application of sulfuric acid** (which acidifies iodine). This method was used for demonstrating cellulose or starch. This staining property was responsible for the coining of the **term amyloid (starch-like). But it is neither starch nor cellulose**.

Microscopy

Q. Write short note on special stains for amyloid.

Hematoxylin and Eosin Stain

- Amyloid deposits are always **extracellular (Fig. 6.25A)** and begin between the cells. In AL form, perivascular and vascular deposits are common.
- Progressive accumulation of **amyloid produces pressure atrophy of adjacent cells**.
- Appears as an **amorphous, eosinophilic, hyaline, glassy, extracellular substance.**
- Many other substances (e.g., collagen, fibrin) also stain eosinophilic with hematoxylin and eosin. Hence, it is necessary to differentiate amyloid from these other hyaline deposits by using special stains.

Staining (Tinctorial) Properties of Amyloid

1. **Congo-red stain:** It is the **special stain used for the diagnosis of amyloidosis**. Amyloid **stains pink or red** with the Congo-red dye **under ordinary light (Fig. 6.25B)**. But **more specific** when viewed **under polarizing microscope**; amyloid gives **apple-green birefringence (Fig. 6.25C)**. This reaction is due to the cross-β-pleated configuration of amyloid fibrils. Can be confirmed by electron microscopy.
2. **Van Gieson stains:** It takes up khaki color.
3. **Alcian blue:** It imparts blue color to glycosaminoglycans in amyloid.
4. **Periodic acid-Schiff (PAS) reaction:** It stains pink.
5. **Methyl violet and cresyl violet:** These metachromatic stains (belong to rosanilin group of basic dyes) give rose pink color to the amyloid deposits whereas surrounding tissue is colored blue.
6. **Iodine solution** produces a dark, mahogany brown color when poured over the cut surface of the organ involved by amyloidosis. It is **transformed to blue violet after application of sulfuric acid**.
7. **Thioflavin T:** It is not specific for amyloid, but amyloid fluoresce when viewed in ultraviolet light.
8. **Immunohistochemical staining:** It can distinguish AA, AL and ATTR types.

MORPHOLOGY OF MAJOR ORGANS INVOLVED

Q. Describe the gross and microscopic features of organs involved in primary/secondary

amyloidosis.

Morphology of major organs involved in primary and/or secondary amyloiodosis is discussed below:

Kidney

Kidney involvement is the **most common** and the **most serious** form of organ involvement.

- **Gross (Fig. 6.24A):** It may be of normal size and color during early stages. In advanced stages, it may be shrunken due to ischemia, which is caused by vascular narrowing induced by
- **Microscopy:** Most commonly renal amyloid is of light-chain (AL in primary amyloidosis) or AA (in secondary amyloidosis type).
  - **Glomeruli:** It is the main site of amyloid deposition**(Fig. 6.24B).**
    - First, focal deposits within **mesangial matrix**, accompanied by diffuse or nodular thickening of the **glomerular basement membranes**.
    - Later, both the mesangial and basement membranes deposits cause **capillary narrowing**. Progressive accumulation of amyloid results in **obliteration of the capillary lumen** and glomerulus shows broad ribbons of amyloid.
- Amyloid may also be deposited in the **peritubular interstitial tissue**, **arteries** and **arterioles**. the amyloid deposit within arterial and arteriolar walls.

Spleen

Q. Describe the gross and microscopic appearance of spleen in amyloid (Sago and Lardaceous spleen).

- **Gross:** It may be normal in size or may cause moderate to marked splenomegaly (200–800 g). It may show one of **two patterns of deposition**.
  - **Sago spleen:** Amyloid **deposits are limited to the splenic follicles**, which **grossly appear like tapioca/sago granule**; hence known as **sago spleen**. Microscopically, the **amyloid is deposited in the wall of arterioles in the white pulp**.
  - **Lardaceous spleen: Amyloid is deposited in the walls of the splenic sinuses** and connective tissue framework in the red pulp. This may result in moderate to marked enlargement of spleen. Fusion of the early deposits give rise to large, map-like areas of reddish color on cut surface. This resembles pig fat (lardaceous) and hence called as **lardaceous spleen**. Microscopically, it shows amyloid deposits in the wall of the sinuses.
- **Light microscopy:** These deposits appear homogenous pink, which when stained with Congo-red and viewed under polarizing microscope, give rise to characteristic green birefringence.

Liver

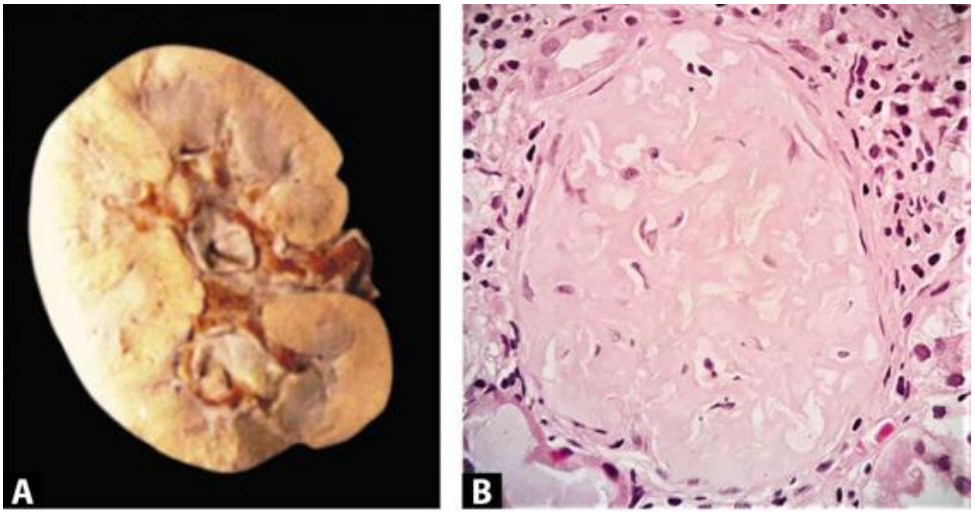
- **Gross:** It may cause moderate to marked enlargement. In advance stages, it appears pale, gray and waxy.
- **Microscopy**
  - Amyloid **first deposits in the space of Disse** and then progressively encroaches on adjacent hepatic parenchymal cells and sinusoids.
  - Progressive accumulation leads to deformity, pressure atrophy and disappearance of liver cells.

Heart

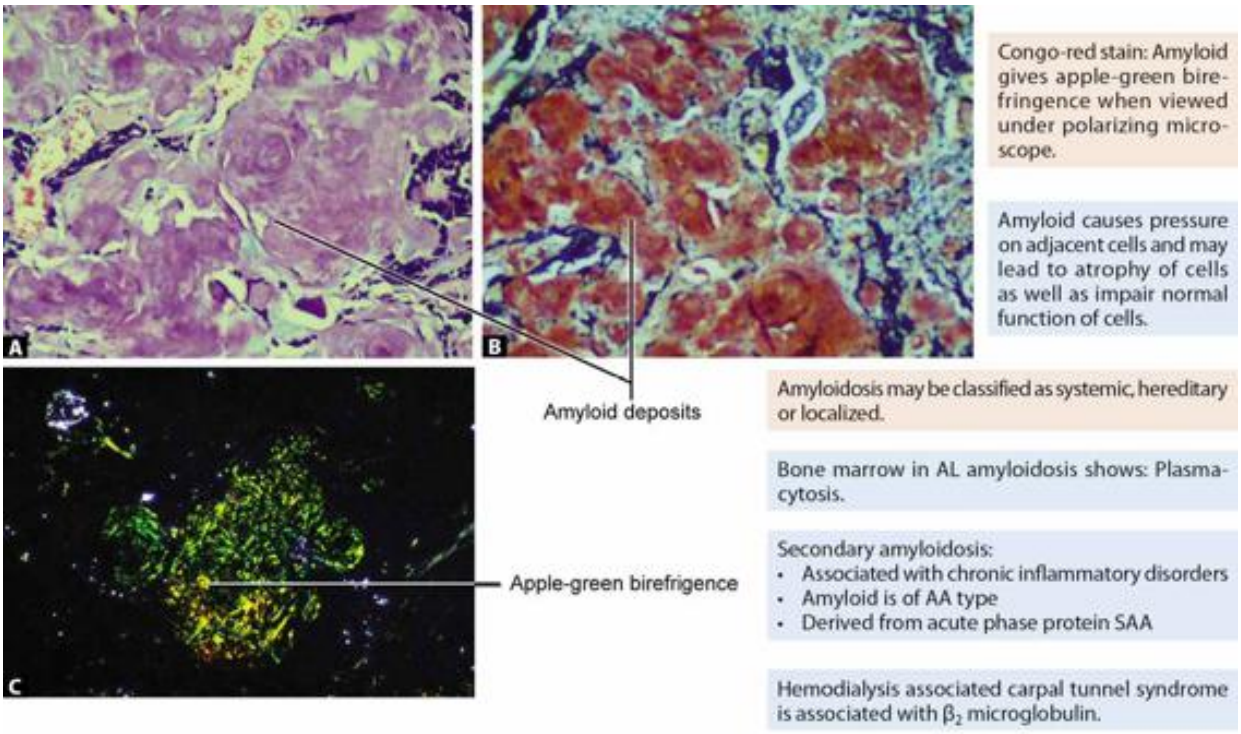
- It may be involved in **systemic amyloidosis (AL type)** or may be the major organ involved in **senile systemic amyloidosis**.
- **Gross:** Heart may be enlarged and firm. Subendocardial deposits may appear as gray-pink like dew-drop.
- **Microscopy**
  - **Myocardium:** Amyloid is deposited between the muscle fibers and their progressive accumulation causes pressure atrophy of myocardial fibers.

Other Organs

They may be involved in systemic disease and include adrenals, thyroid and pituitary. Nodular deposits in the tongue may cause **macroglossia**.



Figs. 6.24A and B: Amyloid kidney: (A) Cut section of a amyloid kidney showing waxy appearance; (B) Amyloidosis of kidney showing pink, amorphous extracellular amyloid deposits in the entire glomerulus.



Figs. 6.25A to C: Amyloid deposits in medullary carcinoma of thyroid: (A) Amyloid appear as extracellular, amorphous, eosinophilic substance under H and E stain; (B) Congo-red stain gives red color to the amyloid deposits; (C) Congo-red stain viewed under polarizing microscope gives apple-green birefringence to amyloid deposits.

Clinical Features

- Amyloidosis may not produce any clinical manifestations, or it may produce symptoms related to the sites or organs affected. Clinical manifestations **initially may be nonspecific** (e.g., weakness, weight loss). Specific symptoms appear later and are related to renal, cardiac and gastrointestinal involvement.
- **Renal involvement:** It gives rise to **proteinuria** sometimes massive enough to cause nephrotic syndrome. In advanced cases, the obliteration of glomeruli causes renal failure and uremia.
- **Cardiac amyloidosis:** It may present as **congestive heart failure**, conduction disturbances and arrhythmias.
- **Gastrointestinal amyloidosis:** It may be asymptomatic, or present as **malabsorption, diarrhea and digestive disturbances**. Amyloidosis of the tongue may hamper speech and swallowing.

Diagnosis

Q. Write short note on diagnosis of amyloidosis.

It depends on the **histologic demonstration of amyloid deposits** in tissues.

- **Biopsy:** The most common sites are the **kidney, rectum or gingival tissues** in systemic amyloidosis.
- **Examination of abdominal fat aspirates** stained with Congo-red is quite specific, but has low sensitivity.
- In immunocyte-associated amyloidosis, **serum and urine protein electrophoresis and immunoelectrophoresis** should be done. Bone marrow aspirates may show monoclonal plasmacytosis, even in the absence of multiple myeloma.
- **Scintigraphy with radiolabeled serum amyloid** P (SAP) component is a **rapid and specific test**.

Familial amyloidotic polyneuropathy is due to amyloidosis of nerves caused by deposition of: Mutant transthyretin (TTR).

Medullary carcinoma of thyroid:
- Calcitonin is the tumor marker
- Calcitonin form amyloid ACal

Sago spleen: Amyloid deposits in splenic follicles (white pulp).

Lardaceous spleen: Amyloid deposits in sinusoids of red pulp.

Amyloidosis: Renal failure is a common cause of death with renal involvement.

Prognosis
- Generalized amyloidosis: Poor and poorer in myeloma-associated amyloidosis
- Reactive systemic amyloidosis: Little better

CHAPTER 7

Neoplasia

INTRODUCTION

CC: Define and classify neoplasia. Describe the characteristics of neoplasia including gross, microscopy, biologic, behavior and spread. Differentiate between benign from malignant neoplasms.

Q. Define neoplasia.

Neoplasia literally means new growth, and a new growth formed is known as a neoplasm (Greek, *neo* = new + *plasma* = thing formed). The term "tumor" was originally used for the swelling caused by inflammation, but it is now used synonymously with neoplasm. Oncology (Greek, *oncos* = tumor) is the study of tumors or neoplasms.

Willis definition: "A neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change."

In the present era, a neoplasm can be defined as a disorder of cell growth which is triggered by a series of acquired mutations involving a single cell and its clonal progeny.

Salient Features of Neoplasia

- **Origin:** Neoplasms arise **from cells** that normally maintain a **proliferative capacity**.
- **Genetic disorder:** Cancer is due to **permanent genetic changes** in the cell, known as **mutations**. These mutations may occur in genes which regulate cell growth, apoptosis, or DNA repair.

- **Heritable:** The genetic alterations are passed down to the daughter tumor cells.
- **Monoclonal:** All the neoplastic cells within an individual **tumor originate from a single cell/or clone of cells that has undergone genetic change**. Thus, tumors are said to be monoclonal.
- **Carcinogenic stimulus:** The stimulus responsible for the uncontrolled cell proliferation **may not be identified or is not known**.
- **Autonomy:** In neoplasia, there is **excessive** and **unregulated proliferation** of cells that do not obey the normal regulatory control. The cell proliferation is **independent of physiologic growth stimuli**. But tumors are dependent on the host for their nutrition and blood supply.
- **Irreversible:** Neoplasm is irreversible and **persist even after the inciting stimulus is withdrawn or gone**.
- **Differentiation** (*refer* page 192-3): It refers to the **extent to which the tumor cells resemble the cell of origin**. A tumor may shows varying degrees of differentiation ranging from relatively mature structures that mimic normal tissues (well-differentiated) to cells so primitive that the cell of origin cannot be identified (poorly differentiated).

Oncology: Study of neoplasms.

Six **P**'s of neoplasm:
- **P**urposeless
- **P**rogressive
- **P**roliferation unregulated
- **P**reys on host
- **P**ersists even after withdrawal of stimulus (autonomous)
- **P**ermanent genetic change in the cell

CLASSIFICATION

Q. Define and classify neoplasia. Discuss the histogenesis and biological behavior of tumors.

Tumors are classified as **benign and malignant**, **depending on** the **biological behavior** of a tumor.

1. **Benign tumors:** They have relatively innocent microscopic and gross characteristics.
   - **Remain localized without invasion or metastasis**.

- **Well-differentiated:** Their cells **closely resemble their tissue of origin**.
- **Prognosis:** It is **very good**, can be cured by surgical removal in most of the patients and the patient generally survives.

2. **Malignant tumors: Cancer is the general term** used for malignant tumors. The term "cancer" is derived from the Latin word for *crab*, because similar to a crab, malignant tumors adhere to any part that they seize on, in an obstinate manner.
   - **Invasion:** Malignant tumors invade **or infiltrate into the adjacent tissues or structures**.
   - **Metastasis:** Cancers **spread to distant sites** (metastasize), where the malignant cells reside, grow and again invade.
     - **Exception: Basal cell carcinoma** of the skin, which is histologically malignant (i.e., it invades aggressively), but rarely metastasize to distant sites. **Glioma** is malignant tumor of CNS.
   - **Prognosis:** Most malignant tumors cause death.

Microscopic Components of Neoplasms

Tumors (both benign and malignant) consist of two basic components:

1. **Parenchyma:** It is made up of neoplastic cells. The **nomenclature and biological behavior of tumors** are **based primarily** on the **parenchymal component** of tumor.
2. **Stroma:** It is the **supporting, non-neoplastic tissue** derived **from the host**.
   - **Components: Connective tissue, blood vessels and inflammatory cells** (e.g., macrophages and lymphocytes).
   - **Inflammatory reaction:** Stroma may show inflammatory reaction in and around the tumors. It may be due to ulceration and secondary infection in the tumors especially in the surface of the body. This type of inflammatory reaction **may be acute, chronic or rarely granulomatous reaction**. Some tumors show inflammatory reaction even in the absence of ulceration. It is due to **cell-mediated immunologic response of the host against the tumor** as an attempt to destroy the tumor. For example, **lymphocytes in the stroma is seen in seminoma testis and medullary carcinoma of the breast**.
   - **Importance of stroma:** It is required for growth, survival and replication of tumor (through blood supply) cells.

- **Tumor consistency depends on amount of stroma:**
  - **Soft and fleshy:** These tumors have **scanty stroma**.
  - **Desmoplasia (Fig. 7.1 and *refer* Fig. 23.8): Parenchymal tumor cells may stimulate the formation of an abundant collagenous stroma** → referred to as desmoplasia. For example, some carcinoma in female breast have **stony hard consistency** (or **scirrhous**).

Malignant tumors:
- Invasion/infiltration
- Metastasis

Almost all cancers can metastasize, *except*:
- Basal cell carcinoma of skin
- Glioma of CNS

Neoplasms: Consists of neoplastic **parenchymal elements and non-neoplastic stroma.**

Desmoplasia seen in:
- Some carcinomas (e.g., scirrhous) of female breast
- Cholangiocarcinoma
- Pancreatic cancer some adenocarcinoma of colon
- Linitis plastica (diffuse type of carcinoma of stomach)

NOMENCLATURE OF NEOPLASMS

Q. Discuss the nomenclature and classification of tumors.

Q. Write short note on histogenesis of tumors.

Benign Tumors

They are generally **named by attaching the suffix "oma" to the cell of origin**.

Mesenchymal Tumors

They usually follow the below nomenclature **(Table 7.1)**.

Epithelial Tumors (Figs. 7.2A to D)

Their nomenclature is **not uniform but more complex**. They are classified in different ways:

1. **Cells of origin**
2. **Microscopic pattern**
3. **Macroscopic architecture.**

- **Adenoma:** It is a **benign epithelial tumor arising from glandular epithelium**, although they **may or may not form glandular structures**. **Examples:**
  - **Adrenocortical adenoma:** It shows heterogeneous mass of adrenal cortical cells growing as a solid sheet without any glands. Termed adenoma because the cell of origin is glandular epithelium.
  - **Follicular adenoma of thyroid:** It usually shows microscopically numerous tightly packed small glands **(Figs. 7.2A and** *refer* **24.9)**.

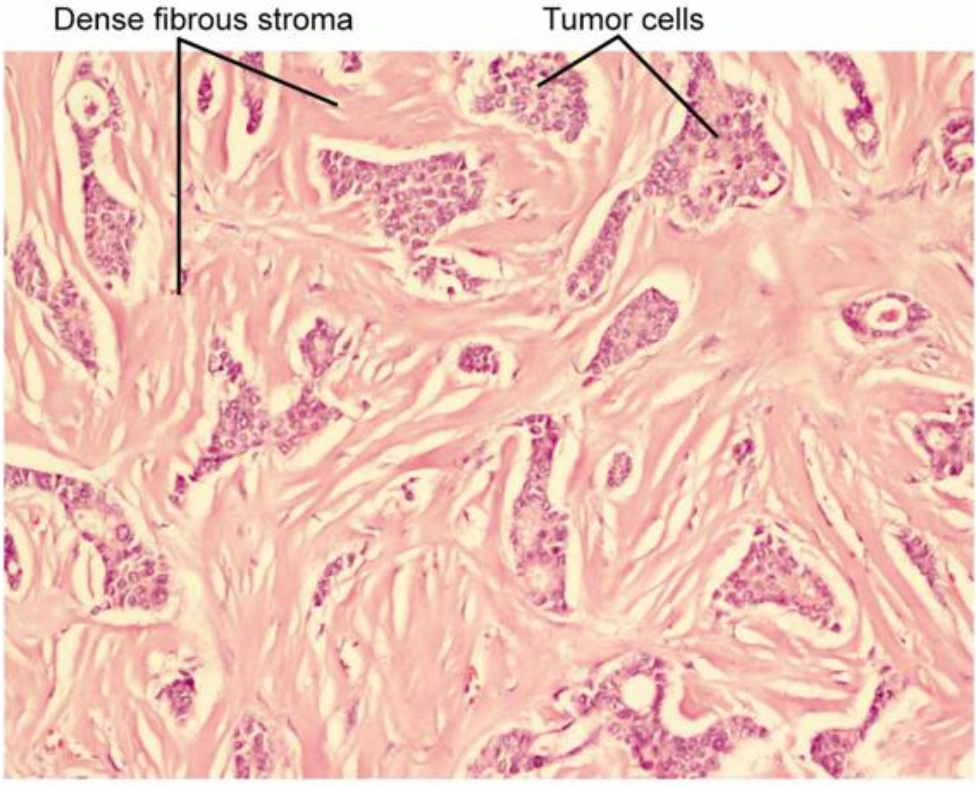
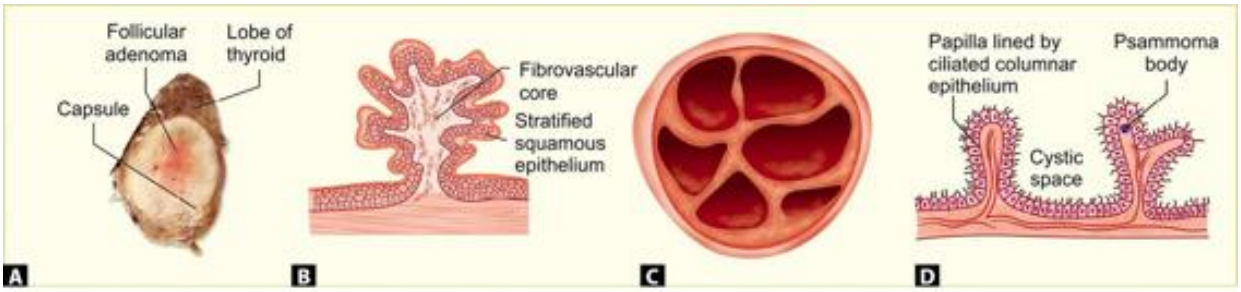


Fig. 7.1: Carcinoma of breast with abundant stroma separating malignant cells.

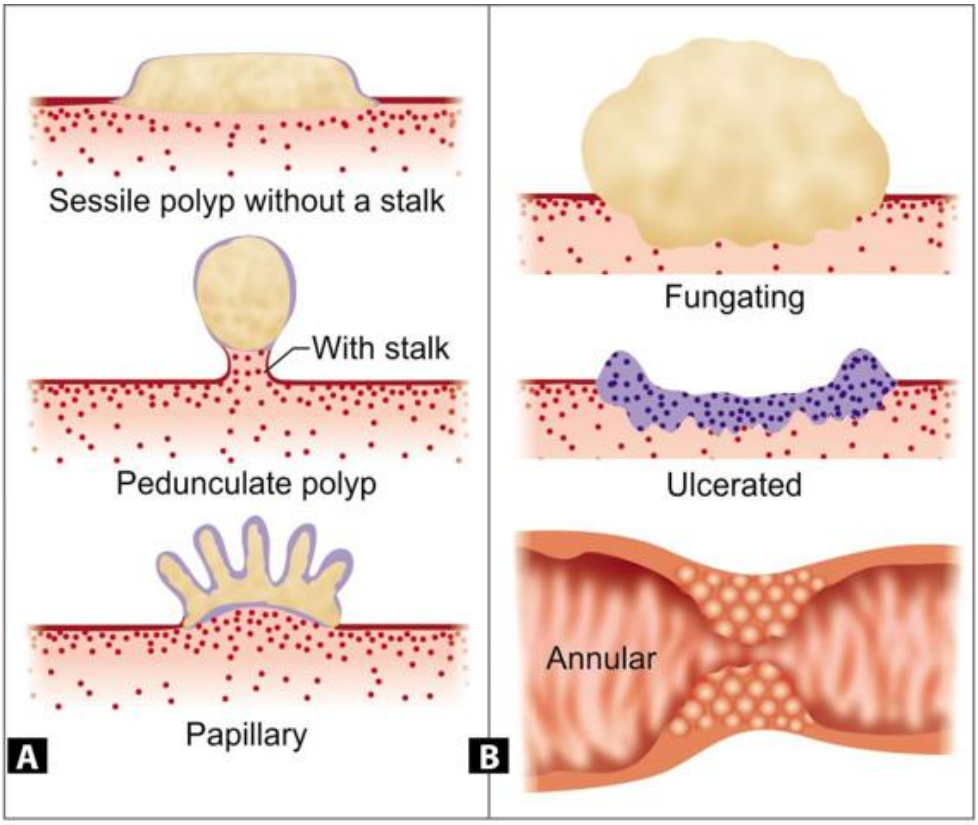
TABLE 7.1: Nomenclature of few benign and malignant mesenchymal tumors.

TABLE 7.1: Nomenclature of few benign and malignant mesenchymal tumors.

Cell of origin	*Benign*	*Malignant*
Fibrous tissue	Fibroma	Fibrosarcoma
Fat cell	Lipoma	Liposarcoma
Blood vessel	Hemangioma	Angiosarcoma
Cartilage	Chondroma	Chondrosarcoma
Bone	Osteoma	Osteogenic sarcoma
Smooth muscle	Leiomyoma	Leiomyosarcoma
Skeletal muscle	Rhabdomyoma	Rhabdomyosarcoma



Figs. 7.2A to D: Morphological (gross/microscopic) appearance of some benign epithelial tumors. (A) Gross appearance of follicular adenoma of thyroid; (B) Squamous papilloma composed of finger-like projections; (C) Cut section showing cystadenoma (e.g., mucinous cystadenoma of ovary); (D) Microscopy of papillary cystadenoma composed of papillae projecting into the cystic cavity.



Figs. 7.3A and B: Gross types of tumors: (A) Usually benign growth pattern; (B) Likely to be malignant growth pattern.

- **Adenomatous polyp of the colon:** They are named because of gross appearance as a polypoidal lesion, which projects above a surface **(***refer* **Figs. 18.40 and 18.41)**.

- **Papilloma:** It is a **benign epithelial neoplasm** that produces microscopically or macroscopically **visible finger-like, exophytic or warty projections** from epithelial surfaces, e.g., squamous papilloma **(Figs. 7.2B and 7.23)**.
- **Cystadenoma:** It is a tumor forming large cystic masses, e.g., serous cystadenoma of ovary **(Fig. 7.2C)**.
- **Papillary cystadenoma:** It is a tumor which consists of papillary structures that project into cystic spaces, e.g., papillary serous cystadenoma of ovary **(Figs. 7.2D and** *refer* **22.19 and 22.21)**.

Polyp (Fig. 7.3A): It is a neoplasm that grossly produces **visible projection above a mucosal surface and projects into the lumen**. It may be either **benign or malignant**. It may have a stalk (**pedunculated** polyp) or may be without a stalk (**sessile** polyp), e.g., polyp of stomach or intestine.

Borderline tumors: The term borderline tumor is used mainly for surface tumors of ovary (e.g., borderline serous or mucinous or endometrioid tumors). These tumors have **behavior intermediate between benign and malignant tumors**.

Malignant Tumors

They are termed as carcinoma or sarcoma depending on the parenchymal cell of origin. The likely malignant macroscopic architecture are shown in **Figure 7.3B**.

Sarcomas

They are **malignant tumors arising in mesenchymal tissue**. These tumors have little connective tissue stroma **(***refer* **Fig. 7.8B)** and are fleshy (Greek, *sar* = **fleshy**), e.g., fibrosarcoma, liposarcoma, osteosarcoma, chondrosarcoma, leiomyosarcoma and rhabdomyosarcoma.

Carcinomas

They are **malignant neoplasms arising from epithelial cell**, which may be derived from any of the three germ layers **(Table 7.2)**.

1. **Undifferentiated malignant tumor:** It is a malignant tumor composed of undifferentiated cells, where the **cell of origin cannot be made out on light microscopic examination**.
2. **Carcinosarcoma:** It is a rare malignant tumor which shows **mixture of carcinomatous and sarcomatous elements.**
3. **Inappropriate terminology for malignant tumor(Table 7.3):** In certain malignant tumors, the terms suffix “oma” is inappropriately used and **sounds like a benign tumor**.

Eponymously Named Tumors

These tumors are named after the person (eponymous) who first described or recognized the tumor **(Table 7.4)**.

Other Tumors

Mixed Tumors (Fig. 7.4)

They are **derived from a single germ layer** but show **divergent differentiation** along two lineages, e.g., **mixed tumor of salivary gland** (pleomorphic adenoma) is derived from a single clone (either myoepithelial or ductal reserve cell) and giving rise to two components, namely epithelial and myoepithelial (stromal elements) cells (*refer* pages 542-3).

Teratomas

Q. Write short note on teratoma.

They are special types of mixed tumors **derived from totipotent germ cells** (normally present in **ovary, testis** and sometimes abnormally present in sequestered embryonic rest in midline). These cells have the capacity to **differentiate into any of the cell types** found in the adult body. Thus, teratoma contains recognizable **mature or immature cells** or tissues representative of more than one germ cell layer and sometimes all three. These cells or tissues are arranged in a helter-skelter fashion. The **tissue derivative from various germ cell layers** may include:

1. **Ectoderm** (e.g., skin, neural tissue, glia)
2. **Mesoderm** (e.g., smooth muscle, cartilage, bone, fat)
3. **Endoderm** (e.g., respiratory tract epithelium, gut, thyroid).

Classification of Teratoma

- **Benign/mature teratoma:** It consists of **all mature and well-differentiated tissue**, e.g., ovarian cystic teratoma (dermoid cyst), in which differentiation is mainly along ectodermal lines → produces a cystic tumor lined by skin with adnexal structure (hair, sebaceous glands) and tooth structures **(***refer* **Figs. 21.12 and 22.25)**.
- **Immature/malignant teratoma:** It consists of **immature or less well-differentiated tissue**.
- **Monodermal teratoma and somatic-type tumors arising from dermoid cyst,** e.g., struma ovarii **(***refer* **Fig 22.26)** and carcinoid developing in ovary.
  - **Teratoma with malignant transformation:** It is the **development of malignant non-germ cell tumors** from one or more germ cell layer

in a teratoma, e.g., squamous cell carcinoma developing in a teratoma of testis.

Hamartomas

TABLE 7.2: Nomenclature of carcinomas.

Germ layer	*Tissue/cell*	*Malignant tumor*
Ectoderm	Epidermis	Squamous cell **carcinoma**
Mesoderm	Renal tubules	Adeno**carcinoma**
Endoderm	Lining of the gastrointestinal tract	Adeno**carcinoma**

TABLE 7.3: List of malignant tumors with suffix "oma".

Inappropriate terminology for malignant tumor	*Site*
Hepatoma	Liver
Melanoma	Skin
Seminoma/dysgerminoma	Testis/ovary
Lymphoma	Lymph nodes and extranodal lymphoid tissue
Mesothelioma	Pleura, peritoneum

TABLE 7.4: Examples of some eponymously named tumors.

Eponymously named tumors	*Cell or tissue of origin*
Burkitt lymphoma	B-cell lymphoma
Ewing sarcoma	Neuroectodermal origin arises in the bone
Grawitz tumor	Renal cell carcinoma arising from renal tubular epithelium
Kaposi's sarcoma	Malignant neoplasm of vascular endothelium
Hodgkin lymphoma	Malignant tumor of post-germinal B-cells
Brenner tumor	Benign tumor arising from surface epithelium of ovary

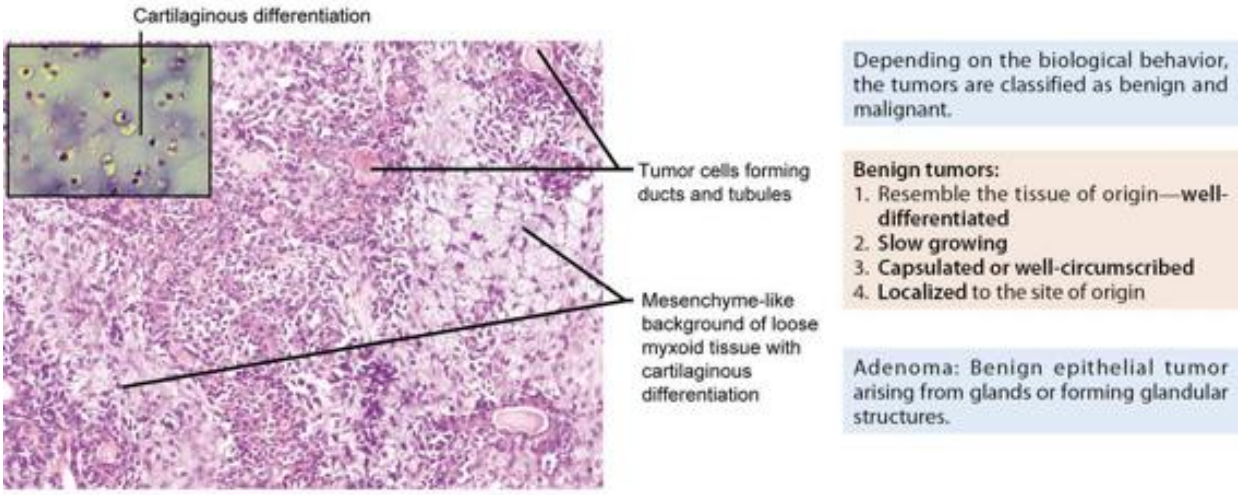


Fig. 7.4: Pleomorphic adenoma showing epithelial cells and myoepithelial cells separated by chondroid matrix. Inset shows cartilage.

Q. Write short note on hamartoma.

Definition: It is a **disorganized mass of benign-appearing cells, indigenous to the** particular **site**.

- Example: Pulmonary chondroid hamartoma consists of islands of disorganized, but histologically normal cartilage, bronchi and vessels (*refer* page 255).

Choristoma

Q. Write short note on choristoma.

Definition: It is an **ectopic island of normal tissue—heterotopic** rest (normal tissue in an abnormal site) and is a congenital anomaly.

- Example: Presence of small nodular mass of normally organized pancreatic tissue in the submucosa of the stomach, duodenum, or small intestine (*refer* page 255).

Embryonal Tumors (Blastomas)

They are the **type of tumors that develop only in children** (usually below 5 years of age), and **microscopically resemble embryonic tissue** of the organ in which they arise **(Table 7.5)**.

Nomenclature of the more common forms of neoplasia is listed in **Table 7.6**.

Papilloma: Benign tumor with visible finger-like projections.

Polyp: Tumor produces visible projection above mucosal surface and protrudes into the lumen.
Polyp: May be benign or malignant.

Sarcoma: Malignant tumor derived from **mesenchymal tissue**.

Carcinoma: Derived from squamous, transitional or glandular (adenocarcinoma) epithelium.

Tumors of the hematopoietic system are indicated by the suffix "emia", e.g., leukemia—malignant proliferation of leukocytes. Exceptions: Anemia is not a neoplasm.

Malignant tumors:
- **Well-differentiated to poorly differentiated**
- **Grow faster**
- **Poorly circumscribed**
- **Invade the surrounding tissue**
- **Metastasize to distant sites.**

Teratoma: Derived from totipotent cells and contains tissues derived from ectoderm, endoderm and mesoderm.

Sites of teratoma:
- Gonads
  - Ovary
  - Testis
- Extragonadal, e.g., mediastinum.

Hamartoma: Benign-appearing, non-neoplastic overgrowth of tissue.

Choristoma: Normal tissue in an abnormal site.

TABLE 7.5: Different types of embryonal tumors and their site.

Type of embryonal tumor	***Site***
Retinoblastoma	Eye
Nephroblastoma or Wilms' tumor	Kidney
Neuroblastoma	Adrenal medulla or nerve ganglia
Hepatoblastoma	Liver

TABLE 7.6: Nomenclature of common tumors.

Tissue of origin	***Benign***	***Malignant***
Composed of single parenchymal cell type		
Tumors of mesenchymal origin		
Connective tissue and	Fibroma	Fibrosarcoma

TABLE 7.6: Nomenclature of common tumors.

Tissue of origin	*Benign*	*Malignant*
derivatives	Lipoma	Liposarcoma
	Chondroma	Chondrosarcoma
	Osteoma	Osteogenic sarcoma
Vessels and surface coverings		
Blood vessels	Hemangioma	Angiosarcoma
Brain coverings	Meningioma	Invasive meningioma
Nerve sheath	Neurofibroma, neurilemmoma	Malignant peripheral nerve sheath tumor
Blood cells and related cells		
Hematopoietic cells		Leukemia
Lymphoid tissue		Lymphoma
Muscle		
• Smooth muscle	Leiomyoma	Leiomyosarcoma
• Striated muscle	Rhabdomyoma	Rhabdomyosarcoma
Tumors of epithelial origin		
Stratified squamous	Squamous cell papilloma	Squamous cell carcinoma
Basal cells of skin or adnexa		Basal cell carcinoma
Epithelial lining of glands or ducts or organs	Adenoma	Adenocarcinoma
	Papilloma	Papillary carcinoma
	Cystadenoma	Cystadenocarcinoma
	Papillary cystadenoma	Papillary cystadenocarcinoma
Urinary tract epithelium (transitional)	Transitional-cell papilloma	Transitional-cell carcinoma
Tumors of melanocyte	Nevus	Malignant melanoma
More than one neoplastic cell type—mixed tumors, derived from one germ cell layer		
Salivary glands	Pleomorphic adenoma (mixed tumor) of salivary origin	Malignant mixed tumor of salivary gland origin

TABLE 7.6: Nomenclature of common tumors.		
Tissue of origin	***Benign***	***Malignant***
More than one neoplastic cell type derived from more than one germ cell layer		
Totipotential cells in gonads or in embryonic rests	Mature teratoma, dermoid cyst	Immature teratoma

Most of the malignant tumors kill, whereas benign tumors are usually not fatal.

CHARACTERISTICS OF BENIGN AND MALIGNANT NEOPLASMS

CC: Differentiate between benign from malignant neoplasm.

Q. Define neoplasia. Describe the characteristics of malignant tumors.

Q. Discuss the differences between benign and malignant tumors.

It is **very important to differentiate** benign from malignant tumors mainly because of the different prognostic outcome. In general, benign and malignant tumors can be distinguished on the basis of four fundamental features, namely: **(1) Differentiation and anaplasia, (2) rates of growth, (3) local invasion, and (4) metastasis**.

Differentiation and Anaplasia

Differentiation

Defined as the **extent to which neoplastic parenchymal cells resemble** the corresponding **normal parenchymal cells**. This includes both morphological and functional differentiation. Differentiation **determines the grade of the tumor**.

Benign Tumors

- **Well-differentiated:** The neoplastic cell **closely resembles the normal cell of origin**. It may be not possible to recognize it as a tumor by microscopic examination of individual cells (e.g., lipoma). Only the growth of these cells into discrete lobules discloses the neoplastic nature of the lesion.
- **Mitoses:** They are **rare** and of normal configuration.

Malignant Neoplasms

- Show a **wide range of differentiation** of parenchymal cells.
- Varies from **well-differentiated to completely undifferentiated**.
- Cancers are usually graded either as well, moderately or poorly differentiated or numerically often by strict criteria as grade 1, grade 2 or grade 3.
- **Well-differentiated** tumors:
  - Well-differentiated adenocarcinomas of colon may form normal-appearing glands **(Fig. 7.5)**.
  - Squamous cell carcinomas may show cells which appear similar to normal squamous epithelial cells **(Fig. 7.6)**.
- **Poorly differentiated tumors:** They consist of **cells** that have **little resemblance to the cell of origin**.
- **Moderately differentiated:** These tumors show differentiation in between the well and poorly differentiated tumors.

Anaplasia

Q. Write short note on anaplasia.

- Anaplasia **literally means "to form backward/backward formation"**, i.e., **reversal of differentiation of cell to a more primitive level**.
- Malignant neoplasms composed of undifferentiated cells are called as anaplastic tumors.
- **Lack of differentiation** (both structural and functional) **is called as anaplasia** and is **characteristic of malignancy**.
- The degree of anaplasia in a cancer cell correlates with the aggressiveness of the tumor.

- Thus, more anaplastic the tumor, the more aggressive it becomes.

Microscopic features of anaplasia (Figs. 7.7 and 7.8):

Q. Write short note on morphology of malignant cells.

- ***Pleomorphism:*** It is defined as **variation in the size and shape of cells and cell nuclei**. It is a feature of malignancy. Thus, cells within the same malignant tumor range from large cells (many times larger than the neighbor cells), to extremely small (primitive appearing) cells.
- ***Abnormal nuclear morphology:***
  - **Extremely hyperchromatic nuclei** of tumor cells are **due to abundant chromatin** and **increased amount of DNA per cell** compared to that of a normal cell. Microscopically, these **nuclei stain darkly (hyperchromatic nuclei**).
  - **Nuclear shape and size is variable** and may be irregular. **Chromatin is coarsely clumped** and distributed along the nuclear membrane. **Large prominent nucleoli** are usually seen.
  - **Mitoses:** Presence of mitotic figures **indicates the higher proliferative activity** of the parenchymal cells.
    - **Number of mitotic figures:** Compared to benign and few well-differentiated malignant tumors, undifferentiated tumors usually show abundant (many) mitotic figures **(Figs. 7.8A and B)**.

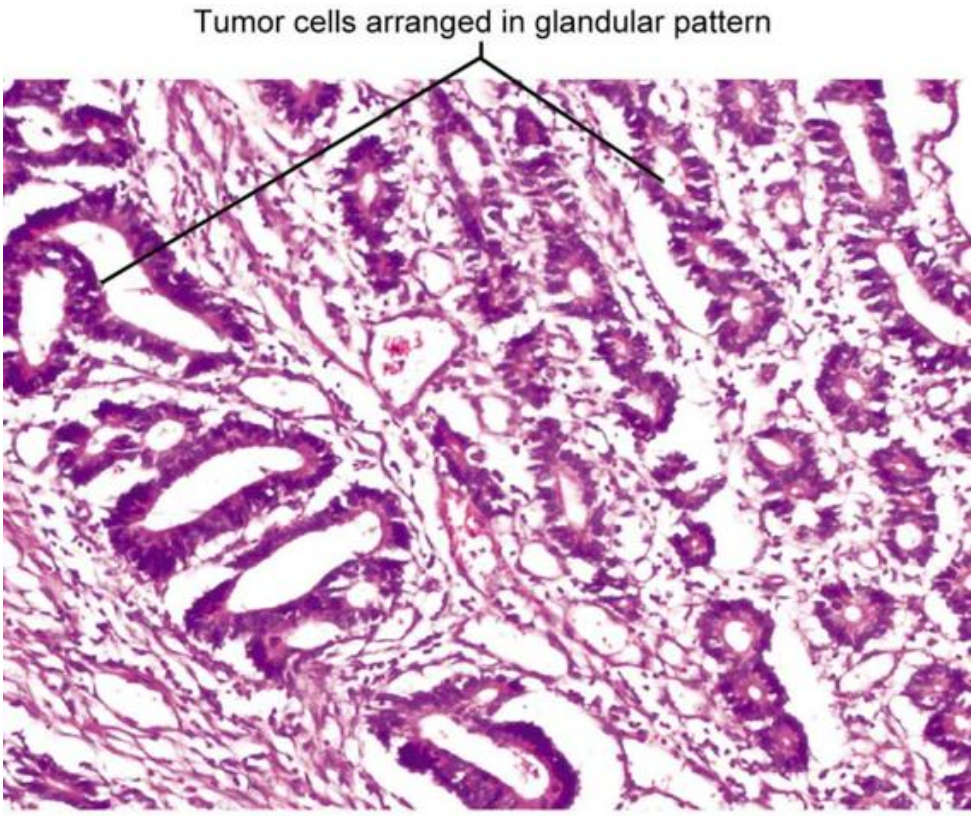


Fig. 7.5: Well-differentiated adenocarcinoma of the colon. It shows cancerous glands that are irregular in shape and size invading the muscular layer of the colon.

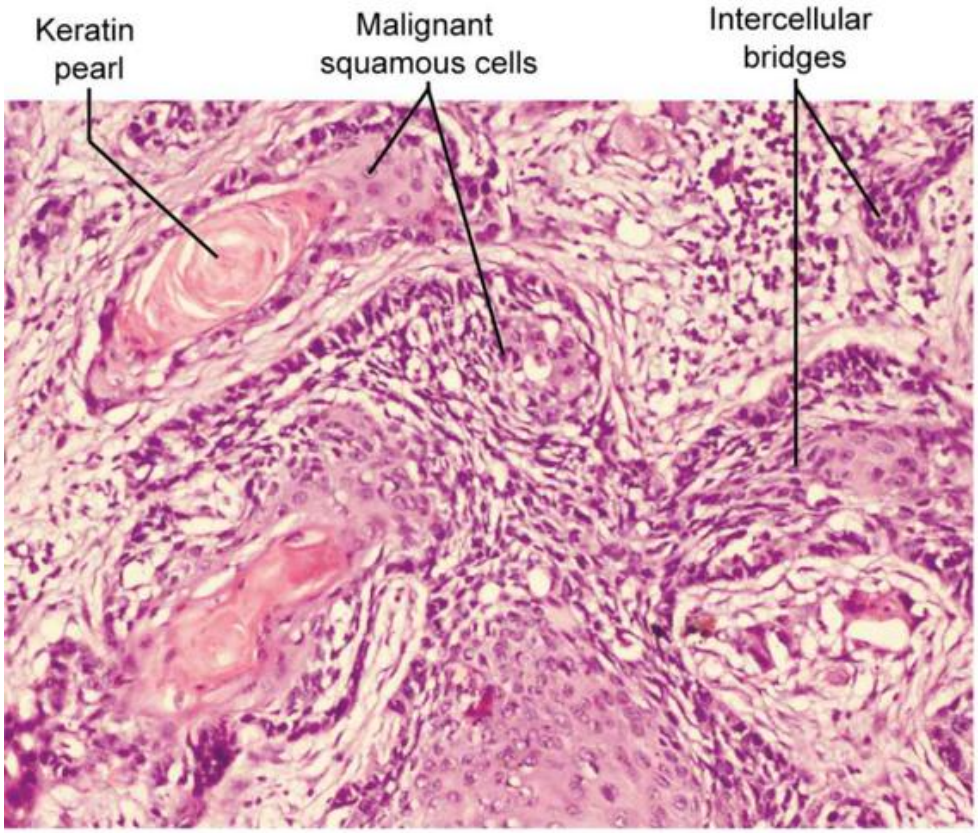
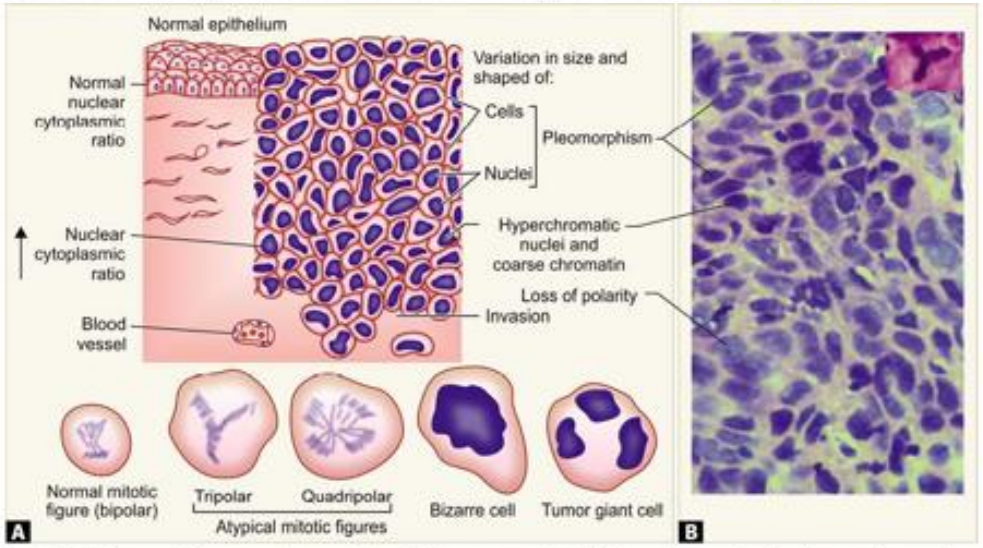
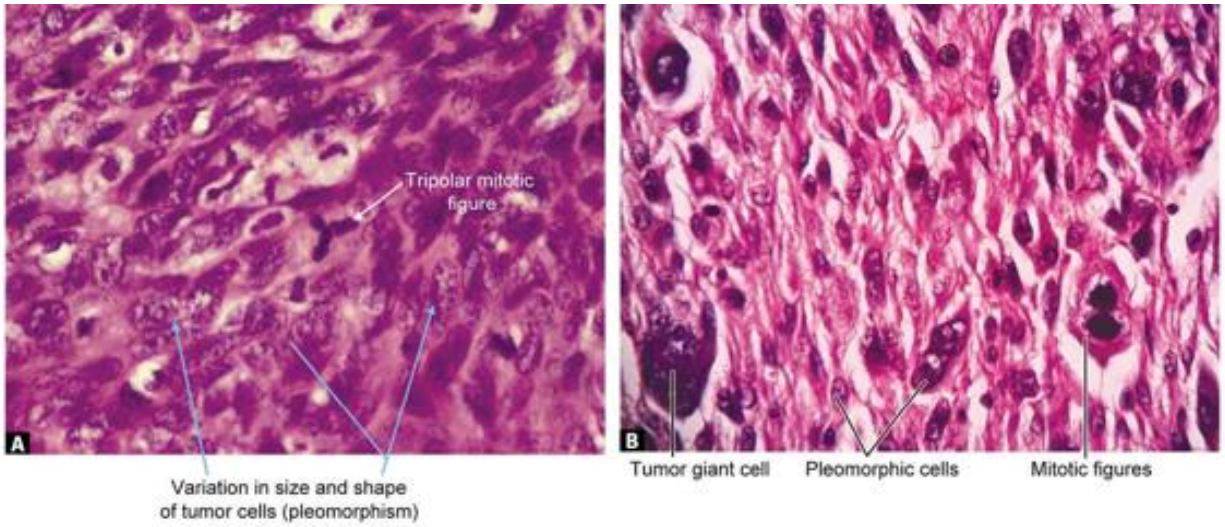


Fig. 7.6: Well-differentiated squamous cell carcinoma of the skin. The tumor consists of cells which are similar to normal squamous epithelial cells, with intercellular bridges and keratin pearls.



Figs. 7.7A and B: Microscopic features of anaplasia. (A) Diagrammatic; (B) Photomicrograph showing nuclear and cytoplasmic pleomorphism, hyperchromatic nuclei, high nuclear cytoplasmic ratio and loss of polarity. Inset of B shows tripolar mitotic figure.



Figs. 7.8A and B: (A) Poorly differentiated carcinoma consisting of tumor cells showing variation in size of cells and nuclei. One tumor cell in the center show an abnormal tripolar spindle; (B) Sarcoma showing cellular and nuclear pleomorphism, tumor giant cells and mitotic figure.

- **Atypical (abnormal) mitotic figures (Figs. 7.8A and B):** Normal mitosis produces bipolar spindles, and one cell divides into two. When the mitotic spindles are more than two, it is called as atypical. Presence of atypical bizarre mitotic figures is an important morphological feature of malignancy **(Figs. 7.8A and B)**.

- **Nuclear cytoplasmic (N:C) ratio*:*** In a normal cell, N:C ratio is 1:4 or 1:6. In a malignant cell, the nuclei are enlarged, become disproportionately large for the cell, and the nuclear-to-cytoplasm ratio may be **increased (Figs. 7.8A and B)** and may reach even up to 1:1.
- **Loss of polarity: Orientation of cells to one another** is known as polarity. The anaplastic cells lose the normal polarity → markedly disturbed orientation (architecture) of tumor cells.
- **Growth pattern:** Malignant neoplasms usually show disorganized growth. The tumor cells may form sheets of cells, arranged around blood vessels, papillary structures, whorls, rosettes, etc. Malignant tumors often show central ischemic necrosis due to compromised blood supply.
- **Bizarre cells**, **including tumor giant cells*:*** Some tumors may show **bizarre cells with a single large polymorphic nucleus** and others having **two or more large, hyperchromatic nuclei (Figs. 7.8A and B)**.
- **Necrosis and apoptosis:** Many rapidly growing malignant tumors undergo large central areas of ischemic necrosis and/apoptosis.

Functional Changes

Well-differentiated tumors usually retain the functional characteristics. Function may be in the form of secretion and vary depending on the tumor type.

- **Secretion of normal substances:**
  - **Hormones:** Benign tumors and **well-differentiated carcinomas of endocrine glands frequently secrete the hormones** characteristic of their cell of origin (e.g., steroid hormones from an adrenocortical adenoma).
  - **Normal product**, e.g., **well-differentiated squamous cell carcinomas** produce keratin → form characteristic **epithelial pearls (***refer* **Fig. 7.6)**.
- **Fetal proteins:** Some tumors may secrete fetal proteins, **which are not produced by comparable normal cells in the adult**, e.g., carcinoembryonic antigen (CEA) by adenocarcinomas of the gastrointestinal tract.
- **Ectopic hormones:** Tumors may produce **substances** which are **not indigenous to the tissue of origin** (*refer* pages 235-6), e.g., bronchogenic carcinomas may produce ACTH, parathyroid-like hormone, etc.

Differences between carcinoma and sarcoma **(Table 7.7)**.

Rates of Growth

Q. Write short note on rate of growth of tumors.

Factors Determining the Rate of Growth

- **Degree of differentiation:**
  - **Benign tumors** are well-differentiated and **usually grow slowly.**
  - **Most malignant tumors grow more rapidly.**
- **Dependency:** Growth also depends on:
  - **Hormonal stimulation**, e.g., uterine leiomyomas may suddenly grow during pregnancy and may undergo atrophy after menopause.
  - **Adequacy of blood supply.**
- **Balance between cell production and cell loss:** This in turn is determined by three main factors:

- **Doubling time of tumor cells:** It is the **time required for the total cell cycle**, i.e., cell to double by mitosis.

Q. Write short note on differences between carcinoma and sarcoma.

TABLE 7.7: Differences between carcinoma and sarcoma.

Features	***Carcinoma***	***Sarcoma***
Definition	Malignant tumor of epithelial origin	Malignant tumor of mesenchymal origin
Meaning of the term	"Carcinoma" came from the Greek word "karkinos" which means crab and "oma" which means growth	"Sarcoma" came from the Greek word "sarc" meaning flesh and "oma" which means growth
Site of origin	Mostly from inside lining of colon, breast and lung or prostrate	Arise from musculoskeletal system, such as bones, muscle and connective tissues
Incidence	More common cancer (more than 90% of cancers)	Less common (less than 1%)
Age	More common in middle and old age	Can occur at any age
Rate of growth	Usually not very rapid	Usually rapid
Route of spread	Initially lymphatics and later hematogenous	• Spread by satellite nodules • Usually hematogenous and lymphatic spread is rare
Site of metastasis through blood	Liver, lung, brain, bone and adrenals	May spread to lungs
Gross appearance	• Varies depends on the subtype (e.g., cauliflower-like in squamous cell carcinoma) • Carcinomas infiltrate all nearby structures (nerves, veins and muscles)	Fleshy, grow in ball-like masses and tend to push nearby structures such as arteries, nerves and veins away
Hemorrhage and necrosis	Usually not extensive	May be extensive
Microscopy	Pattern varies and parenchymal cells may be arranged in glands, acini, sheets, cords, papillae depending on the subtype	Tumor cells are arranged in different pattern depending on the subtype
Radiosensitivity	High	Radioresistance

TABLE 7.7: Differences between carcinoma and sarcoma.		
Features	***Carcinoma***	***Sarcoma***
Prognosis	Depends on the location and stage	Depends on location and stage
Examples	Carcinoma breast, squamous cell carcinoma of skin and mucus membranes, carcinoma stomach and colon	Osteosarcoma, chondrosarcoma, liposarcoma

- **Growth fraction:** It is the **proportion of cells in the proliferative or replicative pool** within the tumor.
- **Rate of tumor cell death:** Rate of growth dependson balance between cell production and cell loss.When **both the rate of cell production and the rate of cell loss** (by apoptosis) **is high,** it is termed as **high cell turnover.**

Local Invasion

Benign Tumors

- **Localized:** Most benign tumors **grow as expansile masses** that remain localized to their site of origin.
  - **No infiltration** into adjacent tissue or capsule (if present).
  - **No metastasis**.
- **Capsule (Fig. 7.9):** It is a rim of compressed connective tissue derived mainly from the extracellular matrix of the surrounding normal tissue.
  - **Capsule → makes tumor palpable and movable mass →** can be surgically enucleated.
  - **Benign tumors without capsule (unencapsulated)**, e.g., hemangiomas, uterine leiomyoma.

Malignant Tumors

Q. Write briefly on pagetoid spread and give example.

- **Lack of capsule:** Malignant tumors are poorly demarcated from the surrounding normal tissue and lack true capsule.
- **Invasion (Figs. 7.10 and 7.11): Two most reliable features** that differentiate malignant from benign tumors are **local invasion and**

metastases.

Local invasion:

- **Invasion of adjacent tissue/organ:** The cancers may invade and destroy the adjacent tissues/organ.
- **Tissues that resists invasion:** They include mature cartilage (e.g., epiphysis) and elastic tissue of arteries.
- **Pagetoid infiltration***:* It is invasion within epithelium and is seen in Paget's disease of the nipple **(***refer* **Figs. 23.12 and 23.13)**.
- **Invasion of blood vessels and lymphatics**
- **Perineural invasion** [e.g., cancer of prostate and pancreas **(Fig. 7.11 B** and *refer* **Fig. 21.7D inset)**, adenoid cystic carcinoma of salivary glands].

Consequences of invasion into the organ/tissue of origin:

- Makes **surgical resection difficult**.
- **Functional insufficiency** may occur, if the much of normal tissue is replaced by cancer, e.g., hepatocellular carcinoma may cause liver insufficiency.
- **Compromise vital regions:** Brain tumors (e.g., astrocytomas, glioblastoma) may infiltrate and compromise vital regions.
- **Life-threatening location** (e.g., intestinal obstruction due to carcinoma of colon).

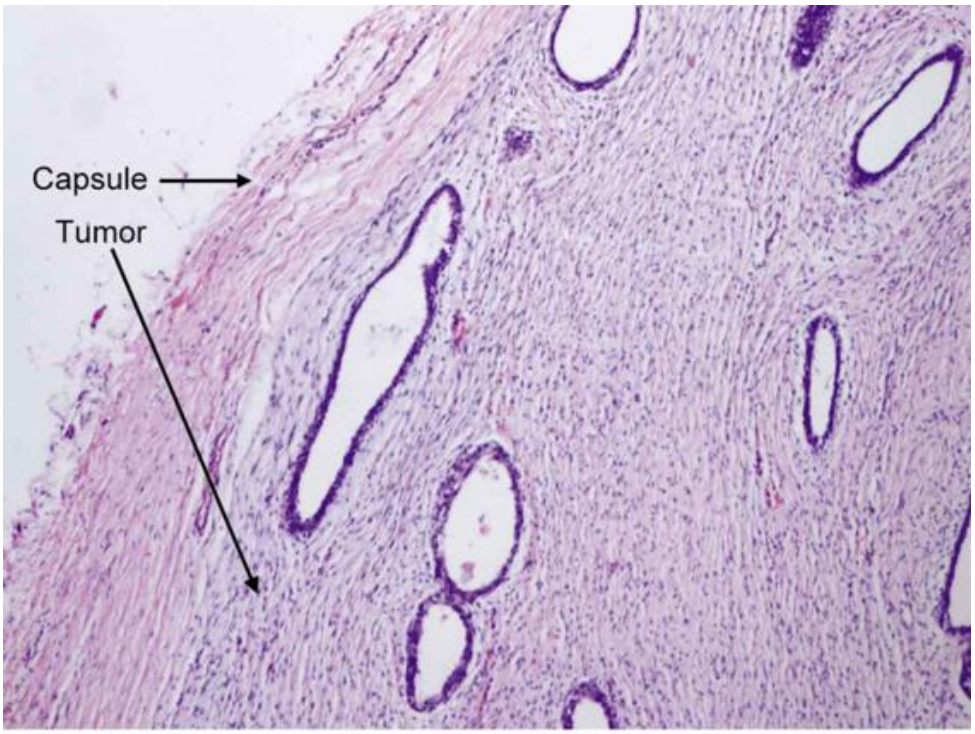


Fig. 7.9: Microscopic appearance of fibroadenoma of the breast with a well-defined fibrous capsule.

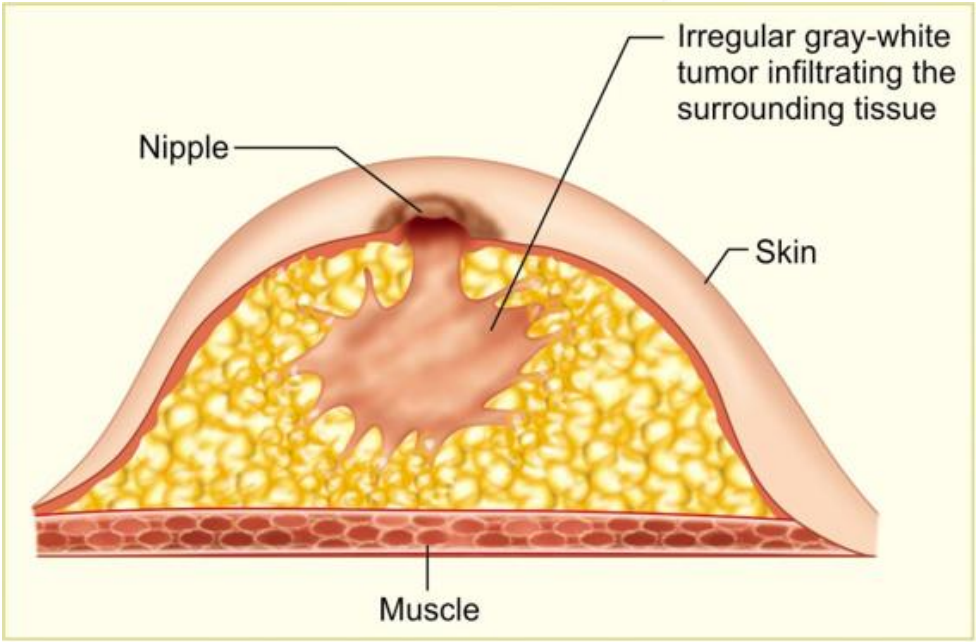
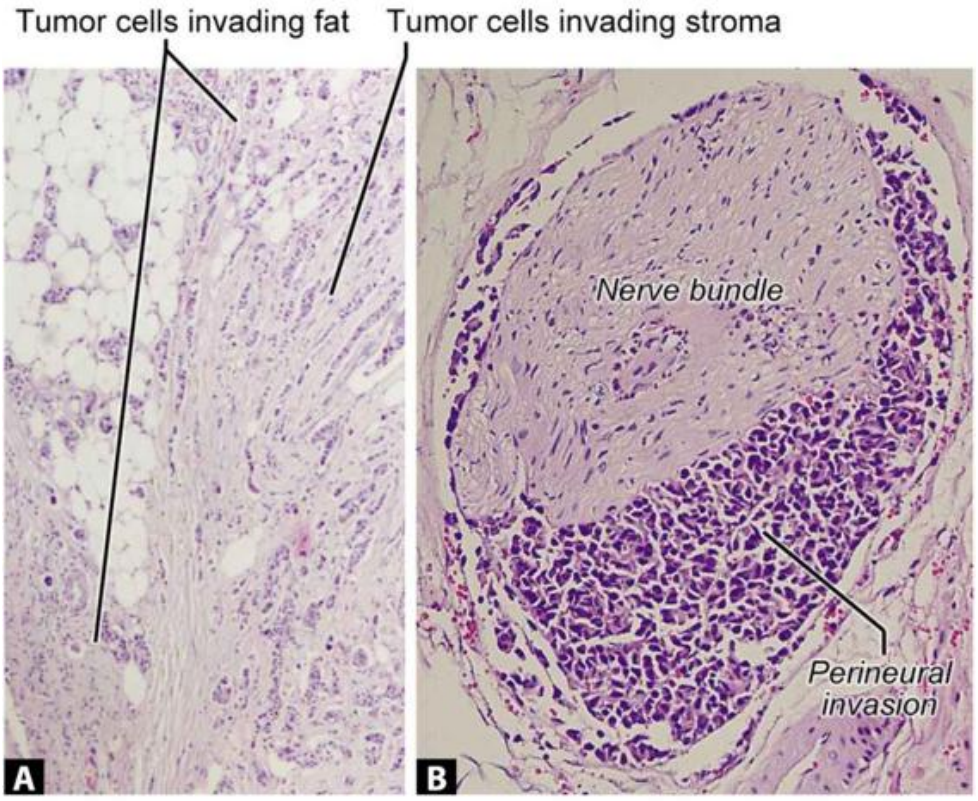


Fig. 7.10: Diagrammatic representation of cut section of an invasive ductal carcinoma of the breast.



Figs. 7.11A and B: (A) Microscopic appearance of breast carcinoma showing invasion of breast stroma and fat by nests and cords of tumor cells; (B) Perineural invasion in prostatic carcinoma.

Atypical mitotic figures: Produce tripolar, quadripolar, or multipolar spindles and these aberrant mitoses are incapable of complete cell division.

Anaplasia may be due to either backward differentiation or **failure of differentiation**.

Anaplasia: Some cancers arise from stem cells present in tissues. In these tumors, failure of differentiation rather than dedifferentiation (backward differentiation) is responsible for the undifferentiated appearance.

Neoplasms may secrete:

- **Normal hormones or products**
- **Fetal proteins**
- **Ectopic hormones**

Latent period: Time period between the exposure of the cell to the carcinogenic agent (initiation) till the tumor becomes clinically detectable.

Purpose of debulking the tumor with surgery or radiation: To shift tumor cells from resting phase (G_0) into the cell cycle and these cells become susceptible to chemotherapy.

Differences between benign and malignant tumors depends on:

- **Differentiation**
- **Rate of growth**
- **Local invasion**
- **Metastasis**

Tissue relatively resistant to invasion: Cartilage and elastic tissue.

DYSPLASIA

Q. Write short essay/note on dysplasia.

The **cells that show cytological features of malignancy** and the term dysplasia is used for these changes. It literally means disordered growth and thus consists of disordered cellular growth and maturation. Dysplasia mostly occurs in epithelial tissues. Normally, the cells of an epithelium show uniform size, shape and nuclei and these cells are arranged in a regular fashion, e.g., a squamous epithelium consists of plump tall basal cells that progress to flat superficial cells.

Features of dysplasia: Dysplasia represents the morphologic expression of a disturbance in growth regulation. However, unlike cancer cells, dysplastic cells are not entirely autonomous, and with intervention, the cells can revert to normal. In dysplasia, the normal pattern is disturbed and it shows following changes:

- **Cellular** and nuclear **pleomorphism** characterized by loss in the uniformity of the individual cells.
- **Large hyperchromatic nuclei.**
- **High/increased nuclear-to-cytoplasmic ratio.**
- **Loss of polarity (architectural orientation),** i.e., disorderly arrangement of cells from basal layer to the surface layer, e.g., in dysplastic squamous epithelium the normal progressive maturation may fail in part or entirely, leading to replacement of the epithelium by basal-appearing cells with hyperchromatic nuclei.
- **Increased mitotic activity:** Mitotic figures are normally confined to the basal layer. In dysplasia, mitotic figures are more than in the normal

tissue and may be seen at all levels (instead of only basal cells), including surface cells.

Sites of Dysplasia

- **Squamous epithelium:** Most common sites of dysplastic changes are seen in the squamous epithelial lining of the **uterine cervix (***refer* **Figs. 22.2 and 22.3 and pages 740-1), respiratory tract and skin**. Dysplasia most common in hyperplastic squamous epithelium and in areas of squamous metaplasia, such as in the bronchus or the cervix.
- **Other epithelium:** Dysplasia can occur in epithelium other than squamous epithelium.
  - **Columnar mucosa:** Dysplasia can occur in the columnar mucosal cells of the colon **in ulcerative colitis**
  - Ductal lining cells in **breast**
  - Metaplastic epithelium of Barrett esophagus
  - Prostate glands: Prostatic intraepithelial neoplasia
  - Urothelium of the bladder

Asymptomatic: In this stage, they are usually asymptomatic.

Classification, Fate, and Significance

- **Dysplasia** can be classified as **(1) mild**, **(2) moderate,** and **(3) severe dysplasia** depending on the thickness of epithelium involved by the dysplastic cells.
  - **Mild-to-moderate dysplastic changes**, which do not involve the entire thickness of epithelium **may be reversible**, if the cause is removed. The **dysplasia may be a precursor to malignant transformation, but dysplasia need not progress to cancer**.
  - Like metaplasia, dysplasia may develop as a response to chronic irritation or prolonged/persistent inflammation. It may regress or disappear after removal of the inciting stimulus, e.g., dysplasia in bronchial epithelium may regress after cessation of smoking or dysplasia in cervix due to human papilloma virus (HPV) may regress following disappearance of HPV from the cervix.
  - Dysplastic changes may be seen adjacent to foci of invasive carcinoma. In some situations, such as in long-term cigarette smokers and individuals with Barrett esophagus, severe epithelial dysplasia may antedate the appearance of cancer.
- **Carcinoma in situ:** When dysplastic changes are marked and involve the entire or full thickness of the epithelium, it is a preinvasive neoplasm and is termed as carcinoma in situ (*refer* below). But the lesion does not penetrate the basement membrane of the lining epithelium.
- **Once the dysplastic/tumor cells breach the basement membrane,** the **tumor is said to be invasive** and carcinoma in situ may take years to become invasive. Most in situ tumor, with time penetrate the basement membrane and invade the subepithelial stroma.

Metaplasia: It is reversible change in which one type of differentiated cell is replaced by another type of differentiated cells (*refer* pages 44-5). It is a cellular adaptation that develops in association with tissue damage, repair and regeneration, e.g., gastroesophageal reflux damages the squamous epithelium of the esophagus which is replaced by glandular (gastric or intestinal) epithelium, columnar epithelium of endocervix is replaced by stratified squamous epithelium. Malignancy may develop in these metaplastic epithelium.

CARCINOMA IN SITU

Q. Write short note on carcinoma in situ.

Some carcinomas evolve from a **preinvasive stage** called as carcinoma in situ (*refer* pages 739-42 of Chapter 22).

Definition: Carcinoma in situ is defined as:
- A **preinvasive epithelial neoplasm**.
- Shows **all the cytological features of malignancy**.
- **Involves the entire thickness of the epithelium**.
- Remains **confined within the epithelial basement membrane**.

The tumor cells cannot reach the potential routes of metastasis, such as blood vessels and lymphatics until the basement membrane has been breached or invaded.

Dysplasia: Potentially reversible condition having intact basement membrane.

Carcinoma in situ: Lesion in which:
- Dysplastic changes involve the entire thickness of the epithelium
- Basement membrane is intact.

METASTASIS

Q. Define metastasis and describe its mechanism.

Q. Write short essay/note on mode/pathways of spread of malignant tumors/Discuss the different modes of metastasis with examples.

Definition: Metastases are tumor deposits discontinuous with the primary tumor and located in a distant tissue. This **process is known as metastasis** and the resulting **secondary deposits are called metastases**.

Significance

- Metastases clearly **identifies a tumor as malignant** because benign neoplasms never metastasize. **Exceptions** include two malignant tumors, which are locally invasive, but rarely metastasize.

- **Gliomas** (malignant neoplasms of the glial cells) in the central nervous system.
- **Basal cell carcinomas of the skin**.

- Metastases **strongly reduce the possibility of cure of cancer.**
- Metastatic spread is the most **common cause of cancer death.**

Factors favoring metastasis: (1) Poorly differentiated tumor, (2) more rapidly growing tumor, and (3) large primary tumor.

Morphological Appearance

- Microscopically, **metastases resemble the primary tumor**. But occasionally, they may be so anaplastic that their cell of origin cannot be made out.
- **Unknown primary: Sometimes metastases may appear without any clinically detectable primary tumor** and even the microscopic examination of metastases may not reveal the characteristics features of primary site tumor, e.g., metastases from adenocarcinoma may be so anaplastic that there is no evidence of any gland formation. In such situations, **electron microscopic examination, immunohistochemistry** by specific tumor markers will be **helpful to establish the primary tumor**.

Pathways of Spread

Invasiveness of cancers allows them to penetrate blood vessels, lymphatics and body cavities. It provides an opportunity for spread/dissemination of cancers through the following pathways:

Lymphatic Spread

Q. Write short note on lymphatic spread of malignant tumors.

- **Most common pathway** of spread for carcinomas.
- **Regional node involvement:** The walls of lymphatics in the region of cancer are readily invaded by cancer cells and form a continuous growth within the lymphatic channels (lymphatic permeation). Once the tumor

cells gain access into the lymphatic vessels, they may detach to form tumor emboli and are carried to the regional draining lymph nodes. In the lymph node, the **tumor emboli** enter through afferent lymphatics at its convex surface and **lodge and grow in the subcapsular sinus**. **Subsequently, the entire lymph node** may be replaced by the metastatic tumor.

- **Pattern of lymph node involvement follows the natural routes of lymphatic drainage**.
- **Sentinel lymph node** biopsy is done to know the presence or absence of metastatic lesions.

Q. Write short note on skip and retrograde metastasis.

- **Skip metastasis:** When **local lymph nodes are bypassed** and **lymphatic metastases develop in lymph nodes distant from the site of the primary tumor**; these are called "skip metastasis", e.g., **abdominal cancers** may be **first detected by an enlarged supraclavicular node**. **Virchow's lymph node** is metastasis to supraclavicular lymph node from cancers of abdominal organs (e.g., cancer stomach).
- **Retrograde metastasis: Tumors spreading against the flow of lymphatics** may cause metastases at unusual sites, e.g., **carcinoma prostate metastasizing to supraclavicular lymph node**.
- **Microscopic pattern of deposits:**
  - Initially, **tumor cells** are deposited in the **marginal sinus and later extend throughout the node**.
  - **Micrometastases** (microscopic involvement of lymph nodes) **consist of single tumor cells or very small clusters.**
- **Significance of lymph node metastases: Prognostic value**, e.g., in breast cancer, involvement of axillary lymph nodes is very important for assessing prognosis and for type of therapy. However, all regional nodal enlargements need not be due to metastasis because necrotic products of tumor and antigens may produce sinus histiocytosis.

Hematogenous Spread

Q. Write short note on hematogenous spread of malignant tumors.

Hematogenous spread is usual for sarcomas but is also found in carcinomas. Blood-borne metastasis usually occurs in **osteosarcoma, choriocarcinoma** and **renal cell carcinoma.**

- **Vessels invaded:** Cancer cells easily invade **capillaries and venules**, but thick-walled arterioles and arteries are relatively resistant.
- **Tumors with affinity for venous invasion:**
  - **Renal cell carcinoma:** It can invade the renal vein and grow in a snake-like fashion up the inferior vena cava, sometimes reaching the right side of the heart.
  - **Hepatocellular carcinoma:** It may invade branches of portal and hepatic vein and grow within the main venous channels.
- **Pattern of involvement:** With venous invasion, the pattern of metastases follows the venous flow.
- **Target organ for metastasis:**
  - **Liver and lungs:** They are the **most frequently involved** organs; liver, because **all portal area drains to the liver**. Tumors which penetrate systemic veins, eventually drain into the vena cava. Since **all caval blood flows to the lungs**, it is the other common site for secondaries by hematogenous spread.
  - Through pulmonary veins, cancer cells from the primary lung cancer and metastatic deposit in the lungs may be carried to the left side of the heart. From here the tumor emboli may be carried in systemic circulation to form secondary masses elsewhere in the body.
  - **Bone metastasis:** Cancer metastasizing to bone-prostate, lung, breast, liver, intestine, kidney and thyroid.
    - **Vertebral column is a common site** and the spread is through the paravertebral plexus, e.g., carcinomas of the thyroid and prostate.
    - Radiograph appearance of bone metastasis
      - **Osteolytic lesion:** It is characterized by radiolucencies (e.g., lung cancer) and may lead to pathological fractures and hypercalcemia.
      - **Osteoblastic lesion:** It is characterized by radiodensities (e.g., prostatic cancer, breast, thyroid) and increased serum alkaline phosphatase due to reactive bone formation.
  - **Other common sites: Brain—**most common primary is lung cancer, kidney and adrenals.
  - **Organs relatively resistant** (e.g., **skeletal muscle and spleen**).

MORPHOLOGY

Gross Appearance (Fig. 7.12)

Appear as multiple round nodules of varying sizes found throughout the organ.

Microscopy

The metastatic deposits generally resemble the structure of primary tumor.

Seeding of Body Cavities and Surfaces

Q. Write short note on transcoelomic spread.

1. **Transcoelomic spread:**
   a. **Malignant tumor arising in organs adjacent to body cavities** (e.g., ovaries, gastrointestinal tract, and lung), **may seed body cavities**. The malignant cells may exfoliate or shed from the organ surfaces into the body cavities and cytological examination of this fluid may show malignant cells.

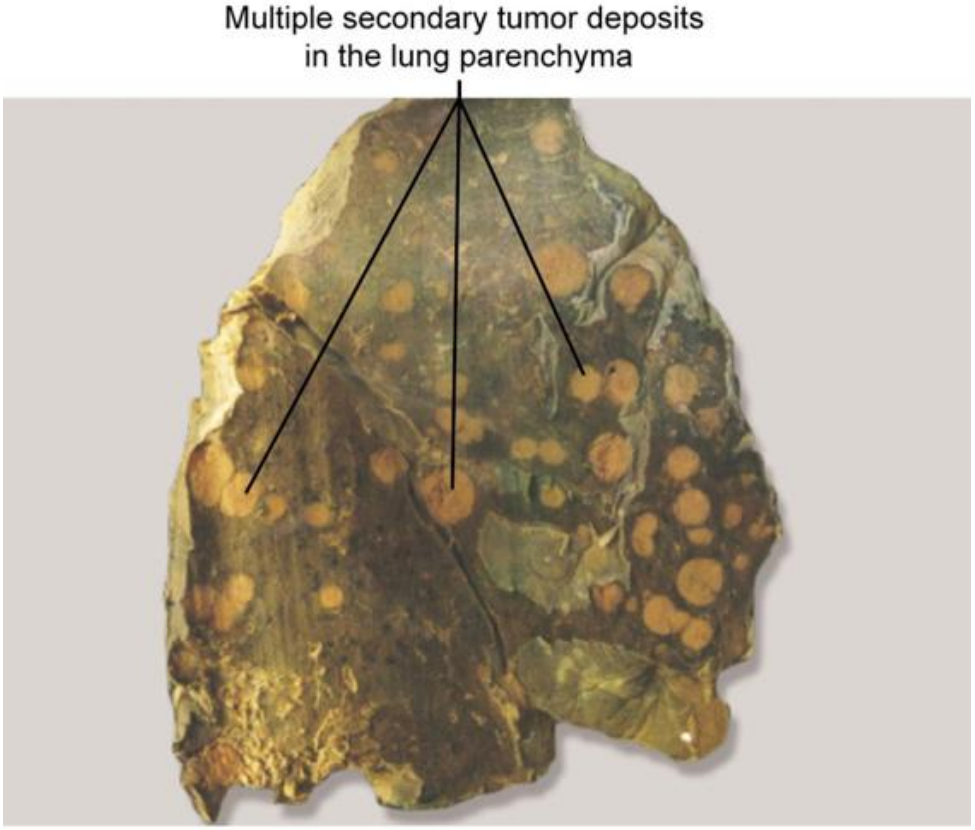


Fig. 7.12: Lung with multiple metastatic cancer.

b. Body cavities include peritoneal (most common), pleural cavities (common), pericardial (occasionally), joint space and subarachnoid space.
   i. **Peritoneal cavity:** Example: (1) **Ovarian tumors,** such as primary carcinomas of surface epithelial origin and (2) **malignant GI tract tumors** may spread to involve peritoneal cavity → ascites.
   ii. **Pleural cavity:** Peripherally situated **lung tumors** → pleural effusions.
   iii. **Cerebrospinal fluid: Glioblastoma** commonly spread through CSF in the subarachnoid space to the spinal cord.

2. **Spread along the epithelial lined spaces:** It is not common, e.g.,
   - **Carcinoma endometrium may spread to ovary** (or vice versa) **through fallopian tube**.
   - **Carcinoma of kidney may spread to lower urinary tract via ureters**.

Direct Transplantation

- Tumor cells may be directly transplanted (e.g., by surgical instruments like scalpel, needles, sutures) or implantation by direct contact (e.g., transfer of cancer of lower lip to the corresponding opposite site in the upper lip).
- Even though this method is theoretically possible, they are **rare**.

Differences between benign and malignant tumors are summarized in **Table 7.8**.

Metastasis is the process and the resulting **secondary deposits are called metastases.**
Metastases: First important criteria for malignancy.
Pathways of metastases: Lymphatics, hematogenous, spread along body cavities, direct transplantation, and rarely **along epithelial lining**.
A historical emphasis on lymphatic spread for carcinomas and hematogenous spread for sarcomas may not always be true and both can spread by any route.
Lymph nodes: First line of defense in malignant tumors and most common site for metastases.

Q. Describe the differences between benign and malignant tumors. Mention the four characteristics of benign neoplasms.

TABLE 7.8: Differences between benign and malignant tumors.

Characteristics	*Benign*	*Malignant*
A. MICROSCOPIC FEATURES		
1. Differentiation/anaplasia	Well-differentiated	Well to poorly differentiated. Anaplasia is characteristic
2. Pleomorphism	Usually not seen	Commonly present
3. Nuclear morphology	Usually normal	Usually hyperchromatic, irregular outline and pleomorphic
4. Nucleoli	Usually absent	Usual and prominent
5. Mitotic activity	Rare and if present they are normal bipolar	High and may be abnormal or atypical (tripolar, quadripolar, multipolar)
6. Tumor giant cells	Not seen	May be seen and show nuclear atypia
7. Nuclear cytoplasmic (N:C) ratio	Normal (1:4 to 1:6)	Increased (may be as much as 1:1)
8. Polarity	Maintained	Usually lost
9. Chromosomal abnormality	Not found	Usually seen
B. GROSS FEATURES		
1. Border/capsule	Mostly circumscribed or encapsulated	Usually poorly defined
2. Areas of necrosis and hemorrhage	Rare	Common, often found microscopically
C. CLINICAL FEATURES		
1. Rate of growth	Usually slow	Relatively rapid
2. Local invasion	Usually well-demarcated without invasion/ infiltration of the surrounding normal tissues	Locally invasive, infiltrate surrounding normal tissue
3. Metastasis	Absent	Frequent
4. Biological behavior/prognosis	Usually prognosis is good	Prognosis is poor; usually death due to local invasion or metastatic complications

Sentinel lymph node: Useful for: 1. Breast cancer, 2. Malignant melanoma and 3. Cancer of vulva.
Exfoliation of malignant cells through serosa occurs in malignant surface tumors, e.g., ovarian cancer.

Sentinel lymph node is the first node in a regional lymphatic drainage **that receives lymph flow from the primary tumor**.

Tumor with strong propensity for vascular invasion:
1. **Renal cell carcinoma**
2. **Hepatocellular carcinoma.**

Hematogenous metastasis to bone: Vertebra is the most common site involved through paravertebral venous plexus.

Bone metastasis: May be either osteoblastic (radiodense) or osteolytic (radiolucent).
Osteoblastic metastasis: Increased alkaline phosphate and is seen in **prostatic cancer**.
Osteolytic metastasis:→hypercalcemia→pathologic fracture.

Extranodal metastasis: Bad prognostic sign.

Drop metastasis: Medulloblastoma invades ventricles and spreads through CSF into spine.

Pseudomyxoma peritonei: Abundant mucin in the peritoneal cavity producing a gelatinous

neoplastic mass occasionally seen in mucus-secreting appendiceal/ovarian carcinomas.

INVASION–METASTATIC CASCADE (MOLECULAR EVENTS IN INVASION AND METASTASIS)

Q. Discuss the mechanism of invasion and metastasis.

Q. Write short note on metastatic cascade.

Invasion and metastasis are characteristic of malignant tumors.

Definition: Invasion–metastatic cascade constitutes the **entire sequence of events from** the beginning of **invasion** to the development of **metastasis**.

Phases

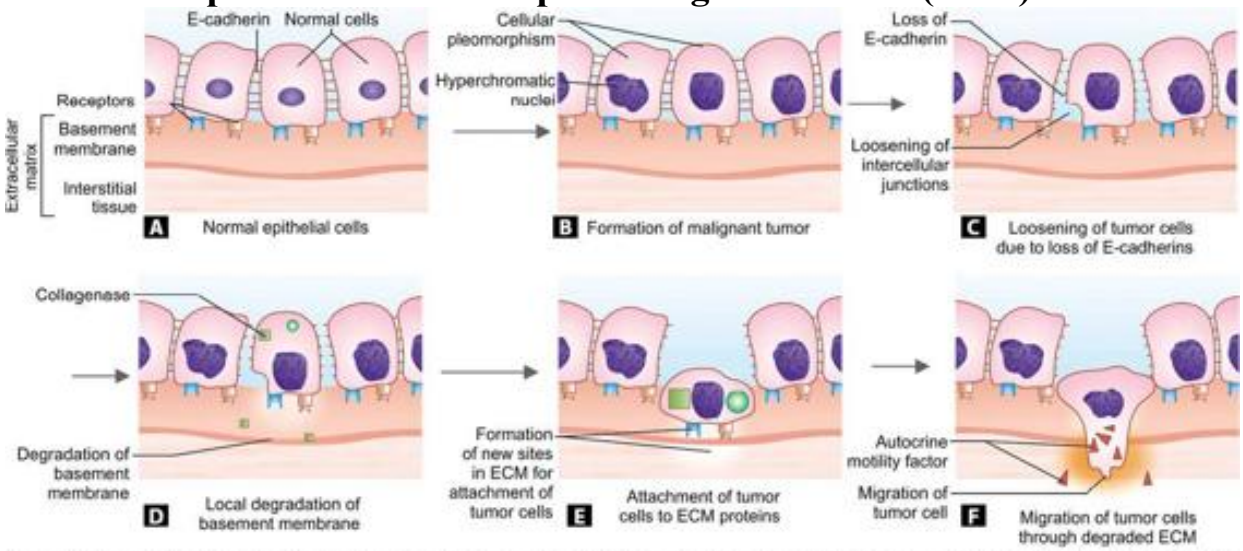
Invasion–metastatic cascade is a **complex multistep process**. It can be divided into two main phases, namely: (1) **invasion of the extracellular matrix (ECM)** and (2) **metastasis (vascular dissemination and homing of tumor cells)**.

Invasion of Extracellular Matrix (Figs. 7.13A to F)

Tumor cells must interact with ECM (includes basement membrane and interstitial tissue) at several steps in the invasion–metastatic cascade. Invasion of the ECM is an active process and consists of four steps:

1. **Loosening of tumor cells: Normal cells** are **attached to each other by** adhesion molecules namely: **E-cadherins**.
   - **Reduced/loss of E-cadherin function:** It is observed in most epithelial cancer (e.g., adenocarcinomas of the colon and breast) → **loosening of tumor cells**. The separated cells get **detached from the primary cancer**.
2. **Local degradation/proteolysis of basement membrane and interstitial connective tissue:** Extracellular matrix is of two types, namely: (1) Basement membrane and (2) interstitial connective tissue.
   - **Secretion of degrading enzymes:** Malignant **tumor cells** and **stromal cells** (e.g., fibroblasts and inflammatory cells) **in the cancers secrete**/induce **many proteolytic enzymes** that **degrade ECM**.

These enzymes include: **Matrix metalloproteinases (MMPs), cathepsin and urokinase plasminogen activator (u-PA)**.



Figs. 7.13A to F: Various steps in the invasion of extracellular matrix in invasion–metastasis cascades: (A) Normal cells; (B to F) Tumor cells loosen and detach from each other because of reduced adhesiveness. The tumor cells bind components of the extracellular matrix and secrete proteolytic enzymes that degrade the extracellular matrix. With binding to proteolytically generated new binding sites in the ECM, tumor cell migration follows. The tumor cells reach the nearby vessels to start the next phase, namely metastasis.

- **Local degradation of basement membrane and interstitial connective tissue:** This is achieved by proteolytic enzymes.

3. **Changes in attachment/adhesion of tumor cells to ECM proteins:** Normal epithelial cells have receptors (e.g., integrin) for basement membrane components (e.g., laminin and collagen) and are located at their basal surface.
   - **Generation of new sites:** Local degradation of basement membrane generates **new and strange sites** in the basement membrane.
   - **Adhesion of tumor cells to ECM:** The receptors on tumor cells attach to the new sites in the basement membrane.
   - **Stimulation of tumor cell migration:** It follows attachment/adhesion of tumor cells to ECM proteins.
4. **Locomotion/migration of tumor cells through degraded ECM:** It is a multistep process.
   - **Locomotion/migration** drives the tumor cells **forward through the degraded basement membranes** and zones of proteolysis in the **interstitial connective tissue matrix**.
   - **Locomotion involves many receptors and signaling proteins**. The locomotion is potentiated by tumor cell-derived cytokines, such as **autocrine motility factors** (AMF) and other molecules.

- **Migration through interstitial tissue:** The tumor cells invade and traverse through the surrounding interstitial connective tissue and ultimately **reach nearby blood and lymphatic vessels.** Cells gain access to the circulation by penetrating the basement membrane of vessels.

Metastasis (Vascular Dissemination and Homing of Tumor Cells)

Following the invasion of surrounding interstitial tissue, malignant cells may spread to distant sites by metastasis. Metastasis is **multistep process** by which tumor produces a secondary growth at a distant site or location. It has several steps **(Fig. 7.14)**.

1. **Penetration of vascular or lymphatic channels (intravasation into the lumen of vessels):** Malignant cells penetrate the basement membrane of blood vessels or lymphatic channels.
2. **Invasion of the circulation and formation of tumor emboli:** In the circulation, tumor cells are susceptible to destruction by several mechanisms. These include mechanical shear stress, apoptosis stimulated by loss of adhesion (termed *anoikis*), and innate and adaptive immune defenses. Survived tumor cells within the circulation, may clump with platelets to form platelet-tumor aggregates. This may enhance tumor cell survival and implantability. Tumor cells may also bind and activate coagulation factors and form emboli.
3. **Transit through the circulation.**
4. **Arrest within circulating blood or lymph:** It occurs at distant location away from primary tumor. At the site of arrest, tumor cells adhere to endothelial cells.
5. **Exit from the circulation into a new tissue site:** Location at which circulating tumor cells leave the capillaries to form secondary deposits depends on the anatomic location and vascular drainage of the primary tumor and the tropism of particular tumors for specific tissues. Exit occurs through the basement membrane of lymphatics or blood vessel. The site at which circulating tumor cells leave the vessel or lymphatics must repeat the same events involved in invasion but in a reverse order.

6. **Formation of micrometastases:** Tumor cells lodge at a distant new site to form micrometastases, e.g., for favored sites of metastasis:

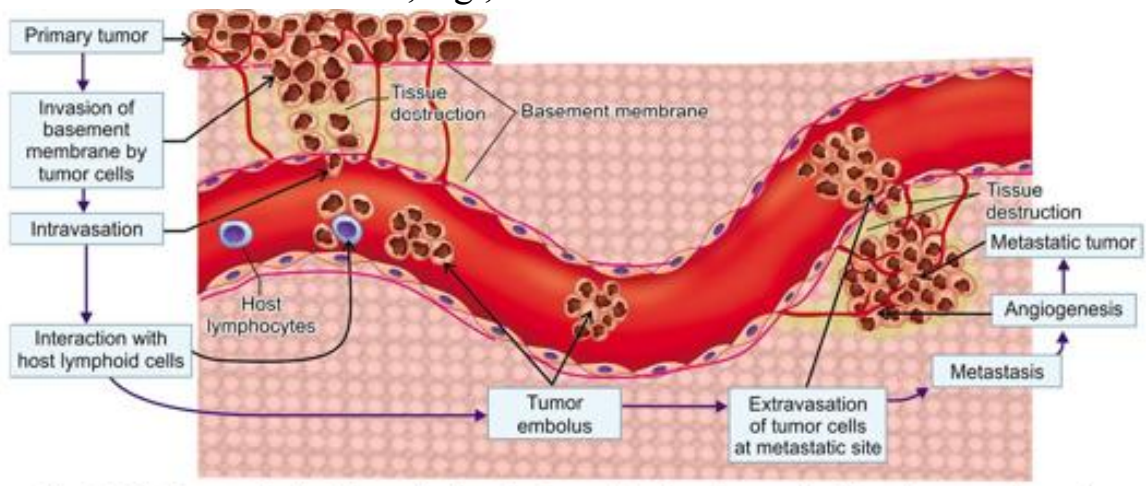


Fig. 7.14: Various steps involved in vascular dissemination and homing of tumor cells during the metastatic cascade.

- Prostatic carcinoma to the bone.
- Bronchogenic carcinomas to the adrenals and to the brain.
- Neuroblastomas to the liver and bones.

7. **Angiogenesis.**
8. **Local growth of micrometastases into macroscopic tumor**.

Invasion: Second most important criteria for malignancy.

Loss of adhesive molecules → invasion.

Loss of E-cadherin: Leads to loosening of tumor cells.

Degradation of ECM: By proteolytic enzymes secreted by tumor cells and stromal cells.

Invasion steps:

1. Loosing of tumor cells
2. Local degradation of ECM
3. Attachment of ECM proteins
4. Migration of tumor cells

Metastasis is the process of deposition of tumor deposits away from primary.

Metastases: Some organs or tissues may be unfavorable soil for the growth of tumor deposit (e.g., spleen and skeletal muscle).

Two features that differentiate benign from malignant:
1. Local invasion

ENVIRONMENTAL FACTORS AND CANCER

Environmental factors are important **risk factors for most cancers.**

- **Smoking:** Cigarette smoking (*refer* pages 262-2) is an important factor involved in cancer of the lung, mouth, pharynx, larynx, esophagus, pancreas and bladder.
- **Alcohol abuse:** Alcohol abuse (*refer* pages 262-3) is a risk factor for carcinomas of the oropharynx (excluding lip), larynx and esophagus, and can produce alcoholic cirrhosis which is a risk factor for hepatocellular carcinoma. Alcohol and tobacco together increases the risk of cancers in the upper airways and digestive tract.
- **Infectious agents:** *Human papilloma virus* (HPV) spreads through sexual contact and is etiological factor for carcinoma of cervix as well as some head and neck cancers.
- **Obesity:** It is associated with cancer risk.
- **Hormones:** Exposure to estrogen stimulation, if unopposed by progesterone, increases the risk of cancers of the breast and endometrium.
- **Carcinogens:** They may be present in food (e.g., grilled meat, high-fat diet, alcohol), water (e.g., arsenic), environment [e.g., ultraviolet (UV) rays, asbestos], drugs medications (e.g., methotrexate), etc.

Q. Write short note on diet and cancer.

- **Diet and cancer:** Though not proved, it may be a risk factor for colorectal carcinoma, prostate carcinoma and breast carcinoma. **Three factors in the diet are probably involved in the development of cancer:**
  - **Exogenous carcinogen in diet:** *Aflatoxin* causes a specific mutation in codon 249 of the *TP53* gene and is involved in the development of hepatocellular carcinomas. The role of food additives, artificial sweeteners, and contaminating pesticides in the genesis of cancer is not known.
  - **Endogenous synthesis of carcinogens from dietary components:**
    - **Nitrosamines and nitrosamides: It was implicated mainly in the genesis of gastric cancer.** Nitrosamines and nitrosamides in the diet can induce gastric cancer. These compounds are formed in the

stomach from nitrites and amines or amides from the digested proteins in the diet. Sources of nitrites include sodium nitrite (added as food preservative), and nitrates (present in common vegetables) and these are reduced to nitrosamine and nitrosamides in the gut by bacterial flora.

- **High animal fat intake:** This along with consumption of red meat and low dietary fiber intake has been implicated in the causation of carcinoma colon. Probably high fat intake increases the bile acids level in the gut. This modifies intestinal flora and favors the growth of microaerophilic bacteria. Bile acid metabolites produced by the action of these bacteria may be carcinogenic.

- **Lack of protective factors:**
  - **High-fiber diet** may have a protective role in carcinoma colon. This may be due to (1) increased bulk of stool and reduced transit time, which reduces the exposure of mucosa to probable carcinogens, and (2) certain fibers in the diet may bind to carcinogens and protect the mucosa. However, it is not proved.
  - Correlation between total **dietary fat intake** and breast cancer is also not clear.
  - **Antioxidant:** Fruits and vegetables, consumption of vitamin C and E, β-carotenes and selenium which have antioxidant properties, have been presumed to have anticarcinogenic effect. However, there is no convincing evidence that antioxidants act as chemopreventive agents. Retinoids are effective agents in the therapy of acute promyelocytic leukemia, and there are reports mentioning the associations between low levels of vitamin D and cancer of the colon, prostate and breast.
  - Epidemiologic studies suggest that a **folate-rich diet** decreases the risk of colorectal cancer.

In conclusion, dietary influences on cancer development are highly controversial. There is no definitive evidence to indicate that a particular diet can cause or prevent cancer. Association has been mentioned that physical activity decreases the risk of developing cancer of breast and colon whereas obesity increases the risk for endometrial, esophageal and kidney cancer.

PRECANCEROUS CONDITIONS/PRECURSOR LESIONS

Q. Write short note on precancerous lesions/premalignant neoplasms.

Precancerous conditions (precursor lesions) are **non-neoplastic disorders** in which there is a **well-defined association with an increased risk of cancer**. However, in majority of these lesions no malignant neoplasm develops except that they have an increased risk, e.g.,

1. **Chronic atrophic gastritis** of pernicious anemia.
2. **Solar or actinic keratosis of the skin, Bowen's disease of the skin.**
3. **Chronic inflammation:** Chronic ulcerative colitis (carcinoma colon), cirrhosis of liver (hepatocellular carcinoma), *H. pylori* gastritis (gastric cancer and lymphoma), chronic irritation from jagged tooth or ill-fitting denture (cancer of the oral cavity) and old burn scar—Marjolin's ulcer (squamous cell carcinoma).
4. **Leukoplakia** (erythroplakia) of the oral cavity, vulva and penis.
5. Barrett esophagus.
6. Squamous metaplasia and dysplasia of bronchial mucosa observed in chronic smokers. Intralobular and intraductal carcinoma of the breast, carcinoma in situ of cervix.
7. Endometrial hyperplasia and dysplasia in women with unopposed estrogen stimulation.
8. Precancerous benign tumors: Few forms of benign tumors may transform into malignant, e.g., villous adenoma of the colon, as it increases in size, becomes malignant.
9. Benign develops occasionally into malignant: Most benign tumors do not become malignant. However, occasionally it may arise from benign tumors, e.g.,
   - Leiomyosarcoma beginning in a leiomyoma.
   - Carcinoma developing in long-standing pleomorphic adenomas.

- Malignant peripheral nerve sheath tumor in patients with neurofibromatosis.

10. Congenital abnormalities may predispose to cancer, e.g., the undescended testis is more prone to neoplasms than the normally located testis.
11. Immunodeficiency states: Patients with deficits in T-cell immunity have increased risk for cancers mainly those due to oncogenic viruses.

Increased risk of cancer is seen in:
- Chronic ulcerative colitis
- Chronic atrophic gastritis
- Solar keratosis
- Leukoplakia
- Barrett esophagus

Precancerous/preneoplastic lesions of GI tract are listed in **Box 7.1**.

Q. Write a short note on preneoplastic (precancerous) conditions/lesions of GIT.

BOX 7.1: Precancerous lesions of gastrointestinal tract.

Esophagus
- Barrett's esophagus, achalasia, post-ingestion of caustic soda lye (alkaline burn)

Stomach
- *H. pylori*, especially if treated with PPI without eradication of *H. pylori*
- Gastric polyps
- Ménétrier's disease (hypertrophic gastropathy)
- Achlorhydria, pernicious anemia
- Post-gastrectomy, post-vagotomy, post-gastrojejunostomy

Small intestine
- Hereditary cancer syndromes, FAP (especially post-cholecystectomy), Peutz-Jeghers syndrome

Colon (large intestine)
- Hereditary cancer syndromes, FAP, Gardner's, HNPCC (Lynch syndrome)
- Inflammatory bowel disease, especially chronic extensive ulcerative colitis
- Sporadic adenomatous polyps, especially villous and tubulo-villous polyps

GENETIC LESIONS IN CANCER

Genetic changes in cancer may be **minute or large** enough to be identified in a karyotype.

Driver and Passenger Mutations

Driver mutations: A relatively small number of genetic changes are fundamental to oncogenesis. Mutations thatproduce malignant phenotype are referred to as "driver mutations". These are mutations which change the function of cancer genes and directly contribute to the development or progression of a cancer. Initiating mutation is the first driver mutation that starts a cell on the path to malignancy. Usually, they are **acquired** and occasionally inherited. The driver mutations tend to be tightly clustered within cancer genes. Tumor cells may acquire driver mutations through several means such as point mutations and nonrandom chromosomal abnormalities (e.g., gene rearrangements, deletions, and amplifications). It is typically maintained in all of the cells of the subsequent cancer. However, only one mutation usually do not fully transform the cell into cancer cell. Hence, development of a cancer requires that the "initiated" cell undergo a number of additional driver mutations, each of which also contributes to the development of the cancer.

Passenger mutations: Loss-of-function mutations in genes that maintain genomic integrity is a common early step in malignancy, particularly in solid tumors. Mutations which lead to genomic instability increase the chances of acquiring driver mutations (that are needed for malignant behavior) and also greatly increase the frequency of mutations that have no phenotypic consequences (called "passenger" or "hitchhikers" mutations). Passenger are acquired mutations and do not change the cellular behavior. In contrast to driver mutations, they occur at random and are sprinkled throughout the genome. It is found that particularly in cancers caused by exposure to carcinogen (e.g., melanoma and lung cancer related to smoking), the number of passenger mutations is more common than driver mutations. Passenger mutations may become driver mutations if selective pressure on the tumor changes (e.g., treatment with an effective therapeutic drug).

Karyotype Abnormalities in Tumors

These may be due to **abnormalities in: (a) structure or (b) number (aneuploidy)** in which whole chromosomes may be gained or lost.

Structural Abnormalities

Common structural abnormalities are: (1) balanced translocations, (2) deletions, (3) gene amplifications and (4) point mutations.

Balanced Translocations

Associated with **hematopoietic** and **mesenchymal neoplasms**.

Method of activation of proto-oncogenes: Balanced translocation can activate proto-oncogenes by two ways: (1) Overexpression or (2) forming chimeric gene.

- **Overexpression** → loss of normal regulatory control on these genes, e.g., In Burkitt lymphoma(*refer* **Fig. 12.27**), **most common translocation t(8;14)(q24; q32)** → converts MYC proto-oncogene into MYC oncogene → **overexpression of MYC protein** (oncoprotein) → **uncontrolled cell proliferation** and **inhibition** of apoptosis.
- **Forming chimeric gene** → chimeric proteins → cellular proliferation, e.g., ***Philadelphia*** (Ph) ***chromosome*** in chronic myelogenous leukemia. **Balanced reciprocal translocation** between long arm of chromosomes 9 and 22, i.e., **t(9;22)(q34;q11.2)** → **shortened chromosome 22—** Philadelphia chromosome (*refer* **Figs 12.16**). ***ABL*** (Abelson murine leukemia virus) proto-oncogene from chromosome 9 **joins** th*e* ***BCR*** (breakpoint cluster region) on chromosome 22 → produces a new **chimeric (fusion) gene → called BCR-ABL oncogene → causes cell division** and **inhibition of apoptosis.**

Deletions

- Chromosomal deletions are more **common in nonhematopoietic solid tumors** and are the second most structural abnormality found in tumor cells.
- Deletion is **common with tumor suppressor gene** and causes **loss of particular tumor suppressor gene protein**. Example: Deletion of *RB* gene (involving chromosome 13q14) is associated with retinoblastoma.

Gene Amplification

- Gene amplification is a chromosomal alteration in which there are an **increased number (several hundred copies) of gene copies**.
- Proto-oncogenes may be converted to oncogenes by gene amplification.
- Gene amplification → produces several hundred copies of the proto-oncogene in tumor cells → **overexpression** of gene product **(normal proteins)**.
- It has been found mainly in **human solid tumors**.
- Gene amplification can produce two patterns**(Fig. 7.15)**:
  - **Extrachromosomal multiple**, **small**, **structures (called "double minutes"**/dmins)
  - Chromosome alterations referred to as **homogeneous stainable regions (HSR)** if increased copies of gene are integrated within chromosomes. Increased copies of gene may be inserted into new chromosomal location, which may be distant from the normal location of the involved genes. HSR appear as homogeneous in G-banded karyotype.

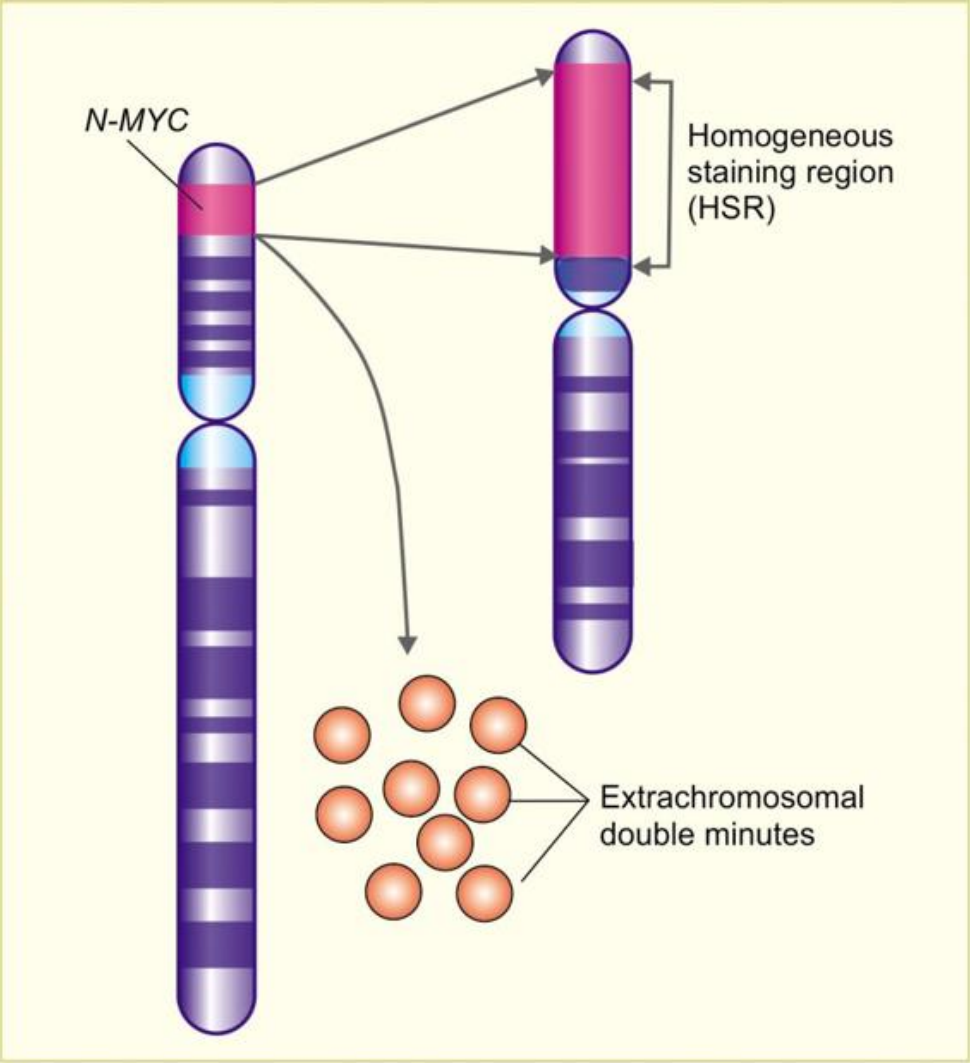


Fig. 7.15: Amplification of the *N-MYC* gene in human neuroblastomas seen either as extrachromosomal double minutes or as a chromosomally integrated, homogeneous staining region (HSR).

- Examples: (1) *N-MYC* **gene amplified in neuroblastomas** and associated with poor prognosis. (2) **HER2/Neu (also called ERBB2) amplification in breast cancer**.

Chromothripsis

Dramatic chromosome "catastrophes" are called chromothripsis (literally means chromosome shattering).

Chromothripsis is found in about 1–2% of cancers, up to 25% of osteosarcomas and other bone cancers and in gliomas. It probably develops as a single event in which **dozens to hundreds of chromosome breaks**

occur within part or across the entirety of a single chromosome or several chromosomes. These catastrophic events may simultaneously activate oncogenes and inactivate tumor suppressors leading to carcinogenesis.

Point mutations (*refer* below).

Numerical Abnormalities

Aneuploidy

Aneuploidy is the **presence of chromosome numbers that is not multiple of haploid number** (i.e., multiples of 23). It is common in cancers particularly in carcinomas.

Minute/Subtle Changes

- Genetic changes in cancer may be subtle and **cannot be detected by karyotyping**. These include: Point mutations or insertions and deletions.
- **Point mutation is characterized by substitution of a single nucleotide base by a different base in a gene** (*refer* page 240). It may change the code in a triplet of bases and lead to the replacement of one amino acid by another in the gene product. **Point mutation is a common mechanism of oncogene activation**.Examples: Point mutations in one of the *RAS* genes (*HRAS*, *KRAS* or *NRAS*) are observed in—85% of pancreatic cancers and 45% of colon cancer, point mutations of ***RET*** in leukemia and ***BRAF*** in melanoma.

Epigenetic Modifications and Cancer

Definition: Epigenetics is a **reversible, heritable mechanisms that control gene expression independent of DNA base sequences and occurs without mutation**. It is unrelated to gene nucleotide sequence. **Epigenetics** is the mechanism that control gene expression.

- Epigenetic changes **involve histone modification** and **DNA methylation**, both of which affect gene expression.
- **In normal cells**, the **majority of the genome is not expressed**, because **they are silenced by DNA methylation and histone modifications**. Apart from DNA mutations, epigenetic aberrations are also responsible for the malignant properties of cancer cells.

- Epigenetic modifications are usually passed on to daughter cells and may occasionally result in changes in gene expression. **In cancer cells,** there is global DNA hypomethylation and selective localized hypermethylation, e.g.,
  - **Silencing genes by hypermethylation**: (1) **Tumor suppressor gene *p53*, *BRCA1*** in breast cancer and ***VHL* in renal cell carcinomas** and (2) DNA repair genes: Mismatch-repair gene ***MLH1* in colorectal cancer**.
  - **Hypomethylation** → can **cause chromosomal instability**, derepression of growth regulatory genes, and overexpression of antiapoptotic genes → induce tumors.
  - **Changes in histones:** Cancer cells may show changes in histones near genes that influence cellular behavior.

Unlike DNA mutations, epigenetic changes are potentially reversible by drugs that inhibit DNA- or histone-modifying factors.

Noncoding RNAs and Cancer

It is observed that many genes do not encode proteins. Instead, their products play important regulatory functions.

One class of **genes, which do not encode proteins** but their products **play important role in gene regulation**, is small RNA molecules. They are small noncoding, single-stranded RNAs → called as microRNAs (miRs).

- **Role in carcinogenesis**: A**mplifications and deletions of miR loci** have been observed in many cancers. The miRs that promote tumor development are often called as ***onco-miRs***. miR-200 are important in invasiveness and metastasis; and miR-155, is overexpressed in many human B-cell lymphomas. Deletions affecting certain ***tumor suppressive miRs***, such as miR-15 and miR-16, are frequent genetic lesions in chronic lymphocytic leukemia. Mir34 family of miRNAs are activated by *p53* gene.
- **Mode of action:** (1) Increased expression of oncogenes or (2) reduced expression of tumor suppressor genes.

Mechanisms of mutations in tumor cells:
- Point mutations
- Balanced translocations
- Deletions
- Gene amplifications

MOLECULAR BASIS OF CANCER

CC: Describe the molecular basis of cancer.

Fundamental Principles

1. **Cancer is a genetic disease** and arises through a series of somatic alterations in DNA that result in uncontrolled proliferation of cells with altered DNA.
2. **Nonlethal genetic damage** (mostly in DNA) **known as mutation is essential for carcinogenesis**, because lethal damage cause death of cells. Mutation may be:
   - **Inherited** in the germline and occurs in certain families.
   - **Acquired** by the action of environmental agents (e.g., chemicals, viruses or radiation) and result in sporadic cancers.
3. **Tumors are monoclonal,** i.e., they originate from a clonal proliferation of a **single type of progenitor cell that has undergone genetic damage**.
4. **Carcinogenesis is a multistep process that occurs over time. This is the result of accumulation of complementary mutations.**
   - **Cancer hallmarks:** This **represents phenotypic properties of malignant neoplasms.** This includes excessive growth, local invasiveness and the ability to form distant metastases. These cancer hallmarks are due to genomic alterations which change the expression and function of key genes and thereby impart a malignant phenotype. Driver and passenger mutation (*refer* below).
5. **Four classes of normal regulatory genes** are the **main targets of genetic damage**.
   - **Growth-promoting proto-oncogenes** (*refer* below).
   - **Growth-inhibiting tumor suppressor genes:** They normally **prevent uncontrolled growth** (*refer* page 211).
   - **Genes involved in DNA repair** (*refer* page 222).
   - **Genes that regulate programmed cell death** (apoptosis) (*refer* page 218).
6. **Failure to differentiate:** The cancer cells arrest at a stage before their terminal differentiation and may retain their stem cell properties.

Four types of genes involved in neoplasia:
1. Oncogenes
2. Tumor suppressor genes
3. DNA repair genes
4. Genes involved in apoptosis

Loss-of-function mutation: Mutation that results in reduced or abolished protein function.
Gain-of-function mutations: Less common and causes abnormal activity of protein. It can take two forms:
1. Increase in a protein's normal function (e.g., excessive enzymatic activity) and
2. Impart a completely new activity unrelated to the affected protein's normal function.

STEPS IN NORMAL CELL PROLIFERATION

Normal cell follows a controlled proliferation. The different sequential steps are:

1. **Growth factors binding** to its **specific cell surface receptor**.
2. **Transient and limited activation** of the **growth factor receptor** → activates signal-transducing proteins on the inner aspect of the cell membrane. Following this signaling, the receptor reverts to its resting state.
3. **Intracellular signal transduction:** Most of the signal-transducing proteins are located on the inner aspect of the plasma membrane. They **receive external signals** and get activated (by binding of growth factor to its growth factor receptors) and **transmit the growth signal across the cytoplasm → to the nucleus of the cell**. The most important signal-transducing protein belongs to **RAS family and ABL**.
4. **Transcription:** Activation of nuclear regulatory factors → initiates DNA transcription.
5. **Cell cycle:** Entry and progression of the cell into the cell cycle → resulting in cell division.

HALLMARKS OF CANCER

Q. Enumerate steps of malignant transformation of a cell.

Normal cell may undergo malignant transformation by corrupting any one of the normal steps involved in cell proliferation.

1. Increased action of positive growth regulators.
2. Loss of function of negative growth regulators.
3. Altered cellular metabolism
4. Loss of normal apoptosis pathways.
5. Loss of replicative senescence.
6. Increased angiogenesis.
7. Ability to invade and metastasize (*refer* pages 201-3).
8. Evasion of the host immune response.

Deregulated cell proliferation: Increased action of positive growth regulators (oncogenes, i.e., Ras, Myc) and loss of function of negative growth regulators (suppressor oncogenes, i.e., Rb, *p53*) leads to aberrant cell cycle control including loss of normal checkpoint responses.

Increased Action of Positive Growth Regulators: Oncogenes

Q. Define proto-oncogenes and write short note oncogene. List the different products of oncogenes.

Q. Describe the mechanism of activation of oncogene giving suitable examples.

Proto-oncogenes are **normal cellular genes**, which encode a number of nuclear proteins that **regulate normal cell proliferation, differentiation and survival**. Proto-oncogenes have multiple roles, but all act at some level in signaling pathways involved in proliferation of cells.

Oncogenes and oncoproteins: Mutation of normal cellular genes known as proto-oncogenes produces genes that lead to tumor formation and these **altered/mutated versions of proto-oncogenes are termed as oncogenes.** These oncogenes promote autonomous cell growth in cancer cells. These oncogenes usually produce increased encoded **gene product called oncoprotein** and cause tumors. These mutations are called as **"gain-of-function," mutations (Fig. 7.19) because they can transform cells even in the presence of a normal copy of the same gene.** Thus, oncogenes are

dominant over their normal counterparts and behave as dominant genes. Mutation of a single allele is sufficient to produce a pro-oncogenic effect.

- **Oncogenes** have the ability to **promote cell growth in the absence** of external normal growth-promoting/**mitogenic signals/stimuli**.
- **Products** of oncogenes → are called **oncoproteins**, which resemble the normal products of proto-oncogenes.
- **Oncoprotein production** is **not under normal regulatory control** → cells proliferate without the usual requirement for external signals and are freed from checkpoints → **growth becomes autonomous**. **Oncoproteins act like accelerators that speed the replication of cells and their DNA. In contrast, tumor suppressors act as brakes that slow or arrest this process.**

Classification of oncogenes: Oncogenes can be classified **(Table 7.9)** according to the function of gene product (oncoprotein) as:

- Growth factors
- Growth factor receptors
- Signal transduction proteins
- DNA-binding nuclear regulatory proteins/transcription factors
- Cell cycle regulators.

Growth Factor Oncoproteins

- Normal cell proliferation requires stimulation by growth factors.
- Neoplasm may be associated with **excessive production of growth factors** by oncogenes.

TABLE 7.9: Categories of oncogenes and examples of associated tumors.

Category of oncogene	*Proto-oncogene*	*Examples of associated tumors*
Growth Factors		
PDGF-β chain	PDGFB	Astrocytoma
Fibroblast growth factors	HST1	Osteosarcoma
	FGF3	Cancer of stomach, bladder, breast and melanoma
HGF	HGF	Hepatocellular carcinomas, thyroid cancer
Growth Factor Receptors		
EGF-receptor family	ERBB1 (EGFR)	Adenocarcinoma of lung
ALK receptor	ALK	Adenocarcinoma of lung certain lymphomas, neuroblastoma
Receptor for neurotrophic factors	RET	Multiple endocrine neoplasia 2A and B, familial medullary carcinoma thyroid
Receptor for KIT ligand	KIT	Gastrointestinal stromal tumors, seminomas, leukemias
FMS-like tyrosine kinase 3	FLT3	Leukemia
PDGF receptor	PDGFRB	Gliomas, leukemias
Signal Transduction Proteins		
GTP-binding (G) proteins	KRAS	Tumors of colon, lung and pancreas
	HRAS	Tumors of bladder and kidney
	NRAS	Melanomas, hematologic malignancies
RAS signal transduction	BRAF	Melanomas, leukemias, colon carcinoma
Nonreceptor tyrosine kinase	ABL	Chronic myelogenous leukemia, acute lymphoblastic leukemia
JAK/STAT signal transduction	JAK2	Myeloproliferative neoplasms, acute lymphoblastic leukemia
Notch signal transduction	NOTCH1	Leukemias, lymphomas, breast carcinoma
Nuclear Regulatory Proteins/Transcription Factors		
Transcriptional activators	MYC	Burkitt lymphoma
	NMYC	Neuroblastoma
Cell Cycle Regulators		
Cyclins	CCND1 (Cyclin D1)	Mantle cell lymphoma, multiple myeloma, breast and esophageal cancers
Cyclin-dependent kinase	CDK4	Glioblastoma, melanoma, sarcoma

Proto-oncogenes: Discovered by Harold Varmus and Michael Bishop.

- **Action of growth factor oncoprotein:** These oncoproteins may act by one of the two ways: (1) Paracrine or (2) autocrine action.
- Example: In **glioblastomas** (malignant glial cell tumors) the tumor cells itself secrete **excess platelet-derived growth factor (PDGF**) and express **PDGF receptor tyrosine kinases.**

Growth Factor Receptor Oncoproteins

Normally, when the growth factor binds to the growth factor receptors, it produces transient dimerization (activity). **Constitutive** (unrestrained) **dimerization** of growth factor receptors → produces continuous mitogenic signals to the cell, **even in the absence of the growth factor**.

Mechanism of activation of **receptor tyrosine kinases**: Growth factor receptors can be constitutively activated in tumors by multiple mechanisms,

including point mutations, gene rearrangements and gene amplifications.

1. **Point mutation:** ***ERBB1*** point mutation in a subset of lung adenocarcinomas.
2. **Gene amplification:** ***ERBB2*** **(also called** ***HER-2/Neu***) gene is amplified in certain **breast cancers**.
3. **Gene rearrangements:** They activate other receptor tyrosine kinases (e.g., tyrosine kinase ALK). Example: A deletion on chromosome 5 results in fusion of part of the *ALK* gene with part of another gene called *EML4* in a subset of lung adenocarcinomas resulting in *EML4-ALK* fusion gene.

Signal-transducing Oncoproteins

GTP-binding (G) Proteins: Normal RAS Cycle (Fig. 7.16)

- RAS proteins (product of *RAS* gene) are attached to the cytoplasmic aspect of the plasma membrane by farnesyl (also the endoplasmic reticulum and Golgi membranes).
- Normally, RAS proteins orderly cycle between inactive state [RAS proteins bound to guanosine diphosphate (GDP)] and active signal-transmitting state (RAS is bound to GTP).
- Stimulation of cells by growth factors activate RAS. The active GTP state is short-lived because an enzyme GTPase hydrolyzes GTP → GDP.

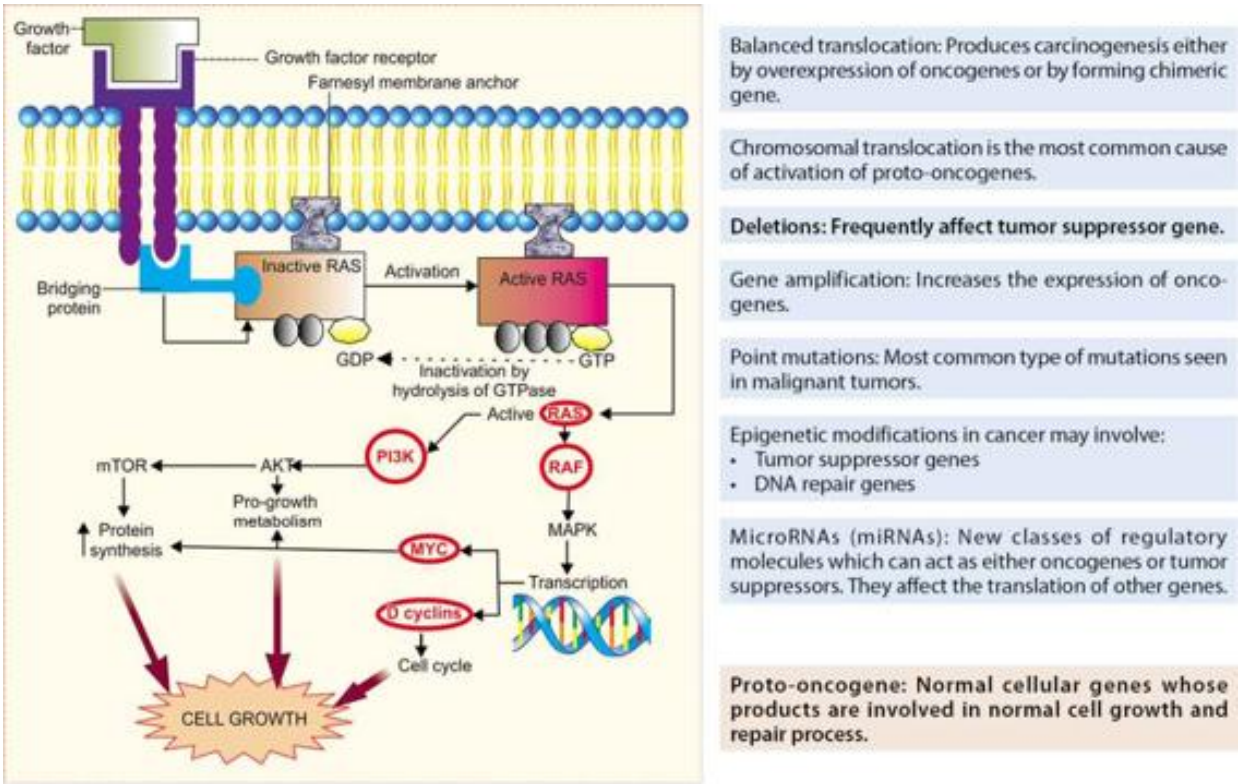


Fig. 7.16: Normal RAS cycle and growth factor signaling pathways in cancer. RAS is anchored to the cell membrane by the farnesyl moiety and is essential for its action. When a growth factor binds to growth factor receptor, inactive (GDP bound) RAS become activated to a GTP bound state. The active GTP state is short-lived because an enzyme GTPase hydrolyses GTP to GDP. Activated RAS in turn transduces proliferative signals to the nucleus. Growth factor receptors, RAS, PI3K, MYC and D cyclins are oncoproteins. These are activated by mutations in various cancers.

(mTOR: mammalian target of rapamycin)

- Activated RAS stimulates downstream regulators of cell proliferation by two pathways: (1) RAF/ERK/MAP kinase pathway and (2) PI3 kinase/AKT pathway, which in turn send the signal to the nucleus resulting in cell proliferation.

RAS oncogenes

Q. Write short note on RAS oncogene.

RAS proto-oncogene can be converted to RAS oncogene by mutation (mainly point mutation). The mutated *RAS* is trapped in its activated GTP-bound form → results in continuous proliferation of cells.

- **Tumors with *RAS* mutations**: Human genome contains three types of *RAS* genes.
  - ***KRAS:* Mutation in adenocarcinomas of colon, lung and pancreas**
  - ***HRAS:* Mutations in bladder and kidney tumors**

- ***NRAS:*** **Mutations in melanoma, hematopoietic tumors.**

BRAF mutations: BRAF is a serine/threonine protein kinase belonging to MAPK family. Similar to activating RAS mutations, activating point mutations in BRAF activate transcription factors. Mutations of BRAF is seen in hairy cell leukemias, melanomas, benign nevi and in few cancers of colon. Activation of the PI3K by point mutations also occurs in many cancers.

Nonreceptor tyrosine kinase—ABL

ABL is a non-receptor-associated tyrosine kinase which functions as signal transduction molecule. ***ABL*** is a proto-oncogene and has a tyrosine kinase activity.

- In **chronic myelogenous leukemia**, ***ABL*** proto-oncogene from chromosome 9 **joins** the ***BCR*** on chromosome 22 **(***refer* **Fig. 12.16)**. It produces a new **chimeric (fusion) gene called *BCR-ABL*, thus converting *ABL* proto-oncogene into oncogene → oncoprotein** (e.g., **p210) → causes cell division** and **inhibition of apoptosis**.
- Point mutation of ABL: In acute lymphoblastic leukemia.

DNA-binding Nuclear Regulatory Proteins (Transcription Factors)

Q. Write short note on MYC oncogene.

- All signal transduction pathways stimulate nuclear transcription factors, which bind DNA and regulate transcription of genes.
- Transcription is a process in which RNA is synthesized from DNA. **Transcription factors stimulate growth-promoting genes which activate cell cycle**.
- Tumor may develop due to mutations of transcription genes. The mutation results in oncogenes like ***MYC***, ***MYB***, ***JUN***, ***FOS*** and ***REL*****,** whose products (oncoproteins) are transcription factors that regulate the expression of growth-promoting genes, such as cyclins. ***MYC*** **is most commonly involved in human tumors**.

MYC Oncogene

- **MYC proto-oncogene is expressed in all cells during normal cell proliferation. MYC activates the expression of several genes involved in cell growth.** These include D cyclins (involved in cell cycle progression) and rRNA genes and rRNA processing (increases the assembly of ribosomes needed for protein synthesis). It also upregulates gene expression that results in metabolic reprogramming and the Warburg effect. Because of their several effects, *MYC* is considered a **master transcriptional regulator of cell growth**, e.g., rapid growth in Burkitt lymphoma has chromosomal translocation involving *MYC* and has highest level of *MYC*.
- **In few tumors, *MYC* upregulates expression of telomerase (responsible for unlimited replication**—the immortalization of cancer cells).
- **MYC is one of transcription factor which can reprogram somatic cells into pluripotent stem cells** thereby leading to immortalization of cancer cells.

Mechanism of deregulation of MYC

1. **Genetic alterations of *MYC*** itself causes overexpression of the *MYC* protein.
2. **MYC translocations** (e.g., *C-MYC* in Burkitt lymphoma).
3. **MYC amplifications** (e.g., some carcinoma of breast, colon, lung, etc). Functionally identical *N-MYC* gene amplification in neuroblastomas and *L-MYC* genes amplification in small cell cancers of the lung.
4. **Oncogenic mutations of upstream signaling pathways:** These may cause increased levels of MYC protein by increasing *MYC* transcription, increasing *MYC* mRNA translation, and/or stabilizing MYC protein.

Cyclins and Cyclin-dependent Kinases (CDKs)

All growth-promoting stimuli, finally, causes the entry of quiescent cells into the cell cycle. The cell cycle is regulated by cyclins and cyclin-dependent kinases.

Role of Cyclins in Normal Cell Cycle

Q. Write short note on role of cyclins in the cell cycle.

- The various phases of the cell **cycle are regulated by cyclins** (named so because of cyclic nature of their production and degradation) and **cyclin-dependent kinases** (CDKs).
- The CDK–cyclin complexes phosphorylate essential proteins which activate the cell cycle, following which the cyclin levels decline quickly.
- Of the several (more than 15) distinct set of cyclins; **cyclins D, E, A** and **B** are important which appear sequentially (one after another) during the cell cycle.
- While cyclins and CDKs drive the cell cycle, **negative control over the cell cycle** is achieved by **silencing the CDKs by their inhibitors (CDKIs)**.

TABLE 7.10: Role of cyclins and cyclin-dependent kinases in regulation of cell cycle.

Type of cyclin and cyclin-dependent kinase (CDK)	***Phase of cell cycle***
Cyclin **D**/CDK**4** Cyclin **D**/CDK**6** Cyclin **E**/CDK**2**	Regulation of transition from $\mathbf{G_1}$ to **S** phase by phosphorylation of RB protein
Cyclin **A**/CDK**2** Cyclin **A**/CDK**1**	Active in **S** phase
Cyclin **B**/CDK**1**	Essential for transition from $\mathbf{G_2}$ to M phase

Role of cyclins and CDKs in regulating cell cycle are mentioned in **Table 7.10**.

Alteration in Cell Cycle Control Proteins in Cancer

Cell cycle has two main checkpoints: (1) At the G_1/S transition and (2) at the G_2/M transition. Both are tightly regulated by a balance of growth promoting and growth suppressing factors and also by sensors of DNA damage. If DNA-damage sensors are activated, they arrest the progression of cell cycle and allow the repair of DNA by DNA-repair genes. If cell damage cannot be repaired, these sensors initiate apoptosis of the cells. Defects in the G_1/S checkpoint are more important in cancer than G_2/M checkpoint. The major cancer-associated mutations that affect the G_1/S checkpoint are mainly divided into two groups.

1. **Gain-of-function mutations in D cyclin genes and CDK4:** These oncogenes **promote G_1/S progression.**
   - **D cyclin genes:** These include D1, D2 and D3 and they undergo mutations in cancer by chromosomal translocations (e.g., lymphomas) and gene amplification (e.g., solid tumors).
   - **Amplification of the *CDK4* gene:** It is observed in melanomas, sarcomas and glioblastomas.
2. **Loss-of-function mutations in tumor suppressor genes that inhibit G_1/S progression:** CDKIs which inhibit cyclin D/CDK complexes are mutated or silenced in some malignant tumors, e.g., deletion or inactivation of CDKN2A (p16) in pancreatic carcinomas, glioblastomas, esophageal carcinoma, acute lymphoblastic leukemias, and non-small-cell lung carcinomas. Apart from this, two most important tumor suppressor genes, *RB* and *TP53,* encode proteins which prevent G_1/S progression.

Above mutations with activation of cyclin D or CDK4 and mutational inactivation of CDK inhibitors cause proliferation of cells by hyperphosphorylation and inactivation of RB. This causes release of E2F transcription factors which leads to the expression of genes required for progression from G_1 to S phase.

Oncogene: Mutated or overexpressed version of proto-oncogene. They function autonomously without requiring normal growth-promoting signals. They are not under normal regulatory control.

Oncoproteins: Products of oncogenes that cause uncontrolled proliferation of cells by several mechanisms.

Growth factor oncoproteins—PDGF: Excessively produced in glioblasoma.

Growth factor receptor oncogene-ERBB2 (Her-2/Neu) is overexpressed in: Breast carcinoma, non-small-cell lung carcinoma, ovarian carcinoma

RAS family of oncoproteins is an example of signal-transducing proteins

Point mutation of *RAS* genes is the most common, frequent and dominant cause of human tumors.

***N-MYC* amplification is associated with: Neuroblastoma. L-MYC in small cell carcinoma of lung.**

Transition from G_1 to S phase of the cell cycle is controlled by: Cyclin D.

Cyclin D is the first cyclin to increase in the cell cycle.

Loss of Function of Negative Growth Regulators (Tumor Suppressor Genes)

Q. Write short note on tumor suppressor genes and cancers produced by their mutations.

Tumor suppressor is a protein or gene which is associated with suppression of any of the various hallmarks of cancer. As discussed earlier, **oncogenes stimulate proliferation of cells,** whereas **the products of most tumor suppressor genes apply brakes and prevent uncontrolled cell proliferation.** Tumor suppressor proteins form a network of checkpoints and act as negative growth regulators. They prevent uncontrolled growth. Abnormalities in these tumor genes lead to loss of function of negative growth regulators, i.e., **failure of growth inhibition.** So, a second mechanism of carcinogenesis results from failure of negative growth regulator (growth inhibition), due to deficiency of normal tumor suppressor genes and their products.

General Characteristic Features of Tumor Suppressor Genes

Q. Define cancer suppressor gene and cancers produced by their mutations.

- **Mechanism of action:** Most tumor suppressors inhibit cell growth through one or other mechanism. Mutations that affect tumor suppressor genes usually cause a "loss-of-function."
  - **Apply brakes to cell proliferation:** Many tumor suppressors (e.g., two important tumor suppressor genes *RB* and *p53*) are part of a regulatory network and they **apply the brakes on cell cycle progression and DNA replication.** They recognize genotoxic stress from any source and prevent proliferation of these cells. Thus, an oncogene in normal cells with intact tumor suppressor genes may result in quiescence, or permanent arrest of cell cycle (oncogene-

induced senescence), rather than uncontrolled proliferation. These cells may ultimately undergo apoptosis. Abnormalities in these genes lead to failure of growth inhibition.

- **Other mechanisms:** Some tumor suppressors prevent cellular transformation through other mechanisms. These include **by altering cell metabolism** (e.g., the serine-threonine kinase STK11) or **by maintaining genomic stability** (e.g., the DNA repair factors *BRCA1* and *BRCA2*).

- **Mutations of tumor suppressor genes may be hereditary and spontaneous**.
- **Loss of heterozygosity:**
  - Usually, for tumor to develop, **both normal alleles of tumor suppressor genes must be inactivated (damaged/mutated)**.
  - Heterozygous state (one allele normal and other allele inactive) is sufficient to protect against cancer.
  - Cancer develops when the cell loses heterozygosity (known as loss of heterozygosity—**LOH**) for the normal tumor suppressor gene by **deletion or somatic mutation**. Tumor can develop when the cell becomes homozygous (both alleles are inactive) for the mutant allele. Thus, mutated tumor suppressor genes usually behave in a recessive fashion. However, sometimes, loss of a single allele of a tumor suppressor gene can lead to cell proliferation. When loss of gene function is caused by damage to a single allele, it is called haploinsufficiency.
- **Groups of tumor suppressor genes:** (a) Governors and (b) Guardians.
  - **Governor gene** mutations → remove the brake for cellular proliferation → neoplasia, e.g., *RB* gene.
  - **Guardian genes** sense the genomic damage and prevents proliferation of cells with genetic damage or if damage is too severe to be repaired → induces apoptosis, e.g., *p53*.

Retinoblastoma Gene (RB Gene)

Q. Write short note on Knudson's two-hit hypothesis.

***RB (RB1)* gene** was the **first discovered tumor suppressor gene**, which is present **on chromosome locus 13q14**. **Inactivation of *RB* gene was found**

in retinoblastoma, which is a rare malignant childhood tumor derived from the retina. Retinoblastoma may occur either as a hereditary or sporadic form.

Knudson's two-hit hypothesis of oncogenesis: It explains the inherited and sporadic occurrence of an identical tumor. According to Knudson's hypothesis:

- **Two mutations (hits),** involving **both alleles** of tumor suppressor gene **are required to produce the tumor**.
- **In familial cases, one mutation (first hit) takes place in the germline** and **second hit after birth.**
- In **sporadic cases both mutations** (two hits) **develop after birth**.

RB Gene and Retinoblastoma

Q. Write short note on retinoblastoma gene.

1. **Familial/hereditary retinoblastoma**: It constitutes about 40% of retinoblastoma and two hits occurs as follows:
   - **First hit**: Affected **children inherit** cells with **one defective copy** (mutated allele) of the ***RB* gene in the germline** (one hit) and one normal copy of *RB* gene (the child is heterozygous at the *RB* locus). The product of normal *RB* gene is sufficient to prevent tumor.
   - **Second hit**: **Retinoblastoma develops when** the remaining normal *RB* allele is inactivated (mutated) due to **spontaneous somatic mutation** (second hit). Because only a single somatic mutation is sufficient for loss of *RB* function in familial retinoblastoma, (it is **transmitted as an autosomal dominant trait**).

Patients with **familial retinoblastoma** have also **increased risk** of developing **osteosarcoma** and other soft-tissue sarcomas.

2. **Sporadic retinoblastoma:** It forms about 60% of cases. The child has two normal *RB* alleles in all somatic cells. To develop retinoblastoma, **both normal *RB* alleles must undergo mutation** and it **needs two hits**.

Normal cell cycle has two gaps (*refer* pages 75 and **Fig. 3.11**):

1. **Gap 1** (G_1) between mitosis (M) and DNA replication (S). Gap 1 is **very important checkpoint**, because once the cells cross this checkpoint they are compelled to complete mitosis. In G_1 phase, **signals determine whether the cell should enter the cell cycle**, **exit** the cell cycle either

temporarily (known as quiescence), or permanently (known as senescence). ***RB* plays a key role in this decision process**.

2. **Gap 2** (G_2) between DNA replication (S) and mitosis (M).

Functions of the RB gene (Fig. 7.17)

Q. Write short note on role of RB in the cell cycle.

Q. Write short note on function of retinoblastoma gene.

***RB* gene is governor of cell cycle** and plays a key role in **regulating the cell cycle** and also controls cellular differentiation.

State of *RB* gene product: *RB* gene product is a DNA- binding protein expressed in all cells. It is present either in an **active hypophosphorylated** state (in quiescent cells) or **inactive hyperphosphorylated** state (in cells passing through the G_1/S cell cycle phase).

Active *RB* gene regulates G_1/S checkpoint of cell cycle: Cell cycle is tightly controlled by cyclins and cyclin-dependent kinases (CDKs), which form cyclin-CDK complexes.

Before DNA replication, the cell must pass through G_1/S check which is regulated by *RB*.

- **Initiation** of DNA replication (S phase) requires activation of cyclins D/CDK4, cyclin D/CDK6 and cyclin E/CDK2 complexes. High levels of these complexes lead to hyperphosphorylation and inhibition of RB. This releases E2F transcription factors which causes the expression of genes that are required for progression of cell from G_1 to S phase.

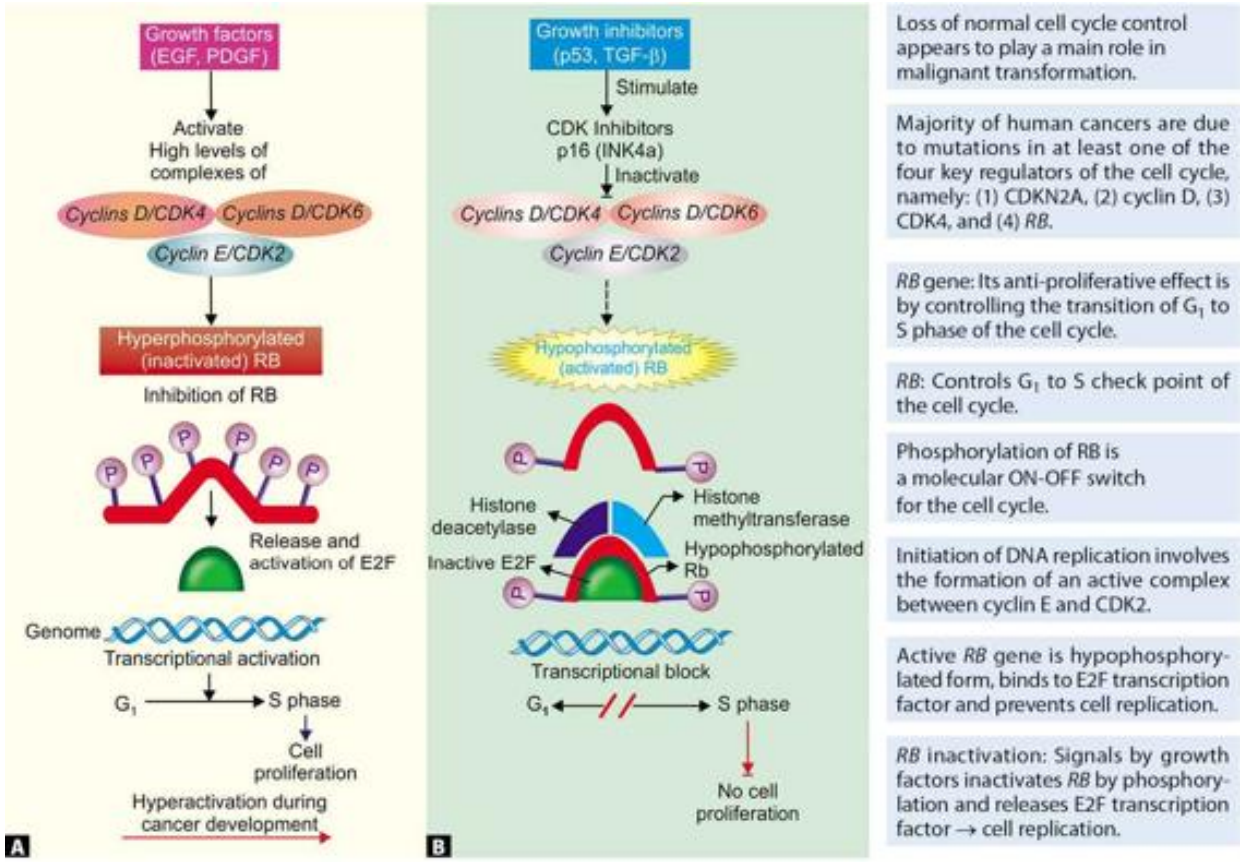


Fig. 7.17: Function of *RB* in regulating the G_1-S checkpoint of the cell cycle: (A) When *RB* is phosphorylated by the cyclin D–CDK4/6 complexes, it releases E2F. The latter then activates transcription of S-phase genes; (B) Hypophosphorylated active *RB* combines with the E2F transcription factors along with histone deacetylases and histone methyltransferases, and inhibits progression from G_1-S phase of cell cycle.

- ***RB* blocks E2F-mediated transcription**: During early G_1 phase, **active hypophosphorylated** RB binds to E2F family of transcription factors. Two methods of blocking transcription are:
  1. **Sequesters E2F** and prevent it from interacting with other transcription activators.
  2. Recruits two enzymes (histone deacetylases and histone methyltransferases) that **block the transcription**.

Inactivation of *RB* gene: Growth factor (mitogenic) signaling → upregulate the activity of the CDK/cyclin complexes → conversion of active hypophosphorylated RB into inactive hyperphosphorylated RB.

- **Consequence of inactivation of *RB* gene:** Inactivation of RB release the break and frees the transcription factor E2F from RB → DNA replication → progression of cell cycle.

Reactivation of *RB* gene*:*** During M phase phosphate groups are removed from hyperphosphorylated RB by cellular phosphatases → regeneration of active hypophosphorylated RB.

Method of Inactivation of RB Gene and Associated Tumors

1. **Loss-of-function mutations involving both *RB* alleles.** It may be:
   - **Germline mutation,** e.g., in **retinoblastomas** and **osteosarcomas**.
   - **Acquired mutation,** e.g., in **glioblastomas, small-cell carcinomas of lung,** breast cancers and bladder carcinomas.
2. **Other mechanism:** The active hypophosphorylated RB state may be shifted to an inactive hyperphosphorylated RB state. This may be due to (1) gain-of-function mutations that upregulate CDK/cyclin D activity or (2) by loss-of-function mutations that abolish/cancel the activity of CDK inhibitors (p16/INK4a).
3. **Viral oncoproteins that bind and inhibit RB (E7 protein of HPV)** may occur even without *RB* mutation, e.g., E7 protein of human papilloma virus (HPV) bind to the hypophosphorylated RB → prevents binding of RB protein with E2F transcription factors → free E2F causes progression of cell cycle → cervical carcinomas **(Fig. 7.21).**

In the majority of cancers at least one of four key regulators of the cell cycle, namely: (1) p16/INK4a, (2) cyclin D, (3) CDK4, or (4) RB is dysregulated.

TP53 Gene (Guardian of the Genome)

Q. Write short note on p53/TP53 gene and its role in neoplasia.

TP53 is a **tumor suppressor gene located on small arm of chromosome 17(17p13.1)**. Its protein product *p53* is present in almost all normal tissues. Loss-of-function mutations in *TP53* is the most common mutations observed in more than 50% of cancers. *TP53* mutations occur at variable frequency with almost every type of cancer, including the three leading causes of cancer death namely carcinomas of the lung, colon and breast.

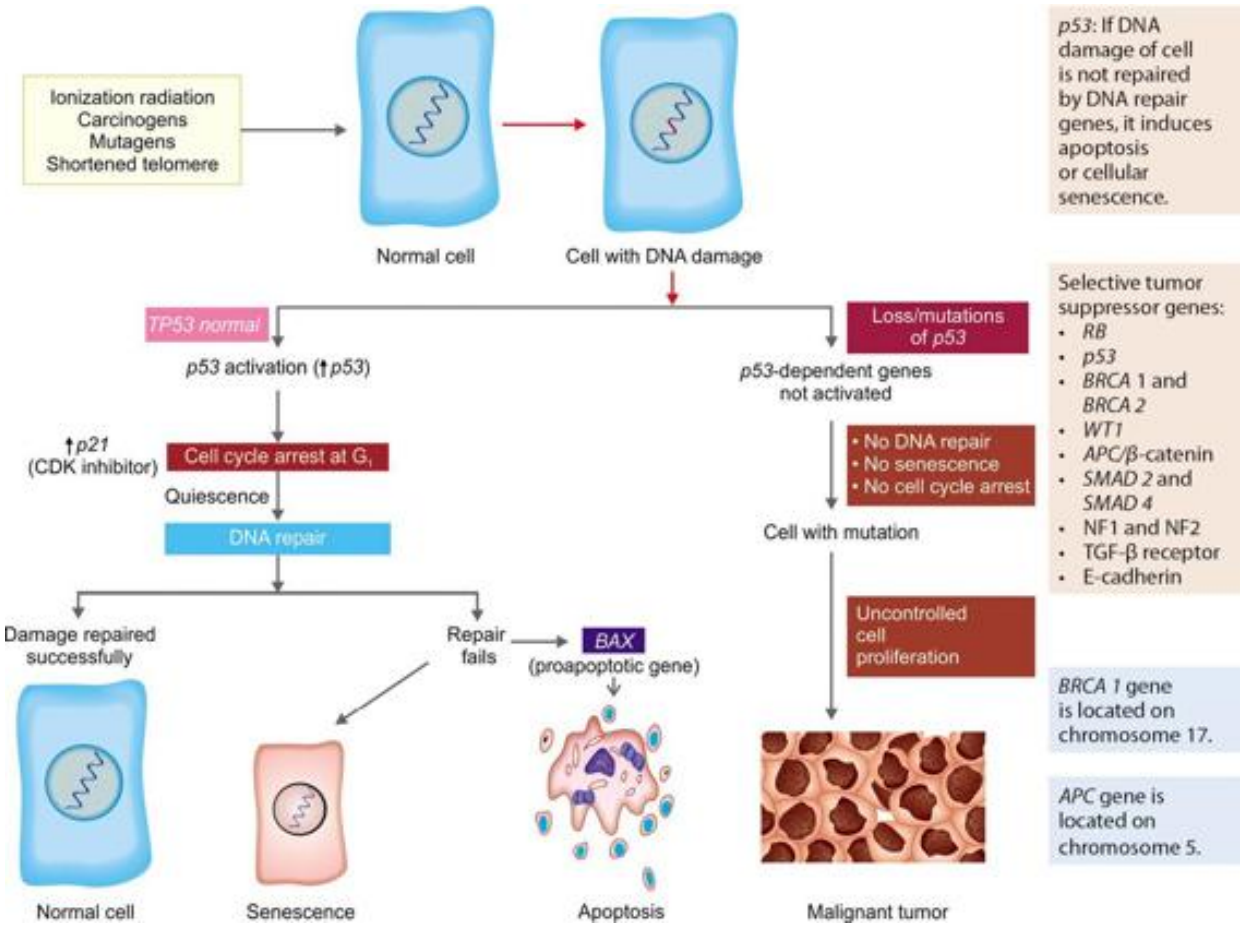
Functions of *p53* (Fig. 7.18)

Q. Why p53 gene is called "Guardian of genome".

Guardian of the genome: It functions as **critical gatekeeper genes**. It plays **main role in maintaining the integrity of the genome** and thus known as **guardian of the genome** or **"molecular policeman."**

Role of TP53

TP53 has critical role in the prevention of cancer development and *p53* serves as focal point of large network of signals which sense cellular stress, DNA damage, shortened telomeres, hypoxia and stress caused due to increased pro-growth signaling (e.g., cells with mutations in *RAS* and *MYC* genes).



p53 gene located on small arm of chromosome 17(17p13).

p53: DNA oncogenic viruses (e.g., HPV) encode proteins that bind to *p53* and blocks its function.

Fig. 7.18: Role of *p53* in maintaining the integrity of the genome. DNA damage activates normal *p53* and arrests the cell cycle in G_1 and induces repair of DNA. Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, *p53* triggers either apoptosis or senescence. In cells with loss or mutations of *p53*, DNA damage does not induce cell cycle arrest or DNA repair or senescence, and cells with mutation proliferate to form malignant neoplasms.

- **In nonstressed, healthy/normal cells, *p53* is maintained at low levels** by MDM2 (murine double minute). MDM2 is an E3 ubiquitin (Ub) ligase that conjugates *p53* to Ub and degrades *p53*.
- **In stressed cells**, *p53* is released from the inhibitory effects of MDM2 **and *p53* becomes activated.** Activation of *p53* may occur through two mechanisms that depend on the nature of the stress.
  - **DNA damage and hypoxia:** Stress due to DNA damage or hypoxia activates two related protein kinases, namely: (1) ataxia-telangiectasia mutated (ATM) and (2) ataxia-telangiectasia and Rad3 related (ATR). ATM gene was first identified as the germline mutation in patients

with ataxia-telangiectasia (inability to repair certain kinds of DNA damage, and have increased incidence of cancer). Activated ATM and ATR stimulate the phosphorylation of *p53* and MDM2. This disrupts the binding and degradation of *p53* by MDM2 and leads to activation and accumulation of *p53*.

- **Oncogenic stress:** It may be induced by activation of oncoproteins such as RAS. These stresses produce sustained signaling via pro-growth pathways (e.g., MAPK and PI3K/AKT pathways). These signals produce cellular stress and lead to increased expression of p14/ARF (encoded by the *CDKN2A* tumor suppressor gene). p14/ARF binds MDM2 and releases *p53* and resulting in raised *p53* levels in the cell.

Prevention of neoplastic transformation

Activated *p53* prevents neoplastic transformation of cell by three interconnected mechanisms:

1. **Transient/temporary *p53*-induced cell cycle arrest:** If there is **damage to DNA**, transient, rapid cell cycle arrest occurs late in the G_1 phase. It is brought-out partly by *p53*-dependent transcription of the *CDKN1A* gene (encodes the CDK inhibitor *p21*). *p21* in turn inhibits CDK4/D cyclin complexes and maintain RB in an active, hypophosphorylated state. This blocks the progression of cells from G_1 phase to S phase. This cell cycle arrest gives the cells time to repair DNA damage. **If DNA damage is repaired,** the signals that caused stabilization/activation of *p53* disappears. This results **in fall in the levels of *p53*** and **releases the block in cell cycle** and return of cells to a normal state.
2. ***p53*-induced senescence (permanent cell cycle arrest):** Senescence is defined as a state of permanent cell cycle arrest. Senescence may be stimulated in response to different types of stresses (e.g., unopposed oncogene signaling, hypoxia and shortened telomeres). The senescent cells are prevented from forming tumors.
3. ***p53*-induced apoptosis (programmed cell death): Cells with irreversible DNA damage undergo *p53*-induced apoptosis** and is the protective mechanism against development of cancer. *p53* stimulates transcription of several pro-apoptotic genes (e.g., *BAX* and *PUMA*) resulting in apoptosis of cells via the intrinsic (mitochondrial) pathway.

Method of Inactivation of *TP53* Gene and Associated Tumors

Most cancers have defect in *TP53* gene.

1. **Acquired** loss-of-function **mutation in both** (biallelic) *TP53* **alleles** in somatic cells is most common.
2. **Germline mutations in one *TP53* allele:** It is less common. Individuals may **inherit one mutated/defective *TP53* allele and one additional "hit" in the other normal *TP53* allele** will produce malignant tumors. For example, Li–Fraumeni syndrome has germline mutations in one *TP53* and these individuals usually develop cancer at younger age, have 25-fold greater chance of developing a malignant tumor by age 50 and are more prone to develop multiple primary tumors of varying types.
3. **Mutations of proteins that regulate *p53* function:** *TP53* encodes the **protein *p53*, the function** of which is **tightly regulated at several levels by other proteins.** Thus, many **tumors without *TP53* mutations have mutations of proteins that regulate *p53* function.** For example, MDM2 and related proteins of the MDM2 (enzyme that ubiquitinylates *p53*) family degrade *p53* leading to a functional deficiency of *p53*. These proteins are frequently overexpressed in cancers with normal *TP53* alleles.
4. **Blocking of *p53* function:** Similar to RB, the **transforming proteins of many DNA viruses bind and degrade *p53*** even without mutation in *p53*. For example, viral oncoprotein E6 of high-risk human papilloma viruses (HPVs) promote *p53* degradation and cause cervical carcinoma and a subset of squamous cell carcinomas of the head and neck.

Consequences of loss of p53 function

- DNA damage goes unrepaired.
- Driver mutations accumulate in oncogenes and other cancer genes.
- Cell blindly follows a dangerous path leading to malignant transformation.

Therapeutic Implications of *TP53*

- **Wild type versus mutated *TP53:*** Irradiation and chemotherapy used for the treatment of cancer, mediate their effects by causing damage to the

DNA and producing apoptosis of tumor cells. Tumors with **wild type *TP53*** (wild type refers to the most common form or phenotype in nature) alleles are **more susceptible for apoptosis than tumors with mutated *TP53* alleles.** For example, childhood acute lymphoblastic leukemias which have wild type *TP53* alleles respond to radio and chemotherapy; whereas lung cancers and colorectal cancers with mutated *TP53* allele, are relatively resistant to chemotherapy and irradiation.

- **Consequences of mutated *TP53:*** Tumor cells with mutated *p53* **have a tendency to acquire additional mutations** at a high rate and are **resistant to any mono/single therapy** (radiation/conventional chemotherapy/molecularly targeted therapy).

Other *p53* family members: These include *p63* and *p73*. *p53* is universally expressed, whereas *p63* and *p73* show more tissue specificity. For example, *p63* is required for the differentiation of stratified squamous epithelium and p73 has powerful pro-apoptotic effects after DNA damage produced by chemotherapeutic drugs.

Location, function and tumors associated with few selected tumor suppressor genes are presented in**Table 7.11**.

Figure 7.19 shows gain of function in oncogene and loss of function mutation in tumor suppressor gene.

Tumor suppressor gene *p53* induces cell cycle arrest at: G_1 to S phase

Transition from G_2 to M phase of the cell cycle is controlled by: Cyclin B.

Fixed time is required for steps of cell cycle: S and M phase

Cellular content of DNA is doubled during S phase of the cell cycle.

Correct sequence of cell cycle is: G_0-G_1-S-G_2-M.

Note: Gene symbols are italicized but not their protein products.

Tumor suppressor genes: Protect the cells against unregulated proliferation.
Tumor suppressor gene: For tumor formation both copies of genes must be inactivated.

RB gene: First discovered tumor suppressor gene.
***RB* gene is present on chromosome 13q14.**
RB gene product is *RB* protein.

Patients with *RB* mutations have increased risk:
1. Retinoblastoma

2. Osteosarcoma
3. Soft-tissue sarcomas

Q. Write short note on familial syndromes/inherited cancers.

TABLE 7.11: Few tumor suppressor genes and associated familial syndromes and sporadic cancers.

Gene (protein)	*Function*	*Familial syndromes*	*Sporadic cancers*
Inhibitors of mitogenic signaling pathways			
APC (Adenomatous polyposis coli)	Inhibits Wnt signaling	Familial colonic polyps and carcinomas	Carcinomas of stomach, colon, pancreas; melanoma
NF1 (Neurofibromin-1)	Inhibits RAS/MAPK signaling	Neurofibromatosis type 1	Neuroblastoma
PTEN (Phosphatase and tensin homologue)	Inhibits of PI3K/AKT signaling	Cowden syndrome	Carcinomas and lymphoid tumors
SMAD2, SMAD4 (SMAD2, SMAD4)	TGF-β signaling pathway	Juvenile polyposis	Carcinoma of colon and pancreas
Inhibitors of cell cycle progression			
RB (Retinoblastoma protein)	G_1/S transition during cell cycle	Familial retinoblastoma syndrome	Retinoblastoma; osteosarcoma, carcinomas of breast, colon, lung
Inhibitors of "pro-growth" programs of metabolism and angiogenesis			
VHL (Von Hippel–Lindau protein)	Hypoxia-induced transcription factors(e.g., HIF1α)	Von Hippel–Lindau syndrome	Renal cell carcinoma
Inhibitors of invasion and metastasis			
CDH1 (E-cadherin)	Cell adhesion, inhibition of cell motility	Familial gastric cancer	Gastric carcinoma, lobular carcinoma of breast
Enablers of genomic stability			
TP53 (*p53* protein)	Cell cycle arrest and apoptosis in response to DNA damage	Li–Fraumeni syndrome	Majority of cancers
DNA repair factors			

TABLE 7.11: Few tumor suppressor genes and associated familial syndromes and sporadic cancers.

Gene (protein)	***Function***	***Familial syndromes***	***Sporadic cancers***
BRCA1(Breast cancer-1), *BRCA2* (breast cancer-2)	Repair of double-stranded breaks in DNA	Familial carcinoma of breast and ovary; carcinomas of male breast; chronic lymphocytic leukemia (*BRCA2*)	Rare
Unknown mechanisms			
WT1 (Wilms tumor-1)	Transcription factor	Familial Wilms tumor	Wilms tumor, certain leukemias

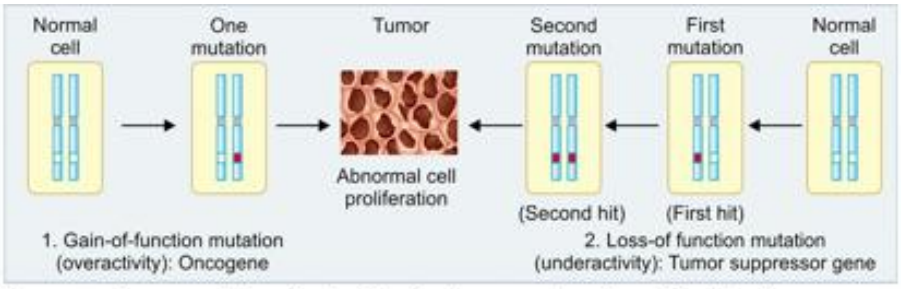


Fig. 7.19: Diagrammatic representation of gain of function (oncogenes) and loss of function (tumor suppressor gene).

Most common secondary malignancy in a patient with retinoblastoma is osteosarcoma.

DNA oncogenic viruses (e.g., HPV) encode proteins (e.g., E7) that bind to RB → blocks RB function.

TP53 gene product is protein *p53*.
p53: Guardian of the genome.

Non-mutated *TP53* is also called as the 'wild type' of *TP53* gene and is associated with reduced risk of cancers.
Consequences of loss of function of *p53*:
- DNA damage remains unrepaired.
- Mutations accumulate in dividing cells.
- Cell undergoes malignant transformation.

p53 is activated whenever there is damage to cellular DNA. Activated *p53* causes G_1 arrest of a cell allowing time for the DNA repair by DNA-repair genes.
p53 can be inactivated by oncogenic viruses, such as HPV.
p53: Tumors with normal *p53* respond better to chemotherapy and radiotherapy than those with mutated *p53*. Tumors with *p53* mutations are relatively resistant to treatment.
Activated *p53* controls genes involved in cell cycle, DNA repair, cellular senescence and apoptosis.
p53 directs the cell with unrepaired DNA to undergo death by apoptosis.
p53 induces cell arrest at: G_1-S phase.

Altered Cellular Metabolism in Cancer Cells (Warburg Effect)

- Cancer cells have different needs than their normal counterpart. Their proliferative rate generally exceed that of normal cells. Cancer cells must quickly synthesize the structural components (e.g., protein, lipid, etc.) that are required for rapid cell growth (that is to sustain their mitotic activity).
- **With adequate oxygen supply**, cancer cells undergo a metabolic switch to **aerobic glycolysis**. They develop a **distinctive form of cellular metabolism characterized by increased amount of glucose uptake and increased conversion of glucose to lactose (fermentation) via the glycolytic pathway.** This *aerobic glycolysis* is called the ***Warburg effect.*** It was described in 1930 by Otto Warburg and is not cancer specific, but observed in growing cells and it becomes "fixed" in cancer cells. It is seen because tumor cells have **M2 isoform of pyruvate kinase enzyme**.
- **The aerobic glycolysis provides metabolic intermediates that are needed for the synthesis of cellular components in rapidly dividing tumor cells.** This cannot be met with normal mitochondrial oxidative phosphorylation.
- **Clinical utility:** The "glucose-hunger" of tumors is made use for visualization of tumors in **positron emission tomography (PET) scanning.** In PET scanning, patients are injected with ^{18}F-fluorodeoxyglucose (a8-FDG- a non-metabolizable derivative of glucose) which is preferentially taken up into tumor cells (and also actively dividing normal cells, e.g., bone marrow cells). **Most tumors are PET-positive,** and markedly positive are the rapidly growing tumors.

Autophagy

Q. Write short note on autophagy.

Autophagy ("self-eating") is the process in which lysosomal enzymes digest the own components of the cell during stress. It is a survival mechanism which occurs during a **state of severe nutrient deficiency. By this mechanism**, the starved cell can live by eating its own contents (e.g.,

organelles, proteins, and membranes) and by recycling these contents to provide nutrients and energy. If this adaptive mechanism fails, the cells die.

Autophagy in cancer: Role of autophagy in the development and progression of cancer is complex and represents a double-edged sword. Several autophagy genes (e.g., Beclin-1 and some *Atg* genes) that promote autophagy, act as tumor suppressors and are deleted or mutated in many cancers. Tumor cells may accumulate mutations which deranges the pathways that induce autophagy and allows the cancer cells to grow without triggering autophagy. Tumor cells may corrupt the autophagy process to provide nutrients for continued growth and survival. On the other hand, autophagy can protect cancer cells if they are deprived of nutrients or oxygen because of therapy or insufficient blood supply.

Oncometabolism

Another new mechanism of oncogenesis is mutations in enzymes that participate in the Krebs cycle and is called as *oncometabolism*. One of them being mutations in enzyme isocitrate dehydrogenase (IDH).

Acquired mutation in IDH results in the mutated protein (oncoprotein) that catalyzes the production of high levels of *oncometabolite* namely 2-hydroxglutarate (2-HG). 2-HG in turn acts as an inhibitor of several other enzymes that are members of the TET family, including TET2. TET2 is one of several factors that regulate DNA methylation, which is an epigenetic modification that controls normal gene expression. Loss of TET2 activity leads to abnormal DNA methylation which in turn leads to misexpression of currently unknown cancer genes, which drive cellular transformation and oncogenesis. Oncogenic IDH mutations have been found in many cancers (e.g., cholangiocarcinomas, gliomas, acute myeloid leukemias, sarcomas).

Loss of Normal Apoptosis Pathways

Apoptosis is a programmed cell death and **is one of the normal protective mechanism by which a cell with DNA damage (mutation) undergoes cell death.** Many types of signals such as DNA damage, potent oncoproteins such as MYC, and loss of adhesion to the basement membrane (termed anoikis), can initiate apoptosis. Mutations in the genes that regulate apoptosis may result in accumulation of neoplastic cells. Apoptotic genes

that protect against apoptosis (anti-apoptotic) are often overexpressed in cancer cells, whereas those that promote apoptosis (proapoptotic) tend to be underexpressed or functionally inactivated by mutations.

- Abnormalities of *apoptosis-regulating genes* may result in less death and increased survival of the cells. These abnormalities may be gain-of-function mutations in genes whose products suppress apoptosis and loss-of-function mutations in genes whose products promote cell death. The *apoptosis-regulating genes* can behave as **proto-oncogenes** (loss of one copy is enough) or **tumor suppressor genes** (loss of both copies required).

Pathways of Apoptosis (refer Fig. 2.22)

Two different molecular cascades activate apoptosis (*refer* pages 34-6).

1. Extrinsic (death receptor) Pathway

It is initiated when certain ligands (e.g., CD95L, TNF, FasL) bind to death receptor expressed on the surface of plasma membrane.

2. Intrinsic (mitochondrial) Pathway

It is activated by various stimuli (e.g., withdrawal of survival factors, stress and injury). This pathway is most commonly disabled in cancer.

- Activation of intrinsic pathway → leads to **increased permeability of the mitochondrial outer membrane → releases cytochrome c and SMAC (second mitochondrial activator of caspases)** → initiate apoptosis.
- Integrity of the mitochondrial outer membrane is controlled by: (1) **proapoptotic, and (2) antiapoptotic proteins**.
  - **Proapoptotic proteins *BAX* and *BAK*** → increase mitochondrial permeability → initiate apoptosis.
  - **Antiapoptotic proteins *BCL2*, *BCL-XL* and *MCL1*:** Belong to ***BCL2*** family of proteins, inhibit the action of proapoptotic proteins.
  - **Regulator of balance between proapoptotic and antiapoptotic proteins:** It is achieved by **BH3-only proteins** and includes **BAD, BID and PUMA.**

Methods of Evasion of Apoptosis and Associated Tumors

Tumor cells may escape or undergo reduced apoptosis. **Reduced apoptosis** may be **due to activation of either antiapoptotic proteins or reduced proapoptotic activity**.

1. **Activation of antiapoptotic *BCL2*** [e.g., **follicular lymphomas** (about 85%) show a characteristic chromosomal translocation, t(14;18), causing **overexpression** of the **antiapoptotic *BCL2* protein**. Neoplastic B lymphocytes are **protected from undergoing apoptosis** and survive for long periods].
2. **Reduced levels of proapoptotic *BAX*:** The *p53* induces apoptosis of cells that are unable to repair DNA damage partly by transcriptional activation of *BAX*. **Mutation of *p53* leads to reduced levels of *BAX*** resulting in decreased apoptosis.

Chemotherapeutic drugs can cause: Both necrosis and apoptosis.

BCL2: An antiapoptotic gene activated by t(14;18) translocation in majority of follicular B-cell lymphoma.
BCL2 gene family: Constitutes antiapoptotic genes.
BAX gene: Apoptotic gene.

Loss of Replicative Senescence

Q. Write briefly on telomerase activity.

All cancers contain immortal cells with unlimited capacity to replicate (cellular immortalization). Probably three interrelated factors appear to be involved in the immortality of cancer cells: (1) loss of senescence; (2) loss of mitotic crisis; (3) the capacity for self-renewal.

Loss of Senescence

Most **normal cells** have a limited capacity to undergo **cell division** (replication) **for about 60–70 times.** After this, the cells cannot divide (arrest of growth) and become senescent by permanently leaving the cell cycle and without any cell division. Cancer cells evade the process of senescence and retain the ability to reproduce. The senescence is probably

associated with upregulation of tumor suppressors (e.g., *p53* and INK4a/p16). These tumor suppressors maintain RB in a hypophosphorylated state that favors cell cycle arrest. RB-dependent G_1/S cell cycle checkpoint is disrupted in almost all cancers by a wide variety of acquired genetic and epigenetic aberrations.

Loss of Mitotic Crisis

Cells resistant to senescence have increased capacity to replicate. However, these are not immortal and finally undergo **mitotic crisis** and die. This is due to progressive shortening of **telomeres. Telomeres** (*refer* page 46) **are the special structures** present **at the ends of chromosomes**. **During each cell division**, a small section of the **telomere** is not duplicated resulting in **progressive shortening**, which is responsible for the limited replicative property of a cell. The shortening of telomere is prevented by an enzyme called telomerase.

Activation of Telomerase

Telomerase is expressed at very low levels in most somatic cells and with each cell division their telomeres shorten. Thus, any cells that escape from senescence die in mitotic crisis. However, if cells in crisis reactivate telomerase, these cells can restore their telomeres and survive. The cells damaged by oncogenes and tumor suppressor genes during crisis are at high risk for malignant transformation. Cancers may arise from stem cells which express telomerase. Whatever the mechanism, telomere is maintained in almost all types of cancers, and in 85–95% of cases it is due to upregulation of telomerase.

Self-renewal

Tissue stem cells and germ cells express telomerase. Hence, they are resistant to mitotic crisis, and avoid the genetic and epigenetic alterations that trigger senescence. The long-lived stem cells have the capacity for self-renewal (*refer* page 74), i.e., each time a stem cell divides at least one of the two daughter cells remains as a stem cell. Since cancers are immortal and have limitless proliferative capacity, they also may contain cells that can

self-renew, and are called as *cancer stem cells*. It is not clear whether cancer stem cells arise from the transformation of tissue stem cells or from the conversion of conventional somatic cells to transformed cells. In chronic myelogenous leukemia (CML), tumor cell subset with the *BCR-ABL* fusion gene has all the properties of a normal hematopoietic stem cell. Thus, CML appears to arise from a transformed hematopoietic stem cell.

Increased Angiogenesis

- Under homeostatic conditions, there is a balance between factors that favor new blood vessel formation (angiogenic factors/angiogenic promoters) and those that hinder it (antiangiogenic factors/angiogenesis inhibitors).
- **Solid tumors,** even though have all the genetic aberrations that are required for malignant transformation; their **growth requires increased supplies, of nutrients and oxygen.** This in turn, requires proliferation of blood vessels (i.e., vascularization of tumors). In growing cancers, **angiogenic factors promote angiogenesis** during which vessels sprout from previously existing capillaries (*refer* pages 77-8). Thus, angiogenesis is an essential feature of malignancy**.** However, these vessels are not entirely normal. They are leaky and dilated and have a haphazard pattern of connection.

Effects of Neovascularization on Tumor Growth

- **Perfusion** supplies required nutrients and oxygen and remove waste products.
- Newly formed endothelial cells **secrete growth factors** [e.g., insulin-like growth factors (IGFs), platelet derived growth factors (PDGF)] which stimulate the growth of adjacent tumor cells.
- Permits access of tumor cells to these abnormal vessels and **contributes to metastasis**.

Mechanism of Angiogenesis

- During early phase of development, most tumors do not induce angiogenesis and tumors remain in a stage of vascular quiescence and

starved of nutrients. During this phase, the tumor remains small or in situ, probably for years, till an ***angiogenic switch*** terminates this stage.

- **Molecular basis of the angiogenic switch**: This may be due to increased production of angiogenic factors and/or loss of angiogenic inhibitors. The source of these factors may be the tumor cells or inflammatory cells (e.g., macrophages) or other stromal cells associated with the tumors.

Mediators of Tumor Angiogenesis

- **Family of VEGFs**: Relative lack of oxygen due to hypoxia triggers angiogenesis through the actions of HIF-1α (an oxygen-sensitive transcription factor) on the transcription of the proangiogenic factor VEGF and bFGF. Gain-of-function mutations in RAS, MYC and MAPK signaling also upregulate VEGF expression and stimulate angiogenesis.
- **Mutations involving tumor suppressors and oncogenes**: In cancers, this tilts the balance in favor of angiogenesis. For example, normal *p53* stimulate the synthesis of the angiogenesis inhibitor thombospondin-1 and suppresses the expression of proangiogenic molecules such as VEGF. Mutation of these genes favor angiogenesis.
- **Angiopoietins**: Angiopoietin-2 is a family of vascular growth factor which favors formation of tumor blood vessel, stabilizes growing blood vessels and stimulates pericytes to surround the developing blood vessels.

Invasion and Metastasis

Refer pages 201-2.

Evasion of Immune Surveillance

NC: Describe immunology and the immune response to cancer.

The immune system distinguishes self from non-self (foreign) molecules and is very effective against infectious agents. Paul Ehrlich first suggested that tumor cells also can be recognized as "foreign/nonself" and the immune system can elicit protective immunologic responses against the tumor cells. The immune system may suppress tumor development or destroy these tumor cells. Later Lewis Thomas and Macfarlane Burnet formalized the concept that **normal function of the immune system is to constantly "scan" the body for any emerging malignant cells and destroy them.**

This mechanism was termed as immune surveillance. This concept was supported by many observations such as (i) demonstration of tumor-specific T cells and antibodies in cancer patients; (ii) the correlation of outcome of the cancer patients with extent and quality of immune infiltrates in cancers; (iii) the increased incidence of certain cancers in immunodeficient individuals (e.g., patients with AIDS) than in immunocompetent individuals; and (iv) the success of immunotherapy in several cancers.

Principles: Several factors may govern the outcome of interactions between tumor cells and the host immune system. The basic principles involved are as follows:

- **Expression of antigens by cancer cells:** Cancer cells express a variety of antigens that stimulate the host immune system. These antigens may play an important role in preventing the emergence of cancers.
- **Immune response is ineffective:** Though the antigens expressed by cancer cells can elicit an immune response, it may be ineffective against established tumors. In fact, in some tumors immune response may actually promote cancer growth, due to acquired changes in cancer cells which make them escape from anti-tumor responses and may encourage pro-tumor responses.
- **Effective new immunotherapies:** By understanding the mechanisms of immune evasion and "immunomanipulation" by cancer cells, it was possible to invent effective new immunotherapies that act by reactivating latent host immune responses.

Tumor Antigens

Tumor antigen is an antigenic substance produced in tumor cells. The three main classes of tumor antigens are (i) products of mutated genes, (ii) overexpressed or aberrantly expressed proteins, and (iii) tumor antigens produced by oncogenic viruses.

1. **Products of mutated genes:** Cancer is a genetic disorder caused by mutations in oncogenes and tumor suppressor genes. Most of these mutations are acquired rather than inherited. These mutations may generate new protein sequences (neoantigens) that the immune system has not come across. Hence, the immune system can recognize them as foreign and react against it.

2. **Overexpressed or aberrantly expressed proteins:** Sometimes the unmutated proteins expressed by tumor cells also can stimulate the host immune response.
   - **Self-antigen: Tyrosinase** is an enzyme involved in biosynthesis of melanin and is expressed only in normal melanocytes and melanomas. The immune system is capable of responding against this normal self-antigen.
   - **Cancer-testis antigens:** These are encoded by genes that are silent in all adult tissues except germ cells in the testis, hence termed testis antigens. This antigen is present in the testis but is not expressed on the cell surface in a form that can be recognized by $CD8^+$ T cells, because sperm do not express MHC class I molecules. Thus, these antigens are tumor specific and are capable of stimulating anti-tumor immune responses.
3. **Tumor antigens produced by oncogenic viruses**: Viral proteins can be expressed in cancer cells that are transformed by oncogenic viruses. The most potent of these antigens are proteins produced by cells infected with DNA viruses [e.g., human papilloma virus (HPV) and Epstein-Barr virus (EBV)]. The cytotoxic T lymphocytes (CTLs) can recognize viral antigens and play important roles in surveillance against virus-induced tumors. CTLs can recognize and kill virus-infected cells. It is observed that multiple cancers associated with oncogenic viruses, such as HPV-associated cervical carcinoma and EBV-related B cell lymphomas, develop at significantly higher rates in individuals with defective T cell immunity (e.g., patients infected with HIV).

Effective Immune Responses to Tumor Antigens

- Immune reactions to cancers are initiated by the death of individual cancer cells. The death of cancer cells may be due to dysregulated growth, metabolic stresses, and hypoxia due to insufficient blood supply. Death of tumor cells release “danger signals” (damage associated molecular patterns) and they stimulate innate immune cells, including resident phagocytes and antigen presenting cells.
- Probably some of the dead tumor cells are phagocytosed by dendritic cells. These dendritic cells migrate to draining lymph nodes and present tumor neoantigens in the context of MHC class I molecules, a process named as **cross-presentation**. The displayed tumor antigens are

recognized by antigen-specific $CD8^+$ T cells, which become activated, proliferate, differentiate into active CTLs. These CTLs migrate to the site of the tumor, recognize and kill tumor cells presenting neoantigens.

Immune Evasion by Cancers

Immune system is capable of recognizing and destroying cancer cells. When a tumor become clinically significant size, it must be composed of cells that are either not recognized by the host immune system or that the tumor cells express factors that suppress the host immunity.

Cancer immunoediting: Immune system can identify and control nascent tumor cells in a process called cancer immunosurveillance. Immune system can also promote tumor progression through chronic inflammation, immunoselection of poorly immunogenic variants, and suppressing antitumor immunity. Together, the dual host-protective and tumor-promoting actions of immunity are referred to as cancer immunoediting. Cancer immunoediting consists of **two type of processes**

1. **Host-protective action:** A process by which immune system **protect the host against primary tumor development** and/or
2. **Tumor-promoting action:** It **enhances tumor escape either by manipulating tumor immunogenicity or weakening antitumor immune responses**. By cancer immunoediting, the immune system can promote the selection of the tumor subclones that are capable of avoiding host immunity or even manipulate the immune system for their own malignant purposes.

In immunocompetent patients, several mechanisms may avoid the response by immune system to tumors. These include selective outgrowth of antigen-negative variants, loss or reduced expression of histocompatibility molecules, and immunosuppression mediated by expression of certain factors (e.g., TGF-β, PD-1 ligands) by the tumor cells.

Cytotoxic T cells (CTLs): CTLs are the **most important cells involved in defense of the host against tumors**. Hence, tumor cells undergo a variety of alterations so that they can avoid CTL responses. These alterations include (i) acquired mutations in β2-microglobulin that prevent the assembly of functional MHC class I molecules, and (ii) increased expression of proteins

that inhibit CTL function. These proteins activate immune checkpoints which are regulators of the immune system.

Immune checkpoints are **inhibitory pathways that normally are crucial for maintaining self-tolerance** which prevents the immune system from attacking cells indiscriminately. They also control the size and duration of immune responses so as to minimize collateral tissue damage. However, some cancers can protect themselves from attack by stimulating immune checkpoint targets. There are several immune checkpoint pathways, involving different ligands and receptors involved in immunoevasion by tumors. For example, one of the immune checkpoints involves a protein called PD-L1 (programmed cell death ligand 1). PD-L1 ligand is usually expressed on the surface of tumor cells. When PD-L1 ligand engages itself to its receptor, PD-1, on CTLs, the CTLs become unresponsive and lose their ability to kill tumor cells.

Blocking of checkpoints: The checkpoints can be blocked by using appropriate antibodies. This will shut off anti-tumor immunity and release the brakes on the immune response. Currently checkpoint blockade therapies are being used in a variety of solid tumors (melanoma, lung cancer, bladder cancer, and others), and in some hematologic malignancies (e.g., Hodgkin lymphoma). Since these checkpoints are blocked to prevent responses to self-antigens, the patients treated with checkpoint blockade therapy may develop various autoimmune manifestations (e.g., colitis), most of which can be controlled with anti-inflammatory agents.

Chimeric antigen receptors (CARs): Recently personalized tumor vaccines are prepared against neoantigens identified in the tumors of individual patients and new kinds of adoptive immunotherapy are also used. One of the examples of adoptive immunotherapy is the development of patient-derived CTLs that are engineered to express CARs. CARs have extracellular and intracellular domains. The extracellular domains consist of antibodies that bind tumor antigens on the surface of tumor cells and intracellular domains which deliver signals that activate CTLs. CAR T cells kills the tumor cells and are used for treating certain leukemias (e.g., B cell acute lymphoblastic leukemia). However, CAR T cells can produce serious complications due to cytokines released from the activated CTLs.

GENOMIC INSTABILITY

DNA Repair Genes

Q. Write briefly on genomic instability.

We swim in environmental agents that are mutagenic (e.g., chemicals, radiation, sunlight). Thus, DNA is under relentless assault from many environmental agents (exogenous stresses) as well as internal stresses such as reactive oxygen species (ROS), etc., that can damage cellular DNA. However, cancers are relatively rare outcomes of these encounters. Reasons for this is that the cells maintain genomic stability through different mechanisms that detect and repair DNA damage, cause the death of cells with irreparable damage, oncogene-induced senescence and immune surveillance. As discussed earlier, *TP53* tumor suppressor gene protects the genome from oncogenic damage, (1) by arresting cell division to provide time for repair of DNA damage caused by environmental mutagens, and (2) by initiating apoptosis in irreparably damaged cells. Genes which repair DNA are called as **DNA repair genes which protect the integrity of the genome**.

- Normally, DNA repair genes repair nonlethal damage in other genes including proto-oncogenes, tumor suppressor genes and genes that regulate apoptosis. Mutations of these DNA repair genes do not directly transform cells. **Loss-of-function mutations** (disability) **involving DNA repair genes** contribute to carcinogenesis (neoplastic transformation) indirectly by **impairing the ability of the cell to recognize and repair nonlethal genetic damage in other genes**. These affected cells acquire mutations at an accelerated rate, a state referred to as a ***mutator phenotype*** **and it is marked by genomic instability.**
- Genomic instability may be due to either **microsatellite instability** (single or oligo-nucleotide mutations) or more commonly due to **chromosomal instability** leading to aneuploidy (abnormal number of chromosomes in a cell).

Types of DNA Repair Genes (Flowchart 7.1)

1. **Mismatch repair:** After DNA replication is complete; **mismatch repair genes act as spell checkers or proofreaders. They excise and replace the mismatched nucleotides**. Defect in these genes → mismatched nucleotide errors gradually accumulate in the genome. These **errors may involve proto-oncogenes and tumor suppressor genes**.

Hereditary nonpolyposis colon cancer syndrome: HNPCC syndrome (Lynch syndrome) is characterized by **familial predisposition to the development of carcinomas of the colon** affecting predominantly the cecum and proximal colon. **It is due to defects in DNA mismatch repair gene**.

- **Microsatellite instability**: One of the characteristics of patients with mismatch-repair defects is microsatellite instability. **Microsatellites are tandem repeats of one to six nucleotides found throughout the genome**. Normally the length of these microsatellites remains constant. In individuals with **HNPCC**, **these satellites are unstable and increase or decrease in length** in tumor cells, creating alleles not found in normal cells of the same patient. Minisatellites are longer tandem repeat units (n = 15–500 nucleotides) also termed variable number of tandem repeats (VNTR) because number of repeats in a given minisatellite varies widely among individuals.
- Each affected patient inherits one defective copy of a DNA mismatch-repair gene and acquires the second hit of DNA mismatch-repair gene in colonic epithelial cells. Thus, **mode of inheritance of DNA-repair genes is like tumor suppressor genes**.

2. **Nucleotide excision repair: Example—xeroderma pigmentosum.**
   - It is an **inherited disorder** of defective **nucleotide excision repair gene**.
   - These patients have an **increased risk** for the development of **skin cancers following exposure to the UV light** present in sunrays.
   - **UV radiation causes cross-linking of pyrimidine residues, preventing normal DNA replication**. Such DNA damage is normally **repaired by the nucleotide excision repair system**.
3. **Recombination repair:** Recombination is a process in which random crossing over of double-stranded DNA occurs between two parental homologous chromosomes. This occurs by breakage of homologous DNA molecules and rejoining of the parts in new combinations. It is a

necessary process in meiosis and involves exchange of genetic information. Recombination also occur during mitosis at a predictable rate. Exposure to ionizing radiation significantly increases the rate of breakage in chromosomes. Usually, these breakages are accurately repaired by recombination repair genes. Disorders associated with recombination repair genes include Bloom syndrome, ataxia-telangiectasia, and Fanconi anemia. *BRCA1* and *BRCA2* are mutated in familial breast cancer and both are associated with many proteins involved in the homologous recombination repair pathway.

DNA repair genes: Enzymes causing excision of dimers include endonuclease, exonuclease and polymerase ligase.

DNA repair genes: Inherited mutations are associated with increased risk of cancer.

Xeroderma pigmentosum: Defect in the nucleotide excision repair gene → increased risk for cancer of skin exposed to UV light.

Microsatellites: Tandem repeats of one to six nucleotides found in the genome.

DNA contains several repeat sequences of three nucleotides (trinucleotide). If repeat sequences are directly adjacent to each other they are called as tandem repeats.

Syndromes associated with defects in recombination repair gene: (1) Bloom syndrome, (2) Ataxia-telangiectasia, (3) Fanconi anemia.
They have hypersensitivity to DNA damaging agents (e.g., ionizing radiation).

ETIOLOGY OF CANCER (CARCINOGENIC AGENTS)

CC: Enumerate carcinogens and describe the process of carcinogenesis.

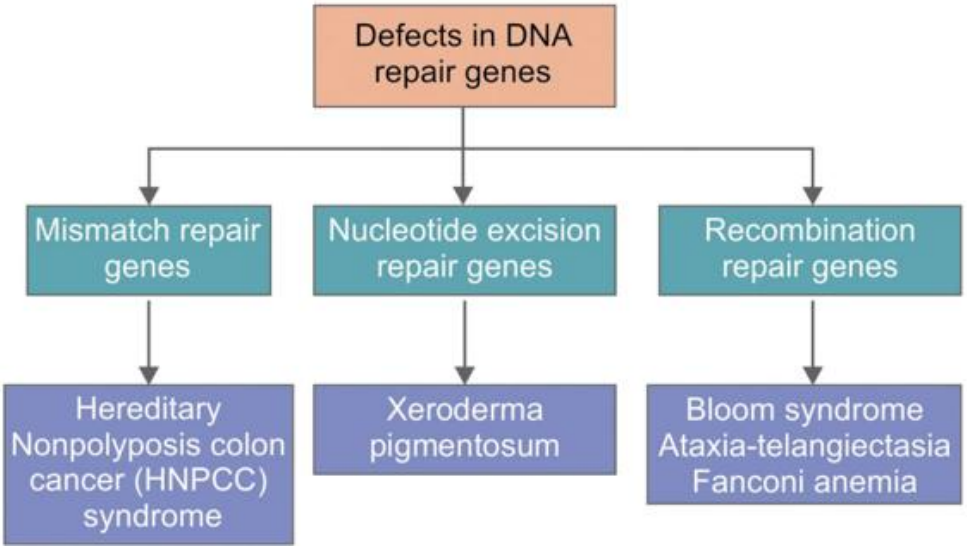
Q. Classify different carcinogenic agents. List major chemical carcinogens. Discuss chemical carcinogens and their role in carcinogenesis.

Q. Classify carcinogens/enumerate the types of carcinogens. Describe carcinogenesis in detail.

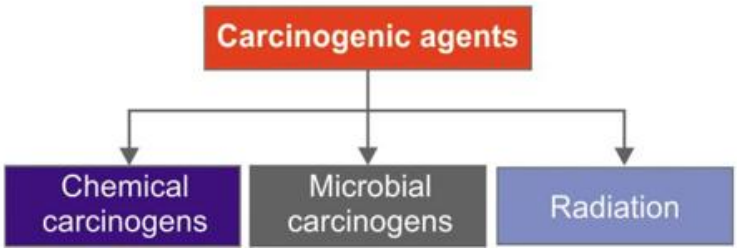
Definition: A **carcinogen** is an **agent known or suspected to cause tumors** and such agents are said to be **carcinogenic** (cancer causing).

Carcinogenic agents (Flowchart 7.2): (1) chemicals, (2) microbial agents, and (3) radiation.

Flowchart 7.1: Different types of DNA repair gene defects and associated conditions.



Flowchart 7.2: Major types of carcinogenic agents.



Chemical Carcinogenesis

Q. List major chemical carcinogens and describe in detail chemical carcinogenesis.

Sir Percival Pott (London surgeon) **first related scrotum skin cancer in chimney sweeps to a specific chronic chemical exposure to soot**. Based on this, a rule was made that chimney sweep members must bathe daily and this public health measure controlled scrotal skin cancer. Japanese investigators (Yamagiva and Ichikawa) experimentally produced skin cancers in rabbits

by using coal tar. Subsequently, hundreds of chemical carcinogens were discovered.

Classification of chemical carcinogens: **Chemical carcinogens** may be classified into two categories: Direct acting and indirect acting. Major chemical carcinogens are listed in **Box 7.2**.

Direct-acting Agents

Direct-acting chemical agents **do not require metabolic conversion** to become carcinogenic, but most of them are **weak carcinogens**. Some of the drugs (e.g., alkylating agents) used to cure, control, or delay recurrence of some cancer (e.g., leukemia, lymphoma), may produce a second form of cancer (e.g., acute myeloid leukemia) later.

Alkylating Agents

- **Source: Many cancer chemotherapeutic drugs** (e.g., cyclophosphamide, cisplatin, busulfan) are alkylating agents.
- **Mechanism of action:** Alkylating agents **contain electron-deficient atoms** that **react with electron-rich atoms in DNA**. These drugs not only destroy cancer cells by damaging DNA, but also injure normal cells.
- **Cancers produced:** Solid and hematological malignancies.

Indirect-acting Agents (Procarcinogens)

Q. Write short note on polycyclic hydrocarbons.

These chemicals **require metabolic activation** for conversion **to an active ultimate carcinogen**.

1. Polycyclic Aromatic Hydrocarbons

They are the **most potent** and extensively studied indirect-acting chemical carcinogens. Examples: Benzo[a]pyrene, 3-methylcholanthrene, and dibenzanthracene.

- **Source:**
  - Originally derived from coal tar and fossil fuels.

- Cigarette smoke: Polycyclic aromatic hydrocarbons are formed during high-temperature combustion of tobacco in cigarette smoking → responsible for lung cancer in cigarette smokers.
- Animal fats: It may produce it during the process of broiling meats.
- Smoked food: Smoked meats and fish.

- **Mechanism of action:**
  - Polycyclic hydrocarbons are metabolized by cytochrome P450-dependent mixed function oxidases to electrophilic (have electron-deficient atoms) epoxides.
  - Epoxides react with proteins and nucleic acids (DNA, RNA), e.g., polyvinyl chloride (used in plastic industry) is metabolized to an epoxide → causes hepatic angiosarcomas.
  - **Cancers produced**: The specific type of cancer produced depends on the route of administration, e.g., cancers in the **skin**, **soft tissues**, **lung and breast**.

BOX 7.2: Major chemical carcinogens.

DIRECT-ACTING CARCINOGENS

1. ***Alkylating Agents***
   - β-Propiolactone
   - Anticancer drugs (cyclophosphamide, chlorambucil, nitrosoureas, etc.)
   - Dimethyl sulfate
   - Diepoxybutane
2. ***Acylating Agents***
   - 1-Acetylimidazole
   - Dimethylcarbamoyl chloride

INDIRECT-ACTING CARCINOGENS (PROCARCINOGENS)

1. ***Polycyclic and Heterocyclic Aromatic Hydrocarbons***
   - Benz[*a*]anthracene
   - Benzo[*a*]pyrene
   - Dibenz[*a,h*]anthracene
   - 7,12-Dimethylbenz[*a*]anthracene
   - 3-Methylcholanthrene
2. ***Aromatic Amines, Amides and Azo Dyes***
   - 2-Naphthylamine (β-naphthylamine)
   - Benzidine
   - 2-Acetylaminofluorene
   - Dimethylaminoazobenzene (butter yellow)

NATURAL PLANT AND MICROBIAL PRODUCTS

- Aflatoxin B_1
- Griseofulvin

- Betel nuts

OTHERS

- Nitrosamine and amides
- Vinyl chloride
- Metals: Nickel, chromium
- Insecticides, fungicides
- Asbestos

2. Aromatic Amines and Azo Dyes

They are indirect-acting carcinogens.

- **Source:**
  - In the past, the aromatic amines (β-naphthylamine) and azo dyes were used in the aniline dye and rubber industries.
  - Azo-dyes were used for coloring food (e.g., butter and margarine which give yellow color, scarlet red for coloring cherries).
- **Mechanism of action**:
  - They are not carcinogenic at the point of application.
  - Both aromatic amines and azo dyes are mainly metabolized in the liver.
  - The aromatic amines are converted to active carcinogens in the liver. However, can be detoxified immediately by conjugation with glucuronic acid in the liver.
  - The conjugated metabolite is excreted in the urine and deconjugated in the urinary tract by the enzyme glucuronidase. The urothelium is thus exposed to the active carcinogen (reactive hydroxylamine) which may cause bladder cancer.
- **Cancers produced: Bladder cancer** (β-naphthylamine and benzidine) and **liver tumors** (azo dyes).

Natural Microbial Product

Aflatoxin B_1

- **Source:** Aflatoxin B_1 is a natural product of ***Aspergillus flavus***, a mold which grows on **improperly stored grains and peanuts**.
- **Mechanism of action:** Metabolized to an **epoxide** and bind to DNA and also produces **mutations of *p53* gene**.

- **Cancers produced:** Powerful liver carcinogen → **hepatocellular carcinoma**.

Others

- **Nitrosamines**: They are potent carcinogens.
  - **Source: Before the advent of refrigerator**, nitrites were added as **a preservative for meats and other foods.**
  - **Mechanism of action: Nitrites react with amines and amides** in the diet and are metabolized by commensal bacteria within the gut and converted to **carcinogenic nitrosamines**.
  - **Cancers produced**: Mainly **gastrointestinal neoplasms**.
- **Metals**: Compounds like arsenic, nickel, lead, cadmium, cobalt, chromium and beryllium can produce cancer. Most metal-induced cancers occur due to occupational exposure.
- **Asbestos**: Inhalation of asbestos fibers → results in asbestosis, pleural plaques, **mesothelioma** and **carcinoma of the lung**. Mesothelioma may involve pleura as well as peritoneum.

Detection of carcinogenicity of a chemical: Mutagenicity testing of chemical is done by **Ames test**. The appearance of frameshift mutations and base-pair substitutions in a culture of **bacteria of a *Salmonella* species** indicates that the chemical tested is carcinogenic.

Mechanism of Action of Chemical Carcinogens

Molecular targets of chemical carcinogens: Most chemical carcinogens are mutagenic. A **mutagen** is an agent, which can **permanently alter the genetic constitution** of a cell.

- All direct and ultimate carcinogens (of indirect carcinogens) **contain highly reactive electrophilic groups → form adducts with DNA**, **RNA and proteins**.
- **Genes affected**: Any gene may be affected but commonly involved are proto-**oncogenes** (*RAS*) **and tumor suppressor genes** (*p53*).

Multistep Hypothesis (Fig. 7.20)

Q. Write short essay on multistep carcinogenesis.

Chemical carcinogenesis is a **multistep** process. Once the tumor process is started, it does not require the continued presence of the carcinogen.

Two major steps involved in chemical carcinogenesis are:

1. Initiation

It is the **first important step** that develops from **exposure of cells** to a sufficient dose of a **carcinogenic agent** (initiator).

- **Reaction with DNA**: All initiators are highly reactive electrophiles (electron-deficient atoms) and can react with nucleophilic (electron-rich) sites in the cell. Sites of reaction of initiation are DNA, RNA and proteins.
- **Effect of initiation**: Initiators **produces nonlethal permanent (irreversible) alterations or damage to DNA (mutations)** in a cell. If damage is lethal or severe it causes cell death.

Q. Write short note on Promoters in carcinogenesis.

Q. Differences between initiators and promoters.

2. Promotion

- **Promoters**: They are **noncarcinogenic** agents and **cannot directly damage DNA** (mutation).
- **Cell proliferation:** Promoters stimulate the initiated (with permanent DNA damage-mutated) **cells to enter into the cell cycle** → cell proliferation. Unlike initiators, the **cellular changes produced by promoters are reversible**.
- **Produce changes only on initiated cell: Tumors develop only if the promoter is applied after the initiator** and not the reverse way.
  - **Examples of promoters include:** Phorbol esters, hormones, phenols and drugs.
- **Continuous proliferation** of initiated cells → **leads to secondary genetic abnormalities** → tumor growth becomes independent of the initiator or the promoter (i.e., autonomous). **Many accumulated mutations finally immortalize the cells.**

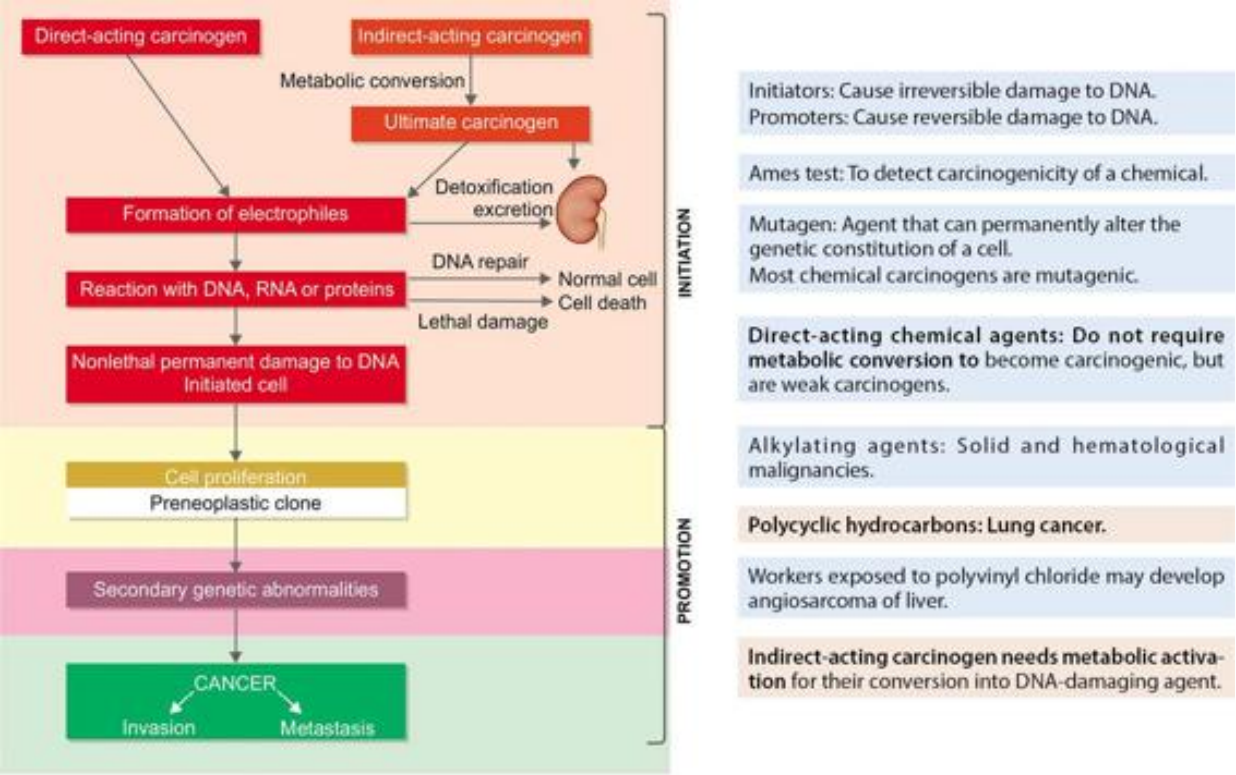


Fig. 7.20: Multistep theory of chemical carcinogenesis.

- Final result of the different steps is the development of neoplasm → invasion → metastases.

Examples: The morphologic sequence of hyperplasia, dysplasia and carcinoma in situ found in epithelium (e.g., skin, cervix and colon) indicate multistep carcinogenesis.

Microbial Carcinogenesis

Q. Define and classify oncogenic viruses. Explain the mechanism of tumor production by viruses.

Q. Classify/list oncogenic viruses. Discuss oncogenesis by human papilloma viruses (HPV).

Viruses that cause tumors are called as oncogenic viruses. Many viruses have been proved to be oncogenic in animals, but only a few have been associated with human cancer.

Classification (Flowchart 7.3): They are mainly classified depending on the genetic material into: (1) oncogenic **RNA viruses,** and (2) oncogenic **DNA**

viruses.

Oncogenic RNA Viruses

Q. Define carcinogenesis. Discuss the role of RNA viruses in tumorigenesis.

Q. Explain the mechanism involved in tumor production by viruses.

Human T-cell Leukemia Virus Type 1

It is a retrovirus.

- **Major target** for neoplastic transformation: **$CD4^+$ T lymphocytes.**
- **Tumor caused**: Adult **T-cell leukemia/lymphoma**—develops after a long latent period (20–50 years).
- **Mode of infection**: (1) Sexual intercourse, (2) blood products, and (3) breastfeeding.

Mechanism of Oncogenesis

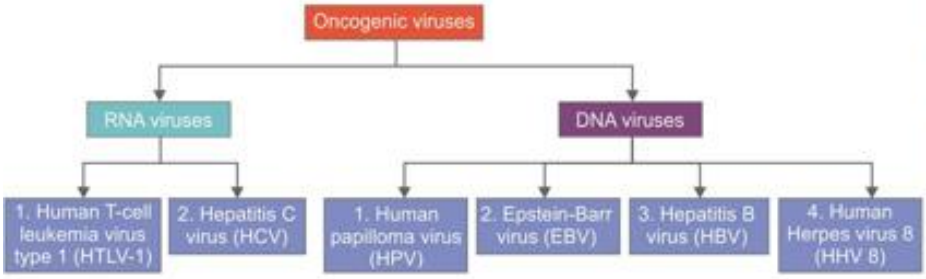
It is a **multistep process**.

HTLV-1 infects $CD4^+$ T-cells. **HTLV-1 does not contain oncogene and its genes cannot integrate** into the host genome.

HTLV-1 contains *TAX* gene and actions of its product ***TAX* protein** are:

- Required for viral replication and cellular transformation.
- Activates other genes involved in T-cell proliferation and differentiation.
- *TAX* protein also stimulates secretion of GM-CSF by $CD4^+$ T-cells → stimulates nearby macrophages to produce T-cell mitogens → polyclonal proliferation of CD^{++} T-cells.
- *TAX* inactivates ***p53*** and other genes controlling cell cycle → **increased risk of developing mutations and genomic instability** in proliferating $CD4^+$ T-cells.

Flowchart 7.3: Classification and types of oncogenic viruses.



- Accumulation of mutations and chromosomal abnormalities → **monoclonal neoplastic proliferation of** $CD4^+$ **T-cell**.

Oncogenic DNA Viruses

Five DNA viruses can cause cancer. HCV is not a DNA virus and found to be associated with cancer.

Human Papilloma Virus (HPV)

Q. Write short note on oncogenesis by human papilloma virus.

- **Cell infected:** Human papilloma viruses (HPV) infects **only the immature squamous cells** but **its replication occurs in the maturing, nonproliferating squamous cells.** Thus, their full productive life cycle occurs only in squamous cells. The physical state of the virus differs in different lesions.
- **Types of HPV and associated lesions (Table 7.12):** More than 70 genetically different types of HPV have been identified. They are divided into **low-risk and high-risk HPVs** (*refer* pages 738-39).

Q. Write a short note on high-risk human papilloma viruses.

Mode of action (Fig. 7.21)

Episomal form: In **benign lesions** such as benign warts, condylomata and most **precancerous lesions**; the HPV genome is present as nonintegrated, free (episomal) viral DNA.

Integration: In **cancers**, the HPV genome is integrated into the host genome and is essential for malignant transformation. **Integration results in overexpression of the two viral genes *E6* and *E7*.** Protein products of E6 and E7 (oncoproteins) are important for the oncogenic effects of HPV.

- **Actions of E7 protein:**
  - **Inactivation of tumor suppressor *RB* gene: E7 protein binds** to the hypophosphorylated (active) form of **RB protein → releases its inhibitory effect on cell cycle progression** E7 proteins from high-risk HPV types have a higher affinity for RB than E7 proteins from low-risk HPV types.
  - **Inactivation of inhibitors of cell cycle** [e.g., inactivation of CDKIs (**CDKN1A/*p21*** and CDNK1B/*p27*) → **activates cell cycle]**.
  - **Activation of cyclins (activators of cell cycle):** These include cyclins E and A → facilitates G_2/M transition → **activation of cell cycle.**

TABLE 7.12: Various viruses implicated in human tumors and associated lesions.

Type of virus	*Lesions*
ONCOGENIC RNA VIRUSES	
• Human T-cell lymphotropic virus type-1	Adult T-cell leukemia/lymphoma
• Hepatitis C virus	Hepatocellular carcinoma
ONCOGENIC DNA VIRUSES	
1. Human papilloma virus	
A. Low-oncogenic risk HPV—benign lesions of squamous epithelium	
◆ HPV types 1, 2, 4 and 7	Benign squamous papilloma (wart)
◆ HPV-6 and HPV-11	Condylomata acuminata (genital warts) of the vulva, penis and perianal region
	Laryngeal papillomas
B. High-oncogenic risk HPV—malignant tumors	
◆ HPV types 16 and 18	Squamous cell carcinoma of the cervix and anogenital region
	Oropharyngeal cancers (tonsil)

TABLE 7.12: Various viruses implicated in human tumors and associated lesions.	
Type of virus	***Lesions***
2. Epstein-Barr virus	Burkitt lymphoma (requires cofactor-malaria)
	Nasopharyngeal cancer
3. Hepatitis B virus	Hepatocellular carcinoma
4. Human herpes virus-8	Kaposi's sarcoma
	Pleural effusion lymphoma, multicentric Castleman disease
5. Merkel cell polyomavirus	Merkel cell carcinoma

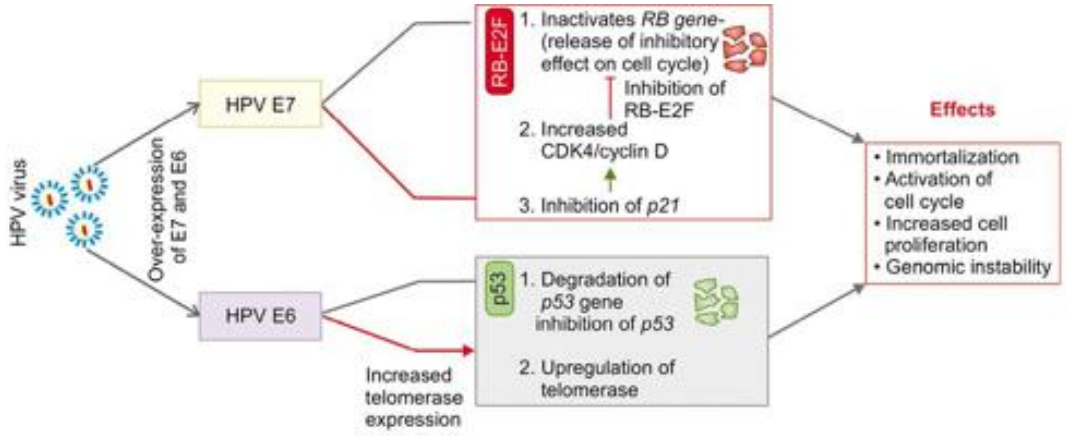


Fig. 7.21: Mode of action of HPV proteins E6 and E7 on the cell cycle.

- **Oncogenic activities of E6:** The E6 protein complements the effects of E7.
  - **Inactivation of tumor suppressor *p53* gene:** E6 binds and degrades *p53* → degrades *BAX* (a proapoptotic factor) → **prevents apoptosis**. E6 from high-risk HPV types has a higher affinity for *p53* than E6 from low-risk HPV types.
  - **Activation of telomerase:** E6 stimulates the expression of TERT (the catalytic subunit of telomerase) → prevents replicative senescence and **cell proliferation** continues (immortalization of cells).
- **Combined action of E6 and E7:** They induce centrosome duplication and **genomic instability**.

Epstein-Barr Virus (EBV)

Q. Write short note on Epstein-Barr virus, diseases caused and cancers.

EBV is a human **herpesvirus, which infects B lymphocytes**. Patients may manifest as a short-lived **infectious mononucleosis** or develop few human cancers. The **list of cancers** produced include:

1. **African form of Burkitt lymphoma.**
2. **B-cell lymphomas** in immunosuppressed (e.g., HIV infection or immunosuppressive therapy after organ transplantation).
3. A subset of **Hodgkin lymphoma.**
4. **Nasopharyngeal carcinoma (T-cell tumor).**
5. **Some gastric carcinomas.**
6. **Rare forms of T-cell lymphomas and natural killer (NK) cell lymphomas.**
7. Very rarely sarcomas.

Pathogenesis: EB virus infects B lymphocytes by binding to the membrane receptor CD21 (CR2). The **infection of B-cells may be either productive** (lytic) **or latent**.

- **Productive/lytic infection**: It **develops only in a few patients** and results in death of infected cells → release of virions → infection of other B-cells.
- **Latent infection**: It **occurs in majority of the cases**. The virus becomes latent inside the B-cells → are transformed or "immortalized" so that they are capable of proliferation indefinitely. **Immortalization of B lymphocyte is the hallmark of EBV infection**. **Molecular basis of B-cell immortalization** is related to **two EBV-coded genes** and **viral cytokines**.
  1. **LMP1** (latent membrane protein 1): It **acts as an oncogene** → activates the NF-κB and JAK/STAT signaling pathways → **promote B-cell survival** (prevents apoptosis by activating **Bcl-2**) and **proliferation**.
  2. **EBNA2** (Epstein-Barr nuclear antigens 2): **It stimulates transcription of many host genes**, including genes that drive the cell cycle (e.g., cyclin D) and the *SRC* family of proto-oncogenes.

3. **Viral cytokine (vIL-10):** It is pirated from host genome, **prevent macrophages and monocytes from activating T-cells and killing viral infected cells**.

African form of Burkitt lymphoma: It is a **B-cell neoplasm** and is the **most common childhood tumor in central Africa** and New Guinea. A morphologically similar lymphoma occurs sporadically throughout the world.

- **Mechanism of endemic Burkitt lymphoma**: **Normally, EBV infects B-cells and stimulate B lymphocyte proliferation which is controlled by suppressor T-cells.** Sequence of events in the pathogenesis of endemic African Burkitt lymphoma are:
  - **Accompanying infections** (such as **malaria** or other infections) **impairs immune competence → lack of an adequate suppressor T-cell response** → allows **uncontrolled proliferation of B-cell**.
  - **EBV-infected B-cells expressing LMP-1 are eliminated by immune system.**
  - **Lymphoma develops** only **when there are chromosomal translocations** that activate the ***c-MYC*** **oncogene** (*refer* **Fig. 12.27**) → results in **uncontrolled proliferation** of a malignant clone of B lymphocytes.
- **Mechanism of nonendemic Burkitt lymphoma**: All tumors possess the t(8;14) or other translocations that dysregulate c-*MYC*.

Hepatitis B and C Viruses

HBV is a DNA virus whereas **HCV is RNA virus**. There is a strong association between chronic infection with HBV and HCV (chronic hepatitis and cirrhosis) with primary **hepatocellular carcinoma**.

Mechanism: The oncogenic effects of both HBV and HCV are **multifactorial**.

- **Immunologically mediated chronic inflammation**: It causes **death of the hepatocytes.**
- **Compensatory liver cell regeneration**: It is aided by a several growth factors and cytokines produced by **activated immune cells of inflammation**.
- **Genomic damage and mutation:** It is due to mediators (e.g., reactive oxygen species) produced by activated immune cells.

HBV: HBV genome contains a viral regulatory gene known as ***HBx***. Various actions of *HBx* are:

- Direct or indirect **activation of many transcription factors and signal transduction pathways**.
- **Inactivation of *p53***
- **HBV DNA can be integrated within the human genome** and can **cause multiple deletions,** which may harbor unknown tumor suppressor genes.

HCV: HCV genome, such as the **HCV core protein**, may **activate many growth-promoting signal transduction pathways** and cause tumor. HCV is associated with **lymphoplasmacytic lymphoma**.

Human Herpes Virus 8 (HHV 8)

It is a DNA virus, which **infects the spindle cells of Kaposi sarcoma** and also **lymphocytes**.

Neoplasm produced:

- **Kaposi sarcoma:** It is a **vascular neoplasm**, which is the **most common neoplasm, associated with AIDS**. HHV 8 has also been found in Kaposi sarcoma from HIV-negative patients.
- **B-cell lymphoid malignancies**: Two uncommon lymphoid malignancies, namely **primary effusion lymphoma** and **multicentric Castleman disease** are associated with HHV 8.

Mechanism

- **HHV 8 viral genome encodes proteins, which interfere with the *p53* and *RB* tumor suppressor pathways**.
- HHV 8 also **encodes gene products, which downregulate class I major histocompatibility complex (MHC) expression** → infected cells **escape recognition by cytotoxic T lymphocytes**.

Various viruses implicated in human tumors are listed in **Table 7.12.**

Bacteria

Helicobacter pylori

Diseases caused by *H. pylori* are: (1) **peptic ulcers**, **(2) gastric adenocarcinomas, and (3) gastric lymphomas.**

Gastric adenocarcinomas

Mechanism: It is similar to that of HBV and HCV-induced hepatocellular carcinoma.

- **Chronic inflammation**: *H. pylori* causes chronic inflammation (chronic gastritis) → followed by gastric atrophy → intestinal metaplasia → dysplasia → cancer.
- **Genes**: *H. pylori* causing gastric adenocarcinoma contains **cytotoxin-associated A (*CagA*)** gene can penetrate into gastric epithelial cells → initiation of signals → unregulated growth factor stimulation.

Gastric lymphoma

H. pylori produces lymphoma of **B-cell origin** and are called as lymphomas of mucosa-associated lymphoid tissue, or **MALTomas**. *H. pylori* is associated with t(11;18) translocation in MALT lymphomas.

Fungi

Fungi may cause cancer by producing toxic substances (mycotoxins). **Aflatoxin B_1** produced by *Aspergillus flavus* is a potent carcinogen responsible for **hepatocellular carcinoma.**

Parasites

Two parasites which can causes tumor are:

1. ***Schistosoma*** is strongly implicated in **carcinoma of urinary bladder (usually of squamous cell type)**. The ova of the parasite can be found in the affected tissue.

2. ***Clonorchis sinensis*** (Chinese liver fluke) lodges in the bile ducts → produces an inflammatory reaction, epithelial hyperplasia and sometimes **adenocarcinoma of the bile ducts (cholangiocarcinoma)**.

Hormones

Hormones in the body may act as cofactors in carcinogenesis.

Estrogen

- **Endometrial carcinoma**: It may develop in females with estrogen-secreting **granulosa cell tumor of ovary** or those receiving exogenous estrogen.
- **Adenocarcinoma of vagina**: Increased frequency of adenocarcinoma of vagina is observed in **daughters of mothers who received estrogen during pregnancy**.
- **Abnormal vascularity of tumor: Estrogens can make existing tumors abnormally vascular** (e.g., adenomas and focal nodular hyperplasia).

Androgenic and anabolic steroids: They may cause hepatocellular tumors.

Hormone-dependent Tumors

- **Prostatic carcinoma** usually responds to administration of estrogens or castration.
- **Breast carcinomas** regress following oophorectomy.

Microbial carcinogens: Viruses > bacteria > parasites

Oncogenic DNA viruses:
1. Human papilloma virus (HPV)
2. Epstein-Barr virus (EBV)
3. Hepatitis B virus (HBV)
4. Kaposi sarcoma herpes virus (KSHV), also called human herpes virus 8 (HHV-8)
5. Merkel cell polyomavirus causing Merkel cell carcinomas

EBV: African form of Burkitt lymphoma

EBV-related oncogenesis: Evasion of immune system is the key step.

LMP-1 gene plays a role in oncogenesis induced by: Epstein-Barr virus.

Burkitt lymphoma: EBV is not directly oncogenic, but acts as a polyclonal B-cell mitogen → favors **t(8;14) translocation** → activate the *c-MYC* oncogene → release the cells from normal growth regulation.

Hepatitis C virus: Oncogenic RNA virus.

HHV8:
- Kaposi sarcoma
- Primary effusion lymphoma
- Multicentric Castleman disease

Kaposi sarcoma:
- Vascular neoplasm
- Most common neoplasm, associated with AIDS.

Neoplasms due to *Helicobacter pylori:*
- Gastric adenocarcinoma
- MALT lymphoma

Helicobacter pylori: Gene involved is CagA
Aspergillus flavus produces aflatoxin B: Hepatocellular carcinoma.
Clonorchis sinensis: Cholangiocarcinoma.
Schistosoma: Squamous cell carcinoma of urinary bladder.

Estrogen: Endometrial adenocarcinoma.

Radiation Carcinogenesis

Q. Write short note on radiation induced cancers.

Radiation is a **well-known carcinogen**.

Latency: **Extremely long latent period** is common and **it has a cumulative effect**. Radiation has also **additive or synergistic effects with other** potential **carcinogenic agents**.

Types of radiation: They are divided into two types, namely (1) ultraviolet (UV) rays of sunlight, and (2) ionizing electromagnetic and particulate radiation.

Ultraviolet Rays

They are derived from the sunlight.

Tumors caused: **Skin cancer,** namely (1) **squamous cell carcinoma**, (2) **basal cell carcinoma,** and (3) **malignant melanoma**. They are more

common on parts of the body regularly exposed to sunlight and ultraviolet light (UVL).

Risk Factors

The amount of damage incurred depends on:

- **Type of UV rays**
- **Intensity of exposure**
- **Protective mantle of melanin**
  - **Melanin** absorbs UV radiation and has a **protective effect**.
  - Skin cancers are more common in fair-skinned people and those living in geographic location receiving a greater amount of sunlight (e.g., Queensland, Australia, close to the equator).

Pathogenesis

- UV radiation leads to → **formation of pyrimidine dimers in DNA**, which is a type of DNA damage which is responsible for carcinogenicity.
- **DNA damage is repaired by the nucleotide excision repair pathway.**
- With **excessive sun exposure**, the DNA **damage exceeds the capacity of the nucleotide excision repair pathway** and **genomic injury becomes mutagenic and carcinogenic**.
- **Xeroderma pigmentosum**: It is a rare **hereditary autosomal recessive disorder** characterized by **congenital deficiency of nucleotide excision repair DNA**. These individuals **develop skin cancers** (basal cell carcinoma, squamous cell carcinoma and melanoma) due to impairment in the excision of UV-damaged DNA.

Ionizing Radiation

Electromagnetic (X-rays, γ-rays) and particulate (α particles, β particles, protons, neutrons) radiations are all carcinogenic.

Cancers Produced

- **Medical or occupational exposure**, e.g., **leukemia and skin cancers**
- **Nuclear plant accidents:** Risk of **lung cancers**.
- **Atomic bomb explosion**: Survivors of atomic bomb explosion (dropped on Hiroshima and Nagasaki) → increased incidence of **leukemias** →

mainly acute and chronic myelogenous leukemia after about 7 years. Subsequently, increased mortality due to **solid tumors** (e.g., breast, colon, thyroid and lung).

- **Therapeutic radiation**: (1) **papillary carcinoma of the thyroid** follows irradiation of head and neck and (2) **angiosarcoma of liver** due to radioactive thorium dioxide used to visualize the arterial tree.

Mechanism: Hydroxyl free radical injury to DNA.

Tissues which are relatively resistant to radiation-induced neoplasia: Skin, bone and the gastrointestinal tract.

UV rays causes skin cancer:
1. Squamous cell carcinoma
2. Basal cell carcinoma
3. Malignant melanoma

Lymphoid tissue: Most sensitive to radiation.

Bone: Least sensitive to radiation.

UV radiation: Induces formation of pyridine dimers in DNA leading to mutations.

Acute leukemia: Most frequent malignant tumor caused by radiation.

Total body radiation: Lymphopenia is the first hematological feature.

Xeroderma pigmentosum is caused due to abnormalities in: Nucleotide excision repair.

Ionizing radiation: Damages DNA.

Ionizing radiation: Causes genetic damage by—
1. Chromosomal breakage
2. Translocations
3. Point mutations

Neoplasms associated with therapeutic radiation:
1. Papillary carcinoma of thyroid
2. Angiosarcoma of liver

CLL: Not associated with ionizing radiation.

LABORATORY DIAGNOSIS OF CANCER

Q. Write short note on laboratory diagnosis of cancer.

Confirmation of lesion as neoplastic usually requires cytological and/or histopathological examination of the suspected organ or tissue. Different laboratory methods available for the diagnosis of malignant tumors are:

Morphological Methods

Histopathological Examination

Histopathological diagnosis is based on the microscopic features of neoplasm and by this method of examination, **accurate diagnosis can be made in majority of cases.**

- **Clinical data**: It should be provided for accurate pathologic diagnosis e.g.,
  - Radiation causes changes in the skin or mucosa that mimic changes seen in cancer.
  - Sections taken from the site of a healing fracture can mimic an osteosarcoma.
- **Adequate and representative** area of the **specimen** should be sent.
- **Proper fixation**.

Frozen Section

Q. Write short essay/note on frozen section and its uses.

In this method, **tissue is hardened rapidly by freezing** (hence termed frozen section) and **sections are cut by special instrument** called **freezing microtome or cryostat** (microtome in a refrigerated chamber). Its **uses are**:

- **Rapid diagnosis:** Frozen section is used for quick histologic diagnosis (within minutes) and useful for determining the nature of a tumor (benign or malignant) lesion, especially when the **patient is still on the operation table**. It will help to determine whether a lesion is a neoplasm and, if so, whether it is benign or malignant..
- **Evaluation of the margins of an excised cancer** to know whether excision of the neoplasm is complete. If resection margins are inadequate, additional tissue can be removed immediately, without the need for a subsequent operation.

- **Demonstration of fat** mainly in non-neoplastic lesions.
- To know the lymph node (sentinel) status in carcinoma. Knowing the extent of regional tumor metastases may be useful in deciding the further surgery.
- **To determine whether additional workup is necessary** for a particular tissue specimen while it is still fresh. For example, if the metastatic tumor found in a lymph node is recognized as a poorly differentiated carcinoma, special fixation and electron microscopy may be needed for proper diagnosis.

Various Techniques for Tissue Sampling

- **Needle biopsy**: It is called as cutting-needle or core- needle or drill biopsy. Using cutting needle, a core of tissue1– 2 mm wide and 2 cm long is obtained for histologic examinations or special studies that permit evaluation of architectural structure. Tissue obtained is small and interpretation may be difficult.
- **Endoscopy biopsy:** It is performed through endoscopy. Usually performed for lesions in gastrointestinal, respiratory, urinary and genital tracts.
- **Incision biopsy:** In this representative tissue sample is obtained by incising the lesions. Incisional biopsy (along with fine-needle aspiration) is often the method of choice for inoperable lesions, or too large for excision, or when excision causes functional or cosmetic impairment.
- **Excision biopsy**: In this entire abnormal lesion is surgically removed. It provides generous amounts of tissue for diagnosis and may be surgical therapy for some tumors (e.g., small- to medium-sized breast cancers.

Pitfalls in biopsy interpretation: These include inadequate tissue sampling and artifacts due to procedure itself (e.g., thermal damage caused by an electrocautery or laser).

Cytological Examination

It is performed on many tissues and usually done for identifying neoplastic cells.

Methods of Obtaining Cells

Exfoliative cytology: It is the **study of spontaneously exfoliated (shed) cells** from the lining of an organ into a body cavity. Discussed in detail on pages 279-81.

Fine-needle Aspiration Cytology

It involves **aspiration of cells and attendant fluid with a small-bore needle**. The smears are prepared and stained, followed by microscopic examination of cells. It is **widely used, simple and quick procedure** (For details *refer* pages 281-3).

Liquid-based cytology (thin prep): This is a special technique for preparation of samples that provides uniform monolayered dispersion of cells on smears.

Cytological Characteristics of Cancer Cells

Cancer cells have decreased cohesiveness and show cellular features of **anaplasia**. Cytologically, differentiation can be made between normal, dysplastic, carcinoma in situ and malignant cells.

Disadvantages of Cytological Examination

- **Diagnosis is based on the features of individual cells** or a clump of cells, without the supporting evidence of loss of orientation.
- The **invasion** which is diagnostic of malignant tumor under histology **cannot be assessed** by cytology.

Histochemistry and Cytochemistry

These are stains, which identify the chemical nature of cell contents or their products. H&E staining cannot demonstrate certain specific substances/constituents of cells. This requires some special stains. Common histochemical and cytochemical stains useful in diagnosis of tumors are listed in **Table 7.13**.

Immunohistochemistry

Q. Write short note on immunohistochemistry and its role in the diagnosis of tumors.

It is an immunological method of identifying the antigenic component in the cell or one of its components by using specific antibodies. It is widely used in the diagnosis or management of malignant neoplasms.

Uses of Immunohistochemistry

- **To categorize undifferentiated cancers:** Many malignant tumors of diverse origin resemble each other and are difficult to distinguish on routine hematoxylin and eosin (H&E) sections.
  - Example: Few anaplastic carcinomas, lymphomas, melanomas and sarcomas may look almost similar. They should be accurately diagnosed because of their different modes of treatment and prognosis.
    - In **poorly differentiated carcinoma intermediate filaments** (e.g., cytokeratins) shows positivity **(Table 7.14)**.
    - **Malignant melanomas when unpigmented** (amelanotic melanoma) appear similar to other poorly differentiated carcinomas. They express **HMB-45** and S-100 protein, but negative for cytokeratins.
    - **Desmin** is found in neoplasms of **muscle cell origin**.
- **To determine the origin of poorly differentiated metastatic tumors**: It may be determined by using tissue-specific or organ-specific antigens **(Table 7.15)**.
- **For prognosis or to select the mode of treatment:**
  - Identification of **hormone** (estrogen/progesterone) **receptors in breast cancer** cells is of **prognostic and therapeutic value**. These cancers respond well to antiestrogen therapy and have a better prognosis.
  - Breast cancers with **ERBB2 protein (HER2/neu) positivity have a poor prognosis**.

TABLE 7.13: Common histochemical and cytochemical stains useful in diagnosis of tumors.

Chemical substance	*Name of the stain*

TABLE 7.13: Common histochemical and cytochemical stains useful in diagnosis of tumors.

Chemical substance	*Name of the stain*
Basement membrane/collagen	• Periodic Acid Schiff (PAS) • Reticulin • Masson trichrome • Van Gieson
Glycogen	PAS with diastase
Mucin	Combined Alcian blue-PAS Mucicarmine
Cross-striation	Phosphotungstic acid hematoxylin (PTAH)
Enzymes	• Myeloperoxidase • Acid phosphatase • Alkaline phosphatase

TABLE 7.14: Intermediate filaments and their use in diagnosis of neoplasms.

Type of intermediate filament	*Normal issue expression*	*Diagnostic usefulness*
1. Cytokeratin	All epithelial cells	Carcinoma
2. Vimentin	Mesenchymal cells	Sarcoma
3. Desmin	Muscle cells	Tumors of muscle (e.g., rhabdomyosarcoma)
4. Glial fibrillary acid protein (GFAP)	Glial cells	Glial tumors (e.g., astrocytoma)
5. Neurofilament (NF)	Neurons and neural crest derivatives	Neural tumors (e.g., neuroblastoma)

TABLE 7.15: Lineage-associated immunohistochemical markers useful in establishing the origin of a poorly carcinoma.

Lineage-associated markers	*Associated cancer*
Prostate-specific antigen (PSA) and prostate-specific acid phosphatase (PSAP)	Prostatic carcinoma

TABLE 7.15: Lineage-associated immunohistochemical markers useful in establishing the origin of a poorly carcinoma.

Lineage-associated markers	*Associated cancer*
Carcinoembryonic antigen (CEA)	Colonic carcinoma
Thyroglobulin	Thyroid carcinoma
CA 125	Ovarian cancers
Nuclear **receptors for estrogen and progesterone**	Carcinoma of breast

Immunohistochemical Markers

Q. List tumors in which immunohistochemistry has diagnostic role.

Apart from the various immunochemical markers mentioned above, other markers useful are as follows:

- **Neuroendocrine tumors** show positivity for cytokeratins like carcinomas, but they can be identified by their contents, namely:
  - Chromogranins (proteins found in neurosecretory granules)
  - Neuron-specific enolase (NSE)
  - Synaptophysin.
- **Soft tissue sarcomas:** They show intermediate filament positivity
  - **Vimentin**
  - **Desmin** is positive in smooth or striated muscle fibers
  - **Muscle-specific actin** marker for muscle tissue.
- **Neurofilament proteins: Marker for tumors of neurons, neuroblastomas and ganglioneuroma.**
- **Neuron-specific enolase** in neuroblastomas.
- **Glial fibrillary acidic protein** (GFAP), also intermediate filament expressed in **glial cell neoplasms.**
- **Malignant lymphomas:** Generally positive for **leukocyte common antigen** (LCA, CD45). Markers for lymphomas and leukemias are called cluster designations (CDs) and useful to differentiate T and B lymphocytes, monocytes, and granulocytes and the mature and immature variants of these cells.
- **Vascular tumors** derived from endothelial cells, include benign hemangiomas, and malignant hemangiosarcomas and are positive for

factor **VIII-related antigen** or certain lectins.

- **Proliferating cells:** Cells in cell cycle show positivity for **Ki-67** and proliferating cell nuclear antigen (**PCNA**).

Electron Microscopy

It helps in the **diagnosis of poorly differentiated/undifferentiated cancers**, which cannot identify the origin by light microscopy, e.g., carcinomas show desmosomes and specialized junctional complexes, structures which are not seen in sarcomas or lymphomas.

Flow Cytometry

Q. Write short note on modern techniques in tumor diagnosis.

It quantitatively measures various individual cell characteristics, such as membrane antigens and the DNA content of tumor cells. Flow cytometry is useful for identification and **classification of tumors of T and B lymphocytes** (leukemias and lymphomas) and mononuclear-phagocytic cells.

Circulating Tumor Cells

Detection, quantification, and characterization of rare solid tumors cells (e.g., carcinoma, melanoma) circulating in the blood is emerging as a diagnostic modality though presently in research stage. Few latest devices detect three-dimensional flow cells coated with antibodies specific for tumor cells of interest (e.g., carcinoma cells) in the blood. It will be useful for early diagnosis, to assess the risk of metastasis and assess the response of tumor cells to therapy.

Tumor Markers

Q. Write short note on tumor markers.

Q. List tumor markers giving one example for each.

Tumor markers are **products of malignant tumors** that can be detected in the cells themselves or in blood and body fluids.

Usefulness

- **Detection of cancer,** e.g., **PSA** is the most common and useful tumor markers used to screen **prostatic adenocarcinoma**. High levels of PSA are found in the blood of prostatic carcinoma patients but it also may be elevated in benign prostatic hyperplasia.
- **Determine the effectiveness of therapy.**
- **Detection of recurrence.**

Types of markers (Table 7.16): These may be tumor-associated hormones, oncofetal antigens, specific proteins, mucin and glycoproteins, enzymes and molecular markers.

Molecular Diagnosis

Molecular diagnosis can be done by different techniques such as FISH technique and PCR (polymerase chain reaction) analysis.

a. Diagnosis of Cancer

- **Monoclonal (**malignant) **versus polyclonal (benign):** To differentiate benign (polyclonal) proliferations of T- or B-cells from malignant (monoclonal) proliferations.
- **Chromosomal alterations:** Many **hematopoietic neoplasms** (leukemias and lymphomas) and few solid tumors (e.g., Ewing sarcoma) are characterized by particular translocations that can be detected by FISH technique or by PCR analysis.

Q. Write short note on tumor markers for choriocarcinoma.

Q. Write short note on carcinoembryonic antigen.

Q. Write short note on alpha fetoprotein.

TABLE 7.16: Common tumor markers.

Tumor marker	*Associated tumors*

TABLE 7.16: Common tumor markers.

Tumor marker	*Associated tumors*
1. **Hormones**	
– Human chorionic gonadotropin (hCG)	Trophoblastic tumors, nonseminomatous tumors of testis
– Calcitonin	Medullary carcinoma of thyroid
– Catecholamine	Pheochromocytoma
– Ectopic hormones	Paraneoplastic syndromes **(Table 7.17)**
2. **Oncofetal Antigens**	
– α-fetoprotein (AFP)	Cancer of liver, nonseminomatous germ cell tumors of testis
– Carcinoembryonic antigen (CEA)	Carcinomas of the colon, pancreas, lung and stomach
3. **Mucins and Other Glycoproteins**	
– CA-125 – CA-19-9 – CA-15-3	Ovarian cancer Colon cancer, pancreatic cancer Breast cancer
4. **Isoenzymes**	
– Prostatic acid phosphatase (PAP)	Prostate carcinoma
– Neuron-specific enolase (NSE)	Small-cell carcinoma of lung, neuroblastoma
5. **Specific Proteins**	
– Immunoglobulins	Multiple myeloma and other gammopathies
– Prostate-specific antigen (PSA)	Prostate carcinoma
6. **New Molecular Markers**	
– *p53*, *APC*, *RAS* mutants in stool and serum	Carcinoma colon
– *p53* and *RAS* mutants in sputum and serum	Lung cancer

Elevated AFP:
- Hepatocellular carcinoma
- Germ cell tumor
- Cirrhosis

CA–125 is associated with: Ovarian cancer

b. Prognosis of Cancer

Certain genetic alterations are of prognostic value. They can be detected by routine cytogenetics and also by FISH or PCR assays. Example of poor prognostic feature is amplification of the N-*MYC* gene and deletions of 1p in neuroblastoma and amplification of *HER-2/Neu* in breast cancer.

c. Detection of Minimal Residual Disease

PCR can detect minimal residual disease or the onset of relapse in patients who are treated for leukemia or lymphoma, e.g., detection of *BCR-ABL* transcripts in treated patients with CML.

d. Detection of Hereditary Predisposition to Cancer

Germline mutations in many tumor suppressor genes are associated with increased risk for specific cancers. This will help in prophylactic surgery, and counseling of relatives at risk, e.g., *BRCA1*, *BRCA2* and the *RET* proto-oncogene.

e. For Therapeutic Decision

It is useful in target therapy.

Molecular Profiling of Tumors

Traditional Cancer Typing

Traditionally cancer is diagnosed and classified according to the morphological (histopathology/cytopathology) appearance of the cells and its surrounding tissue. It has limitations such as (i) it relies on a subjective review of the tissue which is dependent on the knowledge and experience of a pathologist, and therefore may not be reproducible, (ii) limited ability to determine the individual recurrence risk of cancer, (iii) insufficient to reflect the complicated underlying molecular events that drive the neoplastic process and (iv) histopathology reports lack or offer very little information regarding the potential drug treatment regime to which a cancer will respond. Traditional pathology reports helps to determine treatment that

leads to better outcomes. However, tumors with identical pathology may have different origins and respond differently to treatment.

Newer Cancer Diagnostics

Molecular profiling of tumors is the identification and documentation of the structure of a specific DNA, RNA, or protein molecule. Till recently, molecular studies of tumors consisted of analyzing individual genes. However, the revolutionary technologies which have achieved completion of the human genome sequence and more reliable methods of gene expression analysis have revolutionized cancer diagnosis and treatment. Present methods like DNA microarray technology can measure the expression **single gene to all genes** in the genome instead of only one gene at a time. By providing a molecular portrait of an individual cancer, this technology will help the clinicians to determine the origin of the cancer, its potential for metastasis, its specific drug responsiveness, and the probability of its recurrence. Classification of cancer typing by molecular profile overcomes the limitations of traditional cancer typing. A molecular profile determines the level of gene expression within the cancer by hybridizing the cellular RNA with known genes. However, RNA is prone to degradation and is a more difficult analyte to work with than DNA in clinical practice.

DNA Microarray

Currently, the most common method for large-scale analysis of RNA expression is based on DNA microarrays. A DNA **microarray** (also known as DNA chip or biochip) is a collection of microscopic DNA spots attached to a solid surface. This provides information on thousands of genes simultaneously. Once the gene expression pattern is determined, this information is compared to the expression profiles of cancers with known outcomes using a predetermined algorithm.

DNA Sequencing

It is the process of determining the precise order of nucleotides within a DNA molecule and is technically simpler than RNA sequencing. **Next-generation sequencing** refers to non-Sanger-based high-throughput DNA

sequencing technologies. Millions or billions of DNA strands can be sequenced in parallel, yielding substantially more throughput and minimizing the need for the fragment-cloning methods that are often used in "Next-generation [NextGen] sequencing" (*refer* page 250) which can be readily performed on virtually any tissue specimen. By nextGen sequencing, the process of whole-genome of individual tumors can be completed in as little as a few weeks. The cancer genome sequencing can identify new mutations in various cancers; describe the full, extensive collection of genetic lesions in individual cancers; and a presence of genetic heterogeneity in individual cancers from area to area. It is particularly useful to tumors, such as lung carcinomas, that are genetically diverse and require a "personalized" approach for successful targeted therapy. However, histopathological examination of tumors provides information about important characteristics of cancers, such as anaplasia, invasiveness, and tumor heterogeneity. These cannot be obtained from DNA sequences. Thus, the most accurate diagnosis and assessment of prognosis in cancer can be arrived by a combination of morphologic and molecular techniques (i.e., molecular profiling of tumors by RNA expression profiling, DNA sequencing, and DNA copy number arrays).

Histopathological specimens: Most commonly used fixative is 10% buffered formaline (formaldehyde).

Diagnosis of neoplasia depends on:

- Clinical investigation
- Imaging
- Laboratory investigations

Principle of exfoliative cytology: Cells normally exfoliate from any surface lining and this exfoliation increases in pathological conditions.

Most common malignant tumor in children: Acute lymphoblastic leukemia.

Most common cause of cancer death in adults: Carcinoma lung.

Uses of immunohistochemistry:

1. Categorization of undifferentiated cancers
2. Determine the origin of poorly differentiated metastatic tumor
3. Select the mode of treatment and to know the prognosis

Carcinoma: Cytokeratin +ve.

Malignant melanoma: HMB-45 +ve (more specific) and S100 +ve (more sensitive) after S100+ve.

BCL2 is a marker for: Follicular lymphoma.

Neuroendocrine tumors:
- Chromogranin +ve
- NSE +ve
- Synaptophysin +ve

Malignant lymphoma: Leukocyte common antigen (LCA, CD45) +ve.

Soft tissue sarcoma:
- Vimentin +ve
- Desmin +ve in tumors of smooth or striated muscle

Electron microscopy: Fixative used is glutaraldehyde

Immunohistochemistry and flow cytometry: Help in the diagnosis and classification of neoplasms.

Tumor markers: Products of malignant tumors.

PSA is specific for prostatic diseases but not specific for prostatic cancer. This has both low sensitivity and low specificity.

CLINICAL ASPECTS OF NEOPLASIA

CC: Describe the effects of tumor on the host including paraneoplastic syndrome.

Q. Clinical features of malignant tumors.

Both benign and malignant tumors may produce clinical features by its various effects on host.

Local Effects

These are due to encroachment on adjacent structures.

- **Compression** (e.g., adenoma in the ampulla of Vater causing obstruction of biliary tract).
- **Mechanical obstruction:** It may be caused by both benign and malignant tumors. Example: Tumors may cause obstruction or intussusception in the GI tract.

- **Endocrine insufficiency:** It is caused due to destruction of an endocrine gland either due to primary or metastatic cancer.
- **Ulceration**, **bleeding and secondary infections:** It may develop in benign or malignant tumors in the skin or mucosa of the GI tract, e.g.,
  - Melena (blood in the stool) in neoplasms of the gut
  - Hematuria in neoplasms of the urinary tract.
- **Rupture or infarction of tumor.**

Functional Effects

These include:

- **Hormonal effects**: It may be observed both in benign and malignant tumors of endocrine glands, e.g., β-cell adenoma of the pancreas may produce insulin → to cause fatal hypoglycemia.
- **Paraneoplastic syndromes:** Nonendocrine tumors may secrete hormones or hormone-like substances and produce paraneoplastic syndromes (explained below).
- **Fever:** It is most commonly associated with Hodgkin disease, renal cell carcinoma and osteogenic sarcoma. Fever may be due to release of pyrogens by tumor cells or IL-1 produced by inflammatory cells in the stroma of the tumor.

Tumor Lysis Syndrome

- It is a group of metabolic complications that can occur after treatment for leukemias such as acute **lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL); lymphomas such as Burkitt lymphoma,** and uncommonly in solid tumors.
- It is caused by **breakdown products of tumor cells following chemotherapy or glucocorticoids or hormonal agent** (tamoxifen).
- The killed tumor cells **release intracellular ions and large amounts of metabolic byproducts into systemic circulation**.
- **Metabolic abnormalities** include:
  - **Hyperuricemia:** Due to increased turnover of nucleic acids.
  - **Hyperkalemia:** Due to release of the most abundant intracellular cation potassium.
  - **Hyperphosphatemia:** Due to release of intracellular phosphate.

- **Hypocalcemia:** Due to complexing of calcium with elevated phosphate.
- **Lactic acidosis.**
- **Hyperuricemia:** It can cause uric acid precipitation in the kidney resulting in **renal failure**.

Cancer Cachexia (Wasting)

Q. Write short note on cachexia.

It is defined as **progressive weight loss accompanied by severe weakness, anorexia and anemia developing in patients with cancer**.

- **Mechanism:** It is poorly understood and may be due to **TNF and other cytokines**, like IL-1, interferon-γ, and leukemia inhibitory factor. They may be produced by macrophages in the tumor or by the tumor cells themselves.

Functional effects of tumor:
- Production of hormones
- Paraneoplastic syndrome
- Fever

Tumor lysis syndrome: Associated with hypocalcemia and NOT hypercalcemia.

Cachexia: TNF-α plays an important role.

Cancer cachexia: Progressive weight loss accompanied by severe weakness, anorexia and anemia.

PARANEOPLASTIC SYNDROMES

Q. Write short note on paraneoplastic syndrome.

Malignant tumors invade local tissue, produce metastasis and can produce a variety of products that can stimulate hormonal, hematologic, dermatologic and neurologic responses.

Definition: Paraneoplastic syndromes are **symptom complexes in cancer patients** which are **not directly related to mass effects or invasion or metastasis or by the secretion of hormones indigenous to the tissue of origin.**

Frequency: Though they occur in 10–15% of patients, it is important because:

1. **May be the first manifestation** of an occult neoplasm.
2. **May be mistaken for metastatic disease** leading to inappropriate treatment.
3. May present clinical problems which **may be fatal**.
4. Certain tumor products causing paraneoplastic syndromes may be **useful in monitoring recurrence** in patients who had surgical resections or are undergoing chemotherapy or radiation therapy.

Some paraneoplastic syndromes, their mechanism and common cancer causing them are listed in **Table 7.17**.

Q. Write short note on tumors which produce paraneoplastic syndromes.
Q. Write short note on paraneoplastic syndromes produced by small cell (oat cell) carcinoma of lung.
Q. Write short note on paraneoplastic syndromes produced by renal cell carcinoma (RCC).

TABLE 7.17: Paraneoplastic syndromes.

Clinical syndromes	*Cause/mechanism*	*Example of associated cancer*
1. Endocrinopathies		
Cushing syndrome	ACTH or ACTH-like substance	Small-cell carcinoma of lung
Syndrome due to inappropriate antidiuretic hormone secretion (SIADH)	Antidiuretic hormone or atrial natriuretic hormones	Small-cell carcinoma of lung
Hypercalcemia	Parathyroid hormone-related protein (PTHRP), TGF-α, TNF, IL-1	Squamous cell carcinoma of lung
		Renal carcinoma
Carcinoid syndrome	Serotonin, bradykinin	Bronchial carcinoid
Hypoglycemia	Insulin or insulin-like substance	Fibrosarcoma
Polycythemia	Erythropoietin	Renal carcinoma, hepatocellular carcinoma
2. Neurologic (neuromyopathic) syndromes		
Myasthenia	Immunological	Bronchogenic carcinoma
3. Cutaneous syndromes		
Acanthosis nigricans	Immunological; secretion of epidermal growth factor	Carcinoma of stomach, lung and uterus
Dermatomyositis	Immunological	Bronchogenic, breast carcinoma
Exfoliative dermatitis	Immunological	Lymphoma
4. Changes in osseous, articular and soft-tissue		
Hypertrophic osteoarthropathy and clubbing of the fingers	Not known	Bronchogenic carcinoma
5. Vascular and hematologic syndromes		
Venous thrombosis (Trousseau syndrome)	Tumor products like mucins which activate clotting	Pancreatic carcinoma
		Bronchogenic carcinoma
Disseminated intravascular coagulation	Procoagulant substance: Cytoplasmic granules (e.g., acute promyelocytic leukemia cells) or mucus (adenocarcinomas)	Acute promyelocytic leukemia, prostatic adenocarcinomas
Nonbacterial thrombotic endocarditis	Hypercoagulability	Advanced mucus secreting adenocarcinomas
6. Renal syndromes		
Nephrotic syndrome	Tumor antigens, immune complexes	Various cancers
7. Amyloidosis		
Primary amyloidosis	Immunological (AL protein)	Multiple myeloma
Secondary amyloidosis	AA protein	Renal cell carcinoma and other solid tumors

(ACTH: adrenocorticotropic hormone; IL: interleukin; TGF: transforming growth factor; TNF: tumor necrosis factor).

Syndrome of inappropriate ADH (SIADH): Most common cause is oat cell carcinoma of lung.

PROGNOSIS

Q. Write short note on prognostic factors of malignant tumors.

The prognosis of malignant tumors vary and is determined partly by the characteristics of the tumor cells (e.g., growth rate, invasiveness) and partly by the effectiveness of therapy.

Prognostic Indices

Prognosis and the treatment of a malignant tumor depend on:

1. **Tumor type**: It is usually identified from the growth pattern of the tumor and its origin by only histopathological examination.
   - **Prognosis depends on the histological type** (e.g., squamous cell carcinoma, melanoma, adenocarcinoma, leiomyosarcoma).
   - Some tumors like lymphomas require further subclassification into Hodgkin and non-Hodgkin's lymphoma, each of which is then further subclassified by the cell type.
2. **Grading of malignant tumors**: It is done by histological examination and is mainly based on the **degree of differentiation** of the tumor cells.
   - In general, there is a **correlation between histologic grade and biologic behavior**.
   - Most grading systems classify tumors into three or four grades of increasing malignancy. Low-grade tumors are well-differentiated; high-grade ones tend to be anaplastic.
     - **Shortcomings: (1) Less correlation with behavior**: In general, in soft-tissue sarcomas, grading is of less clinical value than staging; **(2) subjective:** Grading is subjective and the degree of differentiation can vary in different areas of the same tumor.
3. **Staging of tumors:** It **refers to the extent of spread of a malignant tumor** and is independent of grading. The **mode of treatment is determined by the stage of a cancer** than by its grade.
   - **Criteria:** Staging requires **both histopathological examination** of the resected tumor and **clinical assessment** of the patient [including additional non-invasive techniques like computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET)].
   - The **criteria used** for staging **vary with different organs**. Commonly the staging of cancers is based on:
     - **Size and extent of local growth of the primary tumor** (e.g., in colorectal cancer, the tumor which has penetrated into the

muscularis and serosa of the bowel is associated with a poorer prognosis than with a tumor restricted to superficial mucosa/submucosa).

- **Extent of spread to regional lymph nodes**: Presence of lymph node metastases indicate poor prognosis than without lymph node involvement.
- **Presence of or absence of blood-borne (distant) metastases**: The presence of blood-borne distant metastases is bad prognostic sign and is a contraindication to surgical intervention other than for palliative measures.

TNM Staging Systems (Fig. 7.22)

Q. Grading and staging of cancer.

It is the cancer staging system widely used and it varies for each specific form of cancer. Its general principles are:

- **T** refers to the **size of the primary tumor**.
  - It is suffixed by a number which indicates the size of the tumor or local anatomical extent. The number varies according to the organ involved by the tumor. With increasing size, the primary lesion is characterized as T1 to T4. T0 is used to denote an in situ lesion.
- **N** refers to **lymph node status**.
  - It is suffixed by a number to indicate the number of regional lymph nodes or groups of lymph nodes showing metastases.
  - N0 would mean no nodal involvement, whereas N1 to N3 would denote involvement of an increasing number and range of nodes.
- **M** refers to the presence and anatomical extent of **distant metastases**.
  - M0 signifies no distant metastases, whereas M1 indicates the presence of metastases.

Grading of tumor depends on the degree of differentiation.

TNM staging system widely used.

Prognosis of tumor depends on:
1. Histological type
2. Grade
3. Stage

TNM staging
- T = size of primary tumor
- N = lymph node status
- M = metastatic status

EXAMPLES OF BENIGN TUMORS

Squamous Papilloma

Squamous papilloma is a common benign tumor of squamous epithelium of the skin and mucosa.

It can occur anywhere in the skin and mouth.

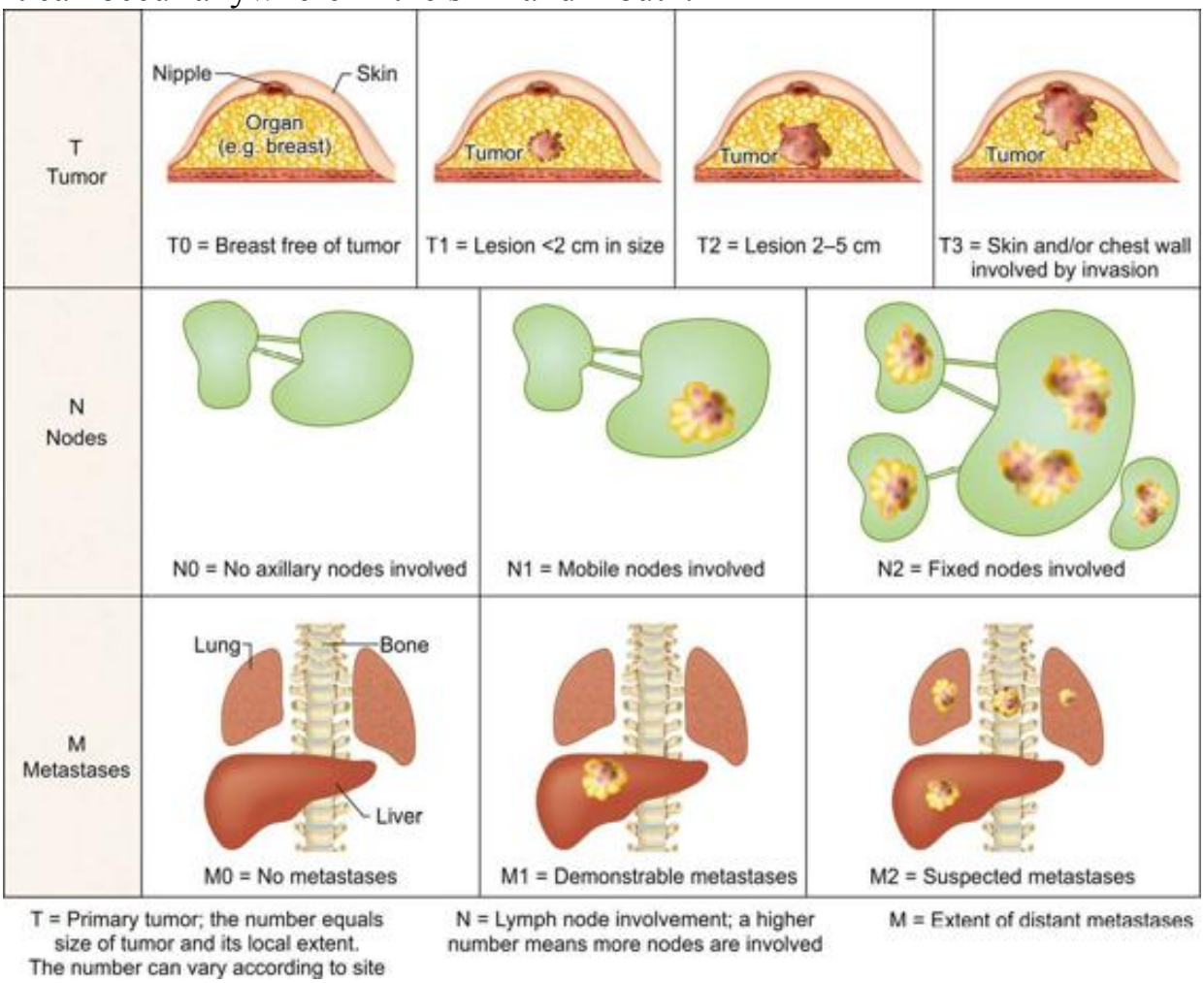
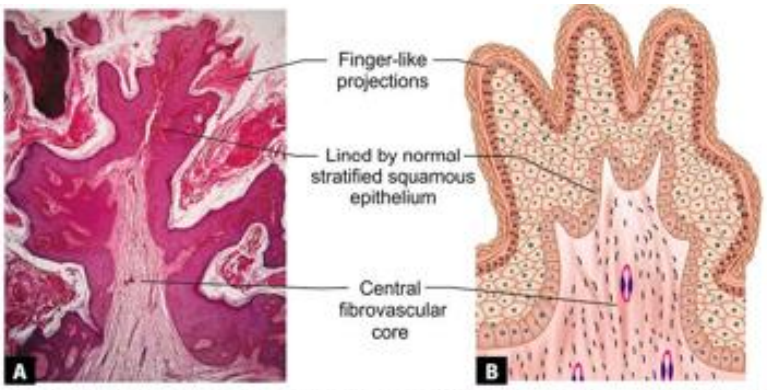


Fig. 7.22: Tumor staging by TNM system (e.g., breast carcinoma).TNM system is just one of many in current use.



Figs. 7.23A and B: Squamous papilloma: (A) H & E and (B) Diagrammatic. Microscopic appearance of squamous papilloma.

Gross: It has exophytic papillary or finger-like projections. The number of papillae varies.

Microscopy (Figs. 7.23A and B): Each papillary (finger-like) projections consists of proliferating squamous epithelia of the epidermis with intact basement membrane and the basal cells. The central region of each papilla is composed of vascularized connective tissue core.

Lipoma and Schwannoma (Neurilemmoma)

For lipoma *refer* page 857 and schwannoma *refer* page 861.

CHAPTER

8

Genetic and Pediatric Diseases

Genetics is the study, which deals with the **science of genes**, heredity and its variation in living organisms. Disorders of childhood are usually of genetic origin. Hence, developmental and pediatric diseases are discussed along with genetic diseases.

GENES

Definition: Gene is defined as a **segment of deoxyribonucleic acid (DNA)** which carries the genetic information. Gene is the basic **physical and functional unit of heredity**. DNA has also segments which do not contain genes.

The human genome contains about **19,000 genes comprising 1.5% of the genome. Each** gene varies in size.

Structure of Gene (Fig. 8.1)

Each gene consists of a specific sequence of nucleotides. **Genes may be silent or active**. When active, the **genes direct the process of protein synthesis**. Genes do not code for proteins directly but **by means of a genetic code**. The **genetic code** consists of a sequence codeword called **codons**. A codon for an amino acid consists of a sequence of three nucleotide base pairs called a **triplet codon**.

Regions of Gene

- **Initiator and stop codons:** The boundaries of a gene are known as **start and stop codons**. The start codon tells when to begin protein production and stop (termination) codons tells when to end the protein production.

- **Coding region:** The **nucleotide sequence between the start and stop codons is** the core region known as **coding region**. This region is divided into two main segments namely—exons and introns. Most of the genes contain both exons and introns, the number of which varies with different genes.
  - **Exons:** This region codes for producing a protein.
  - **Introns:** These are the regions between exons and do not code for a protein (**noncoding region**).
- **Regulatory regions:** These are also noncoding regions which control gene expression.
  - **Promoters** are regions which bind to transcription factors, either strongly or weakly.
  - **Enhancers** are regions which can enhance the effect of a weak promoter.
  - **Silencers** are regulatory regions that can inhibit transcription.

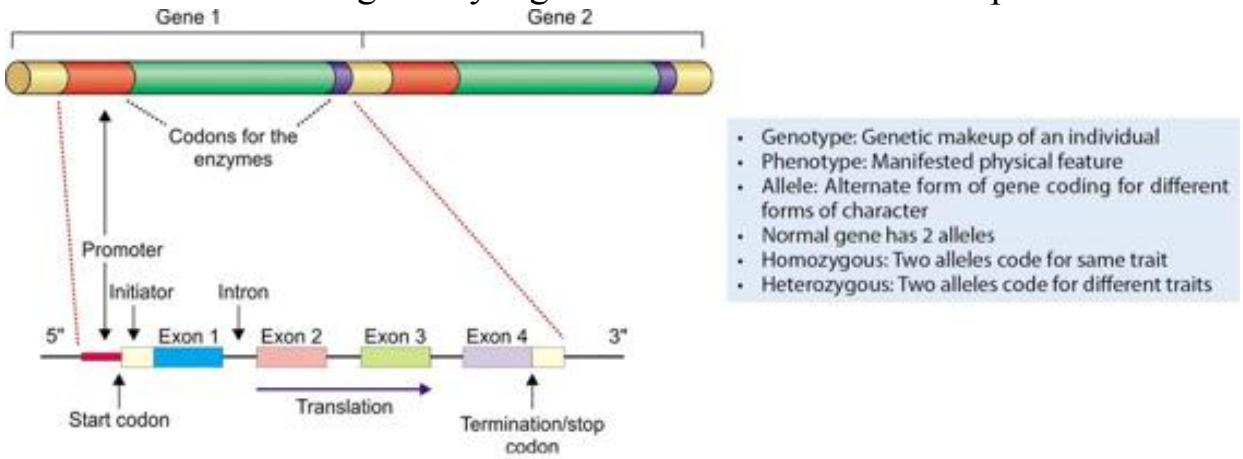


Fig. 8.1: Diagrammatic structure of gene. Start and termination codons mark the limits of the gene. The coding portion of the gene is exons (four in this example), and interspersed with introns.

BOX 8.1: Classification of genetic disorders.

A. Single-gene or monogenic disorders/Mendelian disorders
   - Autosomal dominant
   - Autosomal recessive
   - X-linked dominant
   - X-linked recessive

B. Cytogenetic disorders–chromosomal disorders (aberrations/abnormalities)
   - Numerical aberrations aneuploidy (trisomy, monosomy), polyploidy, and mosaicism
   - Structural aberrations
     - Translocations
       - Balanced reciprocal translocations
       - Robertsonian translocations

- Inversion
- Isochromosome
- Ring chromosome
- Deletions
- Insertions

C. Complex/multifactorial multigenic/polygenic disorders
- Diabetes mellitus
- Hypertension

CLASSIFICATION OF GENETIC DISORDERS

Genetic disorders are classified into three major categories **(Box 8.1)**.

GENETIC ABNORMALITIES

There are many types of genetic abnormalities that affect the structure and function of proteins. This may disrupt the cellular homeostasis and produce disease.

Mutations

Single gene disorders result from mutations in single gene.

Definition: A mutation is defined as a **permanent change in the genetic material (DNA) which results in a disease. The term mutation was coined by Müller in 1927**.

Causes

- **Spontaneous mutation:** Majority of mutations occur spontaneously due to errors in DNA replication and repair.
- **Induced mutation:** Mutations can be caused due to exposure to mutagenic agents like chemicals, viruses, and ultraviolet or ionizing radiation.

Polymorphism: If the **genetic material change/variant does not cause obvious effect upon phenotype, it is termed as polymorphism**. A polymorphism is defined as genetic variation that exists in population with a frequency of >1%.

Classification of Mutations

Depending on the cell affected: Mutations can affect either somatic cells or germ cells.

- **Germ cell mutations:** Mutations that affect the germ cells are transmitted to the progeny/descendants and can produce inherited/hereditary diseases.
- **Somatic cell mutations:** Mutations involving the somatic cells can produce cancers and some congenital malformations. These mutations are not inherited and do not cause hereditary diseases are known as de novo mutations.

Structural Chromosomal Mutations

The rearrangement of genetic material causes structural change. Structural mutations may be (1) visible during karyotyping (*refer* pages 245-6) or (2) submicroscopic (minute/subtle changes). The **submicroscopic gene mutations** can result in partial or complete deletion of a gene or more often, a single nucleotide base. Few examples of genetic mutations are discussed below.

Mutations in Protein-coding Genes

Q. Write short note on point mutation.

Point mutation within coding sequences: Point mutation is characterized by replacement of one **nucleotide base by a different nucleotide base within a gene**. Majority of point mutation occurs in the coding region of a gene.

- **Missense mutations**: If point mutations change the genetic code, it may code for a different amino acid and protein.
  - **Conservative missense mutation**: In this type, the substituted amino acid produces **only little change in the function of the protein**.
  - **Nonconservative missense mutation**: In this type, normal amino acid is replaced by very different amino acid and result in **change in function of protein.** Example, sickle cell anemia in which mutation affect the β-globin chain of hemoglobin **(Fig. 8.2)**. In this, the nucleotide triplet CTC, which encodes glutamic acid, is changed to CAC, which encodes valine. This single amino acid substitution

changes the properties of hemoglobin, giving rise to sickle cell anemia.

- **Nonsense mutation (stop codon):** In this type, point mutation changes an amino acid codon to a **premature termination codon**. Example, in β-globin chain, a point mutation affecting the codon for glutamine (CAG) creates a stop codon (UAG) if U replaces C **(Fig. 8.3)**. This change leads to premature termination of β-globin gene translation → deficiency of β-globin chains → no synthesis of hemoglobin A. It produces a severe form of anemia called β^0-thalassemia.

Frameshift mutation: This may occur due to **insertion or deletion of one or more nucleotides** in the coding regions. If the number of nucleotide bases inserted or deleted is not a multiple of 3, the code will be changed **(Fig. 8.4A)**. This leads to alterations in the reading frame of the DNA strand; hence they are known as frameshift mutation. If the number of base pairs involved is three or a multiple of three, frameshift does not occur. This may synthesize an abnormal protein lacking or gaining one or more amino acids. When deletions involve a large segment of DNA, the coding region of a gene may be entirely removed **(Fig. 8.4B)**.

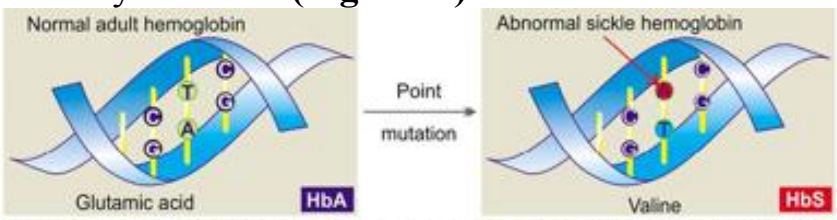


Fig. 8.2: Nonconservative missense point mutation in sickle cell anemia. When adenine replaces thymidine, the amino acid valine replaces glutamic acid in the sixth position of β-globin chain of hemoglobin and give rise to abnormal sickle hemoglobin.

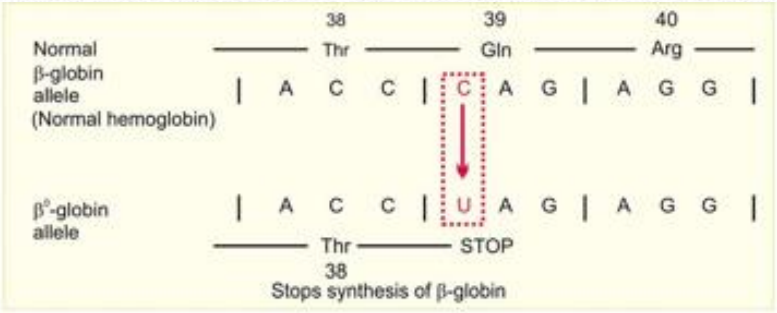
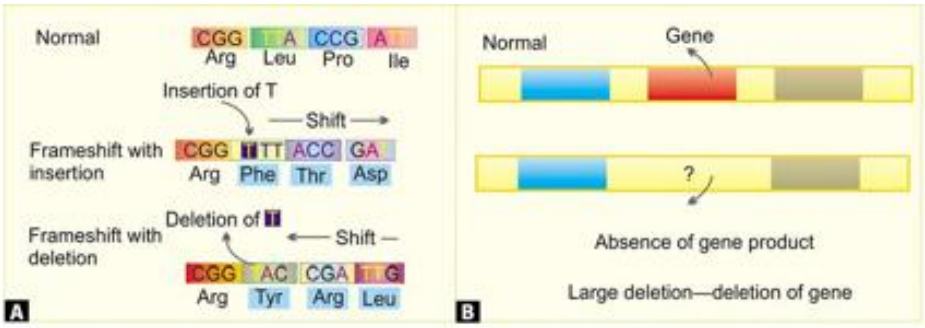


Fig. 8.3: Nonsense point mutation leading to premature chain termination. A point mutation (C replaced by U) in codon 39 changes glutamine (Gln) codon to a stop codon. This stops the synthesis of protein at amino acid 38.



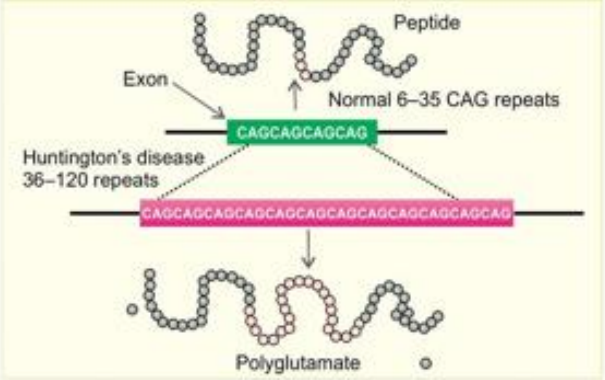
Figs. 8.4A and B: Frameshift mutations. (A) Insertion or deletion of nucleotide bases if not a multiple of 3, the code will be changed; (B) Deletions involving a large segment of DNA in the coding region may entirely remove the gene.

Trinucleotide repeat mutation: The **DNA contains several repeat sequences of three nucleotides (trinucleotide)**. When they are repeated directly adjacent to each other (one right after the other), they are known as **tandem repeats (Fig. 8.5)**. When the repetitive trinucleotide sequences reach above a particular threshold, they can expand (amplify) or contract. The amplification is more common. These trinucleotide-repeat mutation are dynamic (i.e., the degree of amplification increases during gametogenesis).

Functional Effect

Mutations in DNA can lead to either change in the amino acid sequence of a specific protein or may interfere with its synthesis. The consequences vary from those without any functional effect to those which have serious effects.

- **Loss-of-function (LOF) mutations:** These mutations cause the reduction or loss of normal function of a protein. It is usually due to deletion of the whole gene but may also occur with a nonsense or frameshift mutation.
- **Gain-of-function mutations**: These are usually due to missense mutations. In gain-of-function mutation, the protein function is altered in a manner that results in a change in the original function of the gene.
- **Lethal mutations:** These lead to death of the fetus.



Point mutation: Mutation involving a change in a single nucleotide base within a gene

Point mutations:
- Silent mutations: Altered DNA codes same amino acid
- Missense mutations: Altered DNA codes different amino acid
- Nonsense mutation: Altered DNA codes for stop codon and causes premature termination of protein synthesis

Missense mutations: Sickle cell anemia/trait

Nonsense mutation with stop codon: β-thalassemia major

Fig. 8.5: Trinucleotide repeat disorder, e.g., Huntington's diseases. It results from expansion of a CAG triplet repeat from a normal number of 6 to 35 repeats to greater than 36 repeats. This results in expansion of a polyglutamine sequence in the corresponding protein.

Alterations in Protein-coding GenesOther Than Mutations

Apart from alterations in DNA sequence, coding genes also can undergo structural variations. These are copy number changes—*amplifications* or *deletions*—or *translocations*. It may lead to aberrant gain or loss of protein function. Similar to mutations, structural changes may occur in the germline, or be acquired in somatic tissues. Somatically acquired structural alterations (e.g., amplifications, deletions, and translocations) are often seen in cancers. For example, "Philadelphia chromosome"—translocation t (9;22) between the *BCR* and *ABL* genes in chronic myeloid leukemia (*refer* **Fig. 12.16**).

Alterations in Non-coding RNAs

There are very large number of genes that do not encode proteins. The nonencoded products of these genes—so-called "non-coding RNAs (ncRNAs)"—play important regulatory functions [e.g., small RNA molecules called microRNAs (miRNAs) and long non-coding RNAs (lncRNAs)].

MENDELIAN DISORDERS/SINGLE-GENE OR MONOGENIC DISORDERS

Q. Give four examples of each for different Mendelian inheritance.

These genetic disorders result from mutations in single gene.

General Features

- **Location of defective gene: It is on autosomes (autosomal inheritance) or the sex chromosomes (sex-linked inheritance)**.
- **Dominant versus recessive gene: Genes are inherited in pairs—**one gene from each parent. However, the inheritance may not be equal, and **one gene may overpower the other** in their coded characteristic. The **gene that overshadows the other is called the dominant gene; the overshadowed gene is the recessive one**.
- **Homozygote versus heterozygote:** In some autosomal mutations, the disease is partially expressed in the heterozygote and fully expressed in the homozygote, e.g., sickle cell anemia.
- **Codominant inheritance:** Sometimes both of the alleles of a gene pair contribute to the expression of phenotype. It is called codominance, e.g., blood group antigen.

Autosomal Dominant Pattern of Inheritance

General Features

- **Location of mutant gene:** It is on **autosomes**.
- **Required number of defective genes:** Only **one copy**.
- **Sex affected:** Both **males and females** are **equally** affected.
- **Pattern of inheritance:**
  - **Every affected** individual **has** at least one **affected parent.**
  - Normal members of a family do not transmit the disorder to their children.
  - **Risks of transmission to children (offspring):** Affected males and females have an equal risk of passing on the disorder to children.
- **Additional properties:**
  - **Penetrance:** It is the percentage of individuals (with mutation) having clinical symptoms.
    - With **complete penetrance**, **all individuals show clinical symptoms**
    - With **reduced penetrance,** only **some individuals show disease**
    - In **nonpenetrance,** individuals may **not show any symptoms**.

- **Variable expressivity** (qualitatively or quantitatively) of disorder is the term used for variable expression among individuals (even within the same family).
- **Delayed onset:** Symptoms and signs may be delayed and may not appear until adulthood. For example, Huntington's disease.

Q. Name autosomal dominant disorders.

Table 8.1 shows common autosomal dominant disorders.

TABLE 8.1: Example of autosomal dominant disorders.

System affected	*Examples*
Nervous system	• Huntington's disease • Neurofibromatosis • Tuberous sclerosis
Musculoskeletal system	• Marfan syndrome • Osteogenesis imperfecta • Achondroplasia
Hematopoietic system	• Hereditary spherocytosis • von Willebrand disease
Renal system	• Polycystic kidney disease
Gastrointestinal system	• Familial polyposis coli
Metabolic disorders	• Familial hypercholesterolemia

Q. Name autosomal recessive disorders.

TABLE 8.2: Examples of autosomal recessive disorders.

System affected	*Examples*
Inborn errors of metabolism	• Phenylketonuria • Galactosemia • Cystic fibrosis • Homocystinuria • Hemochromatosis • Lysosomal storage diseases (page 250) • Glycogen storage diseases • Wilson disease • α1-antitrypsin deficiency

TABLE 8.2: Examples of autosomal recessive disorders.	
System affected	***Examples***
Hematopoietic system	• Sickle cell anemia • Thalassemias
Skeletal system	• Alkaptonuria
Nervous system	• Friedreich ataxia
Endocrine system	• Congenital adrenal hyperplasia

Autosomal Recessive Pattern of Inheritance

Autosomal recessive disorders constitute the **largest group of Mendelian disorders**.

General Features

- **Location of mutant gene:** It is on **autosome**.
- **Required number of defective gene: Symptoms of the disease appear only when** an individual has **two copies (**both alleles at a given gene locus) of the mutant gene. **When an individual has one mutated gene and one normal gene,** this heterozygous state is called as a **carrier**.
- **Pattern of inheritance:** For a child to be at risk, **both parents must be having at least one copy of the mutant gene.** For example, all inborn errors of metabolism.
- **Sex affected: Females and males are equally affected**.
- **Consanguineous marriage:** It is a common predisposing factor.
- **Risks of transmission:** Siblings have one in four chance of having the trait (i.e., the recurrence risk is 25% for each birth).
- **Expression of disease:** It is more uniform than in autosomal dominant disorders.
- **Penetrance: Complete penetrance** is common.
- **Onset**: It frequently manifest early in life.

Examples of autosomal recessive disorders are shown in **Table 8.2**.

X-linked Pattern of Inheritance

Almost all sex-linked Mendelian disorders are X-linked. Males with mutations involving the Y-linked genes are usually infertile, and hence there

is no Y-linked inheritance. **Expression of an X-linked disorder is different in males and females**.

- **Females:** The **clinical expression** of the X-linked disease is **variable**, depending on whether it is dominant or recessive. **Females are rarely affected by X-linked recessive diseases**; however, they are affected by X-linked dominant disease.
- **Males:** Mutation affecting X-chromosome is **fully expressed** even with one copy, regardless of whether the disorder is dominant or recessive.

X-linked Recessive Traits

General Features

- **Location of mutant gene:** It is on the **X-chromosome** and there is **no male-to-male transmission**.
- **Required number of defective gene: One copy** for the manifestation of disease **in males**, but **two copies** are needed **in females**.
- **Sex affected:** Males are more frequently affected than females; daughters of affected male are all asymptomatic carriers. Affected male does not transmit the disorder to his sons.
- **Pattern of inheritance: Transmission is through female carrier** (heterozygous).
- **Risks of transmission to children (offspring):**
  - An affected male does not transmit the disorder to his sons, but all daughters are carriers.
  - Sons of heterozygous women have 50% chance of receiving the mutant gene.

Examples of X-linked recessive disorders are shown in **Table 8.3**.

X-linked Dominant Disorders

General Features

They are very rare, e.g., vitamin D resistance rickets.

- **Location of mutant gene:** It is on the X-chromosome and there is no transmission from affected male to son.

Q. Name sex-linked inherited disorders.

TABLE 8.3: Examples of X-linked recessive disorders.

System affected	*Examples*
Blood	• Hemophilia A and B • Glucose-6-phosphate dehydrogenase deficiency • Chronic granulomatous disease
Musculoskeletal system	• Duchenne muscular dystrophy
Nervous system	• Fragile-X syndrome
Metabolic disorders	• Diabetes insipidus • Lesch–Nyhan syndrome
Immune systems	• Agammaglobulinemia • Wiskott–Aldrich syndrome

- **Required number of defective gene:** One copy of mutant gene is required for its effect.
  - Often lethal in males and so may be transmitted only in the female line.
  - Often lethal in affected males and they have affected mothers.
  - No carrier state
  - More frequent in females than in males.
- **Risks of transmission to children (offspring):**
  - Transmitted by an affected heterozygous female to 50% of her sons and half her daughters
  - Transmitted by an affected male parent to all his daughters but none of his sons, if the female parent is unaffected.

Mendelian disorders:
1. Genetic disorders due to mutations in single gene.
2. Defective gene may be in the autosome or sex chromosome.

Inheritance pattern of ABO blood group system: Codominant.

Patterns of inheritance for Mendelian disorders:
1. Autosomal dominant
2. Autosomal recessive
3. X-linked dominant
4. X-linked recessive

Penetrance: Percentage of individuals (with mutation) having clinical symptoms.

Autosomal dominant: With reduced penetrance only some individuals show disease.

Autosomal dominant:
- Expression in heterozygous state
- Males and females equally affected
- Both sexes can transmit the disorder

Most common type of Mendelian disorder is autosomal recessive type.

Autosomal recessive:
- Disease develops when both copies of gene are mutated
- Males and females equally affected

Autosomal recessive disorders: Include almost all inborn errors of metabolism.

Autosomal recessive disorder: Both parents must have a mutant gene.

Carrier: Heterozygous state in which an individual has one mutated gene and one normal gene.

Male-to-male transmission is not seen in X-linked dominant disease.

X-linked recessive inheritance: Asymptomatic female carrier transmits mutant gene to 50% of male children.

X-linked dominant inheritance: Female carriers are asymptomatic.

DEVELOPMENTAL DEFECTS

Definition: Developmental defects are a group of abnormalities that occur during fetal life due to errors in morphogenesis.

Q. Write short note on malformations, disruption and deformities with examples.

- **Congenital anomaly (birth defect/congenital defect/congenital disorder):** The term congenital means "born with". All types of the **structural abnormalities** or defects that are present at birth are termed as **congenital anomalies**.
- A **malformation** is a primary (or intrinsic) **structural defect** occurring during the development of an organ or tissue. It may be due to a single gene or chromosomal defect, but are more commonly multifactorial in

origin. Malformations may involve one organ /system or multiple systems.

- **Single system defect** (single abnormality): Single abnormalities may have a genetic or non-genetic basis. Examples, congenital heart defect (such as ventricular or atrial septal defects), anencephaly (absence of the brain), cleft lip and/or palate and neural tube defects.
- **Multiple malformation syndromes (multiple abnormalities):** It consists of defects in two or more systems and is more likely to be due to chromosomal abnormalities.
- **Syndrome:** When a **combination of congenital abnormalities** occur together repeatedly in a consistent pattern due to a single underlying cause, it is termed as "syndrome".

- **Dysmorphology** is the study of **malformations arising from abnormal embryogenesis**.
- **Agenesis** is the complete absence of an organ, e.g., unilateral or bilateral agenesis of kidney.
- **Aplasia** is the absence/failure of development of an organ or tissue to develop or to function normally, e.g., aplasia of lung.
- **Hypoplasia** is underdevelopment or incomplete development of an organ or tissue which does not reach the normal adult size, e.g., microglossia.
- **Atresia** refers to incomplete formation of lumen in hollow viscus, e.g., esophageal atresia.
- Dystrophy is a disorder in which an organ or tissue of the body degenerates.

LYON HYPOTHESIS

Q. Write short note on Lyon hypothesis.

In 1961, Lyon outlined the idea of X-inactivation, now known as the Lyon hypothesis. It states that **only one of the X-chromosomes is genetically active** and:

1. **Other X of either maternal or paternal origin is inactivated** during early stage of embryonic development.
2. **Inactivation of either the maternal or paternal X** occurs **at random** among all the cells **during about 16th day of embryonic life**.

3. **Inactivation of the same X-chromosome persists in all the cells derived from each precursor cell.**

DEMONSTRATION OF SEX CHROMATIN

Q. Write short note on Barr body and sex chromatin.

There are two simple methods:

1. Buccal smear for Barr body (sex chromatin)
2. Leukocytes—nuclear sexing.

Buccal Smear for Barr Body (Fig. 8.6A)

- Barr and Bertram in 1949 identified the presence **of tiny dark granule adjacent to the nuclear membrane**. This granule is known as the **Barr body, or X chromatin or sex chromatin**. It represents one inactive and condensed X-chromosome in a female. The inactive X can be seen in the interphase nucleus as a darkly staining small mass in contact with the nuclear membrane.
- The **number of Barr bodies in a cell depends upon the number of X-chromosome**. It is always one less than the number of X-chromosomes. Thus, normal cells in female (XX) have one Barr body and presence of Barr bodies indicates female genotype. Normal cells in male (XY) have no Barr bodies, because they have only one active X-chromosome. The XXXY cells have two Barr bodies.
- **Demonstration:** Buccal smears are used for demonstration of Barr body are prepared with a thin wooden spatula, by scraping the buccal mucosa. Smears are stained by Papanicolaou stain.

Leukocytes—Nuclear Sexing (Fig. 8.6B)

- Neutrophils in the peripheral smear may also be examined for nuclear sexing. Abnormalities of sex chromosomes can be diagnosed by nuclear sexing.In a normal female (XX), the **neutrophils in a peripheral smear show a drumstick** which is counterpart of Barr body in buccal smear.
- **Absence of drumstick** is observed in **Turner syndrome (**XO), while **one drumstick** is found in males with **Klinefelter syndrome** (XXY).

Y chromosome: Irrespective of the number of X-chromosomes, the presence of a single Y determines the male sex.

Barr body: Attached to inner aspect of nuclear membrane and represents inactivated X-chromosome.

Barr body:
- Absent in normal males
- Normal female has 1 Barr body

CYTOGENETICS

Cytogenetics is a branch of genetics that deals with the **study of the chromosomes**. Karyotype is one of the basic tools of cytogenetics.

Techniques of Cytogenetics

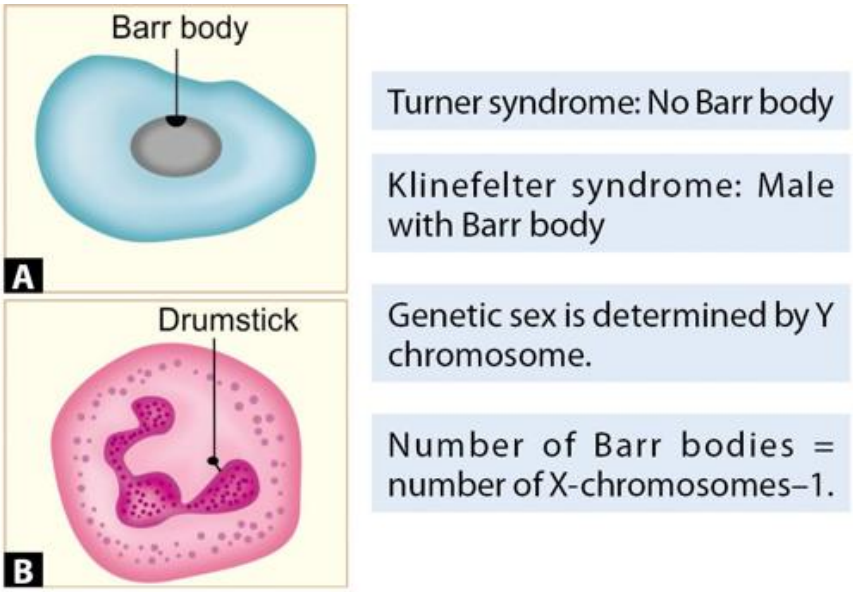
It can be broadly divided into:

- **Conventional cytogenetics:** It is the routine chromosome analysis.
- **Molecular cytogenetics:** Molecular genetics (often called as 'DNA technology') is the study of the genetic material at the level of the individual nucleotide bases of DNA.

Karyotyping

Q. Write short essay/note on karyotyping.

- The **chromosomal constitution of a cell or individual** is known as the **karyotype.** The normal human karyotypes contain 22 pairs of autosomal chromosomes and one pair of sex chromosomes. Normal karyotype for females is denoted as 46,XX and for males as 46,XY.
- **Study of structural patterns** of the chromosomes in a sample of cells is known as **karyotyping**. This includes both **the number and appearance (photomicrograph) of complete set of chromosomes.** Karyotyping requires cells to be in a state of division and arresting this cell division at the metaphase of cell cycle.



Figs. 8.6A and B: (A) Nuclei of intermediate squamous cell with Barr body; (B) Neutrophil with drumstick.

Source of chromosome: To produce karyotype, it is necessary to obtain cells capable of growth and division. Cells for chromosomal study may be obtained from either by culture or directly.

- **Culture:** The source may be fibroblast or cells obtained by amniocentesis (amniotic fluid) or peripheral blood. The more commonly used cell for chromosomal study is **circulating lymphocyte** obtained from the blood sample **cultured in a media**.
- **Direct:** Cells obtained **from bone marrow and chorion villous** biopsy samples may be used without culture.

Staining: There are many staining methods using **specific dyes** to identify individual chromosomes. Most commonly used is **Giemsa stain**.

Classification of Chromosomes in Karyotyping

There are various systems used for study the morphology of the chromosomes.

Denver system of classification*:* In this system, the chromosomes are grouped from A to G according to the length and position of the centromere of the chromosomes.

Paris system of classification: This is a universally accepted classification. According to this, the chromosomes are identified based on the various banding patterns.

Chromosomal Banding

Banding is a method to **study the structure of a chromosome**. In this method, chromosomes are stained by a special stain (e.g., Giemsa) which binds to specific bands of chromosome. Each chromosome shows a characteristic banding pattern (light and dark bands) which will help to identify them.

Techniques: Different banding techniques are:

- **G-banding** (G for Giemsa): It is most commonly used and shows a series of light and dark stained bands**(Fig. 8.7)**. Giemsa stain is specific for the phosphate groups of DNA.
- **Q-banding** (Quinacrine fluorescent stain).
- **R-banding**: It is the reverse of G-banding (the R stands for "reverse").
- **C-banding** (centromeric): This method stains centromeres.
- **T-banding**: It stains the terminal ends of chromosomes (telomeres).
- **High resolution banding:** It provides greater sensitivity.

Karyotype Analysis

Karyotypes are usually described using a standard short hand format in the following order:

- **Total number of chromosomes**
- **Sex chromosome** constitution
- **Description of abnormalities** in ascending numerical order.
  - **Short arm or long arm:** The short arm of chromosome is designated "p" (petit) and the long arm "q" (queue).
  - **Region:** Each arm of the chromosome is divided into two or more regions. The regions are numbered (e.g., 1, 2, 3) from the centromere outward.
    - Bands and sub-bands: Each region is further subdivided into bands and sub-bands, and these are ordered numerically as well. This will help for precise localization of the gene.
- **Structural changes in chromosomes**

Examples: The notation *Xp21.2* (read as " X P two one point two") refers to a chromosomal segment located on the short arm of the X-chromosome, in region 2, band 1, and sub-band 2. 3p22.1 is read as "three P two two point one."

Uses of Karyotyping

- **For diagnosis:** Diagnosis of **genetic disorders** including prenatal diagnosis.
- **To detect the cause of repeated abortions:** Many chromosomal aberrations can cause repeated spontaneous abortions and they can be identified by karyotyping.
- **Prognostic value**: Identification of specific chromosomal anomalies **in certain cancers** will help in predicting the course and prognosis (e.g., Philadelphia chromosome in chronic myeloid leukemia).

Disadvantages of karyotyping (conventional cytogenetics)

- Cannot detect minor (subtle/submicroscopic) deletions/mutations
- Cannot identify gene amplifications
- Metaphasic arrest is difficult in solid tumors

Long and short arm of chromosome are called respectively:q and p.

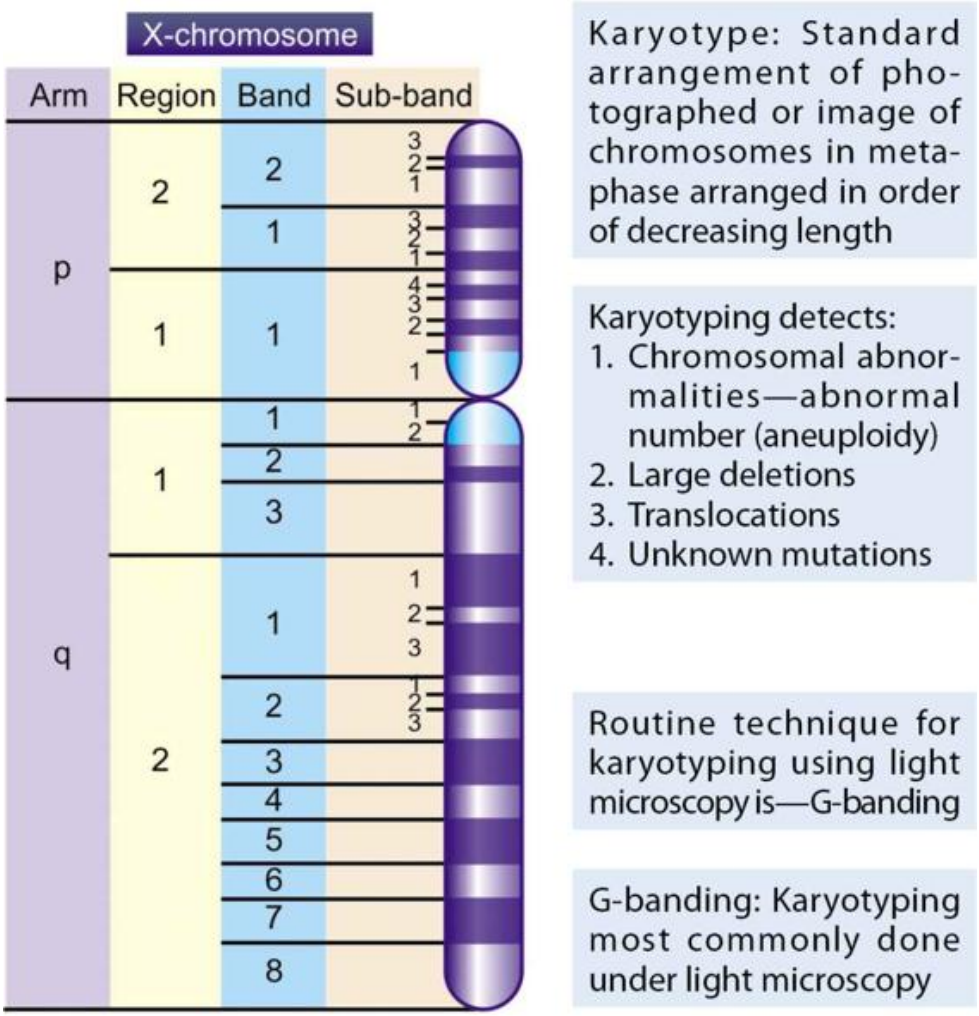


Fig. 8.7: G-banded karyotype (diagrammatic) shows the banding pattern of the X-chromosome with nomenclature of arms, regions, bands, and sub-bands.

Karyotype: Size, shape and number of chromosome.

CHROMOSOMAL ABERRATIONS

Classification (Box 8.1)

1. Numerical chromosomal aberrations
2. Structural chromosomal aberrations

Both may involve either the autosomes or the sex chromosomes.

Numerical Chromosomal Aberrations

Total number of chromosomes may be **either increased or decreased**. The **deviation from the normal number of chromosomes** is called as numerical chromosomal aberrations.

Types of Numerical Aberrations

1. **Aneuploidy:** It is defined as a **chromosome number that is not a multiple of 23** (the normal haploid number-n).
   - **Trisomy:** It is **numerical abnormality with** the presence of **one extra chromosome** (2n + 1). **It may involve either sex chromosomes or autosomes.** For example, **Down's syndrome** (trisomy 21) have three copies of chromosome 21 (47,XX, +21).
   - **Monosomy:** It is the **numerical abnormality** with the **absence or loss of one chromosome** (2n - 1). It may **involve autosomes or sex chromosomes**. For example, **Turner syndrome** 45 XO instead of normal 46,XX.
2. **Polyploidy:** This term used when the **chromosome number is a multiple greater than two of the haploid number** (multiples of haploid number 23).
3. **Mosaicism:** It is the presence of **two or more populations of cells with different chromosomal complement in an individual**.

Structural Chromosomal Aberration

Aberration of structure of one or more chromosomes may **occur during either mitosis or meiosis**. The various structural chromosomal aberrations **(Box 8.1)** include:

Translocations: It is a structural alteration between two chromosomes in which **segment of one chromosome gets detached and is transferred to another chromosome**. It can be:

- **Balanced reciprocal translocations (Fig. 8.8A):** It is characterized by single breaks in each of two chromosomes with exchange of genetic material distal to the break.
- **Robertsonian translocation/centric fusion (Fig. 8.8B):** It is a translocation between two acrocentric chromosomes. The breaks occur close to the centromeres of each chromosome. Transfer of the segments

leads to one very large chromosome and one extremely small one. The small one is because of fusion of short arms of both chromosomes which lack a centromere and is lost in subsequent divisions. This loss is compatible with life, but it may produce abnormal progeny.

Inversion: It involves two breaks within a single chromosome, the affected segment inverts with reattachment of the inverted segment. The genetic material is transferred within the same chromosome. Inversions are usually fully compatible with normal development. Two types of inversions are:

1. **Paracentric inversions (Fig. 8.8C)** result from breaks on the same arm (either the short arm or the long arm) of the chromosome.
2. **Pericentric inversions (Fig. 8.8D)** result from breaks on the opposite sides of the centromere where both the short and long arms are involved.

Isochromosome (Fig. 8.8E): They are formed due to faulty centromere division. Normally, centromeres divide in a plane parallel to long axis of the chromosome. If a centromere divides in a plane transverse to the long axis, it results in pair of isochromosomes. One pair consists of two short arms and the other of two long arms.

Ring chromosome (Fig. 8.8F): It is a special form of deletion. Ring chromosomes are formed by a break at both the ends of a chromosome with fusion of the damaged ends. The consequences depend on the amount of genetic material lost due to the break. Loss of significant amount of genetic material will result in phenotypic abnormalities. It is expressed as *46,XY,r(14)*. Ring chromosomes do not behave normally in meiosis or mitosis and usually result in serious consequences.

Deletion (Figs. 8.8G and H): It is the loss of a part of a chromosome. It is of two types namely: interstitial (middle) and terminal (rare).

Insertion: It is a form of nonreciprocal translocation in which a fragment of chromosome is transferred and inserted into a nonhomologous chromosome. Two breaks occur in one chromosome, which releases a chromosomal fragment. This fragment is inserted into another chromosome following one break in the receiving chromosome, to insert this fragment.

Autosomal monosomy: Not compatible with life.

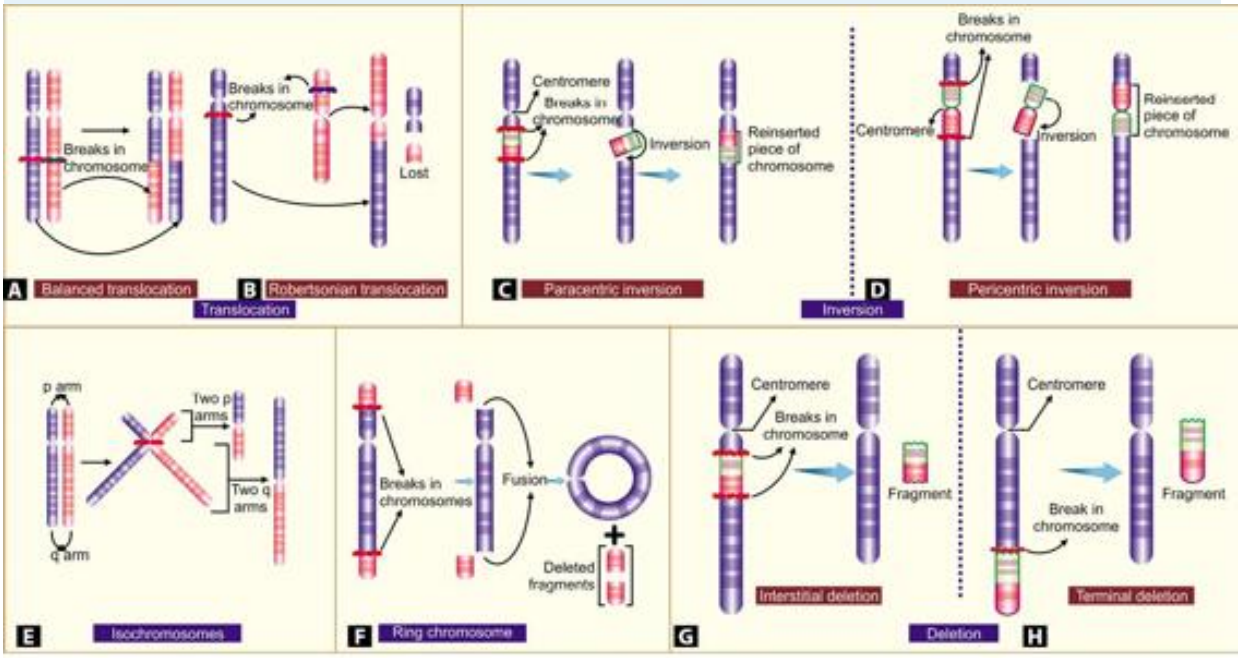
Nondysjunction: Unequal separation of chromosomes during meiosis.

Mosaicism: Nondysjunction during mitosis.

Acrocentric transmission is called: Robertsonian translocation.

Structural chromosomal aberrations:

- Translocation
- Inversion
- Isochromosome
- Ring chromosome
- Deletion
- Insertion



Figs. 8.8A to H: Types of chromosomal rearrangements.

GENOMIC IMPRINTING

All individuals inherit two copies of each autosomal gene. One of these is from maternal and other is from paternal chromosomes. It was earlier thought that there is no functional difference between the alleles derived from the mother or the father. It is found that different clinical features can result, depending on whether a gene is inherited from the father or mother. These differences are due to an epigenetic process, called **imprinting**. Mostly imprinting selectively inactivates either the maternal or paternal allele. Thus, in ***maternal imprinting***, there is silencing of the maternal allele, whereas in ***paternal imprinting***, there is inactivation of paternal allele. Imprinting occurs during gametogenesis in the ovum or the sperm, before fertilization, and then is stably transmitted to all somatic cells through

mitosis. The pattern of imprinting is maintained to variable degrees in different tissues.

MOLECULAR GENETIC DIAGNOSIS

Diagnostic Methods and Indicationsfor Genetic Testing

Q. Laboratory diagnosis of genetic diseases.

Genetic disease may be caused from single base substitutions up to gains or losses of entire chromosomes. These can be detected by various genetic tests.

Timing of Genetic Tests

Depending on the timing of performing, these genetic tests can be divided into four types.

1. **Preimplantation testing:** Done before conception (i.e., when one or two of the parents are carriers of a certain trait) on embryos created in vitro prior to uterine implantation to detect genetic changes in embryos. This is performed when are parents known to be at risk of having a child with a genetic disorder. It eliminates the chance of generational transmission of a familial disease.
2. **Prenatal testing:** These are done after conception and its indications are listed in **Box 8.2**.
   - Genetic test is performed on cells obtained by amniocentesis, chorionic villus biopsy, or umbilical cord blood. About 10% of the free DNA in a pregnant mother's blood is of fetal origin, and new noninvasive prenatal diagnostics tests use this source of DNA.
3. **Newborn and children genetic testing:** It is used to identify genetic disorders just after birth, so that it can be treated early in life. Indications for newborn and child genetic analysis are shown in **Box 8.3**. It is usually performed on peripheral blood DNA.
4. **Genetic test in adults and older individuals:** Its indications are listed in **Box 8.4**.

Indications for Analysis of Acquired Genetic Alterations (Box 8.5)

BOX 8.2: Indications for prenatal testing.

- A mother of advanced age (>35 years) who have increased risk of trisomies
- A parent to carry a balanced chromosomal rearrangement which increases the frequency of abnormal chromosome segregation during meiosis and the risk of aneuploidy in the fertilized ovum
- A fetus with abnormalities detected by ultrasound
- Routine maternal blood screening, indicating an increased risk of Down syndrome or another trisomy.

BOX 8.5: Common indications for analysis of acquired genetic alterations.

1. Diagnosis and management of cancer
   - To detect tumor-specific acquired mutations and cytogenetic alterations, e.g., *BCR-ABL* fusion genes in chronic myelogenous leukemia, or CML
   - To identify specific genetic alterations which helps in choosing therapy, e.g., *HER2* (*ERBB2* amplification in breast cancer or *EGFR* (*ERBB1* mutations in lung cancer)
   - To detect minimal residual disease, e.g., detection of *BCR-ABL* by PCR in CML
2. Diagnosis and management of infectious disease
   - To detect microorganism-specific genetic material for definitive diagnosis, e.g., HIV, mycobacteria
   - To identify specific genetic alterations in the genomes of microbes in case of drug resistance
   - To determine efficacy of treatment, e.g., to assess viral loads in HIV, hepatitis C virus infection

Genetic Tests

Polymerase Chain Reaction (PCR)

It is widely used, powerful tool in the molecular diagnosis of human disease.

Principle: In PCR, the double-stranded DNA of interest is separated into two individual strands. Each strand is then allowed to hybridize with a primer. The specific fragment of DNA is amplified to generate large quantities (thousands to millions of copies) of particular DNA fragments of interest.

- Subsequent analysis can be done by different techniques such as **(1) Sanger sequencing, (2) pyrosequencing, (3) single-base primer**

extension, (4) restriction fragment length analysis, (5) amplicon length analysis and (6) real-time PCR.

Advantages

- **Wide range of samples:** PCR allows analysis of DNA from any cellular source containing nuclei.
- **Small quantity required:** PCR needs very small quantity of genetic material and can amplify DNA from even single cell.
- **Sensitivity:** It has remarkable sensitivity.
- **Rapid:** It produces DNA fragments in a matter of hours.

Disadvantages

- It requires knowledge of the nucleotide sequence of the target DNA fragment.
- It can amplify DNA fragments usually up to 1 kb.

Molecular Analysis of Genomic Alterations

Genetic lesions with large deletions, duplications, or more complex rearrangements cannot easily be assayed by standard PCR methods. Such genomic alterations can be studied by hybridization-based techniques.

1. **Fluorescence in situ hybridization (FISH):** Uses DNA probes which detect and localize sequences specific to particular chromosomal regions.
2. **Multiplex ligation-dependent probe amplification (MLPA):** Blends DNA hybridization, DNA ligation, and PCR amplification to detect deletions and duplications of genome of any size. It detects genetic alteration that are too large to be detected by PCR and too small to be identified by FISH. It can either be performed on dividing cells (metaphase chromosomes) or nondividing cells (interphase nuclei) making it much more versatile than traditional karyotyping.
3. **Southern blotting:** Detects changes in the structure of specific loci.
4. **Cytogenomic array technology: It detects genomic abnormalities without prior knowledge** in contrast to FISH which needs prior knowledge of the one or few specific chromosomal regions suspected of being altered in the test sample.

BOX 8.3: Indications for newborn and children genetic analysis.

- Major/multiple congenital anomalies
- Suspicion of a metabolic syndrome (e.g., phenylketonuria)
- Unexplained mental retardation and/or developmental delay
- Suspected aneuploidy (e.g., features of Down syndrome) or other syndromic chromosomal abnormality (e.g., Turner syndrome)
- Suspected monogenic disease.

BOX 8.4: Indications for genetic test in adolescence and adulthood.

- Inherited cancer syndromes (family history of cancer with a known or suspected inherited predisposition or an unusual cancer presentation)
- Atypically mild monogenic disease (e.g., attenuated cystic fibrosis)
- Family history of an adult-onset of neurodegenerative disorders (e.g., familial Alzheimer disease, Huntington disease)

Next-Generation Sequencing

- **Next-generation sequencing (NGS) consists of several newer DNA sequencing technologies which can produce large amounts of sequence data in a massively parallel manner.**
- NGS can perform previously impossible analyses at extremely low relative cost.
- **Advantage:** Any DNA from almost any source can be used and are well suited to heterogeneous DNA samples. NGS is useful for detecting genetic anomalies of essentially any size scale ranging from SNPs (single nucleotide polymorphisms—SNPs is pronounced "snips") to very large rearrangements including aneuploidy.

Fluorescent in situ hybridization (FISH):
1. Identify known deletions irrespective of size
2. Identify translocation by different probes
3. Identify gene amplification
4. No need of metaphasic arrest

Fluorescent in situ hybridization: Cannot detect unknown chromosomal changes

STORAGE DISEASES

NC: Describe the pathogenesis of common storage disorders in infancy and childhood.

Q. Name storage disorders.

- Lysosomal enzymes are used for the intracellular digestion/degradation of many complex biological macromolecules.
- **Deficiency of lysosomal enzymes:** Inherited deficiency of lysosomal enzyme may cause **incomplete catabolism of its normal macromolecular substrate**. This can lead to the **accumulation of the partially degraded insoluble substrate within the lysosomes**. The inherited disorders resulting from mutations in genes that encode lysosomal hydrolases are known as lysosomal storage disorders.

General Features

- Lysosomal disorders are **transmitted as autosomal recessive disorder**.
- Usually **detected in infants and young children.**
- Hepatosplenomegaly due to accumulation of insoluble intermediate compounds in the mononulear phagocytes.
- CNS involvement is associated with damage to neurons.

Classification of lysosomal storage disorders: They are classified according to the biochemical nature of the metabolite accumulated within the lysosomes. The subgroups include **glycogenoses, sphingolipidoses (lipidoses), sulfatidoses, and mucopolysaccharidoses (MPSs).**

Niemann–Pick Disease

Q. Write short note on Niemann-Pick disease and its enzyme deficiency.

Niemann–Pick disease (NPD) is one of the **lysosomal storage disorders (lipidoses)** that are characterized by lysosomal **accumulation of sphingomyelin** due to an **inherited deficiency of sphingomyelinase**.

- Mode of transmission: **Autosomal recessive.**

Classification of Niemann–Pick Disease

- **Type A:** It is a **severe infantile form** with **almost complete deficiency of sphingomyelinase**. It is characterized by **extensive neurologic involvement**, **massive visceromegaly,** marked accumulations of **sphingomyelin in liver and spleen**, and progressive wasting and **death occurring by 3 years of age**.
- **Type B:** It usually presents with **hepatosplenomegaly** and generally **without involvement of central nervous system**. They **usually survive into adulthood**.
- **Type C:** It is **more common than types A and B**. It is due to **mutations in** two genes namely—***NPC1*** **(Niemann–Pick disease, type *C1*)** and ***NPC2*** **(Niemann–Pick disease, type *C2*)**. It is due to **primary defect in lipid transport**. Commonly manifests in childhood with ataxia, vertical supranuclear gaze palsy, dysarthria, dystonia, and psychomotor regression.

MORPHOLOGY

Deficiency of sphingomyelinase enzyme blocks degradation of the lipid—sphingomyelin → accumulates inside the lysosomes of cells of the mononuclear phagocyte system.

- **Organs involved:** They show **moderate to marked enlargement**.
  - **Brain:** It shows shrunken gyri and widened sulci. Microscopically, the **neurons show vacuolation and ballooning**, which in time leads to cell death and loss of brain substance.
  - **Retina:** It shows a **cherry-red spot**.
  - **Other organs: Spleen (massively enlarged)**, liver, lymph nodes, bone marrow, tonsils, gastrointestinal tract, and lungs.
- **Light microscopy:**
  - **Characteristic storage cell is a macrophage with many, small, uniform vacuoles** (contains sphingomyelin and cholesterol) within the cytoplasm.
  - These lipid-laden foam cells are large (20–90 μm in diameter) and frozen sections—vacuoles take up fat stains.
- **Electron microscopy: The lipid vacuole resembles concentric lamellated myelin figures** which are called **"zebra" bodies**.

Tay–Sachs Disease (GM2 Gangliosidosis: Hexosaminidase β-Subunit Deficiency)

- Tay-Sachs disease is inherited as an **autosomal recessive** trait.
- **Most common** form of GM2 gangliosidosis.

- Characterized by genetic **mutations in** *HEXA* gene on chromosome 15 and a severe **deficiency of** β-subunit **hexosaminidase A** enzymes.
- **Hexosaminidase A enzymes is absent in almost all the tissues.**

MORPHOLOGY

- **GM_2 ganglioside accumulates in many tissues** such as CNS, retina, heart, liver and spleen.
- **Special stains**: Special stains for fat such as oil red O and Sudan black B stain positive with gangliosides.
- **Light microscopy:**
  - **Neurons: Ballooned with many cytoplasmic vacuoles**, each representing a severely distended lysosome filled with gangliosides → followed by destruction of neurons, proliferation of microglia, and accumulation of lipids in phagocytes within the brain substance.
  - **Retina:** Ganglion cells in the retina distended with GM_2 ganglioside, more prominent at the margins of the macula → gives rise to characteristic **cherry-red spot** in the macula. Cherry-red spot is also seen in other storage disorders affecting the neurons.
- **Electron microscopy**: Most prominent features is **prominent lysosomes with whorled configurations which represents onion-skin layers of membranes**.

Clinical Features

- Usually presents between 6 and 10 months of age.
- Clinical features are mainly due to **neuronal involvement in the central and autonomic nervous systems and retina**. Symptoms include progressive motor and mental deterioration, blindness, and increasing dementia.
- Ophthalmoscopy cherry-red spot in the macula.
- Over the span of 1 or 2 years a complete vegetative state is reached. Most children die before 3 years of age.
- **Antenatal diagnosis and carrier detection**: It can be done by enzyme assays and DNA-based analysis.

Gaucher Disease

Q. Write short essay/note on Gaucher disease.

- **Most common lysosomal storage disorder** due to mutation in the gene that encodes glucocerebrosidase (cleaves glucose residue from ceramide).
- **Autosomal recessive mode of transmission.**
- Due to **deficiency of enzyme glucocerebrosidase → results in accumulation of glucocerebroside,** mainly in **lysosomes of macrophage**.
- **Pathological changes** are both due to:
  - **Accumulation of glucocerebroside**
  - **Activation of macrophages** → secretes cytokines such as IL-1, IL-6, and tumor necrosis factor (TNF).

Clinical Subtypes

Q. Write short note on enzyme deficiency in Gaucher disease.

There are three variants namely:

1. **Type I or the chronic non-neuronopathic form:**
   - **Most common type** (about 99% of cases)
   - **Glucocerebrosides** are stored only **in the mononuclear phagocytes throughout the body** mainly in the **spleen and skeletal system**. It does not involve the brain.
2. **Type II or acute neuronopathic Gaucher disease:**
   - **Infantile acute cerebral pattern**
   - Almost **complete absence of glucocerebrosidase** activity in the tissues → progressive **involvement of CNS → death at an early age.**
3. **Type III:** It is **intermediate between types I and II**.

MORPHOLOGY

Q. Write short note on Gaucher cell and its morphology.

Light microscopy: Gaucher cells are hallmark of this disorder and its characteristics are:

- **Enlarged, phagocytic cells** (sometimes up to 100 μm in diameter) distended **with massive amount of glucocerebrosides.**

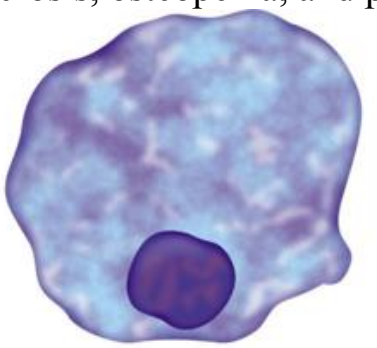
- **Seen throughout the body in virtually all organs**, especially in the **spleen, liver, bone marrow**, lymph nodes, tonsils, thymus, and Peyer's patches.
- **Gaucher cells** have a **fibrillary type of cytoplasm** such as a **crumpled/wrinkled tissue paper (Fig. 8.9)** and one or more dark, eccentrically placed nuclei.
- The **cytoplasm** of Gaucher cells stain intensely **positive with Periodic acid–Schiff.**

Electron microscopy: The fibrillary cytoplasm appears as elongated, distended lysosomes, containing the stored lipid arranged in parallel layers of tubular structures.

Clinical Features

Type I

- Manifests in adult life and follows a progressive course.
- **Spleen is enlarged, sometimes up to 10 kg** and **hypersplenism** may lead to pancytopenia or thrombocytopenia. Hepatomegaly is also seen.
- **Bone marrow: Accumulation of Gaucher cells** produces extensive expansion of the marrow space, bone erosion, focal lytic bone lesions, osteonecrosis, osteopenia, and pathologic fractures.



Gaucher cell: Fibrillary type of cytoplasm like a crumpled tissue paper.

Fig. 8.9: Appearance of Gaucher cells (diagrammatic).

Types II and III

- In patients with CNS involvement, it may produce cerebral dysfunction, convulsions, and progressive mental deterioration. Gaucher cells are seen in the Virchow–Robin spaces.

Lysosomal storage disorders:
- Inherited
- Mutation in genes that code lysosomal hydrolases

Niemann–Pick disease (NPD):
- Lysosomal storage disorders (lipidoses)
- Inherited deficiency of sphingomyelinase
- Lysosomal accumulation of sphingomyelin

Neimann–Pick disease type A and B:
- Diagnosis and detection of carriers by estimation of sphinomyelinase activity in leukocytes/cultured fibroblasts.
- Antenatal diagnosis by enzyme assay or DNA probe analysis

Neimann–Pick disease type 3:
- More common than type A and B
- Mutations in *NPC1* and *NPC2* gene

Tay–Sachs disease is a GM_2 gangliosidosis caused by deficiency of enzyme hexosaminidase, subunit.

Gaucher disease:
- Autosomal recessive
- Deficiency of enzyme glucocerebrosidase
- Accumulation of glucocerebroside, mainly in lysosomes of macrophage

CYTOGENETIC ABNORMALITIES AND MUTATIONS IN CHILDHOOD

NC: Describe the pathogenesis and features of common cytogenetic abnormalities and mutations in childhood.

Trisomy 21 (Down Syndrome)

Q. Write short essay/note on Down syndrome.

- Down syndrome was first described by Dr John Langdon Down.
- It is a **cytogenetic disorder involving autosome**.
- Most **common chromosomal disorder** and is a **leading cause of mental retardation**.
- About **95%** of these individuals have **trisomy 21** (extra copy of chromosome 21), resulting in **chromosome count of 47** instead of normal 46.
- Parents of children with Down syndrome are normal and have a normal karyotype.

Etiology and Pathogenesis

- **Maternal age: Older mothers** (above 45 years of age) have much **greater risk**.

- **Other factors:** Increased incidence may be associated with exposure of mother to pesticides, electromagnetic fields, anesthetic drugs, alcohol and caffeine.

Mechanism of trisomy 21: The **three copies of chromosome 21** in somatic cells cause Down syndrome. It may be due to:

- **Nondisjunction in the first meiotic division of gametogenesis** and is responsible for trisomy 21 **in most (95%) of the patients**.
- Robertsonian translocation (*refer* page 247 and **Fig. 8.8 A**)in about 5% of cases.
- Mosaicism in about 1% of cases.

Clinical Features

Diagnosis of Down syndrome is **usually apparent at the time of birth** by the infant's **characteristic craniofacial appearance (Fig. 8.10)**. The **diagnosis is confirmed by cytogenetic analysis.**

Characteristic features appear as the child grows.

- *Mental status:* Children are **mentally retarded with low IQ** (25–50).
- *Craniofacial features:* Diagnostic clinical features are:
  - **Flat face and occiput, with a low-bridged nose, reduced interpupillary distance and oblique palpebral fissures**.
  - Epicanthal folds of the **eyes impart an oriental appearance** (obsolete term mongolism).
  - Speckled appearance of the iris **(Brushfield spots)**.
  - **Enlarged and malformed ears**.
  - A prominent tongue **(macroglossia)**, which typically **lacks a central fissure** and protrudes through an open mouth.
- *Heart:* **Congenital cardiac anomalies** are responsible for the majority of the deaths in infancy and early childhood. The cardiac defects are:
  - **Septal and AV defect:** These defects may involve atrial septum (atrial septal defect), ventricular septum (ventricular septal defect), and one or more atrioventricular (AV) valves.
  - **Other cardiac anomalies:** Tetralogy of Fallot and Patent ductus arteriosus.

- *Skeleton:* These **children are small** because of shorter bones of the ribs, pelvis, and extremities. The hands are broad and short and show a **Simian crease** (a single transverse crease across the palm). The fifth finger curves inwards.
- *Gastrointestinal tract:* It may show **esophageal/duodenal stenosis or atresia, imperforate anus and Hirschsprung disease** (megacolon).
- *Reproductive system:* **Men are sterile** because of spermatogenesis arrest.
- *Immune system:* Affected children are susceptible to serious infections due to defective immunity.
- *Endocrine system:* **Antithyroid antibodies** may cause hypothyroidism.

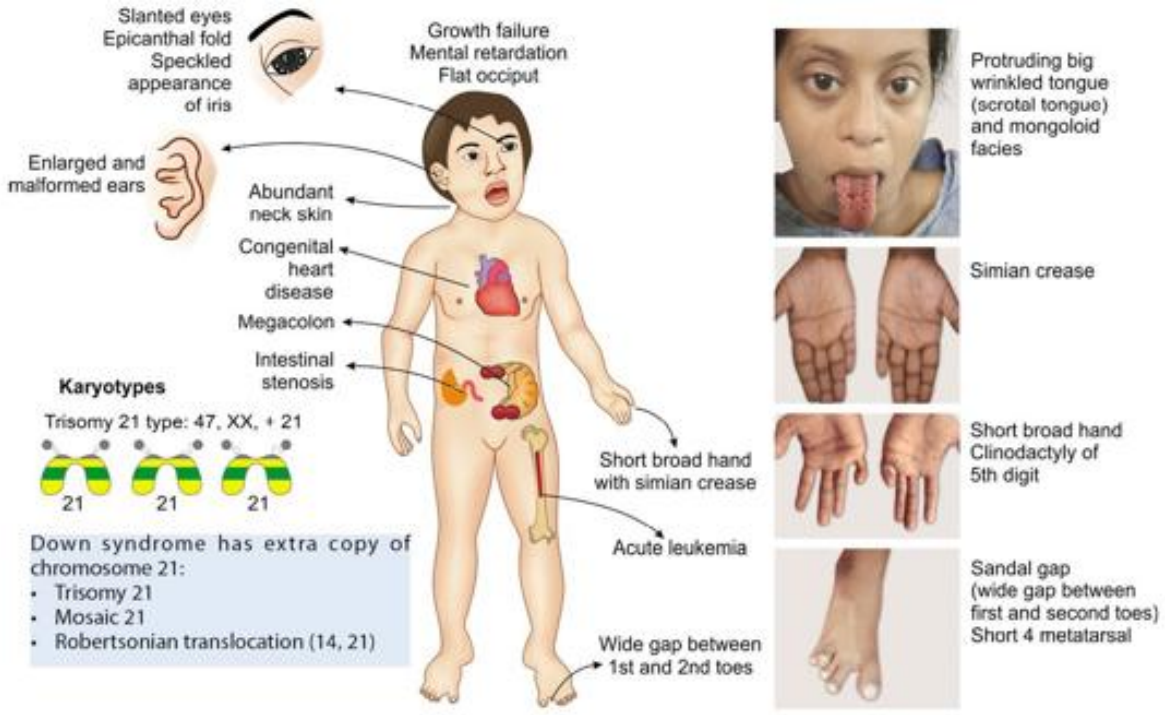


Fig. 8.10: Major clinical features of Down syndrome.

- *Hematologic disorders:* They have increased **risk of** both acute lymphoblastic and acute myeloid **leukemia**. The latter is most commonly acute megakaryoblastic leukemia.
- *Atlantoaxial instability:* It is characterized by excessive movement at the junction of the atlas (C1) and axis (C2) vertebrae, due to laxity of either bone or ligament. Neurological symptoms develop when spinal cord is compressed. Clinically, it may present with easy fatigability, difficulty in walking, abnormal gait, restricted neck mobility, torticollis, etc.

Down syndrome: Alzheimer disease at younger age.

Down syndrome: Non-disjunction of chromosome 21. Most common cause is maternal meiotic non-

dysfunction. Those due to Robertsonian translocation and mosaicism has no relation with maternal age.

Chromosomal abnormality in mongolism (obsolete term) is: Trisomy 21.

Down syndrome: Most common cause of mental retardation.

Klinefelter Syndrome

Q. Write short essay/note on Klinefelter syndrome.

It is a **cytogenetic disorder involving sex chromosomes**.

Definition: Klinefelter syndrome (testicular dysgenesis) is **characterized by two or more X-chromosomes and one or more Y chromosomes.** It is an **important** and **most frequent genetic cause of male hypogonadism**.

It is the most important genetic disease involving **trisomy of sex chromosomes**; it is associated with **reduced spermatogenesis and male infertility**.

Pathogenesis

- **Most of the patients with Klinefelter syndrome have an extra X-chromosome (47,XXY karyotype)**. This complement of chromosomes results from **nondisjunction during the meiotic divisions in one of the parents**.
- A **minority** of them **are mosaic** (e.g., 46,XY/47, XXY) or have more than two X-chromosomes (e.g., 47,XXY/48,XXXY) and one or more Y-chromosomes.
- Regardless of the number of extra X-chromosomes (even up to 4), the Y-chromosome results in a male phenotype.

Clinical Features

Klinefelter syndrome **(Fig. 8.11)** is usually diagnosed after puberty and **hypogonadism is a consistent finding**.

- Most of the patients are **tall and thin** with relatively long legs (eunuchoid body habitus).
- Mental retardation is uncommon, although average IQ is reduced.
- At puberty, **testes and penis remain small** with **lack of secondary male characteristics**.

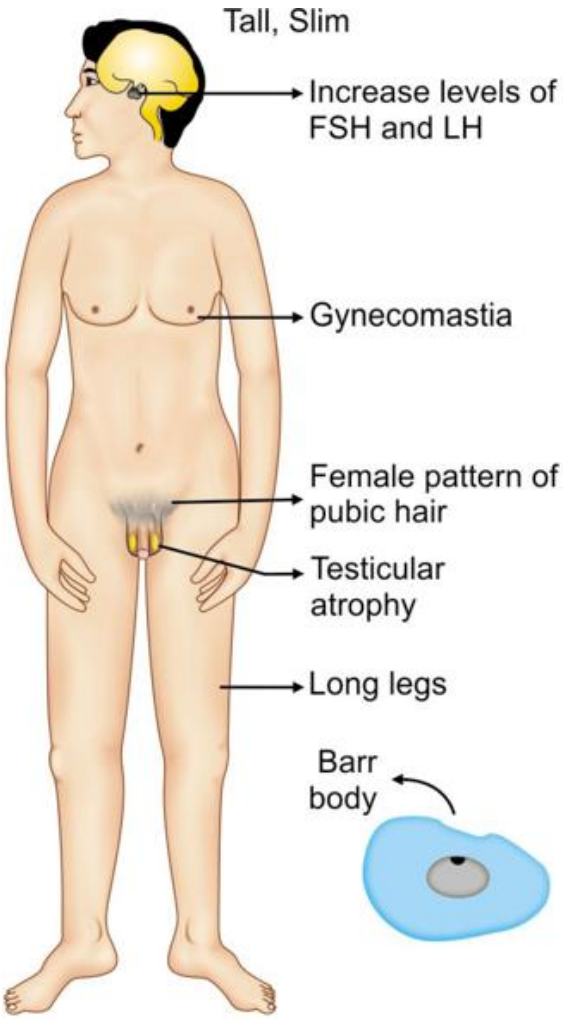


Fig. 8.11: Features of Klinefelter's syndrome.

Klinefelter's syndrome: Two or more extra copy of X chromosomes and 1 Y chromosome.

Classic karyotype of Klinefelter's syndrome is: 47,XXY.

Klinefelter syndrome: An important genetic cause of male infertility.

Klinefelter syndrome: Increased levels of LH and FSH and decreased levels of testosterone.

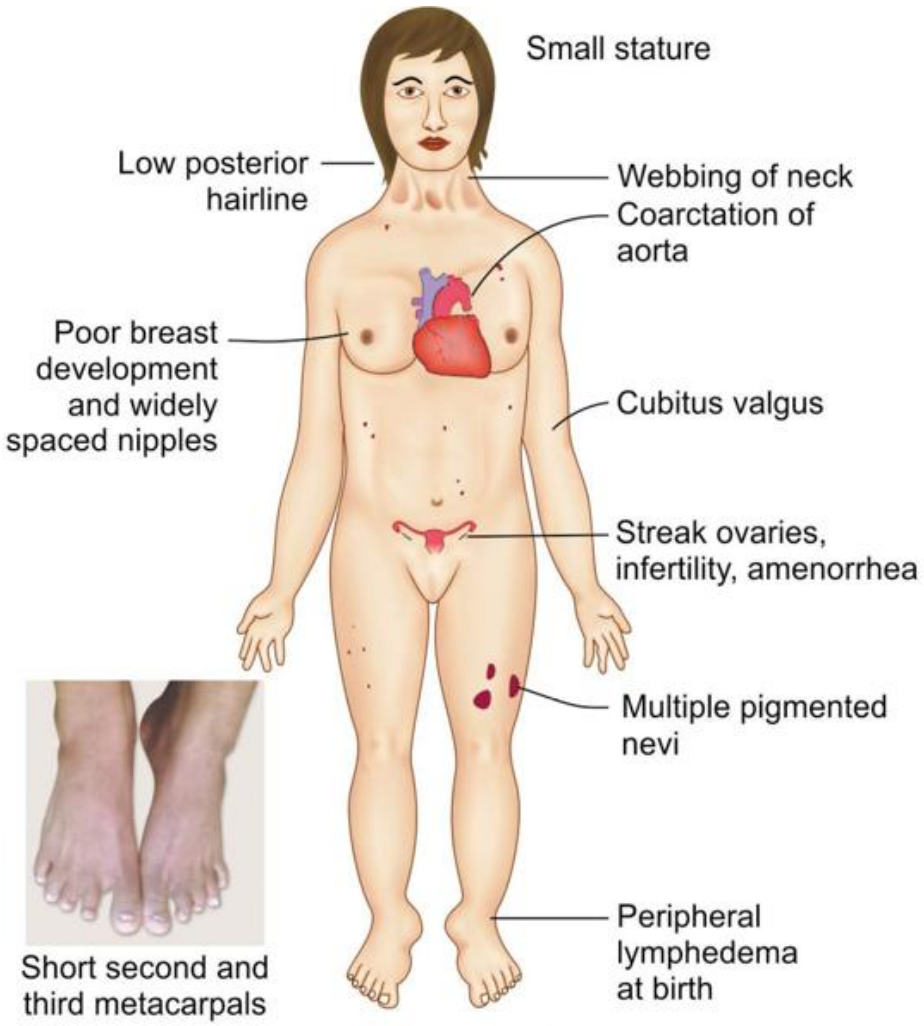


Fig. 8.12: Clinical features of Turner syndrome.

- Female characteristics include a **high-pitched/deep voice, gynecomastia, and a female pattern of pubic hair.**
- **Hypogonadism, reduced levels of testosterone, remarkably high levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH).**
- **Reduced spermatogenesis** → azoospermia → **infertility**. The testis may show atrophy of seminiferous tubules containing pink, hyaline, collagenous ghosts. Leydig cells may appear prominent.
- **Increased incidence of type 2 diabetes and the metabolic syndrome.** Mitral valve prolapse is seen in about 50% of cases.
- **Higher risk for breast cancer, extragonadal germ cell tumors and autoimmune diseases** such as systemic lupus erythematosus.

Turner Syndrome

Q. Write short essay/note on Turner syndrome.

It is a **cytogenetic disorder involving sex chromosomes**.

Turner syndrome **(Fig. 8.12)** is a spectrum of abnormalities that **results from complete or partial monosomy of the X-chromosome** in a **phenotypic female**. It is characterized by hypogonadism and is the most common sex chromosome abnormality in females.

Karyotypic Abnormalities

Three types of karyotypic abnormalities are found in Turner syndrome.

1. **Missing of an entire X-chromosome:** It results in a **45 X karyotype**.
2. **Structural abnormalities of the X-chromosomes:** It include isochromosome of the long arm, translocations and deletions.
3. **Mosaics:** 45,X cell population along with one or more karyotypically normal or abnormal cell types. Examples: (1) 45,X/46,XX; (2) 45,X/46,XY.

The molecular pathogenesis of Turner syndrome is not completely understood.

Clinical Features

Turner syndrome is usually **not discovered before puberty**. It presents with **failure to develop normal secondary sex characteristics.** Important diagnostic features are:

- **Adult women with short stature** (less than 5 ft tall), **primary amenorrhea and sterility**. At puberty, normal secondary sex characteristics fail to develop.
- **Webbed neck**, **low posterior hairline**, **wide carrying angle of the arms** (cubitus valgus), **broad chest** with **widely spaced nipples** and hyperconvex fingernails.
- Other features: **Infantile genitalia, inadequate breast development, and little pubic hair.** The **ovaries** are converted to **fibrous streaks**.
- **Pigmented nevi** become prominent as the age advances.
- **Cardiovascular anomalies** like congenital heart disease particularly coarctation of the aorta.
- **Development of autoantibodies:** About 50% show autoantibodies that react with the thyroid gland → 50% of them may develop

hypothyroidism.

Turner syndrome: Primary amenorrhea (menopause before menarche). It is the most common genetic cause of primary amenorrhea.

Karyotype is 45 X and Barr body is not seen in: Turner syndrome.

Turner syndrome: Webbed neck, streak gonads, and menopause before menarche.

Chromosomes involved in Patau syndrome: Chromosome 13.

TUMORS AND TUMOR-LIKE LESIONS OF INFANCY AND CHILDHOOD

NC: Describe the pathogenesis and pathology of tumor and tumor-like conditions in infancy and childhood.

Introduction

Tumors in infancy and childhood may be benign or malignant. Benign tumors are more common than malignant tumors. However, **malignant tumors constitute the second most common cause of death in children between the ages of 4 and 14 years** (first being the accidents).

Tumor-like lesions: Two tumor-like lesions in infancy and childhood are:

- **Heterotopia or choristoma:** It is defined as the presence of microscopically normal cells or tissues in abnormal locations (*refer* page 191). Examples include presence of (i) pancreatic tissue "rest" in the wall of the stomach or small intestine and (ii) a small mass of adrenal cells in the kidney, lungs, ovaries, or elsewhere. Heterotopic rests usually are of little clinical importance.
- **Hamartoma:** It is a jumbled mass consisting of an excessive but focal overgrowth of cells and tissues native/indigenous to the organ in which it occurs (*refer* page 191). The cellular elements are mature and identical to those found in the remainder of the organ. But they do not have the normal architecture of the surrounding tissue. Distinction between a hamartoma and a benign neoplasm is frequently slight. For example, hemangiomas, lymphangiomas, rhabdomyomas of the heart, and adenomas of the liver are considered as hamartoma by some, whereas others consider them as true neoplasms.

Benign Neoplasms

Any benign neoplasm may develop in the pediatric age group. Only hemangiomas, lymphangiomas, and teratomas are discussed below.

Hemangiomas

They are the **most common neoplasms of infancy**.

Histological types: It may be **cavernous or capillary** hemangiomas (*refer* pages 451-2 of Chapter 16 for morphological features).

Sites: Most hemangiomas in children are found in the **skin**, particularly on the face and scalp.

Appearance: They vary in size and appear as **flat to elevated, irregular, red-blue masses**. The flat, larger congenital capillary hemangiomas are called as **port-wine stains**. Hemangiomas may enlarge as the child grows, but many a times they **may spontaneously regress**. Majority of superficial hemangiomas have only a cosmetic significance. Rarely, hemangiomas may be the manifestation of a hereditary disorder associated with disease within internal organs (e.g., von Hippel-Lindau syndrome). Few cavernous hemangiomas of CNS can occur in the familial setting and the affected families show mutations in one of three cerebral cavernous malformation (CCM) genes.

Lymphangiomas

They represent the lymphatic counterpart of hemangiomas and also called as cystic hygromas. **Unlike hemangiomas,** these lesions do not regress spontaneously and should be resected.

Site: They are poorly circumscribed swellings that are usually present at birth. They may be found on the skin but, more commonly, they are seen in the deeper regions of the head and neck, axilla, mediastinum, floor of the mouth, and retroperitoneum. They may increase in size after birth and may compress the mediastinal structures or nerve trunks in axilla.

Microscopy: They may be unilocular or multilocular. They show cystic and cavernous spaces lined by endothelial cells and surrounded by lymphoid aggregates. The lumen/ spaces usually contain pale straw-colored fluid.

Teratomas

Types (*refer* page 190): They may be

i. Mature teratomas: Benign, well-differentiated cystic lesions
ii. Immature teratomas: Lesions of indeterminate potential or
iii. Malignant teratomas: Usually admixed with another germ cell tumor such as an endodermal sinus tumor.

Sacrococcygeal teratomas: They present as masses near the sacrum and buttocks. They are the most common (about 40%) teratomas of childhood. About 10% of sacrococcygeal teratomas may be associated with congenital anomalies such as primarily defects of the hindgut and cloacal region and other midline defects (e.g., meningocele, spina bifida). About 75% of these tumors are mature teratomas with a benign course. About 12% may be malignant and lethal. The remainder are immature teratomas, and their malignant potential depends on the amount of immature tissue elements present. Microscopically, they teratomas contain multiple tissues derived from more than one germ cell layer.

Age: Most of the benign teratomas are observed in younger infants (4 months of age or younger), whereas children with malignant teratomas are seen in somewhat older.

TABLE 8.4: Common malignant neoplasms of infancy and childhood.

Age: 0–4 Years	*5–9 Years*	*10–14 Years*
• Acute leukemia • CNS tumors • Lymphoma • Nephroblastoma (Wilms tumor) • Neuroblastoma • Retinoblastoma • Hepatoblastoma • Soft tissue sarcoma (e.g., rhabdomyosarcoma) • Teratomas • Miscellaneous	• Acute leukemia • Central nervous system (CNS) tumors • Lymphoma • Wilms tumor Neuroblastoma • Retinoblastoma • Soft tissue sarcoma • Hepatocellular carcinoma • Ewing tumor • Miscellaneous	• Acute leukemia • CNS tumors • Hodgkin lymphoma • Soft tissue sarcoma • Osteogenic sarcoma • Thyroid carcinoma • Hepatocellular carcinoma • Miscellaneous

Malignant Neoplasms

Malignant neoplasms in infancy and childhood are uncommon and their distribution is presented in**Table 8.4**. Most common of these include neoplasms of the hematopoietic system, neural tissue, and soft tissues. This distribution is unlike in that in adults, in whom most cancers are of epithelial origin such as carcinomas of the lung, breast, prostate, and GI tract.

Malignant neoplasms developing during infancy and childhood biologically and histologically differ from those in adults. These differences are:

- **Presence of genetic abnormalities or familial syndromes** that predispose to cancer. For example, short arm deletions of chromosome 11, causing Wilms tumor associated with aniridia, genitourinary malformations and mental retardation (WAGR complex); Loss of the long arm of chromosome 13 is associated with retinoblastoma due to the loss of the Rb tumor suppressor gene.
- **Close relationship between abnormal development (teratogenesis) and tumor induction (oncogenesis)**, suggesting a common stem cell defect.
- Tendency of malignancies to regress spontaneously or to undergo "differentiation" into mature elements. For example, neuroblastoma, which may show differentiation to ganglioneuroblastoma and ganglioneuroma.

Primitive appearing tumor cells: Many malignant tumors in infancy and childhood have unique histological features. They consist of primitive (embryonal) cells rather than pleomorphic-anaplastic microscopic appearance characteristic of malignant tumors in adult.

Small, round, blue-cell tumors: Because of their primitive appearance of cells, many childhood tumors have been collectively called as small, round, blue-cell tumors (Box 8.6). These small cells have small, round nuclei and are arranged in sheets.

BOX 8.6: List of small, round, blue-cell tumors.

- Neuroblastoma
- Lymphoma
- Rhabdomyosarcoma
- Ewing sarcoma (peripheral neuroectodermal tumor)
- Some cases of Wilms tumor

Diagnosis: These tumors usually show distinctive microscopic features by which a definitive diagnosis is possible. However, molecular studies are

becoming increasingly useful, both for diagnosis and prognosis.
Neuroblastoma *(Discussed on pages 818-9)*
Retinoblastoma *(Discussed on pages 884-5)*
Wilms Tumor *(Discussed on pages 709-11)*

CHAPTER 9

Environmental and Nutritional Diseases

ENVIRONMENTAL DISEASES

CC: Enumerate and describe the pathogenesis of disorders caused by air pollution, tobacco and alcohol.

Air Pollution

Air pollution is responsible for a **significant cause of morbidity and mortality**, especially among individuals with pre-existing lung or heart disease. The air that we breathe also contains many potential causes of disease. Airborne microorganisms are responsible for major morbidity and death. More widespread are the air pollutants which include chemical and particulate pollutants. Air pollutants may be found in both outdoor and indoor air.

Outdoor Air Pollution

The outdoor air is contaminated with a mixture of gaseous and particulate pollutants. The most important air pollutants are generated by combustion of fossil fuels, industrial and agricultural processes. The main pollutant that cause human disease are particulate matter, especially **carbon particles**. Others noxious and irritant gasses such as **sulfur dioxide (SO_2)**, **nitrogen dioxide, CO, ozone and lead** are important constituents of polluted air.

Organs affected: The **lungs** are the most common organ affected by air pollution, but air pollutants can affect many organ systems.

Ozone: Tropospheric **ozone** (i.e., ozone near the ground as opposed to in the upper atmosphere) is a gas mainly formed by the action of sunlight on nitrogen oxides (NO_2). Most of the nitrogen oxides (refer below) are released by automobile exhaust. Together with oxides and fine particulate matter, ozone forms the smog (derived from the words *smoke* and *fog*). Ozone is one of the **most common and strong oxidant air pollutants**. Its toxicity is due to production of free radicals, which damage the lining cells of the respiratory tract and the alveoli (produces cough and dyspnea) on acute exposure. Though low levels of ozone can be tolerated by healthy individuals, they are harmful to lung function, especially in patients suffering from asthma or emphysema. Chronic exposure to ozone in smog can cause **deterioration in pulmonary function** and is associated with a slight but significant increase in mortality.

Sulfur dioxide, particles, and acid aerosols: SO_2 are mainly derived from burning fossil fuels especially by coal- and oil-fired power plants and industrial processes burning these fuels. Of these, particles are responsible for the morbidity and death. Particles of size <10 μm in diameter are particularly harmful, because they can reach all the way to the alveoli. In the alveoli, they are phagocytosed by macrophages and neutrophils, release mediators and produce an inflammatory reaction. In contrast, larger particles are less dangerous because they are removed in the nose or are trapped by the mucociliary action in the upper respiratory tract. Sulfur dioxide is highly irritative and may be oxidized to sulfuric acid. **Acute exposure produces bronchoconstriction and inflammation of respiratory tract**.

Oxides of nitrogen: Nitrogen oxides (NOx) are a mixture of several compounds. They are also generated from burning fossil fuels, especially in generating electricity. These gases are oxidants and respiratory irritants. Acute **exposure causes airway hyperreactivity**.

Carbon monoxide: It is a nonirritating, colorless, tasteless, odorless gas. It is produced by the incomplete combustion (oxidation) of organic substances (carbonaceous materials). The sources of CO include automotive engines (exhaust), home oil burners, industries using fossil fuels, and cigarette smoke. The low levels of CO usually found in air may produce impaired respiratory function but are not usually life-threatening. However, individuals working in confined environments with high exposure to fumes

(e.g., tunnel and underground garage workers), may develop chronic poisoning. CO can also be an important cause of accidental and suicidal death. Treatment of such acute CO poisoning (e.g., who attempt suicide or are trapped in fires) consists mainly of the administration of 100% oxygen.

Carboxyhemoglobin concentrations under 10% are common in smokers and usually do not produce symptoms. CO is a systemic asphyxiant with its affinity for hemoglobin that is 200 times more than that of oxygen. CO binding to hemoglobin forms carboxyhemoglobin and prevents oxygen transport. The oxygen from carboxyhemoglobin does not dissociate as readily as in oxyhemoglobin. This produces hypoxia that in turn leads to central nervous system (CNS) depression. Systemic hypoxia develops when the hemoglobin is 20–30% saturated with CO, and unconsciousness and death occur with 60–70% saturation. **In fatal CO poisoning**, the **skin shows a characteristic cherry-red color** due to carboxyhemoglobin in the superficial capillaries. The diagnosis of CO poisoning depends on detection of high levels of carboxyhemoglobin in the blood.

Indoor Air Pollution

In the modern world there is increased potential for pollution of the indoor air. The most common pollutant is **tobacco smoke** and others include CO, nitrogen dioxide (already discussed above under outdoor pollutants), and asbestos (*refer* pages 524-26 of Chapter 17).

Smoke from burning of organic materials: They contain various oxides of nitrogen and carbon particulates which are irritants that predisposes to **lung infections** and also may contain **carcinogenic polycyclic aromatic hydrocarbons**. It is produced by burning carbon-containing material, such as wood, dung, or charcoal in homes for cooking, heating, and light.

Radon: It a radioactive gas derived from uranium. It is present in soil and in homes. Radon **exposure in uranium miners** (especially in smokers) can cause **lung cancer**. However, low-level chronic exposures in nonsmokers home are less likely to increase the risk of lung cancer.

Bioaerosols: They may contain pathogenic microbiologic agents and can cause Legionnaires' disease, viral pneumonia, and the common cold. They may also contain allergens derived from pet dander, dust mites, and fungi and molds that can cause rhinitis, eye irritation, and even asthma.

Metals as Environmental Pollutants

The various heavy metals that can produce harmful effects in humans include lead, mercury, arsenic, and cadmium.

Lead

Source: Exposure to lead occurs through contaminated air, food, and water. Lead is a heavy metal commonly found in the environment of industrialized countries. The major sources of lead in the environment were house paints and gasoline. The use of lead-based paints and leaded gas has been greatly decreased and the main sources of lead in the environment includes mines, foundries, batteries, and spray paints, all of which are occupational hazards. The flaking lead paint in older houses, lead pipes and soil contamination also contains lead.

In adults: Occupational exposure in individuals engaged in lead smelting (releases metal fumes and deposits lead oxide dust). Lead oxide is a constituent of battery grids and an occupational exposure occurs in individuals working in the manufacture and recycling of automobile batteries.

In children: Lead poisoning was related to pica—the habit of chewing on cribs, toys, furniture and woodwork—and eating painted plaster and fallen paint flakes.

Pathophysiology: Lead is easily absorbed through the lungs or, less often, the gastrointestinal tract. Most absorbed lead (80–85%) is taken up by the developing teeth and bone. In these sites, lead competes with calcium, binds phosphates, and has a half-life of 20 to 30 years. Once in the blood, it circulates in the plasma and RBCs and is excreted by the kidneys. About 5–10% of the absorbed lead remains freely diffusible in the blood, and the remainder is distributed throughout soft tissues. Lead can easily cross the blood-brain barrier and concentrations in the brain, liver, kidneys and bone marrow. Lead binds to sulfhydryl groups in proteins and interferes activities of zinc (Zn)-dependent enzymes and also calcium metabolism (lead competes with calcium). This leads to hematologic, skeletal, neurologic, GI, and renal toxicities. In bones, teeth, nails and hair, it forms a tightly bound pool of lead which is harmful.

Effects of chronic exposure to low lead levels: Presently, the level of exposure to lead is markedly reduced due to the several measures taken to prevent exposure to lead (e.g., removal of lead from gasoline, use of titanium instead of lead in paints and control of industrial point sources). The chronic and cumulative exposure to lead is best measured in bone, rather than blood. The level of lead in blood reflect more ongoing exposure. The chronic lead exposure in children may produce mild dysfunction, or may be massive and lethal. In young children, it may produce sensory, motor, intellectual, and psychologic impairments. In more severe cases, it may produce blindness, psychoses, seizures, and coma. Lead-induced peripheral neuropathies in adults generally subside with the elimination of exposure. In children, both peripheral and CNS abnormalities are usually irreversible.

MORPHOLOGY

The major targets of lead toxicity are the blood, bone, nervous system, GI tract, and kidneys.

Bones: In chronic exposure, 90% of the total body lead burden is in the bones. Excess lead interferes with the normal remodeling of calcified cartilage and primary bone trabeculae in the epiphyses. During bone formation, lead and calcium are deposited in the bone. This can be detected radiographically as radiodense "lead lines" and is simple method of detecting increased body stores of lead in children. Lead also inhibits the healing of fractures by increasing chondrogenesis and delaying mineralization of cartilage.

Blood: Anemia is a cardinal and one of the earliest signs of lead intoxication. Lead binds to sulfhydryl groups and interferes with two enzymes involved in heme synthesis in bone marrow erythroblasts. The enzymes inhibited are (i) aminolevulinic acid dehydratase and (ii) delta ferrochelatase. Inability to produce heme adequately causes a **microcytic and hypochromic anemia** like that seen in iron deficiency (*refer* Chapter 11). RBC in lead intoxication shows **prominent basophilic stippling**, reflecting clustering of ribosomes. Lead also inhibits sodium- and potassium-dependent ATPases in cell membranes. This may increase the fragility of red cells causing **hemolysis** (reduced life span). Thus, anemia in lead intoxication is due to ineffective hematopoiesis and accelerated RBC turnover. There is formation of zinc-protoporphyrin instead of heme.

Brain damage: Excess lead is toxic to nervous tissues in adults and children. In adults, peripheral neuropathies are common whereas central nervous tissue is more commonly affected in children.

Lead encephalopathy: In children, brain is the target of lead toxicity. **Lead encephalopathy** is observed in severe cases and the brain shows edema, flattening of gyri and compression of ventricles. Microscopically the changes include edema, congestion, petechial hemorrhages, demyelination of the cerebral and cerebellar white matter, and necrosis of cortical neurons accompanied by diffuse astrocytic proliferation in both the gray and white matter.

- **Clinical features**: Children with lead encephalopathy are irritable and ataxic. They may develop convulsions or display altered states of consciousness (drowsiness to frank coma). Children with low blood lead levels may present with mild CNS symptoms, such as clumsiness, irritability and hyperactivity.

Peripheral motor neuropathy: In adults, the CNS is less-often involved than in children. The most common manifestation of lead neurotoxicity in the adults is **peripheral demyelinating neuropathy**. It typically involving motor neurons (radial and peroneal nerves) innervating the most commonly used muscles. Thus, the extensor muscles of the wrist and fingers are often the first to be affected, followed by paralysis of the peroneal muscles (**wrist drop** and **foot drop**).

GI tract: It is also a site of major clinical manifestations. Lead-induced neuropathy may be responsible for the paroxysms of extremely severe, poorly localized abdominal pain known as **lead colic.**

Kidney: Excretion of lead occurs through kidneys, and acute exposures may cause damage to proximal tubules. **Lead nephropathy** is due to the toxic effect of the lead on the proximal tubular cells of the kidney. It is characterized by aminoaciduria, glycosuria and hyperphosphaturia (Fanconi syndrome). Microscopically, characteristic intranuclear lead inclusions are seen in the nuclei of the proximal tubular cells in lead nephropathy. These inclusions are composed of a lead–protein complex containing more than 100 times the concentration of lead in the whole kidney. Chronic renal damage may result in fibrosis in the interstitium and renal failure.

Laboratory diagnosis: For definitive diagnosis of lead poisoning, intoxication, elevated blood lead and red cell free protoporphyrin levels (>50 μg/dL) or, alternatively, raised blood levels of zinc-protoporphyrin levels or its product are required. In milder cases of lead exposure, anemia may be the only obvious abnormality.

Treatment: Lead poisoning is treated with chelating agents namely ethylenediaminetetraacetic acid (EDTA), either alone or in combination with dimercaprol (BAL). Usually, hematologic and renal manifestations of lead intoxication are reversible whereas the alterations in the CNS are usually irreversible.

Mercury

Mercury binds to sulfhydryl groups (like lead) in certain proteins with high affinity. The kidney is the main target of the toxicity of inorganic mercury and the CNS (brain) is damaged by organic mercury. It can also **damage the GI tract.**

Source: Humans have used **inorganic mercury** since prehistoric times and it has been known to be an occupation-related hazard. It was used as a

pigment in cave paintings, a cosmetic, a remedy for syphilis, and a component of diuretics. It is used as a medicine in homeopathy. In recent years, the main sources of exposure are **organic** (derived from living matter) **mercury** present in contaminated fish and dental amalgams that release mercury vapors. In some areas mercury used in gold mining, or from fertilizer and plastics factory, is discharged into and contaminate the rivers. Inorganic mercury from industrial wastes can be converted to highly neurotoxic organic compounds, such as methyl mercury by bacteria in bays and oceans. Methyl mercury is taken by the carnivorous fish (e.g., swordfish, shark, tuna, pike, and bluefish). Consumption of such fish contaminated with mercury can produce mercury poisoning.

Neurotoxicity: Children exposed in utero showed **delayed developmental milestones** and abnormal reflexes, cerebral palsy, deafness, blindness, and major CNS defects. The developing brain is very sensitive to methyl mercury. There will be cerebral and cerebellar atrophy.

Nephrotoxicity: In the **kidneys**, mercury can produce **acute tubular necrosis (proximal tubular necrosis) and renal failure.**

GIT: Almost 90% of the ingested organic mercurial compounds is readily taken up in the GI tract because of their lipid solubility. Ingested mercury can damage the gut and cause ulcerations and bloody diarrhea.

Arsenic

The toxic properties of arsenic are well known. **Arsenic interfere with several aspects of cellular metabolism. It produces its toxic effects mainly in the GI tract, nervous system, skin, and heart.**

Sources: Arsenic was used as poison by skilled practitioners in Italy. Arsenic compounds have been used as insecticides, herbicides, weed killers, other agricultural products and wood preservatives. Arsenicals may also be found naturally in soil and ground water (in countries, such as Bangladesh, Chile, and China) as a result of naturally occurring arsenic-rich rock formations, from coal burning or from use of arsenical pesticides. It may contaminate the environment by the mining and smelting industries. Arsenic is present in Chinese and Indian herbal medicine.

Toxic effects: These include:

- **Acute arsenic poisoning** is almost always due to accidental or homicidal ingestion. Ingested of large quantities of arsenic causes **severe abdominal pain, diarrhea; cardiac arrhythmias, shock and respiratory distress syndrome**; and acute encephalopathy. GI, cardiovascular and CNS toxicity may be severe and cause death. These effects may be due to the interference with mitochondrial oxidative phosphorylation.
- **Chronic exposure to arsenic**: Chronic arsenic intoxication affects many organ systems (e.g., gastrointestinal, cardiovascular and hematologic systems). It produces nonspecific symptoms such as malaise and fatigue. **Cancers of the skin, respiratory tract** (lung carcinoma) and **gastrointestinal tract** may develop due to industrial and agricultural exposure. Arsenic produces hyperpigmentation and hyperkeratosis of the skin. This may be followed by the development of **basal and squamous cell carcinomas** (but not melanomas). A symmetrical sensorimotor polyneuropathy can also develop. Arsenic-induced skin tumors occur on palms and soles, and may be multiple. Arsenic can cause encephalopathy and peripheral neuropathy (paresthesias, motor palsies and painful neuritis).

Cadmium

Sources: Cadmium is a plasticizer and a pigment and is relatively a modern toxic agent. It is used in manufacturing alloys, producing rechargeable nickel-cadmium batteries and electroplating other metals (e.g., automobile parts and musical instruments). Cadmium oxide fumes are released during welding of steel parts previously plated with a cadmium anticorrosive. Cadmium can contaminate soil and plants directly or through fertilizers and irrigation water. The main routes of exposure are ingestion and inhalation. Both plant- and animal-derived foodstuffs may contain substantial amount of cadmium.

Effects: **Cadmium is mainly toxic to the kidneys and the lungs.** To a lesser extent, it may affect skeletal and vascular system. They may **generate increased production of ROS.** It accumulates in the human body with a half-life of over 20 years. It is rarely recycled.

- Short-term cadmium inhalation: It irritates the respiratory tract and causes pulmonary edema.

- Chronic cadmium exposure: It can lead to obstructive lung disease (emphysema) and renal toxicity. In the kidney, initially it produces proteinuria due to tubular damage (rather than glomerular damage) that may progress to end-stage renal disease. Cadmium can cause skeletal abnormalities due to loss of calcium loss.

Chromium

Chromium (Cr) is used in several industries, such as metal plating and some types of manufacturing. Chronic exposure is highly genotoxic and increases the **risk of lung cancer** and other tumors.

Nickel

Nickel is used in electronics, coins, steel alloys, batteries and food processing.

The most frequent effect of exposure to nickel is contact dermatitis ("nickel itch") and can develop due to contact with metals containing nickel, such as coins and costume jewelery.

Exposure to nickel increases the risk of **lung cancer and cancer of the nasal cavities**.

EFFECTS OF TOBACCO

Q. Write short essay/note on effects of tobacco.

Tobacco is the most common exogenous cause of human cancers and is responsible for about 90% of lung cancers. **Smoking tobacco is the single largest preventable cause of human death**. Apart from death due to various types of cancers, tobacco can cause deaths from cardiovascular and metabolic diseases, deaths from nonmalignant lung diseases and perinatal deaths.

Types of tobacco: Tobacco may be used either for **smoking** or as **smokeless tobacco**.

- **Smoking:** It is the main culprit and may be used in the form of cigarette, beedi, cigar, or pipe smoking, or reverse smoking (smoking a cheroot

with the burning end inside the mouth is practiced in certain regions of India). Regular marijuana use has also been associated with oropharyngeal cancer. Life expectancy is reduced by cigarette smoking and overall mortality is proportional to the amount (dose dependent) and duration of smoking commonly quantitated as "pack-years." Tobacco smoking not only produces personal risk, but passive tobacco inhalation from the environment ("second-hand smoke") can also cause lung cancer in nonsmokers. Cessation of smoking greatly decreases the risk of death from lung cancer.

- **Smokeless tobacco:** It is also harmful to health and is an important cause of oral cancer. It may be used in its various forms such as chewing tobacco or snuff. Betel quid/pan is a type of tobacco chewing that contains several ingredients, such as areca nut, slaked lime, and tobacco which are wrapped in a betel leaf. It is commonly used in India and Southeast Asia, and is associated with marked increase in oral cancer. **Betel quid appears to be the major carcinogen**. However, it may also be related to slaked lime and the areca nut. Other methods of tobacco consumption include snuff dipping and tobacco chewing.

Oral cancers are found on the **buccal and gingival** surfaces in the sites **where tobacco products** are held in **contact** with the mucosa for long periods.

Constituents of Tobacco Smoke

Tobacco contains more than 2,000 substances (potentially noxious) and more than 60 have been identified as carcinogens and few of them along with the type of injury produced by these agents are listed in **Table 9.1**.

TABLE 9.1: Effects of selected tobacco smoke constituents.

Constituent of tobacco smoke	***Effect(s)***
Cigarette, tar	Carcinogenesis
Polycyclic aromatic hydrocarbons	
Benzopyrene	
Nitrosamine	
Nicotine	Release catecholamine, cause depression and promotes tumor

Phenol	Promotes tumor and causes irritation of mucosa
Carbon monoxide (CO)	Impaired oxygen transport and use
Formaldehyde	Toxic to cilia and causes irritation of mucosa
Oxides of nitrogen	

Nicotine is an alkaloid present in tobacco leaves. It does not directly cause tobacco-related diseases, but is strongly addictive and is responsible for tobacco addiction. Nicotine binds to nicotinic acetylcholine receptors in the brain, and release catecholamines from sympathetic neurons. This is responsible for the acute ill effects of smoking namely increase in heart rate, blood pressure, cardiac contractility and output.

Tobacco products and smoking: Any irritating smoked product increases the risk for tumors of the oral cavity. Nicotine in tobacco and other tobacco leaf components cause cancer. **Carcinogens in tobacco can act as initiators, as well as promoters.** Risk increases with amount and duration of tobacco use.

Diseases Caused

Q. Write a short essay/note on diseases caused and cancers associated with smoking.

Respiratory System

The most common diseases caused by cigarette smoking involve the respiratory system mainly lung.

- **Lung cancer:** Components of cigarette smoke, mainly **polycyclic aromatic hydrocarbons and nitrosamines** are directly involved in the development of lung cancer in humans (*refer* pages 527-35 of Chapter 17). Cytochrome P-450 phase I enzymes (CYPs) and phase II enzymes increase the water solubility of the carcinogens, thereby helping in their excretion. However, few intermediates produced by CYPs are electrophilic and combine with DNA to form DNA adducts. If DNA adducts persist, they can cause mutations in oncogenes and tumor suppressors. The risk of developing lung cancer depends on the intensity of exposure and is usually expressed in terms of number of "pack years" (e.g., one pack daily for 20 years is equal to 20 pack years) or cigarettes

smoked per day and duration of smoking habit. Smoking also increases the risk of other carcinogens (e.g., asbestos, uranium). In nonsmokers, passive environmental smoke inhalation is also associated with risk of lung cancer than those who are not exposed to passive smoke. The risk of lung cancer declines after one quits smoking.

- **Chronic bronchitis, emphysema** and **chronic obstructive pulmonary disease:** Contents in **tobacco smoke directly irritate the tracheobronchial mucosa**, producing inflammation and increased production of mucus (bronchitis). Cigarette smoke also recruits leukocytes to the lung, and increases the local production of elastase. This injures lung tissue leading to emphysema. Children living along with adult smokers are susceptible to increased respiratory illnesses and asthma.

Other Systems

Apart from lung cancer, tobacco smoking is a risk factor for many other malignant and nonmalignant disorders of many organ systems.

- **Oral cancers:** Smokeless tobacco along with alcohol consumption is important cause of oral cancer (*refer* pages 540-1). These include **cancers of the lip, tongue and buccal mucosa**. All forms of tobacco use-cigarette, cigar and pipe smoking, and tobacco chewing are associated with oral cancer.
- **Other cancers:** Cigarette smoking is associated with **cancers of the esophagus, larynx, pancreas, bladder, kidney, cervix, bone marrow** (some forms of leukemia), colon and liver. Tobacco consumption interacts with alcohol in multiplying the risk of oral, laryngeal and esophageal cancer.
- **Atherosclerosis and myocardial infarction** (*refer* pages 435 and 458)**:** Cigarette smoking is major risk factor, of atherosclerosis and its major complication is myocardial infarction. Smoking is a risk factor for **atherosclerotic peripheral vascular disease** and **cerebrovascular disease** (ischemic stroke, intracranial hemorrhage). If smoking is combined other risk factors, such as hypertension (elevated blood pressure) and hypercholesterolemia (raised blood cholesterol levels), this multiplies the risk of atherosclerosis and myocardial infarction. The cigarette smoking is a strong risk factor for **atherosclerotic aortic aneurysms**.

- **Buerger disease:** It is an inflammatory and occlusive disease of the lower leg vasculature occurs almost only in heavy smokers.
- **Peptic ulcer disease** (*refer* pages 552-5 of Chapter 18).
- **Maternal smoking:** Increases the **risk of spontaneous abortions and preterm births** and results in **intrauterine growth retardation** (fetal tobacco syndrome).
- **Type II diabetes mellitus**: It may develop in smokers. It may be due to nicotine-related insulin resistance and beta cell apoptosis, increased central obesity and altered metabolism of estrogens and androgens in smokers.
- The **course of tuberculosis** is **more severe** in smokers.

EFFECTS OF ALCOHOL

Alcohol (ethanol) consumption in moderation is not injurious. However, excessive amounts of alcohol can cause marked physical and psychologic damage. Alcohol can cause acute and chronic toxic effects.

After consumption, ethanol is directly absorbed in the stomach and small intestine. Then it is distributed to all the tissues and fluids of the body which is directly proportional to its level in the blood. Less than 10% of the alcohol is excreted directly in the urine, sweat, and breath.

Chronic alcoholism: It is defined as regular intake of sufficient alcohol to injure an individual socially, psychologically or physically. Alcoholism is more common in men, but the number of female alcoholics is gradually increasing. Alcohol abuse (improper/bad use) is a hazardous. It may lead to death either directly or due to accidents caused by drunken driving and alcohol-related homicides and suicides, and as a consequence of cirrhosis of the liver.

Alcohol level in blood: The amount of alcohol exhaled is proportional to the blood level. This is the basis for the breath test used by traffic police for drunken driving. The legal definition of drunk driving is an alcohol concentration of 80 mg/dL in the blood. In most individual, this alcohol concentration may be observed after consumption of three standard drinks [for example, three (12 ounce) bottles of beer, 15 ounces of wine, or 4–5 ounces of 80-proofdistilled spirits]. When the alcohol level reach 200

mg/dL, drowsiness develops, 300 mg/dL level stupor, and with more than this level, coma with respiratory arrest may develop. For most individuals, daily consumption should be <45 g alcohol and any quantity more than this should be discouraged and consumption of 100 g or more/day may be dangerous [10 g alcohol = 1 oz, or 30 mL, of 86 proof (43%) spirits].

Factors affecting alcohol levels in blood: Apart from amount of alcohol consumed other factors also affect the alcohol levels in blood: The **rate of metabolism** affects the alcohol level in blood. Chronic alcoholics develop tolerance to alcohol and they metabolize alcohol at a higher rate than normal. Hence, chronic alcoholics show lower peak levels of alcohol than others for the same amount of alcohol consumed.

Metabolism of alcohol (*refer* **Fig. 19.14):** Discussed on pages 615-8.

Acute Alcoholism

It produces its effects mainly on the CNS. It may also produce reversible **hepatic and gastric damage**. Acute alcohol intoxication may cause fatalities from motor vehicle accidents.

- **Central nervous system:** In the CNS, alcohol is a depressant. In a normal individual, intellectual **behavioral changes** can be observed at low alcohol concentrations (below 50 mg/dL). Levels above 80 mg/dL are usually associated with **slower reaction times and gross incoordination**. Most individuals become comatose at levels above 300 mg/dL, and at concentrations above 400 mg/dL, death from respiratory **arrest**/failure is common.
- **Liver:** Even moderate intake of alcohol produces **accumulation of multiple fat droplets in the cytoplasm** of hepatocytes (fatty change or hepatic steatosis—*refer* pages 23 and 619).
- **Gastric damage:** It may induce **acute gastritis and ulceration**.

Chronic Alcoholism (Box 9.1)

Q. Write a short note on diseases and cancers associated with chronic alcoholism.

Chronic alcoholics usually develop CNS tolerance to alcohol and may easily tolerate blood alcohol levels of 100–200 mg/dL. **Most alcohol-related**

disorders are due to the toxic effects of alcohol itself. Chronic alcoholism can affect any organs and tissues, but main affects are found in the liver and stomach. Chronic alcoholics have significant morbidity and shortened life span. This is mainly due to damage to the liver, GI tract, CNS, cardiovascular system, and pancreas.

Liver: It is the main organ affected by chronic alcoholism. It can produce alcoholic liver disease which includes (i) **fatty change** (steatosis), (ii) **alcoholic hepatitis**, and (iii) **cirrhosis** (*refer* Chapter 19). Cirrhosis is associated with portal hypertension and also is a risk factor for hepatocellular carcinoma. Any alcoholic beverage (beer, wine, whiskey, hard cider, etc.) consumed in excess produces cirrhosis which in turn depends on the total dose of alcohol.

BOX 9.1: Chronic alcoholism and associated diseases (main effects).

- **Liver:** Fatty liver, alcoholic hepatitis, cirrhosis and an increased risk of hepatocellular carcinoma
- **GI tract:** Massive bleeding from gastritis, gastric ulcer, or esophageal varices (associated with cirrhosis). Impaired small intestinal absorption
- **CNS:** Thiamine deficiency resulting in peripheral neuropathies and the Wernicke-Korsakoff syndrome, cerebral atrophy, cerebellar degeneration, and optic neuropathy
- **CVS:** Dilated congestive cardiomyopathy (alcoholic cardiomyopathy), arrhythmias and increased incidence of hypertension
- **Pancreas:** Increased risk of acute and chronic pancreatitis
- **Effects on fetus:** Consumption during pregnancy can cause fetal alcohol syndrome
- **Carcinogenesis:** Increased incidence of cancers of the oral cavity, esophagus, liver, and, possibly, breast in females
- **Malnutrition and deficiencies of B vitamins**
- **Hemopoietic dysfunction:** Megaloblastic anemia, hemolytic anemia
- **Endocrine system:** Feminization, loss of libido and potency, gynecomastia, and testicular atrophy in male alcoholics
- **Bone:** Osteoporosis and aseptic necrosis of bone

Gastrointestinal tract: Chronic alcoholism can cause massive bleeding from **gastritis, peptic/gastric ulcer, or esophageal varices** (complication of cirrhosis). **Reflux esophagitis** is also common. Violent retching may cause tears at the **esophageal–gastric junction (Mallory–Weiss syndrome).** Alcohol inhibits active transport of amino acids, thiamine and vitamin B_{12}.

Nervous system: General cortical atrophy of the brain is common in chronic alcoholics. Most of the characteristic brain diseases in alcoholics are probably due to nutritional deficiency.

- **Wernicke encephalopathy**: It is due to nutritional thiamine deficiency which is common in chronic alcoholics. It is characterized by mental confusion, ataxia, abnormal ocular motility and polyneuropathy.
- **Korsakoff psychosis**: It is also due to thiamine deficiency and is characterized by retrograde amnesia and confabulatory symptoms.
- **Polyneuropathy**: Peripheral neuropathy is common in chronic alcoholics. It is usually due to deficiencies of thiamine and other B vitamins. Its most common complaints include numbness, paresthesias, pain, weakness, and ataxia.
- **Others**: Cerebral atrophy, cerebellar degeneration, and optic neuropathy may also develop.

Cardiovascular effects: Chronic alcoholism produces diverse effects on the cardiovascular system.

- **Alcoholic cardiomyopathy:** It can cause myocardial injury and produce dilated congestive cardiomyopathy called as alcoholic cardiomyopathy (*refer* pages 484-8).
- **Arrhythmias:** Alcoholics are more susceptible to arrhythmias and sudden, fatal arrhythmias may cause sudden death. In this context, moderate amounts of alcohol consumption, or "social drinking" (one to two drinks a day) may increase serum levels of high-density lipoproteins (HDLs) and inhibit platelet aggregation. Thus, it **may provide significant protection against coronary artery disease**(atherosclerosis) and its consequence, myocardial infarction. However, in those who **heavily consume** with attendant liver injury, there may be **decreased levels of HDL and increased** likelihood of **coronary heart disease**.
- **Hypertension:** Chronic alcoholism may also be associated with an increased incidence of hypertension**.**

Pancreatitis: Excess alcohol intake increases the risk of both **acute and chronic pancreatitis** (*refer* Chapter 19). **Chronic calcifying pancreatitis** in alcoholism produces severe pain, pancreatic insufficiency and pancreatic stones.

Effects on fetus: Infants born to mothers who consume alcohol (even in low amounts) during pregnancy (especially during the first trimester) can show a cluster of abnormalities that together constitute the fetal alcohol syndrome. These abnormalities in the newborn consists of microcephaly, growth retardation and facial abnormalities (dysmorphology, neurologic dysfunction), and other congenital anomalies. However, about 6% of the

infants of alcoholic mothers develop the full syndrome. More commonly it is associated with less severe abnormalities, such as mental retardation (reduction of mental functions in older children), intrauterine growth retardation and minor dysmorphic features.

Carcinogenesis: Chronic alcohol consumption is associated with an increased incidence of cancers of the oral cavity, esophagus, larynx, liver (increased in patients with alcoholic cirrhosis), and probably, breast in females than in the general population. The mechanisms of the carcinogenic effect are not known. The risk is markedly more if there is concurrent smoking or the use of smokeless tobacco.

Malnutrition: Alcohol provides substantial energy, but is often consumed at the expense of food (empty calories). Thus, chronic alcoholism may be associated with malnutrition and deficiencies, particularly of the B vitamins (such as thiamine deficiency).

Hemopoietic dysfunction: **Megaloblastic anemia** is not uncommon in alcoholics. It may be due to dietary deficiency of folic acid and decreased absorption of folate by the small intestine in alcoholics. Chronic ethanol intoxication may directly **increase the mean corpuscular volume (MCV) of RBCs**. In patients with alcoholic cirrhosis, the spleen is often enlarged by portal hypertension. In these patients, **hypersplenism** may **cause hemolytic anemia. Transient thrombocytopenia** may develop after acute alcohol intoxication and may **produce bleeding**. Platelet aggregation is impaired by alcohol and may also contribute to bleeding.

Endocrine system: **Male alcoholics** may develop **feminization** and **loss of libido and potency**. They may also develop **gynecomastia** (enlargement of breasts), loss of body hair and a female distribution of pubic hair (female escutcheon). Some of these are due to impaired estrogen metabolism in chronic liver disease. But many of the changes (especially testicular atrophy) may occur even without any liver disease. Chronic alcoholics have lower levels of circulating testosterone. Alcohol has a direct toxic effect on the testes and causes male sexual impairment.

Bone: Chronic alcoholism, especially in postmenopausal women predisposes to **osteoporosis**. Interestingly, it may be noted that moderate consumption of alcohol may be protective against osteoporosis. Male alcoholics are likely to have high incidence of aseptic necrosis of the head of the femur.

Immune system: Chronic alcoholics may be prone to many infections (particularly pneumonias) with micro-organisms that are unusual in the general population (e.g., *Haemophilus influenzae*).

PROTEIN–ENERGY MALNUTRITION

CC: Describe the pathogenesis of disorders caused by protein calorie malnutrition and starvation.

- **Protein–energy malnutrition (PEM)** or **protein–calorie malnutrition** refers to a group of malnutrition where there is inadequate calorie or protein intake.
- Severe PEM is a serious, often lethal disease and usually affects children of low-income countries.
- **PEM include marasmus, kwashiorkor and intermediate states of marasmus–kwashiorkor**.

Marasmus

Q. Write short note on marasmus.

- *Marasmus* is the childhood form of starvation. It develops **due to inadequate intake of protein and calories** and is characterized by emaciation with obvious muscle wasting and loss of body fat. There is **no edema**. The hair is thin and dry.
- The marasmic child does not appear as apathetic or anorexic as with kwashiorkor. Diarrhea occurs frequently and there may be signs of infection.

Kwashiorkor

- **Inadequate protein intake**: Kwashiorkor develops due to an inadequate protein intake with reasonable caloric (energy) intake.
- **Edema**: In kwashiorkor, marked protein deprivation causes hypoalbuminemia leading to **generalized or dependent edema**. Edema is not a characteristic feature of marasmus.
- **Skin lesions**: Children with kwashiorkor have characteristic *skin lesions*. This consists of **alternating zones of hyperpigmentation, and**

hypopigmentation, producing "flaky paint" appearance.

Q. Write short essay/note on flag sign.

- **Hair changes:** These include **loss of color or alternating bands of pale and darker** hair.
- **Other features:** The other features that differentiate kwashiorkor from marasmus are as follows:
  - Presence of enlarged, fatty liver.
  - Development of apathy, listlessness and loss of appetite.
  - Likely presence of vitamin deficiencies.
  - Defects in immunity and secondary infections.

MORPHOLOGY

- Growth failure.
- Peripheral edema in kwashiorkor.
- Loss of body fat and atrophy of muscle more marked in marasmus.

Differences between kwashiorkor and marasmus are listed in **Table 9.2**.

Cachexia

- PEM is a common complication that develops in patients with AIDS or advanced cancers. In these settings it is called as cachexia.
- Cachexia occurs most commonly in patients with cancers of gastrointestinal, pancreatic and lung.
- Characterized by **extreme weight loss, fatigue, muscle atrophy, anemia, anorexia and edema**.

STARVATION

Definition: Starvation is a state of overall deprivation of nutrients.

Causes of starvation are listed in **Box 9.2**.

Metabolic Changes in Starvation

Starvation is a gradual process in which there is decrease in the metabolic rate as storage carbohydrate reserves are metabolized.

1. **Glucose:** Normal body has glucose stores that are sufficient only for one day's metabolic needs. During fasting, insulin independent tissues, such as the brain, blood cells and renal medulla continue to utilize glucose. However, insulin-dependent tissues like muscle do not utilize glucose. To maintain normal blood glucose level, there will be release of glycogen stores from the liver. When the liver glycogen stores are depleted (within a few days), the supply of glucose released into the blood decreases. Subsequently, hepatic gluconeogenesis from other sources, such as breakdown of proteins takes place.

TABLE 9.2: Differences between kwashiorkor and marasmus.

Feature	***Kwashiorkor***	***Marasmus***
Definition	Inadequate protein intake with reasonable caloric (energy) intake	Inadequate intake of both protein and calories
Age	Children 6 months to 3 years	Infants under 1 year
Growth failure	Present	Present
Edema	Localized or generalized	Absent
Liver	Enlarged fatty	Not enlarged

BOX 9.2: Causes of starvation.

- Deliberate fasting (religious or political)
- Famine conditions (country or community)
- Secondary undernutrition: Due to chronic wasting illness/diseases (e.g., infections, inflammatory conditions, liver disease), cancer*

*Cancer cachexia is defined as **progressive weight loss accompanied by severe weakness, anorexia and anemia developing in patients with cancer**. Its m**echanism** is poorly understood and may be due to **TNF and other cytokines**, such as IL-1, interferon-γ, and leukemia inhibitory factor. They may be produced by macrophages in the tumor or by the tumor cells themselves.

2. **Proteins:** Protein stores and the triglycerides of adipose tissue have enough energy for about 3 months. In starvation, the *proteins are* broken down to release amino acids and these amino acids are utilized as fuel for hepatic gluconeogenesis. Protein is used for energy by means of gluconeogenesis because certain tissues prefer glucose as an energy

source. The use of protein for gluconeogenesis produces a negative nitrogen balance.

3. **Fats:** When insulin levels decline during fasting, triglycerides of adipose tissue are broken down to form glycerol and free fatty acids. The fatty acids are converted into ketone bodies in the liver and they are used for energy use in place of glucose by most organs including brain. As starvation continues, overall needs for energy are reduced by slowing metabolism. The tissues that usually need glucose for energy adapt by using ketone bodies produced from fat. Eventually lipolysis of stored fat provides the source of required energy, and the use of protein as an energy source decreases. Through this adaptive response the body strives to conserve lean body mass. Overall, there will be reduction and depletion of the body's protein. Fasting (voluntary starving) is not associated with a high mortality unless it is prolonged. Starvation can continue till a stage when all the body fat stores are exhausted. After the exhaustion of fat stores death will occur.

Manifestations: Starved patient has lax, dry skin, wasted muscles and atrophy of internal organs. In chronic starvation the patient is susceptible to infections **(Box 9.3)**.

Laboratory findings: Characteristic findings in starvation include decreased serum glucose levels and urinary nitrogen excretion, along with elevation in the levels of ketone bodies (with ketonuria) and free fatty acids.

OBESITY

CC: Describe the pathogenesis of obesity and its consequences.

Q. Write short essay/note on obesity.

Definition: Obesity is defined as an accumulation of excess body fat (adipose tissue) that is of sufficient magnitude to impair health.

Prevalence of obesity: Obesity is a major health problem in developed countries and an emerging health problem in developing countries, such as India.

Classification of overweight and obesity by body mass index is presented in **Table 9.3**.

Types of Obesity

The distribution of the stored fat is important in obesity and **according to body fat distribution** obesity is divided into:

- **Central ('abdominal', 'visceral', 'android' or 'apple-shaped') obesity:** This type of obesity shows increased accumulation of fat in the trunk and in the abdominal cavity/intra-abdominal (in the mesentery and around viscera). It is associated with a greater risk for several diseases (e.g., Type 2 diabetes, the metabolic syndrome and cardiovascular disease) than generalized obesity.
- **Generalized' ('gynoid' or 'pear-shaped') obesity:** This type is characterized by excess accumulation of fat diffusely in the subcutaneous tissue.

Etiology

Accumulation of fat in obesity can be considered to be the result of caloric imbalance between the energy consumption (intake of calories) in the diet and energy expenditure through exercise and bodily functions. However, the pathogenesis of obesity is complex and incompletely known.

Genetic Aspects of Human Obesity

- Obesity is a **polygenic disorder** with small contributions from a number of different genes.
- Single-gene (**monogenic forms**) disorders are rare and produce severe childhood obesity. These include mutations in the *leptin gene* and *leptin receptor* gene, mutations of *POMC* (Proopiomelanocortin), *Mc4R* (melanocortin-4 receptor) *genes*.
- A few **genetic conditions in which obesity** is a feature include the Prader–Willi and Laurence–Moon–Biedl syndromes.

Environmental Contributors to Human Obesity

- **Food:** Many environmental factors can influence food intake. Increased consumption of energy-dense foods, larger food portion size, and

increased variety of food, increased availability, reduced cost and increased caloric beverages (soft drinks, juices) promote obesity.

TABLE 9.3: Classification of overweight and obesity by body mass index.

	BMI Kg/m^2
Normal	18.5–24.9
Overweight	25.0–29.9
Obesity—Class I	30.0–34.9
Obesity—Class II	35.0–39.9
Extreme obesity—Class III	≥40

BOX 9.3: Infections associated with starvation.

- Gastroenteritis and gram-negative sepsis
- Respiratory infections, e.g., bronchopneumonia
- Viral diseases, e.g., measles, herpes simplex
- Tuberculosis
- Streptococcal and staphylococcal skin infections
- Helminthic infestations

- **Physical activity:** It can be divided into three categories: (i) exercise (fitness and sports-related activities); (ii) work-related physical activity; and (iii) non-exercise, nonemployment (spontaneous) activity. Increased sedentary behavior, reduced activities of daily living and decreased employment physical activity promote obesity.

Pathogenesis

Body weight regulation (regulation of energy balance) or dysregulation depends on a complex interplay of both humoral (endocrine) and neural mechanism that control appetite and satiety. These neurohumoral mechanisms regulate energy balance and respond to genetic, nutritional, environmental, and psychologic signals. They trigger a metabolic response through the stimulation of centers in the hypothalamus and ultimately influence the effector arms of energy intake and expenditure.

Neurohumoral mechanisms can be subdivided into three components **(Fig. 9.1)**.

Peripheral or Afferent System

Peripheral afferent system can be further subdivided into peripheral appetite suppressing signals and peripheral appetite stimulant signals.

Peripheral Appetite Suppressing Signals

- **Leptin (**Greek term leptos, meaning 'thin'.): It is a **hormone secreted by fat cells** and it stimulates POMC/CART pathway **(Fig. 9.1)** and inhibits NPY/AgRP pathway and **appetite is suppressed** (anorexigenic). Increased leptin **stimulates physical activity**, heat **production** (thermogenesis), and **energy expenditure**.
- **Adiponectin:** It is a hormone (**fat-burning molecule)** and the 'guardian angel against obesity,' and is **produced mainly by fat cells** (adipocytes). Its levels are lower in obese.
- **Resistin:** Primarily produced by macrophages and not fat cells. It **causes insulin resistance.**
- **Gut hormones:** These include ghrelin, PYY, pancreatic polypeptide, insulin and amylin.
  - **Insulin:** It is **secreted by cells of the pancreas** and act centrally to **activate the appetite suppressing pathway**.
  - **Peptide YY (PYY):** It is **secreted by the endocrine cells** (L cells) in the ileum and colon. It **reduces appetite**. Other peripheral **appetite suppressing** signals include glucagon-like peptide 1 (**GLP1**) and oxyntomodulin.
  - **Amylin:** It is a peptide secreted with insulin from pancreatic β-cells.
  - **Obestatin:** It is a peptide produced by the **same gene that encodes ghrelin**. It counteracts the **increase in food intake induced by ghrelin**.

Peripheral Appetite Stimulating Signals

- **Gut hormones:**
  - **Ghrelin:** It is produced by the **oxyntic cells of the fundus of the stomach** and in the **arcuate nucleus of the hypothalamus**. Ghrelin **increases food intake** (orexigenic effect) and **stimulates appetite** by activating the central appetite stimulating NPY/AgRP pathway.

- **Retinol binding protein 4 (RBP4):** Secreted by fat cells. Its actions counteract with those of insulin. Raised levels of RBP4 found in Type 2 diabetes mellitus.

Central Processing

The arcuate nucleus of the hypothalamus processes integrates neurohumoral peripheral afferent signals and generates efferent signals. It consists of:

Central Appetite Suppressing (Anorexigenic Pathway or Leptin Melanocortin Pathway)

In this pathway, **POMC/CART neurons enhance energy expenditure** and weight loss through the production of the anorexigenic α-MSH (α-melanocyte stimulating hormone) by cleavage of POMC by PC1 (prohormone convertase). It exerts its **appetite suppressing action via the activation of the melanocortin receptors 3 and 4 (MC3/4R)** in second-order neurons. Second order neurons in turn produce factors, such as thyroid releasing hormone (TSH) and corticotropin releasing hormone (CRH) that increase the basal metabolic rate and anabolic metabolism, thus **regulate food intake favoring weight loss.**

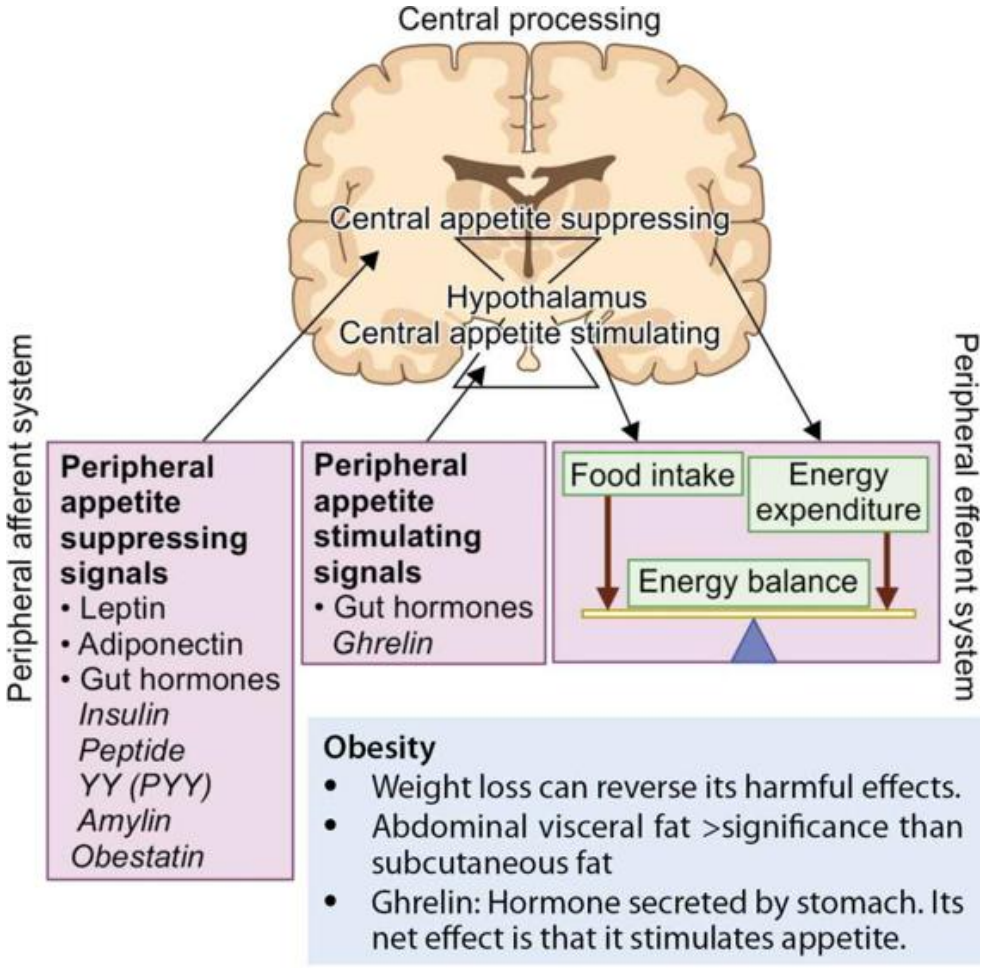


Fig. 9.1: Regulation of energy balance. Peripheral afferent system (appetite suppressing and stimulating signals) influences the activity of the hypothalamus, which is the central regulator of appetite and satiety. Signals from hypothalamus in turn act on peripheral efferent system (food intake and energy expenditure).

Central Appetite Stimulating (Orexigenic) Pathway

It consists of: (i) NPY/AgRP neurons that promote food intake (orexigenic effect) and weight gain, through the activation of Y1/5 receptors in secondary neurons. (ii) Secondary neurons in turn release factors, such as melanin-concentrating hormone (MCH) and orexin, which stimulate appetite. This pathway also decreases energy expenditure.

These pathways interact with each other and communicate with other parts of the brain, and influence the autonomic nervous system and ingestive

behavior. These central pathways are in turn influenced by a variety of peripheral signals discussed above.

Peripheral Efferent System

It is organized into two pathways namely anabolic and catabolic that control food intake and energy expenditure, respectively.

Energy Intake (Food Intake)

- **Food**: The increase in obesity can be related to the type of food consumed (i.e., food containing sugar and fat) and also psychological factors.
- **Control of appetite**: **Signals may affect different aspects of eating behavior**. For example, ghrelin (peptide produced by the stomach) increases hunger but does not affect satiation or satiety. Cholecystokinin causes satiation, but has no effect on satiety. **Leptin act on multiple pathways**, **its deficiency causes increased hunger and reduced satiation**.
- Following a meal, substances, such as cholecystokinin (CCK), bombesin and glucagon-like peptide 1 (GLP1) are released from the small intestine and glucagon and insulin from the pancreas. These hormones are involved in the control of satiety. The control of appetite is extremely complex. Many transmitters in the central nervous system affect appetite:
  - **Appetite inhibitors:** Dopamine, serotonin, γ-aminobutyric acid
  - **Appetite stimulators** (e.g., opioids)
  - **Regulation of food intake by central nervous system**.

Energy Expenditure

It can be divided into resting (or basal) metabolic rate, the thermic effect of food, and physical activity energy expenditure.

- **Resting basal metabolic rate (BMR):** BMR is the energy expenditure and accounts for about 70% of daily energy expenditure, whereas active physical activity contributes to 5–10% of energy expenditure.
- **Thermic effect of food (thermogenesis):** About 10% of ingested energy is spent in the process of digestion, absorption and metabolism of nutrients irrespective of physical activity. This is called as dietary induced thermogenesis which is lower in obese and post-obese individuals.

- **Physical activity:** Obese individuals tend to spend more energy during physical activity as they have a larger mass to move.

Pathologic Consequences of Obesity (Complications of Obesity)

Q. Write a short essay/note on important diseases associated with obesity.

a. **Morbidity and mortality:** Obesity has many adverse effects on health and is associated with an increase in mortality and morbidity. Obese individuals are at risk of early death, mainly from diabetes, coronary heart disease and cerebrovascular disease.
b. **Metabolic complications of obesity:** Central obesity or upper body fat distribution is associated with increased concentration of FFA which can produce several metabolic complications of obesity.
    - **Insulin resistance and Type 2 diabetes mellitus*:* Insulin resistance** is **the decrease/failure of target (peripheral) tissues to insulin action.** The skeletal muscle is the main site of insulin stimulated glucose uptake, oxidation and storage. The liver is the main site of glucose production. Normally, insulin promotes glucose utilization (i.e., glucose uptake, oxidation and storage) as well as to inhibit the release of glucose into the circulation. Insulin resistance can develop in obesity and may produce type 2 diabetes mellitus. **Central/upper body/visceral obesity are found in more than 80% of patients with type 2 diabetes mellitus.**
    - **Dyslipidemia:** Upper body obesity and type 2 diabetes mellitus are associated with an atherogenic lipid profile. Dyslipidemia includes **increased triglycerides, increased low-density lipoprotein (LDL) cholesterol with very-low-density lipoprotein (VLDL) cholesterol**, decreased **high-density lipoprotein (HDL**) cholesterol, and decreased levels of the vascular protective adipokine adiponectin. Dyslipidemia increases the risk of cardiovascular diseases (**atherosclerosis, cardiomyopathy**) in the metabolic syndrome.
c. **Endocrine manifestations of obesity:**

- **Women: Polycystic ovarian syndrome** (PCOS) and menstrual abnormalities.
- **Men:** Reduced plasma testosterone and sex hormone–binding globulin (SHBG), increased estrogen levels and gynecomastia.

d. Mechanical complications of obesity:

- **Osteoarthritis:** Excessive body weight in obesity predisposes to degenerative joint disease (osteoarthritis) and also gout.
- **Venous stasis/varicose veins**
- **Acanthosis nigricans:** It manifests as darkening and thickening of the skinfolds on the neck, elbows and dorsal interphalangeal spaces. It reflects the severity of underlying insulin resistance.
- **Increased friability of skin:** Especially in skinfolds, thereby increasing the risk of fungal and yeast infections.
- **Urinary incontinence.**

e. Pulmonary disease:

- **Obesity hypoventilation syndrome** (Pickwickian syndrome) may also develop.
- **Hypersomnolence:** Develops both at night and during the day. It is often associated with apneic pauses during sleep (sleep apnea), polycythemia and right-sided heart failure (*cor pulmonale*).

f. Cancer:

- Obesity in males is associated with higher mortality from cancer, such as cancer of the prostate, colon, esophagus, rectum, pancreas and liver.
- Obesity in females is associated with higher mortality from cancer of the breasts, endometrium, thyroid, gallbladder, bile ducts, cervix, and ovaries.

g. Gastrointestinal disorders:

- **Gastroesophageal reflux disease**
- **Gallstones:** Higher incidence of gallstones especially cholesterol gallstones.
- **Fatty liver (steatosis) and nonalcoholic steatohepatitis (NASH):** Nonalcoholic steatohepatitis [a component of nonalcoholic fatty liver disease (NAFLD)] can progress to hepatic cirrhosis and rarely to hepatocellular carcinoma.

COMMON VITAMIN DEFICIENCIES

Vitamins are vital organic substances required in limited amounts with key roles in certain metabolic pathways.

Categories

Thirteen vitamins are necessary for health and are categorized as follows:

- **Fat-soluble vitamins:** These include A, D, E and K. Fat-soluble vitamins are stored in the body, but their absorption may be poor in fat malabsorption disorders or in disturbances of digestive functions.
- **Water-soluble vitamins:** All other vitamins (vitamins of the B complex group and vitamin C).

FAT-SOLUBLE VITAMINS

Vitamin A (Retinol)

Vitamin A (retinol) is part of the family of retinoids which is present in food and the body as esters combined with long-chain fatty acids.

Functions

Vitamin A has several metabolic roles. The main functions of vitamin A in human are as follows:

- **Maintenance of normal vision:** It is one of the **major functions of vitamin A.** The visual process involves vitamin A containing pigments.
- **Regulation of cell growth and differentiation:** It is one of the major functions of vitamin A. Retinol and retinoic acid are involved in the **control of proliferation and differentiation of epithelial cells**. Vitamin A and retinoids play an important role in the orderly differentiation of mucus-secreting epithelium. In vitamin A deficiency, mucus-secreting cells are replaced by keratin-producing cells and this process is known as **squamous metaplasia**.
- **Regulation of lipid metabolism**: It is a key regulator of fatty acid metabolism including fatty acid oxidation in fat tissue and muscle,

adipogenesis and lipoprotein metabolism.

- **Host resistance to infections:**
  - **Immune function:** Vitamin A has ability to stimulate the immune system.
  - **Antioxidant:** Retinoids, β-carotene and some related carotenoids act as photoprotective and antioxidant agents.

Deficiency

Q. Write short essay/note on vitamin A deficiency.

Causes: Due to general undernutrition or as a secondary deficiency as a consequence of malabsorption of fats.

Pathologic Effects (Clinical Features) of Vitamin A Deficiency (Fig. 9.2)

- **Effects in the eye:**
  - **Night blindness:** Vitamin A is a component of rhodopsin and other visual pigments. Hence, one of the earliest manifestations of vitamin A deficiency is impaired vision, particularly impaired adaptation to the dark *(night blindness)*.
  - **Xerophthalmia:** Vitamin A is necessary for maintaining the differentiation of epithelial cells. Persistent deficiency produces **epithelial metaplasia** and **keratinization**. In the eyes it produces keratinization of the cornea—**xerophthalmia** (dry eye). Initially, there is dryness of the conjunctiva (**xerosis conjunctivae**) because of the replacement of the normal lacrimal and mucus-secreting epithelium by keratinized epithelium. Subsequently, there is a buildup of keratin debris in small opaque plaques which gives rise to characteristic **Bitot spots.** They may progress to erosion of corneal surface, softening and destruction of the cornea (**keratomalacia**), scarring and **irreversible blindness**.

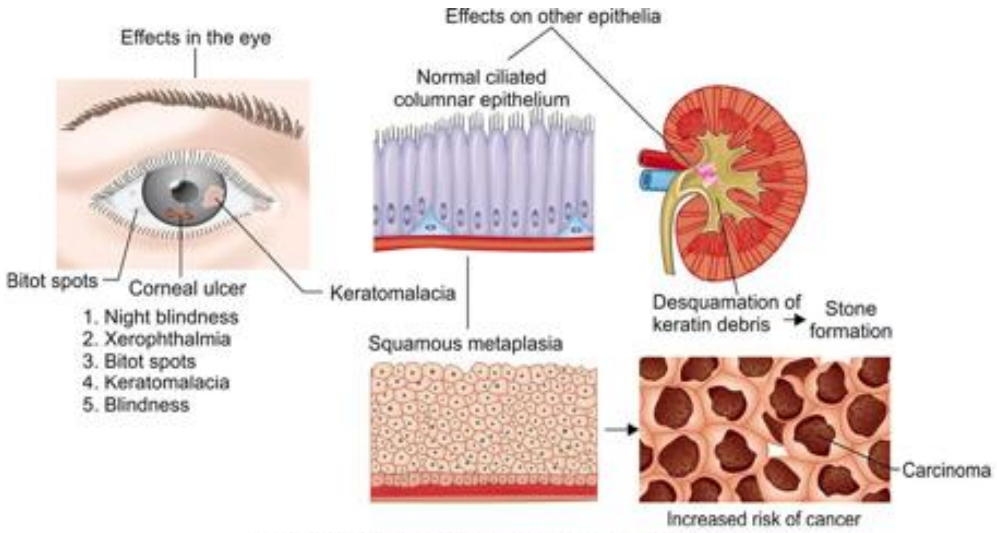


Fig. 9.2: Pathological effects of vitamin A deficiency.

- **Effects on other epithelia:** The epithelium lining the upper respiratory passage and urinary tract also undergoes **squamous metaplasia**. This increases the risk of squamous cell carcinoma.
- **Immune deficiency:** It is responsible for higher mortality rates from common infections, such as measles, pneumonia and infectious diarrhea.
- Follicular hyperkeratosis.

Vitamin D

- Vitamin D is a **fat-soluble vitamin**.
- It is required for the maintenance of adequate plasma levels of calcium and phosphorus to support metabolic functions, bone mineralization and neuromuscular transmission.

Functions

1. **Regulation of plasma levels of calcium and phosphorus:** The main functions of vitamin D on calcium and phosphorus homeostasis are as follows:
   - **Stimulates intestinal absorption of calcium**.
   - **Stimulates calcium reabsorption in the kidney**.
   - **Interaction with PTH in the regulation of blood calcium**.
   - **Mineralization of bone**.
2. **Antiproliferative effects**.

3. **Immunomodulatory:** Vitamin D is involved in the innate and adaptive immune system.

Deficiency

Q. Write a short note on vitamin D deficiency.

Causes

- Impaired cutaneous production due to **limited exposure to sunlight**.
- **Dietary absence:** Diets deficient in calcium and vitamin D.
- **Malabsorption**.

Milder forms of vitamin D deficiency is also called as **vitamin D insufficiency**, leads to an increased risk of bone loss and hip fractures in older adults.

Skeletal Effects of Vitamin D Deficiency

Q. Write short essay/note on rickets and its clinical features.

Rickets in Children (Figs. 9.3A and B)

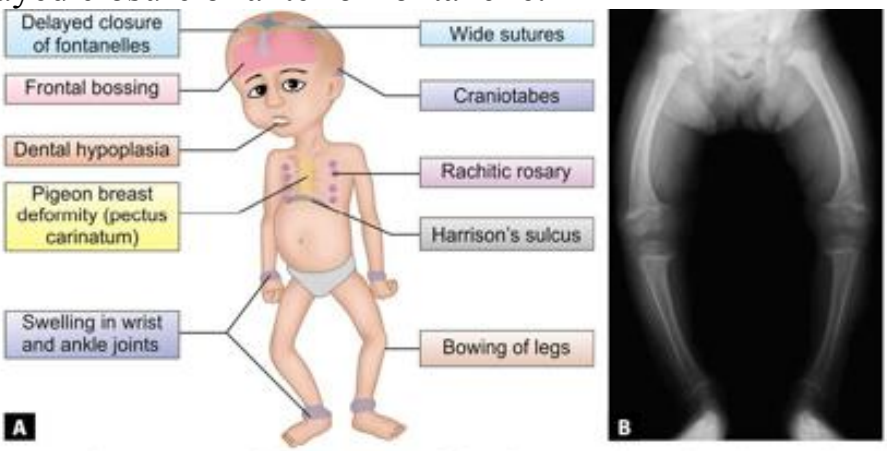
In children, before the closure of epiphyses, vitamin D deficiency causes retardation of growth associated with an expansion of the growth plate known as **rickets.** In the normal growth plate, there are three layers of chondrocytes namely (1) the reserve zone, (2) the proliferating zone, and (3) the hypertrophic zone. Rickets due to impaired vitamin D action is characterized by expansion of the hypertrophic chondrocyte layer. In vitamin D deficiency, the hypophosphatemia due to secondary hyperparathyroidism is responsible for the development of the **rachitic growth plate**.

Gross skeletal changes in rickets: It depends on the severity and duration of the vitamin D deficiency and also the stresses to which individual bones are subjected.

During the nonambulatory stage of infancy:

1. **Head**

- **Craniotabes:** The head and chest are subjected to the greatest stresses. The softened occipital bones become flattened, and the parietal bones buckle inward by pressure; with the release of the pressure, elastic recoil snaps the bones back into their original positions (craniotabes). The **skull appears square and box-like**. Delayed closure of anterior fontanelle.



Figs. 9.3A and B: (A) Features of rickets; (B) X- ray of rickets showing splaying of epiphyses and bow legs.

- **Frontal bossing:** Excess of osteoid produces **frontal bossing** and a **squared appearance of the head**.

Q. Write short note on rachitic rosary.

2. **Chest**
   - **Rachitic rosary: Overgrowth of cartilage or osteoid tissue at the costochondral junction** causes deformation of the chest producing the **"rachitic rosary."**
   - **Pigeon breast/chest deformity:** The weakened metaphyseal areas of the ribs are subject to the pull of the respiratory muscles and thus, bend inward. This creates **anterior protrusion of the sternum** producing **pigeon breast deformity** (pectus carinatum).
   - **Harrison's sulcus/groove:** It is due to indrawing of ribs on inspiration.

During the ambulatory stage:

- **Lumbar lordosis:** This occurs when an ambulating child develops rickets. It is characterized by deformities affecting the spine, pelvis and tibia.
- **Bowing of the legs (Fig. 9.3B):** Due to affect on tibia.

Osteomalacia in Adults

Q. Write short note on osteomalacia.

Vitamin D deficiency in adults is accompanied by hypocalcemia and hypophosphatemia which result in **impaired** (hypo/under/inadequately) **mineralization of bone matrix** proteins, a condition known as osteomalacia. This hypomineralized bone matrix is biomechanically inferior (weak) to normal bone. This **bone is prone to bowing of weight-bearing extremities** and gross skeletal fractures or microfractures which are most likely to affect vertebral bodies and femoral necks.

Proximal Myopathy

It is observed both in children and in adults with severe vitamin D deficiency. It rapidly resolves by vitamin D treatment.

Hypocalcemic Tetany

Calcium is required for normal neural excitation and the relaxation of muscles. Hypocalcemic tetany is a **convulsive state** caused by an insufficient extracellular concentration of ionized calcium.

Nonskeletal Effects of Vitamin D

Vitamin D receptor is also present in various cells and tissues that are not involved in calcium and phosphorus homeostasis. Many cells, such as macrophages, keratinocytes, and tissues, such as breast, prostate and colon can produce 1,25-dihydroxyvitamin D.

- Low levels of 1,25-dihydroxyvitamin D (<20 ng/mL) may **increase in the incidence of cancers of colon, prostate and breast cancers**, but whether vitamin D supplement can reduce cancer risk has not known.

Vitamin C (Ascorbic Acid)

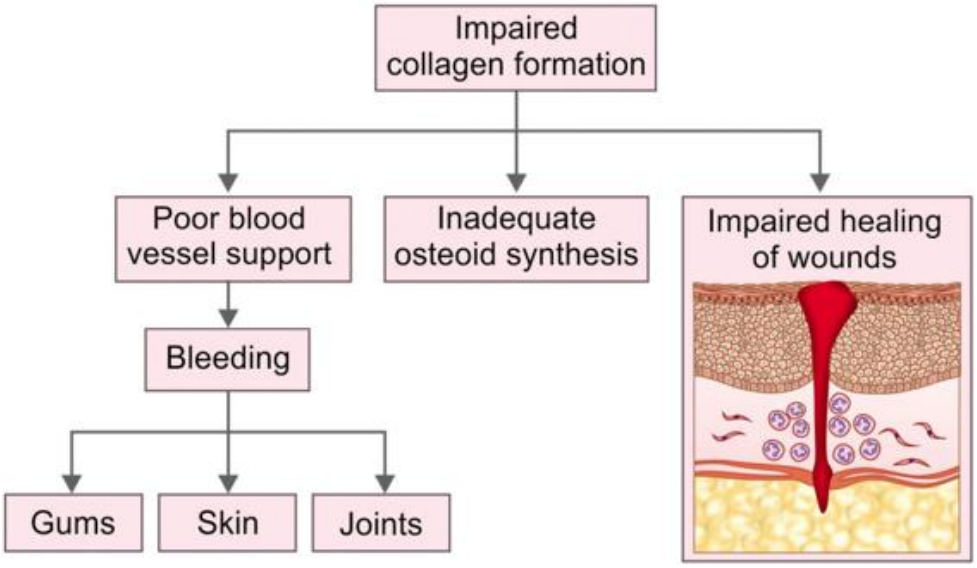
It is a **water-soluble vitamin**.

Functions

- **Hydroxylation of procollagen:** It is necessary for the formation of collagen from procollagen. It is involved in the hydroxylation of proline and lysine in procollagen to hydroxyproline and hydroxylysine in mature collagen.
- **Antioxidant properties:** Ascorbic acid is the most active powerful reducing agent controlling the redox potential within cells. Vitamin C can scavenge free radicals directly and can act indirectly by regenerating the antioxidant form of vitamin E.
- It is involved in intracellular electron transfer.
- **Promotion of nonheme iron absorption**.

Deficiency

Flowchart 9.1: Major consequences of vitamin C deficiency caused by impaired formation of collagen.



Causes

- Ascorbic acid is present in abundance in many foods. Hence, its deficiency is rare.
- Rarely, it may occur as a secondary deficiency, particularly among older persons who live alone, and chronic alcoholics.

Effects of Deficiency (Flowchart 9.1)

Q. Write short essay/ note on scurvy and its pathological findings.

Scurvy: It is characterized by:

- **Bone disease:** More common in growing children. It is characterized by **deranged formation of osteoid matrix**.
- **Hemorrhages:** Marked tendency to bleed into the skin (petechiae, ecchymoses, perifollicular hemorrhages), bleeding into muscles, joints and underneath peritoneum.
- **Delayed wound healing**.
- **Anemia**.
- **Gums**: **Inflamed and bleeding** gums.

Vitamin E

Vitamin E is a collective name for 8 stereoisomers of tocopherols and tocotrienols. The most important dietary form is α-tocopherol.

Functions

- **Antioxidant:** It prevents oxidation of low-density lipoproteins (LDLs) and polyunsaturated fatty acids in cell membranes by free radicals. Other antioxidants (e.g., vitamin C, glutathione) and enzymes maintain vitamin E in a reduced state. Acts in conjunction with other antioxidants, such as selenium.
- It **helps maintain cell membrane structure**.
- It affects DNA synthesis and cell signaling.
- **Anti-inflammatory:** Vitamin E also inhibits prostaglandin synthesis and the activities of protein kinase C and phospholipase A_2.
- **Immune systems**.

Deficiency

- Dietary deficiency of vitamin E is very rare.
- Vitamin E deficiency is seen only in premature infants and in severe and prolonged malabsorption diseases, such as celiac disease, or after small-intestinal resection.

- It can cause **mild hemolytic anemias, ataxia and visual scotomas**.

Vitamin K

Forms of vitamin K: There are two natural forms: Vitamin K_1 (phylloquinone) derived from vegetable (green leafy vegetables, such as kale and spinach) and animal sources (liver), and vitamin K_2 (menaquinone) which is synthesized by bacterial flora in the colon and in hepatic tissue. Phylloquinone can be converted to menaquinone in some organs.

Functions

- **Coagulation:** Vitamin K is a cofactor for carboxylation of glutamic acid which is necessary for the production of carboxyglutamate (gla). Gla residues are found in four of the coagulation factor proteins (II, VII, IX and X). Thus, it is involved in coagulation process.
- **Others:** Other important gla proteins include osteocalcin (in bone) and matrix gla protein (vascular smooth muscle) that are important in **mineralization of bone**. However, the importance of vitamin K for mineralization of bone and prevention of vascular calcification is unknown.

Deficiency

Causes

- In adults:
  - **Chronic small-intestinal disease** (e.g., celiac disease, Crohn's disease).
  - **Obstruction of biliary tracts:** In obstructive jaundice, dietary vitamin K is not absorbed and it is necessary to administer the vitamin in parenteral form before surgery.
  - After small-bowel resection.
  - **Broad-spectrum antibiotics:** They can precipitate vitamin K deficiency by reducing gut bacteria which synthesize menaquinones, and by inhibiting the metabolism of vitamin K.

- **Warfarin and related anticoagulants:** Warfarin-type drugs prevent the conversion of vitamin K to its active hydroquinone form.

- **Deficiency in newborn:** It is because of (1) low-fat stores, (2) low breast milk levels of vitamin K, (3) sterility of the infantile intestinal tract, (4) liver immaturity and (5) poor placental transport.

Effects of Deficiency

- Vitamin K deficiency leads to **delayed coagulation and bleeding**. Hence, the symptoms of vitamin K deficiency are due to hemorrhage.
- **Newborn:** In breastfed newborns it may cause **hemorrhagic disease of the newborn**. Intracranial, gastrointestinal and skin bleeding can occur in vitamin K–deficient infants 1–7 days after birth. Thus, vitamin K (1 mg IM) is given routinely to newborn babies to prevent hemorrhagic disease.

WATER-SOLUBLE VITAMINS—VITAMIN B COMPLEX

Thiamine (Vitamin B_1)

Thiamine was the first B complex vitamin identified and is referred to as vitamin B_1.

Functions

- Thiamine functions as a coenzyme in many α-ketoacid decarboxylation and transketolation reactions. Inadequate thiamin results in inadequate adenosine triphosphate synthesis and abnormal carbohydrate metabolism, respectively.
- May have an additional role in neuronal conduction.

Deficiency

Causes

- Most dietary deficiency of thiamine is due to **poor dietary intake**. Alcoholism, chronic renal dialysis and chronic illnesses, such as cancer are common precipitant factors. High carbohydrate intake increases need for B_1. Alcohol interferes with the absorption of thiamine and with the synthesis of thiamine pyrophosphate.
- Women with **prolonged hyperemesis gravidarum** can develop thiamine deficiency. Maternal thiamine deficiency can lead to infantile beriberi in breastfed children.
- Anorexia.
- Patients.
  - With overall poor nutritional status on parenteral glucose.
  - After bariatric bypass surgery.
  - On chronic diuretic therapy due to increased urinary thiamine losses.

Effects of Deficiency

- **Mild deficiency:** Thiamine deficiency in its early stage is characterized by irritability, decrease in short-term memory, anorexia, fatigue and headaches.
- **More severe deficiency—beriberi:** Prolonged thiamine deficiency causes beriberi. It is classically categorized as **wet or dry or combination of two**. It is the classic deficiency syndrome observed **in individuals consuming polished rice** diet. It shows combinations of peripheral neuropathy, cardiovascular dysfunction and cerebral dysfunction.
  - **Peripheral neuropathy:** Complain of pain and paresthesia associated with diminished reflexes. The neuropathy affects the legs most markedly, and these patients have difficulty rising from a squatting position.
  - **Cardiovascular dysfunction ("wet beriberi"):** Congestive heart failure and low peripheral vascular resistance.
  - **Cerebrovascular dysfunction:**
    - ***Wernicke's encephalopathy:*** Acute appearance of nystagmus, ophthalmoplegia, ataxia and psychotic symptoms. The acute symptoms are reversible when treated with thiamine. However, if untreated, they may be followed by a prolonged and largely irreversible condition, called Korsakoff syndrome.

- ***Korsakoff syndrome:*** Characterized clinically by hallucinations, disturbances of short-term memory and confabulation. The syndrome is common in chronic alcoholics but may also be seen with thiamine deficiency resulting from gastric disorders including carcinoma, chronic gastritis, or persistent vomiting.

- ***Wet beriberi*** presents primarily with cardiovascular symptoms.
- ***Dry beriberi*** presents with a symmetric peripheral neuropathy of the motor and sensory systems with diminished reflexes.

Riboflavin (Vitamin B_2)

- It is important for the metabolism of fat, carbohydrate, and protein. It also plays a role in drug and steroid metabolism including detoxification reactions.
- Serves as a coenzyme for a diverse array of biochemical reactions and as an electron donor.
- The primary coenzymatic forms of riboflavin are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) and are known as *flavoenzymes* (e.g., succinic acid dehydrogenase, monoamine oxidase, glutathione reductase).

Deficiency

Causes: Almost always is due to dietary deficiency and is usually seen in conjunction with deficiencies of other B vitamins.

Effects of Deficiency

Nonspecific and mainly manifests as **lesions of the mucocutaneous surfaces of the mouth and skin.** These include **hyperemia and edema of nasopharyngeal mucosa, cheilosis, angular stomatitis, glossitis and seborrheic dermatitis.** Other lesions include corneal vascularization, normochromic-normocytic anemia and personality changes.

Niacin (Vitamin B_3)

Q. Write short essay/note on niacin deficiency.

- The term *niacin* refers to nicotinic acid and the corresponding amide, nicotinamide and their biologically active derivatives.
- Nicotinic acid and nicotinamide serve as precursors of two coenzymes, nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP) which are important in numerous oxidation and reduction reactions.
- NAD and NADP are active in adenine diphosphate–ribose transfer reactions involved in DNA repair and calcium mobilization.

Deficiency

Pellagra

- Niacin deficiency causes **pellagra.** It is found mostly in populations in which corn is the major source of energy in parts of China, Africa and India.
- **Early symptoms:** Loss of appetite, generalized weakness and irritability, abdominal pain, and vomiting.
- **Early signs:** Bright red glossitis, stomatitis, vaginitis, esophagitis, vertigo and burning dysesthesias.
- **Advanced stages:** Characteristic skin rash develops that is pigmented and scaling that develops in skin areas exposed to sunlight. This rash is known as **Casal's necklace (Fig. 9.4)** because it forms a ring around the neck.
- **Four Ds:** **D**iarrhea (in part due to proctitis and in part due to malabsorption), **d**epression, seizures and **d**ementia (or associated symptoms of anxiety or insomnia) leading to **d**eath and **d**ermatitis are part of the pellagra syndrome.

Pyridoxine (Vitamin B_6)

- *Vitamin B_6* refers to several derivatives of pyridine that include pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), which are interconvertible in the body. The coenzymatic forms are pyridoxal-5-phosphate (PSP) and pyridoxamine-5-phosphate (PMP). 5'-pyridoxal phosphate (PLP) is a cofactor for more than 100 enzymes involved in amino acid metabolism.

- Vitamin B_6 is also involved in synthesis of heme and many neurotransmitters and in the metabolism of glycogen, lipids, steroids, sphingoid bases and several vitamins, including the synthesis of niacin from tryptophan.

Deficiency

- Deficiency usually seen in conjunction with other water-soluble vitamin deficiencies.
- Certain medications, such as isoniazid, cycloserine, penicillamine, l-dopa, ethanol and theophylline can inhibit B_6 metabolism. Pyridoxine should be given concurrently with isoniazid to avoid neuropathy. Because vitamin B_6 interferes with the action of l-dopa, it should not be given with this drug.

Effects of Deficiency

- **Stomatitis, angular cheilosis, glossitis, irritability, depression and confusion** occur in moderate to severe depletion.
- **Microcytic hypochromic** anemia is due to diminished hemoglobin synthesis, since it is the first enzyme involved in heme biosynthesis. It may also produce normochromic-normocytic anemia.
- **In infants: Diarrhea, seizures/convulsions and anemia**.
- **Severe vitamin B_6 deficiency: Peripheral neuropathy** and abnormal electroencephalograms.

Vitamin B_{12}

A group of closely related cobalamine compounds.

Functions

Vitamin B_{12} is indirectly **required for DNA synthesis** in various metabolic steps and its deficiency impairs DNA synthesis. The two active coenzyme forms are deoxyadenosylcobalamin and methylcobalamin.

- Methylcobalamin is the main form of vitamin B_{12} in plasma, and is an essential coenzyme for conversion of homocysteine to methionine and formation of tetrahydrofolate (THF) from methyl THF.

- Vitamin B_{12} is also required for conversion of methylmalonyl-CoA to succinylmalonyl-CoA.

Deficiency

Causes (*refer* Box 11.6)



Fig. 9.4: Casal's necklace (ring around the neck) and dermatitis.

TABLE 9.4: Vitamins and their principal clinical manifestations.

Vitamin	*Clinical finding*
Thiamine	**Beriberi** (dry or wet): Neuropathy, muscle weakness and wasting, cardiomegaly, edema, ophthalmoplegia, confabulation
Riboflavin	Magenta tongue (glossitis), angular stomatitis, seborrhea, cheilosis and seborrheic dermatitis
Niacin	**Pellagr**a: Pigmented rash of sun-exposed areas, bright-red tongue, diarrhea, apathy, memory loss, disorientation
Vitamin B_6	Seborrhea, glossitis, convulsions, neuropathy, depression, confusion, anemia
Folate	Megaloblastic anemia, atrophic glossitis
Vitamin B_{12}	Megaloblastic anemia, loss of vibratory and position sense, abnormal gait, dementia

TABLE 9.4: Vitamins and their principal clinical manifestations.	
Vitamin	***Clinical finding***
Vitamin C	Scurvy: Petechiae, ecchymosis, inflamed and bleeding gums, joint effusion, poor wound healing, fatigue
Vitamin A	Xerophthalmia, night blindness, Bitot's spots, follicular hyperkeratosis, immune dysfunction
Vitamin D	Rickets in children: Skeletal deformation, rachitic rosary, bowed legs Osteomalacia in adults
Vitamin E	Peripheral neuropathy, spinocerebellar ataxia, skeletal muscle atrophy, retinopathy
Vitamin K	Elevated prothrombin time, bleeding

- Dietary inadequacy is a rare cause of deficiency except in strict vegetarians.
- Mostly due to loss of intestinal absorption. These include pernicious anemia, pancreatic insufficiency, atrophic gastritis, small bowel bacterial overgrowth, or ileal disease.

Effects of Deficiency

- **Hematological changes: Megaloblastic anemia** (*refer* pages 300-2) and megaloblastic changes in other epithelia.
- **Neurologic complications: Demyelination of peripheral nerves**, posterior and lateral columns of spinal cord, and nerves within the brain. Altered mentation, depression, and psychoses occur.

Folic Acid

Folates are a group of related pterin compounds. The fully oxidized form is called folic acid which is not found in nature but is the pharmacologic form of the vitamin.

Tobacco: Polycyclic hydrocarbons carcinogens damage DNA. Tar contains carcinogenic agents.

Alcohol: ↑ serum γ-glutamyl transferase (GGT) (alcohol causes hyperplasia of smooth endoplasmic reticulum (SER)
↑ Urine ethyl glucuronide; excellent indicator of recent alcohol ingestion and is better than alcohol level in blood.

Functions

- All folate functions relate to its ability to transfer one-carbon groups.
- The active form of folic acid is tetrahydrofolate (THF) which is the biologic "middleman" involved in metabolic processes which synthesize DNA.

Deficiency

Causes (*refer* **Box 11.6**).

Effects of Deficiency

Megaloblastic anemia (*refer* pages 299-302), diarrhea.

Vitamins and their principal clinical manifestations are summarized in **Table 9.4**.

CHAPTER

10 Basic Diagnostic Cytology

INTRODUCTION

Biopsy was used as the main diagnostic method in pathology for over a century. **Cytology is a branch of science which deals with study of cells and/or tissue fragments** and **cytopathology is a branch of pathology that deals with study and diagnosis of disease at the cellular level**. During 1920, Dr George N Papanicolaou introduced cytology as a method of diagnosis by obtaining cells naturally shed off (exfoliated) into cervical and vaginal secretion. Subsequently use of cytological examination was extended to cells which shed off into other body cavities. Later, similar to *bone marrow aspiration* for diagnosis of hematopoietic diseases, **fine needle aspiration cytology (FNAC)** was used for diagnosis of solid lesions. Though FNAC was started in the 1930s from US, it was in 1960s that it was used mainly in Scandinavian countries of Europe and later it spread to the rest of the world.

Diagnostic cytology consists of the interpretation of cells from the human body that either exfoliate (desquamate) spontaneously from epithelial surfaces (*Exfoliative Cytology)*, or are obtained from various organs/tissues by different clinical procedures *(Interventional Cytology)*. In surgical pathology, the diagnosis is based on changes in tissue architecture. In contrast to surgical pathology, cytopathology deals primarily with **morphology observed in single cells or small groups of cells without regard to stroma**. Pathologist who deals with and reports cytological material is called cytopathologist.

ROLE (USES) OF CYTOLOGY

CC: Describe the diagnostic role of cytology and its application in clinical care.

Role of cytopathologist: Cytopathology has numerous applications. It is usually used for screening, diagnosing and management of lesions that may represent cancer or its precursors. Advantages include cost-effectiveness, rapid turnaround time, and a diagnosis with minimal patient risk. Cytopathology is also helpful in other conditions. Most important applications of cytopathology are:

1. **Diagnosis and management of cancer:** In oncology, diagnosis is essential for proper management of a cancer patient.
   a. **For diagnosis:**
      - Cytopathology is a *valuable* ***adjunct*** **to histopathology for the diagnosis** of benign, dysplastic and malignant lesions. For example, imprint smears for the diagnosis of lymphomas.
      - Provides a **preliminary diagnosis of cancer** which can be confirmed by histopathology. For example, detection of malignant cells in ascitic fluid in a patient with ovarian tumor.
      - ***Primary method*** **of diagnosis**, e.g., detection of malignant cells in an FNAC of breast is considered sufficient for planning the management and may avoid the need for diagnostic surgical biopsy.
      - **Detection of carcinoma in situ:** Used for the early detection and diagnosis of clinically silent carcinoma in situ, e.g., in uterine cervix.
   b. **Management of cancer:**
      - Cytopathology may help **in assessing** ***response to therapy,*** e.g., cervical smears examination after radiotherapy in carcinoma cervix.
      - ***Follow-up*** of previously diagnosed cancer, e.g., in detection of metastasis or recurrence of cancer.
2. **Diagnosis of benign neoplasms:** Cytopathology **helps to distinguish between benign and malignant neoplasms.** For example, to distinguish fibroadenoma of the breast from carcinoma.
3. **Intraoperative consultation**: Intraoperative cytology is the procedure by which the cells are obtained during operation. **It complements the histopathological diagnosis.** For example, imprint smears along with

frozen section for breast tumors or resection margin. **Various** intraoperative cytological **techniques include:**

   a. **Touch (imprint) cytology:** Smears are prepared by forcefully pressing the clean glass slide to the cut surface of the organ. Discussed on page 284.
   b. **Scrape cytology:** Smears are prepared from material obtained by scraping the cut surface of the biopsy specimen with clean scalpel.
   c. **Crush preparation (crush cytology):** Small fragments of fresh unfixed surgical tissue are crushed between two slides. This technique is mainly used in neuropathology. Discussed on page 284.

4. **Diagnosis of specific infections:** A variety of bacterial, viral, protozoal and fungal infections can be diagnosed by cytopathology. For example, tubercle bacilli in lymph node aspirates, herpetic inclusions and trichomonas in cervico-vaginal smears, fungal hyphae in touch preparations from draining sinuses.
5. **Diagnosis of non-neoplastic and inflammatory lesions:** It helps in diagnosis of conditions which do not routinely require surgical intervention. For example, Hashimoto's thyroiditis.
6. **Cytogenetics:** Cytological specimens can be used for chromosomal studies including leukocyte and tissue cultures and for demonstrating sex-chromatin (e.g., buccal smear for Barr body).
7. **Hormonal assessment in females**: Vaginal smears reflect changes in female sex hormonal levels, e.g., to confirm the onset of menopause.
8. **Identification of cell of origin:** The cytoplasmic characteristics may help in identifying the cell of origin **in malignant tumors**, e.g., keratinization in squamous cell carcinoma, mucin in mucin secreting adenocarcinoma, melanin pigment in malignant melanoma**.**
9. **Others:** It is useful for DNA cytometry and DNA ploidy analysis.

METHODS FOR OBTAINING SPECIMENS

CC: Describe the basis of exfoliative cytology including the technique and stains used.

Two categories of methods are involved in diagnostic cytology to obtain cells for microscopic examination namely—exfoliative and interventional.

Exfoliative Cytology

Q. Write short essay/note on exfoliative cytology.

Exfoliative cytology is the **study of spontaneously/naturally exfoliated (shed) cells from the epithelial lining of an organ into a body cavity or body fluids** such as urine, sputum, etc. Rate of exfoliation is increased in disease-states and yields a larger number of cells for study.

Abrasive cytology: Apart from exfoliation, cells for study may also be obtained by **scraping, brushing, or washing** various mucosal surfaces. In abrasive cytology viable cells are artificially obtained by **abrasive techniques** from the surface of an organ. The instrument used to obtain the cells includes **scraper or spatula** (cervical, oral cavity-buccal smear for sex chromatin) and cytological brush (bronchial, gastrointestinal tract). The cells may also be obtained by **washing or lavage** (peritoneal, bronchoalveolar), where small amount of saline or similar solution is instilled into the target organ and aspirated. The cells obtained by abrasive technique do not show degenerative changes or necrosis, in contrast to that of exfoliative cytology. **When the cells are collected with an instrument, the cells are either rinsed into a preservative solution, or simply directly spread onto slides**.

Various sources of exfoliated cells used in cytopathology are listed in **Box 10.1**.

Gynecologic Exfoliative Cytology

This involves microscopic examination of cells exfoliated from the female genital tract and the smears from the female genital tract have been **traditionally known as 'Pap smears' (Papanicolaou smear)**. There are different methods of obtaining exfoliated cells and smears are prepared from the material obtained.

- ***Lateral vaginal smears (LVS):*** The material is obtained by scraping the upper third of the lateral walls of the vagina and is ideal for hormonal assessment.
- ***Vaginal 'pool' or 'vault' smears:*** The material is obtained by scraping or aspiration from the posterior fornix of the vagina and used for detection of endometrial and ovarian cancer.

- ***Cervical smears:*** The material obtained by Ayre's spatula (*refer* **Fig 10.1**) from the portio of the cervix is ideal for detection of intraepithelial lesions (*refer* pages 738-41) and carcinoma of uterine cervix.

BOX 10.1: Sources of exfoliated cells.

Surface of mucosal or epithelial lining

Cells may be shed naturally or obtained by artificial exfoliation

1. *Gynecologic exfoliative cytology* (*female genital tract*)
   - Lateral vaginal smears (LVS)
   - Vaginal 'pool' smears
   - Cervical smears
   - Combined (fast) smears
   - Triple smears (CVE)
   - Endocervical/endometrial aspiration
2. Non-gynecologic exfoliative cytology
   1. Respiratory tract
      - Sputum
      - Bronchial washings/brushing/bronchoalveolar lavage (BAL) by bronchoscopy
   2. Gastrointestinal tract
      - Endoscopic lavage/brushing
   3. Urinary tract
      - Urinary sediment (in voided urine)
      - Bladder washings
      - Retrograde catheterization
      - Prostatic massage (secretions)
   4. Other samples
      - Buccal smears (for sex chromatin) by scrapping

Body fluids

Usually cells are shed naturally into body fluids

- Effusions: Pleural, peritoneal, pericardial
- Cerebrospinal fluid (CSF)
- Synovial fluid
- Seminal fluid (semen)
- Amniotic fluid
- Hydrocele fluid
- Nipple discharge

- ***Combined (Fast) smears:*** The material is obtained by a combination of vaginal pool and cervical scrapings. They are recommended for routine population screening for cervical cancers and endometrial cancers.
- ***Triple (cervical-vaginal-endocervical or CVE) smears:*** These smears contain three distinct samples representing the ectocervix, vagina and endocervix on three separate areas of the same slide. Though they are

recommended for routine screening and help in localization of lesions (if present) but are difficult to prepare.

- ***Endocervical and endometrial smears:*** They are prepared by aspirating the contents of the endocervical canal and endometrial cavity respectively.

Applications of Pap Smear

Pap smears in gynecological sites may be used for hormonal evaluation, and identification of non-neoplastic, benign, and malignant tumors.

Hormonal evaluation

Assessment of hormonal status is carried out on smears from lateral vaginal wall. However, vaginal 'pool' or fast smears may also be used.

Non-neoplastic (benign) cellular changes

In cervicovaginal cytopathology, reporting is done using The Bethesda System (TBS) so that uniformity is maintained in the evaluation.

Non-specific inflammatory changes are commonly observed in gynecological smears and these changes may reflect acute or chronic inflammatory process.

Specific inflammatory changes: They may be seen with a variety of infectious agents and their detection helps to identify these agents as cause of the disease.

- **Bacteria:** *N. gonorrhoeae* (a gram negative, intracellular, diplococcus) and *Gardnerella vaginalis (*a gram-negative bacteria).
- **Virus:** *Human papillomavirus (HPV)* is sexually-transmitted infections and the smears from HPV-infected individuals show aggregates of rounded to oval squamous cells with 'koilocytotic changes.' **(***refer* **page 740 and Figs. 22.3A to F).** Genital infection by *herpes simplex virus (HSV)* can affects the squamous epithelium of the vulva, vagina and cervix. HSV-infected cells show enlarged nuclei with watery, 'ground-glass' chromatin, multinucleation with internuclear moulding, and intranuclear acidophilic inclusions.
- **Fungus:** Moniliasis (infection by *Candida albicans)* is the common fungal infection and associated with diabetes, pregnancy and use of

antibacterial agents (which alter the vaginal flora). In the smears, Candida (*refer* page 98) may appear in two forms namely—the *yeast form* (unicellular) appears as round to oval budding organisms within conspicuous capsules, and the *fungal form* (pseudohyphae) as thin, elongated, pseudo septate, bamboo-like filaments.

- **Parasites:** *Trichomonas vaginalis* is a protozoan, which appears in the background as a fuzzy, gray-green, round or elliptical structure 8–20 μm in size, containing a small vesicular nucleus. *Entamoeba histolytica* in trophozoite form appears in Pap smears as basophilic, round to oval structures, 15 to 20 μm in size, with ingested RBCs.

Neoplastic epithelial cell abnormalities

Detection of carcinoma of the uterine cervix and its precursor lesions is considered as the most important application of exfoliative cytology in female genital tract. These include:

- **Pre-invasive and invasive squamous cell abnormalities**: Pre-invasive intraepithelial neoplastic process of uterine cervix usually precedes the fully-developed invasive squamous cell carcinoma and can be detected by cytologic examination. Classification of cervical precancerous lesions and cervical cancer screening are discussed on pages 739-41, and page 743 respectively. **The Bethesda system** (TBS) divides squamous cell abnormalities into four categories and its reporting of squamous cell abnormalities is presented in **Table 10.1.**
- **Glandular cell abnormalities:** They can be detected in the smears and The Bethesda system is also used for glandular abnormalities.

Automation in Cervical Cytology

Presently, automated devices such as PapNet for primary screening are available in the market. Automation can perform routine pre-screening of many Pap smears, reduces the workload of cytopathologists and also provides quality assurance. It can be applied to both conventional Pap smears and to *Thin-Preps*.

Non-gynecologic Exfoliative Cytology

Respiratory Tract

Cellular material from the respiratory tract may be obtained by different techniques.

TABLE 10.1: The Bethesda System (TBS) of reporting squamous cell abnormalities.

Squamous cell abnormalities	***Salient features***
Atypical squamous cells of undetermined significance (ASCUS)	Cellular changes falling short of intraepithelial lesion
Low-grade squamous intraepithelial lesion (LSIL)	Includes CIN-I and cellular changes associated with HPV infection
High-grade squamous intraepithelial lesion (HSIL)	Includes CIN grade II, III and CIS.
Squamous cell carcinoma	Frank features of carcinoma with tumor diathesis (dirty, necrotic background)

(CIN: cervical intraepithelial neoplasm; CIS: carcinoma in situ; HPV: human papilloma virus)

- Exfoliated cells (sputum) by spontaneous expectoration
- Cells obtained by bronchial wash and by abrasive techniques [brush, bronchoalveolar lavage (BAL)].
- **Fine needle aspiration cytology:** Either by transbronchial needle aspiration (TBNA) or ultrasound-guided fine needle aspiration cytology (USG FNAC).

Sputum (sampling of exfoliated cells)

Sputum contains mucus admixed with exfoliated cells from the entire respiratory tract. By examining the morphology of these cells, it is possible to identify the underlying pathological process. Sputum is the most commonly examined sample from respiratory system and is easy to collect and examine. Sputum is collected in the early morning produced by a spontaneous deep cough to bring up material from small airways and alveoli. If the patient is not able to produce sputum then expectoration (cough reflex) can be artificially induced by 20 minutes aerosol-inhalation of cough-stimulating substances [e.g., vapor of 15% NaCl (hypertonic saline) and 20% propylene glycol heated at 37°C]. Sputum should be collected in a clean container and should be kept in a petri dish on white background. It is recommended to examine fresh sputum sample. If any tissue fragment or blood-mixed material is present, it should be taken and multiple smears are prepared. However, if it cannot be examined immediately, the sputum

sample can be collected in 70% ethyl alcohol solution as a fixative. Both alcohol-fixed and air-dried smears should be kept for staining.

- **Advantages**: Easy to obtain/collect the sample; simple, non-invasive; its cellular content is representative of the entire respiratory tract.
- **Disadvantages**: Large number of cells have to be scrutinized; not possible to locate exact localization of the lesion within the respiratory tract and considerable degeneration of the cells develops.

Bronchial specimens

- **Bronchial aspirate and wash:** Using bronchoscope, the bronchial secretion can be aspirated directly. For bronchial wash, 3–5 mL of balanced salt solution or normal saline is introduced through the bronchoscope and the fluid is re-aspirated. The fluid is centrifuged and multiple smears are prepared and stained.
- **Bronchial brush:** Using fiberoptic bronchoscope, the lesion is localized/directly visualized and the brush is applied to the lesion through the instrument. Multiple smears are prepared and fixed in 95% ethanol or the brush is rinsed in a collection solution by the clinician and sent to the laboratory. Since, the direct visualization of the lesion is possible, the bronchial brush gives excellent diagnostic cellular yield.
- **Bronchoalveolar lavage:** Bronchoalveolar lavage (BAL) is a powerful cytological technique in pulmonary medicine. BAL should be performed before performing any other techniques such as bronchial wash/brush or biopsy. First, 20 mL of normal saline is flushed through bronchoscope in the desired segment of the lung. Then through gentle suction lavage specimen is collected.

Gastrointestinal Tract

Cytology plays a significant role in the diagnosis of various lesions of gastrointestinal tract (GIT) mainly due to efficient, improved technology of the sampling method and visualization techniques. Various endoscopic procedures that can be performed in GIT include esophagoscopy, gastroscopy, duodenoscopy, colonoscopy, sigmoidoscopy and endoscopic retrograde cholangiopancreatography (ERCP).
The common techniques used in GI tract include **endoscopic brush cytology, salvage cytology, endoscopic FNAC, endoscopic ultrasound-**

guided (EUS) FNAC, balloon technique and FNAC by endoscopic retrograde cholangiopancreatography.
Lesions in the oral cavity may be sampled by scraping the surface with wooden and metal tongue-depressors.

Urinary Tract

Urinary cytology is considered as a useful adjunct to the cystoscopy. It is an inexpensive, rapid and highly reliable technique for screening of cancer of lower urinary tract. The major advantage of urine cytology is that it helps to detect the malignant lesions that are either not visible by cystoscopy or difficult to see on cystoscopy.
Specimen collection technique for urine is mainly of three types:

1. Voided urine specimen
2. Catheterized urine specimen
3. Bladder wash, ureter wash, urethral wash and brush cytology

Voided urine specimen: It is the specimen of choice for screening in patients with specific symptoms such as hematuria. The urine sample should be collected 3–4 hours after last micturition. It is better to avoid first morning voided urine sample because it shows considerable amount of degeneration in the cells due to prolonged stagnation. Fresh randomly voided urine sample should be collected in a clean container and should be sent to a laboratory immediately for processing to avoid cellular degenerative changes. About 50 mL of urine is sufficient for cytological examination. The urine is centrifuged and the sediment is examined.
Catheterized urine: Catheterized urine sample yields better cellularity and shows less degenerative changes. It is used for follow-up of patient with neoplastic lesion of the lower urinary tract.
Bladder wash (irrigation) and brush cytology: Bladder wash and brush are indicated in cases of suspected urothelial neoplasm or follow-up of a case of known neoplastic lesion. This technique gives good cellularity and better preservation of the cells.

Processing

The urine sample should be processed immediately. The processing methods include:

- **Simple centrifugation**: About 50 mL urine is centrifuged for 10 minutes at 1,200 RPM (revolution/rounds per minute). From the sediment, multiple smears are prepared and these are fixed in alcohol and stained.
- **Cytocentrifugation**: When the amount of urine is less than 50 mL, it can be processed by cytocentrifugation.
- **Liquid-based technique**: In this technique, the cytological material is filtered or processed through a machine, such as ThinPrep Processor (Cytyc Corp, Boxborough, MA) or SurePath PREP system (TriPath Imaging, Inc, Burlington, NC). LBC technique gives better morphology, cell preservation and better cellularity. However, it is expensive technique.
- **Cell block**: If urine sample shows any fleshy tissue, a cell block preparation can be done.

Body Fluids

Body fluids may be divided into two types:

1. Effusions in body cavities
2. Other body fluids (usually of small volume)

Effusions

Excessive accumulation of fluid in any of the three body cavities (pleural, pericardial and peritoneal) is termed as "effusion". Effusion is always a pathological process. An effusion in the peritoneal cavity is also called as ascites. Effusion can be transude or exudate (Differences between transudate and exudate are presented in **Table 3.3**).

This differentiation is important because malignant effusions are invariably exudates with a protein content greater than 3 g/dL. The effusion samples are obtained mainly to identify malignant cells and wherever possible for its classification. In benign effusions, cytological findings are mostly non-specific.

Cerebrospinal Fluid (CSF)

CSF examination is important both in non-neoplastic and neoplastic diseases of central nervous system (CNS).

Samples are obtained by lumbar puncture. It is discussed on pages 415-6 and *refer* **Table 27.1**.

Components in Routine Cytology

Different components/steps involved in routine cytology laboratory technique are:

- *Specimen collection*: Unlike histopathology, the specimen collection is different in cytology.
- *Fixation*: Different fixatives are used for various cytology samples.
- *Processing*: The processing of the cytology sample is different. It mainly depends on the type of sample and facilities available in the laboratory.
- *Staining*: The common stains used in cytology laboratory are Papanicolaou's and May–Grunwald–Giemsa (MGG) stain.

TECHNIQUES IN EXFOLIATIVE CYTOLOGY

Sample Collection

The common types of samples received in cytology laboratory are presented in **Box 10.1**.

Cervical Cytology

The cervical cytology sample is usually collected by the gynecologist or the trained nurse. The sample should be adequate with optimal cellularity.

Collection of Samples

- Keep the patient in dorsolithotomy position and clean the vagina by wet swab with water.
- Open the vagina by introducing a speculum to visualize the cervix.
- For the *conventional smear*
  - Insert the *Ayre's* spatula **(Fig. 10.1)** within the vagina so that the tip of the spatula fits with the contour of the cervix (i.e., the longer limb of the spatula should fit into the external os).
  - Rotate the spatula in a complete round turn (through 360°) to sample the entire cervix. Gently withdraw the spatula.

- Spread the sample on the spatula on the glass slide so that smears are thin and uniform.

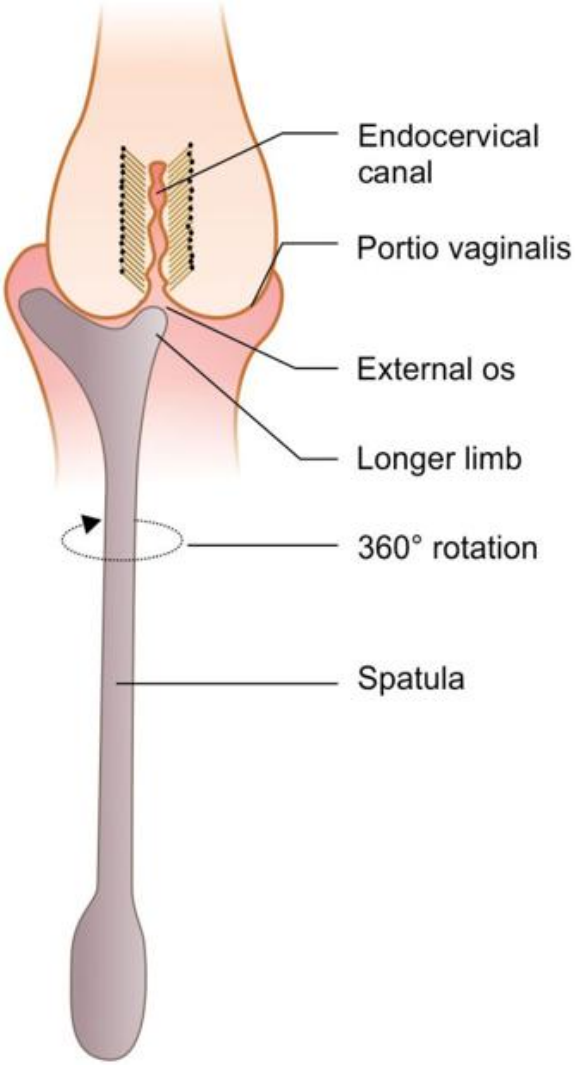


Fig. 10.1: Method of obtaining cervical smear with Ayre's spatula.

- Immediately immerse the slides and fix the smears in the 95% ethyl alcohol fixative in a Coplin's jar to prevent artefacts in cells caused by drying.

- For **liquid-based cytology** *(LBC) preparation:* LBC is a special technique used for preparation of both gynecologic and nongynecologic samples. The smears will show monolayered dispersion of cells without overlapping or clumping and does not contain background material or debris. There are two types of LBC systems commercially available namely—ThinPrep and SurePath.
  - For LBC, use cervix brush. Introduce the central part of the broom into the endocervical canal so that the shorter bristles of the broom

touch to the ectocervix

- Rotate the broom four to five times in a clockwise direction.
- Withdraw the broom and place it (or rinse it) in the fixative solution in a vial provided by the manufacturer.

Vaginal Smear

The cytology sample is obtained by scraping the lateral walls of the upper third of the vagina (at the level of the cervical external os) with the flat surface of a wooden tongue depressor or spatula and smearing the material directly onto labeled slides.

Endometrial Aspiration Smear

It is obtained by using a sterile cannula and a syringe and the endometrial sample is aspirated.

Collections of samples from respiratory, gastrointestinal, urinary tract and cerebrospinal fluid are discussed on Chapter 14.

Fixation and Fixatives

1. **Routine fixatives:** Similar to histopathology, all cytological material for examination must be properly fixed so that the morphological details of cells are preserved. Smears prepared (either at bedside or in the laboratory) from fluid samples should be immediately placed in a 95% ethanol fixative without allowing them to dry prior to fixation. Drying causes distortion of cells and produces staining artefacts in the cytoplasm. Smears should be transported to the laboratory in the fixative preferably in a screw-capped Coplin jars. Routinely used cytological fixatives for Papanicolaou's and hematoxylin stains are listed in **Box 10.2**.

Methods of fixation: It varies depending on the type of staining used. Different methods of fixation of cytological smears are:

- **Wet fixation:** Cytological smears are fixed immediately (when smears are wet). Usually, smears prepared from exfoliated cells are immersed immediately in a fixative without allowing them to dry. This is called wet-fixation. These smears are then stained with Papanicolaou (Pap) or haematoxylin and eosin (H&E) stains.

- **Dry fixation:** Smears are air dried and fixed in 95% alcohol for 5 minutes (for May–Grunwald–Giemsa stain). When Romanowsky stain is used, exfoliative cytology smears are air-dried and then stained. In Romanowsky staining, fixation is performed during the staining procedure itself.
- **Spray fixation (coating fixative):** In this method, the cytological smears are immediately fixed with a spray fixative (*refer* below). This type of fixative usually contains alcohol (e.g., hair spray).

2. **Coating fixatives:** Coating or spray fixatives are applied as aerosol-sprays or with a dropper to the surface of freshly prepared cytological smears. These are commercially available (e.g., cytospray). However, as an alternative a standard hairspray with a high alcohol content may also be used. The components of spray fixatives usually include ethanol or isopropyl alcohol along with polyethylene glycol (carbowax). Within a few seconds, the smear is dried and carbowax in the spray fixative covers over the slide and prevents the shrinkage of the cell.
   Use: It is used for cervical smears, but should be used for smears prepared from fluid specimen and smears with blood.
3. **Special purpose fixatives:** These include Carnoy's fixative (for hemorrhagic fluid), 10% neutral buffered formalin (for cell block materials), etc.
4. **Preservation of fluid samples:** Fluid samples should be submitted to the laboratory in a fresh state for immediate processing. If a delay is expected in despatch to the laboratory or in processing, the sample is collected in a suitable preservative for 'prefixation' so that cellular morphology is preserved. The best preservative for general use is 50% ethanol in volumes equal to that of the fluid sample. Pericardial, pleural and peritoneal fluids may be pre-fixed with an equal volume of 10% formalin if ethanol is not available.

Processing of Samples in the Laboratory

The commonly used processing techniques include: (i) direct smear, (ii) centrifuge, (iii) cytocentrifuge, (iv) liquid-based preparation, and (v) cell block.

BOX 10.2: Routinely used cytological fixatives for Papanicolaou's and

hematoxylin stains.

- 95% ethyl alcohol (most commonly used)
- 100% methanol
- Denatured alcohol
- Methanol-based preservative for ThinPrep system
- Ethanol-based preservative for SurePath system

Prepared smears: If the smears are prepared at the bedside and wet-fixed in ethanol, they need no further processing in the laboratory prior to staining.

Processing of sputum (direct smear): Pour the received sputum sample to a Petri dish kept on a black background Carefully inspect for any tissue fragments (solid particles) or gray-white substance or bloody material. Pick up the tissue fragments by a clean forceps or wooden applicator sticks and place it on clean glass slides. Strands of ropy mucus are also selected (exfoliated cells adhere to mucus strands). Prepare the smears on the clean glass slide by crushing the particles/mucus and by evenly spreading the material. Four such smears are prepared and immediately placed in 95% ethanol for fixation.

Processing of fluid: Urine, body fluids and lavage—if large volumes of fluid are received, they are allowed to stand in the refrigerator for half to one hour. Then, all except the bottom 200 mL is discarded. The retained bottom fluid is then processed. In specimens showing large amounts of blood, RBCs may be lysed by adding 1 mL of glacial acetic acid for every 50 mL of specimen. Commonly employed techniques for processing of fluids are as under:

Centrifuge: If the fluid is of moderate amount (50–100 mL), it should be processed by centrifugation (e.g., effusion fluid, turbid urine, etc.). The fluid sample is poured into clean, air tight 50 mL centrifuge tubes and centrifuged at 1500 rounds per minute (RPM) for 10 minutes. Discard the supernatant liquid and prepare multiple smears from the sediments.

Cytocentrifuge and membrane filter preparations: These methods are most useful for small volume (0.5–1 mL) fluids of low cell content (e.g., CSF).

Cell block technique: The cell block technique employs the retrieval of small tissue fragments in the sediment and they are processed to form a paraffin block.

Staining of Smears

Three staining procedures commonly used in cytology are: **Papanicolaou and H&E stains are used for wet-fixed smears** while **Romanowsky stains are used for air-dried smears**.

1. **Papanicolaou stain:** It is the best method used for routine cytological smears (wet smears). In this technique, three solutions are used namely —one nuclear stain (Harris' hematoxylin) and two cytoplasmic counter-stains (Orange G or OG-6, and eosin-azure or EA-65 or EA-50). Nuclear stain gives basophilic color to the nucleus whereas two cytoplasmic stains give the orange and cyanophilic tints to cytoplasm respectively.
2. **Hematoxylin and eosin (H&E) stain:** It is essentially similar to that used for histological sections. Harris' hematoxylin is the nuclear stain, and eosin is the cytoplasmic counterstain.
3. **Romanowsky stains:** They are commonly used in hematology and may be used for cytological smear preparations. Leishman's, May–Grünwald–Giemsa (MGG) and Wright's stains are most commonly used.

FINE NEEDLE ASPIRATION CYTOLOGY

Q. Write short essay/note on fine needle aspiration cytology (FNAC) FNAB (fine-needle aspiration biopsy).

Fine needle aspiration cytology is also termed fine needle aspiration biopsy (FNAB) or Aspiration Biopsy Cytology (ABC). In exfoliative cytology, the cytological samples are obtained/smears are prepared and sent to the pathologist by the clinician and the cytopathologist interprets them. In contrast, in FNAC, the cytopathologist often performs the clinical examination of the patient and performs the procedure. Presently, FNAC is a popular and effective technique for a rapid tissue diagnosis.

A. **Applications of FNAC:** FNAC procedure can be performed in any organ of the body or any tissue or swelling. FNAC is most often used for diagnosis readily or assessment of **palpable superficial mass lesions**. These include: mass in the **breast, enlarged lymph nodes, enlarged**

thyroid, superficial soft tissue masses and salivary gland masses. Presently due to imaging techniques (ultrasound or CT-guided) FNAC can be done on intra-abdominal/pelvic organs (e.g., liver, pancreas, pelvic lymph node), retroperitoneum, prostate, and lung masses.

B. **Advantages of FNAC:** Various advantages of FNAC are listed in **Box 10.3**.

Method of Aspiration

FNAC of Superficial Lesions

Routinely, transcutaneous FNAC of palpable masses is performed without any anesthesia. It is generally advisable to perform FNAC by the cytopathologist.

- The syringe with attached needle is properly fitted to the pistol handle. The swelling/mass is immobilized by holding it between the two fingers of the operator. The overlying skin is cleaned with an alcohol/antiseptic pad.

BOX 10.3: Advantages of FNAC.

- Simple and less invasive technique
- Outpatient (OPD) procedure and requires no hospitalization
- Rapid, safe and least painful. Reports is available in a matter of hours
- No anesthesia required
- Economical and cost-effective procedure and does not require major equipments
- Wide area of sampling and multiple attempts is possible without inconvenience
- High sensitivity and specificity
- Avoids need of tissue biopsy in case of inoperable malignancy or infective conditions (Extremely reliable and useful)
- Almost all the ancillary techniques can be done on material obtained by FNAC

- The needle is gently and swiftly introduced into the mass. The needle is moved in to and fro within the mass with gentle negative suction by gently retraction of the plunger of the syringe. Aspiration is terminated by release the suction with the release of the plunger to stop the suction. The cellular material within the needle is adequate for diagnostic purpose.
- At the end of the FNAC, the needle is withdrawn sharply. Then the needle is rapidly detached from the syringe and the plunger is retracted to get enough air within the syringe. The needle is reattached and the air is

pushed to eject the aspirated material in the syringe onto one end of the glass slide.

- Following withdrawal of the needle from the mass, firm pressure should be applied to the site of FNAC for 2 to 3 minutes to prevent hematoma formation.

Contraindications of FNAC is listed in Box 10.4.

BOX 10.4: Contraindications of FNAC.

- Hemophilia or other coagulation disorders
- Aneurysm of blood vessels
- Hydatid cyst

Complications of FNAC are presented in Box 10.5.

BOX 10.5: Complications of FNAC.

- Hematoma
- Infraction of lymph node and thyroid
- Accidental rupture of aneurysm or spleen
- Surgical emphysema
- Anaphylaxis in hydatid cyst
- Biliary peritonitis and perforation of bowel
- Needle tract seedling of cancer cells (if thick bore needle is used for the procedure)

Fine needle sampling (FNS) is done without using any syringe. In this, there is no artificial negative suction is applied, and the material gets aspirated into the needle with the help of negative capillary pressure.

FNAC of Deep-seated Lesions

Fine needle aspiration cytology of the deep-seated masses/lesions is almost similar to that of FNAC of superficial masses/lesion. FNAC of deep-seated lesions need radiological guidance and clinical details before performing FNAC of radiological guided lesions. Depending on the clinical case, radiologist decide the mode of FNAC of deep-seated lesion. The guidance used are ultrasonography (USG), computerized tomography (CT) or fluoroscopy. The advantages of USG-guided FNAC is that it is easy to perform, less expensive and rapid technique. Computerized tomography-

guided FNAC is useful for small lesion which needs precise localization. However, it is a time consuming and expensive procedure. The needle used for USG and CT-guided FNAC is longer. Local anesthesia is required for radiological-guided FNAC. The rest of the procedure is same as that of superficial FNAC described above.

Preparation of Smears

The material obtained is pushed on the clean glass slide a little away from the corner. Smear is prepared by gently pressing another clean glass slide over the material and moving the upper slide over the lower slide so that there is uniform spreading of the material. Multiple smears are prepared and both **air dried** and **wet-fixed** (alcohol-fixed) smears should be kept for staining.

Fixation: Air-dried smears are used for May-Grünwald-Giemsa (MGG) staining. For routine Pap staining wet fixation is done using 95% ethanol, methanol or isopropyl alcohol. The wet-fixed smears need at least 30 minutes of fixation. Commercially available spray fixatives can also be used as an alternate to wet fixation (*refer* page above).

Staining of the Smear

- **Air dried smear:** These smears are stained by MGG, Romanowsky stain or Diff-Quik stain. Romanowsky stain consists of a mixture of methylene blue and Azure A, B or C.
- **Wet fixed smear:** These alcohol (95% ethanol, methanol or isopropyl alcohol) fixed smear are stained either by Pap stain or hematoxylin and eosin stain.
- **Rapid staining:** Diff-Quick stain is a commercially available stain used for rapid staining which stains the smear within 2 minutes. Rapid Pap stain can also be done within 3 minutes.

Application of Ancillary Studies onCytologic Materials

Almost all ancillary studies (e.g., immunocytochemistry, flow cytometry, and molecular biology) can be applied on cytologic materials.

Special and Ancillary Techniques

All the ancillary techniques used in histopathology can also be performed on FNAC sample. Most routinely performed include cell block for immunocytochemistry and flow cytometry for immunophenotyping. Various special and ancillary techniques are as follows:

- **Special stains:** These are performed on wet-fixed smears. The special stains used in cytology include alcian blue, mucicarmine and PAS (for mucin and carbohydrates); methyl violet or Congo red (for amyloid); and bacterial and fungal stains (for microbial agents).
- **Microbiological studies:** FNAC material may also be used for cultures such as viral, fungal, mycobacterial and bacterial culture. When there is a clinical suspicion of infection, usually an additional aspirate is obtained and it is delivered into a sterile culture tube.
- **Cell block:** Aspirated material may be centrifuged and the cell button obtained can processed similar to histopathology and paraffin blocks can be prepared. The sections can also be used for ancillary techniques.
- **Immunocytochemistry:** Immunocytochemical stains can be performed on smears or cell block sections by using panel of antibodies similar to immunohistochemistry.
- **Image analysis and morphometry:** They determine the cellular parameters such as nuclear cytoplasmic (N/C) ratio, nuclear area, shape and size of nuclei and nucleoli, etc. This will help quantitation and cytodiagnosis.
- **Flow cytometry:** This is helpful in determination of ploidy status and S phase fraction of tumor cells, thereby enhancing the diagnostic and prognostic information.
- **Electron microscopic (ultrastructural) studies:** These can also be performed on FNAC materials**.**
- **Molecular techniques:** They help in detection of oncogenes like *ERBB-2* in breast cancer, *BCL-2* in lymphomas, DNA sequence analysis, gene expression profiling and fluorescence in situ hybridization (FISH) can all be performed on FNAC material.

FNAC APPEARANCE OF SOME COMMON LESIONS

CC: Observe a diagnostic cytology and its staining and interpret the specimen.

Granulomatous (Tuberculous) Lymphadenitis(Fig. 10.2)

- Smears are cellular and composed of epithelioid cells in cohesive clusters forming granulomas.
- Epithelioid cells have elongated (oblong/slipper shaped) nuclei and abundant pale cytoplasm.
- Multinucleated Langhans type of giant cells may be seen.
- The background shows lymphocytes and eosinophilic granular caseous necrotic material.
- Ziehl-Neelsen stain for acid-fast bacilli may show positivity for the tubercle bacilli.

Pleomorphic Adenoma (Fig. 10.3)

- Epithelial cells in clusters, sheets and singly scattered.
- Regular ovoid nuclei with bland nuclear chromatin and well-defined cytoplasm.
- Fibrillary chondromyxoid ground substance.
- Spindle-shaped cells seen in stromal matrix.

Fibroadenoma Breast (Fig. 10.4)

- Moderately cellular smears
- Large, branching monolayered sheets of uniform benign ductal epithelial cells having **antler horn** configuration.
- Numerous single bare bipolar nuclei of benign type.
- Fragments of loose fibromyxoid stroma.

Infiltrating Duct Carcinoma Breast (Fig. 10.5)

- Highly cellular smear consisting of clusters of loosely cohesive malignant cells of variable size and shape.
- High nuclear cytoplasmic ratio; nuclei are enlarged, hyperchromatic with irregular nuclear membranes.
- Absence of single bare nuclei of benign type.

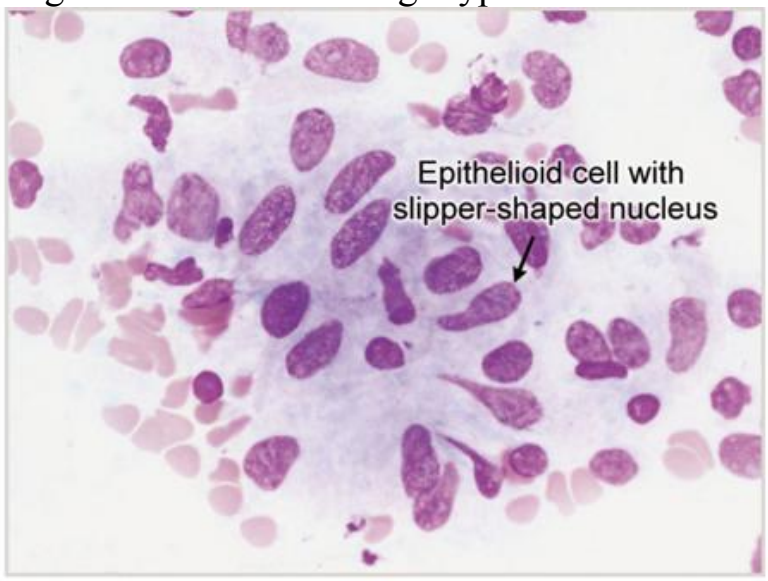


Fig. 10.2: Tuberculous lymph node aspirate with granuloma composed of epithelioid cells showing slipper-shaped nuclei and abundant pale cytoplasm.

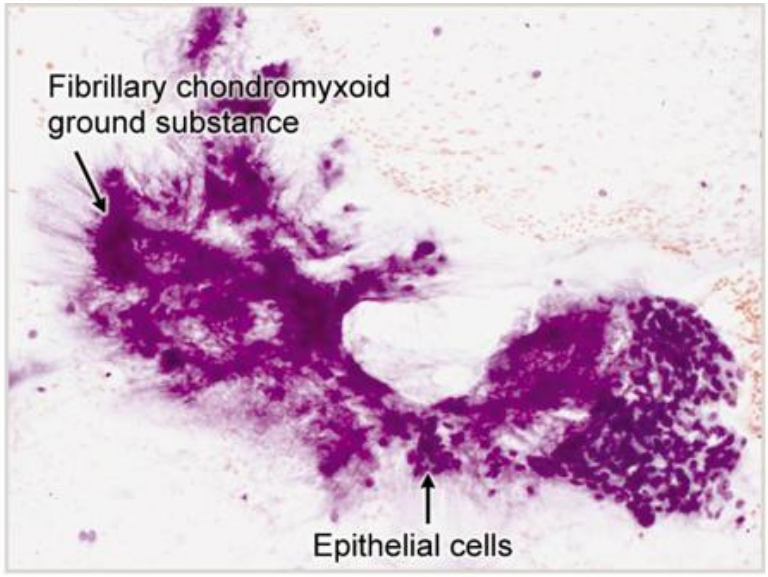


Fig. 10.3: FNAC of pleomorphic adenoma of salivary gland composed of sheets of epithelial cells in a fibrillary myxoid stroma.

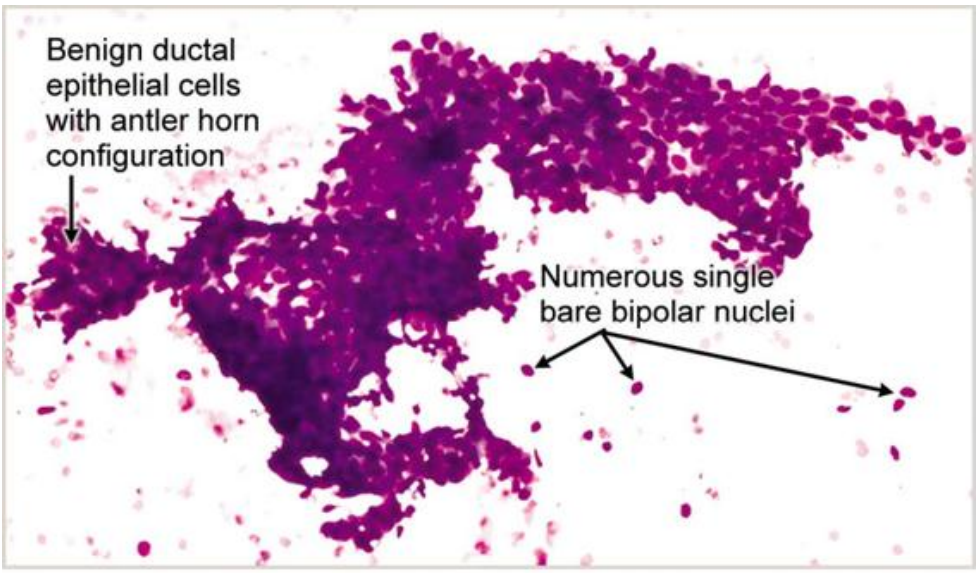


Fig. 10.4: FNAC breast of fibroadenoma consisting of monolayered sheets of benign ductal cells with antler horn configuration.

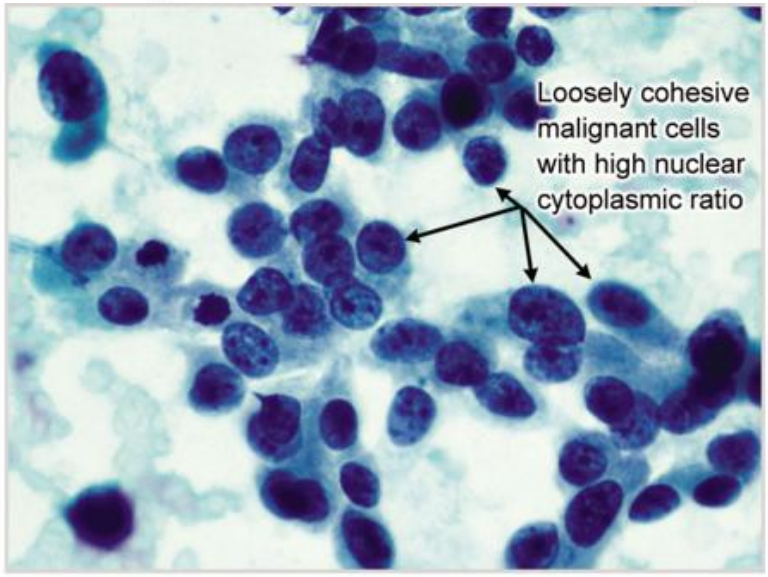


Fig. 10.5: FNAC breast showing malignant cells in infiltrating duct carcinoma.

- The background may show necrosis.

OTHER CYTOLOGY TECHNIQUES

Imprint Cytology

In imprint cytology, cells are obtained by touch preparations from cut surfaces of fresh unfixed surgically excised mass. Imprint smears can also be

obtained from draining sinuses or ulcerated areas. For surgically resected specimens (e.g., lymph nodes), first the specimen is bisected or sliced. Then smears are prepared by gently touching or pressing freshly exposed surface (without smearing) onto a glass slide. Imprint smears cannot be prepared from specimens that are fixed. Imprint smears may be wet-fixed or air-dried and stained accordingly. The advantage of the imprint smear is that the cell distribution recapitulates tissue architecture to some extent, thereby aids in interpretation. Imprint cytology is used for the intraoperative diagnosis of malignancy as a complement to frozen-section, and is also valuable as an adjunct to histopathology in the typing of lymphomas.

Crush Smear Cytology

In crush smear cytology, the tissue particles are crushed to prepare smears. This is used in the diagnosis of brain tumours.

Biopsy Sediment Cytology

In biopsy sediment cytology, the smears are prepared from sediment obtained by centrifugation of fixatives/fluids in which surgical biopsy specimens are received by the laboratory. This method may be useful in the rapid diagnosis of bone tumors because histological sections of bone needs many days for processing due to necessity of decalcification.

2 SECTION

Hematology and Clinical Pathology

Section Outline

11. Introduction to Hematology and Disorders of Red Blood Cell
12. Disorders of White Blood Cell, Lymph Node, and Spleen
13. Disorders of Hemostasis
14. Clinical Pathology
15. Blood Banking and Transfusion

CHAPTER
11

Introduction to Hematology and Disorders of Red Blood Cell

INTRODUCTION

Hematology is defined as the **study of** normal and pathologic aspects of blood and **blood cells. Hematopoiesis** (hemopoiesis) is the continuous, regulated process of blood cell production or formation.

Hematopoietic (hemopoietic) system: It consists of all organs and tissues involved in hematopoiesis, and these are divided into **myeloid tissue and lymphoid tissue.** The pluripotent **hematopoietic stem cell (HSC)** is the progenitor of all the cells in blood and gives rise to cells of both myeloid and lymphoid system.

1. **The myeloid tissue** consists of bone marrow (medullary cavity) and the cells derived from it, which include:
   - **Red blood cells** (RBCs/erythrocytes)
   - White blood cells (WBCs/leukocytes, except lymphocytes): WBCs consist of:
     - Granulocytes: Neutrophils, eosinophils and basophils are collectively called granulocytes because of their different types of cytoplasmic granules. However, the term granulocyte is often referred to only neutrophils.
     - Monocytes
     - Lymphocytes (even though included under WBCs; they are lymphoid derived).
   - Platelets (thrombocytes)

   Compensatory hyperplasia: In adults during pathological states, whenever there is an increased demand for blood cells, the bone marrow undergoes compensatory hyperplasia. This results in replacement of the fatty marrow by hematopoietic tissue.

- **Extramedullary hematopoiesis:**
  - Normally, the cells of the myeloid lineage arise in the central bone marrow (medullary cavity). If the increased demand of blood cells is not met with compensatory hyperactivity of marrow alone, hematopoietic islands appear in liver and spleen (resulting in hepatosplenomegaly) and even in lymph nodes.
  - The myeloid lineage blood cells arising outside the marrow elsewhere in the body are designated as **extramedullary hematopoiesis (agnogenic myeloid metaplasia)**.

2. **The lymphoid tissue** consists of thymus, lymph nodes and spleen. The common lymphoid progenitor cell gives rise to B cell, T cell and natural killer (NK) cell precursors. They mature to form respective lymphoid cells.

The above division of hematopoietic elements as myeloid and lymphoid tissue is mainly for understanding their pathology. It is not always possible to draw clear demarcation between the diseases affecting them.

Functions of Blood Cells

Formed elements of blood are red cells, white cells and platelets. The main functions of blood cells are presented in **Table 11.1**.

HEMATOPOIESIS

CC: Describe hematopoiesis and extramedullary hematopoiesis.

Sites of Hematopoiesis

Different sites of hematopoiesis during various phases of life are shown in **Table 11.2.**

Terminology Used in Hematopoiesis

The meaning of the terms used with reference to hematopoiesis is shown in **Table 11.3.**

Normal Development of Blood Cells (Hematopoiesis)

The hematopoietic system is a hierarchy of cells in which pluripotent hematopoietic stem cells proliferate and differentiate. After several steps, HSCs finally give rise to mature blood cells. This hierarchy **(Figs. 11.1 and 11.2)** consists of:

1. Hematopoietic stem cells
2. Progenitor cells
   a. Multipotent progenitor cells
   b. Committed (unipotent) progenitor cells
3. Precursor cells
4. Maturing and mature cells.

TABLE 11.1: Main functions of formed elements of blood.

Type of blood cell	*Main function*
Red blood cells	Delivery of oxygen to tissues
White blood cells	Defense against infectious organisms
Lymphocytes	Immune regulation
Platelets	Hemostasis

TABLE 11.2: Different sites of hematopoiesis during various phases of life.

Phase	*Period*	*Site*
Yolksac (mesoblastic) phase	First 3 months of gestation	Yolk sac
Hepatic phase	4–9 months of gestation	Liver (chief site till birth), spleen (minor site)
Medullary (myeloid) phase	By 3rd week after birth up to puberty	Bone marrow throughout the skeleton
	Adults	Active marrow is limited to the ends of long bones and flat bones

Hematopoietic Stem Cells (HSC)

These are small, undifferentiated mononuclear cells that can generate all the blood cell lineages.

HSCs possess two fundamental properties:

- **Self-renewal:** HSCs are capable of cell division to give rise to more stem cells.
- **Differentiation:** HSCs can differentiate and give rise to two kinds of lineage-specific multipotent **progenitor** cells, the **common myeloid** and the **common lymphoid progenitors.**

Note: Apart from blood cells, the stem cells may be able to differentiate into diverse tissue types (e.g., neuronal, muscle, liver, vascular cells). This change in the differentiation of a cell from one type to another is known as **transdifferentiation**, and the capacity of a cell to transdifferentiate into diverse lineages is referred to as **developmental plasticity**.

Progenitor Cells

Upon commitment to development, the HSCs enter the next compartment known as **progenitor cell compartment**. This compartment consists of mainly two types of cells.

1. **Multipotent progenitor** (lineage specific) **cells**
2. **Committed (unipotent) progenitor cells**

Both multipotent and unipotent cells in the bone marrow possess the ability to give rise to clones (groups) composed of specific kinds of mature cells when grown in culture and are called as **colony forming units** (CFU).

Multipotent progenitor cells: These are of two types namely—early progenitor with myeloid potential and early progenitor with lymphoid potential.

Early progenitor with myeloid potential further divide to produce mainly two types of multipotent progenitor cells with restricted differentiation. They are:

- CFU b/Mg/E (multipotent) cells which give rise to three types of committed (unipotent) progenitor cells.
  - CFU-E (colony-forming unit-erythrocyte) cells. It is most sensitive to the action of erythropoietin.
  - CFU-Mg (colony-forming unit-megakaryocyte) cells
  - CFU-Baso (colony-forming unit-basophil) cells
- CFU-Mix which differentiate into three types of committed (unipotent) progenitor cells.

- CFU-G (colony-forming unit-granulocyte) cells, precursors of neutrophils.
- CFU-M (colony-forming unit-macrophage) cells, precursors of monocytes and macrophages.
- CFU-Eo (colony-forming unit-eosinophil) cells, precursors of eosinophils.

Early progenitor with lymphoid potential cell in turn gives rise to three **progenitor cells.**

- **Pro-T cells** which differentiate into T cells.
- **Pro-NK cells** which differentiate into NK cells.
- **Pro-B cells** which differentiate into B cells.

Morphologically, the progenitor (both multipotent and unipotent) cells and stem cells cannot be distinguished from one another on morphological appearance or cytochemistry except by immunological techniques.

Precursor Cells

When the immature hematopoietic cell acquires recognizable morphological, cytochemical or immunological feature of a single lineage, it is called as precursor cell. The next step in hematopoiesis is maturation of unipotent progenitor cells to precursor cells. The earliest morphologically recognizable precursor cell of each lineage is termed by adding the suffix "**blast" to the type of lineage** (e.g., lymphoblast to lymphoid lineage).

Mature Cells

The precursor cells finally give rise to mature blood cells, which are released from the marrow into the circulation.

TABLE 11.3: Different terminologies used with regards to hematopoiesis.

Terminology	
Pluripotent	Ability to generate all mature hematopoietic cells
Multipotent	Ability to produce a limited range of differentiated cell lineages appropriate to their location
Unipotent	Restricted ability to differentiate and generate one specific cell type

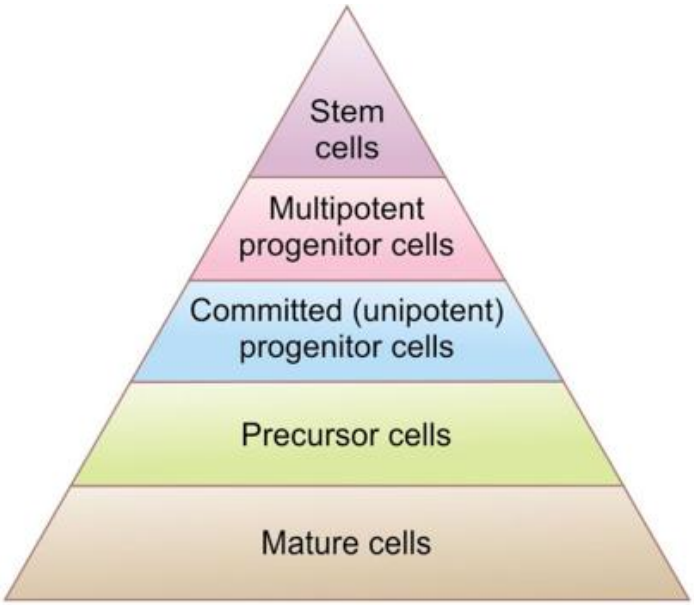


Fig. 11.1: Cell hierarchy in hematopoiesis.

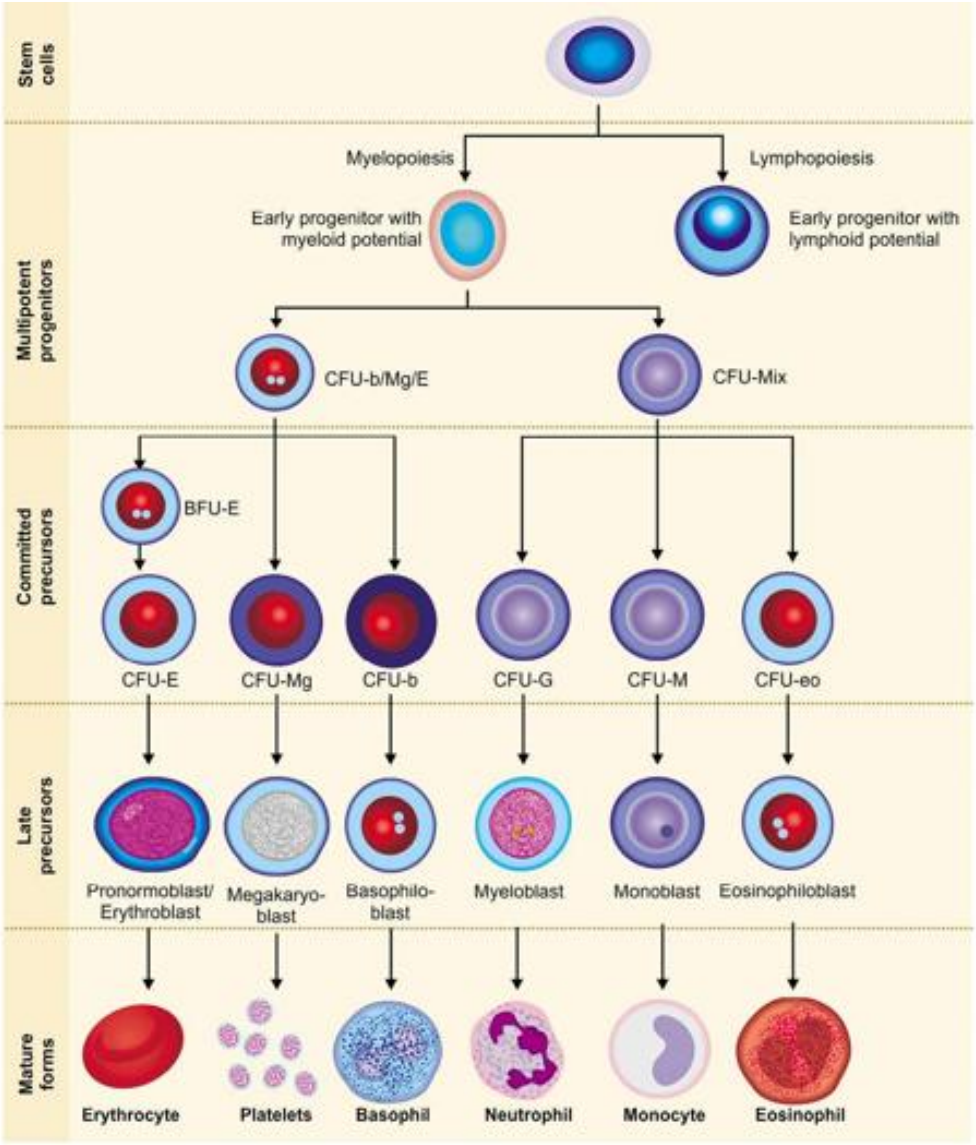


Fig. 11.2: Different stages of hematopoiesis.
(BFU: burst forming unit; CFU: colony forming unit).

Regulation of Hematopoiesis

The growth of different hematopoietic cells is regulated by a number of hematopoietic growth factors, which in general are called cytokines.

Most important growth factors acting on various cells are mentioned below:

- **Stem cells:** Stem cell factor (also called c-KIT ligand), IL-6 and FLT3-ligand.
- **CMP progenitor cells:**
  - Multipotent committed progenitors
    - Granulocyte-macrophage colony-stimulating factor (GM-CSF)

- Thrombopoietin
- IL-3, IL-5, IL-6 and IL-11

- Committed progenitors
  - **Erythropoietin (EPO):** It is a glycosylated protein synthesized mainly by kidney and minor part from liver. It is produced in response to hypoxia. EPO acts on the erythroid precursors through EPO receptors. This stimulates proerythroblasts to proliferate and differentiate to produce RBCs.
    - Granulocyte colony-stimulating factor (G-CSF)
    - Thrombopoietin

Q. Write short note on contents of bone marrow

- **Lymphopoiesis** is regulated by several interleukins (most important being IL-1, 2, 4, 5, 6, 7, 9).

Components of bone marrow are listed in **Box 11.1**.

ANEMIA

CC: Define and classify anemia.

DEFINITION

Q. Define anemia.

- Anemia is defined as the **decrease** below normal limit (below the reference level for the age and sex of the individual) of the **hemoglobin concentration, erythrocyte count or hematocrit** (ratio of packed red cells to total blood volume).
- It can also be defined as a **reduction of the total circulating red cell mass** below normal limits.
- Functionally, it is defined as the decrease in the oxygen-carrying capacity of the blood, which leads to tissue hypoxia.

Anemia may be **absolute** (decreased RBC mass), or **relative** (associated with a higher plasma volume). Anemia is conventionally used for absolute anemia.

Classification of Anemia

1. **Morphological classification (Table 11.4):** It is based on:
   - **Red cell size** (normocytic, microcytic, or macrocytic), and
   - **Degree of hemoglobinization** (normochromic or hypochromic).
2. **Etiological classification:** The etiological classification of anemia is presented in **Box 11.2**.

Red Cell Indices

Q. Write short notes on red cell indices.

Red cell indices are useful in morphological characterization and diagnosis of anemias. They are either directly measured or automatically calculated by specialized instruments. Red cell indices include:

Q. Write short note on mean corpuscular volume.

1. **Mean corpuscular volume**
   - Mean corpuscular volume (MCV) is indicative of average volume of the RBC and is expressed in femtoliters (fL).
   - It is used for classification and differential diagnosis of anemias.
   - **Normal range: 82–98 fL.**

$$MCV = = \frac{PCV \times 1000}{\text{RBC count in millions}} = 0.45 \times 1000/5 = 90 \text{ fL}$$

2. **Mean corpuscular hemoglobin**
   - Mean corpuscular hemoglobin (MCH) indicates the amount of Hb (weight) per RBC and is expressed as picograms (1 pg = 10^{-12} g).
   - It is of limited value in differential diagnosis of anemias.
   - **Normal range: 27–32 pg**

     MCH = Hb (in g/L)/RBC (in millions/μL) = 15 × 10/5 = 30 pg

Q. Write short note on mean corpuscular hemoglobin concentration.

3. Mean corpuscular hemoglobin concentration

- Mean corpuscular hemoglobin concentration (MCHC) denotes the average concentration of hemoglobin in the RBC taking volume into account. It is expressed as g/dL (earlier it was expressed as %).

BOX 11.1: Contents of bone marrow.

- Hematopoietic cells
  - Myeloid series
  - Erythroid series
  - Megakaryocytes
  - Other cells: Lymphocytes, plasma cells
- Fat tissue
- Vessels
- Nerves
- Reticuloendothelial cells
- Stroma

Q. Classify anemia.

TABLE 11.4: Morphological classification of anemia.

Type of anemia	*Microcytic hypochromic*	*Normocytic normochromic*	*Macrocytic*
Size of RBCs	Smaller than normal	Normal	Larger than normal
Central pallor in RBCs	More than 1/3	Normal	Normal
Mean corpuscular volume (MCV)	Reduced (<80 fL)	Normal (82–98 fL)	Increased (>100 fL)
Mean corpuscular hemoglobin concentration (MCHC)	Reduced (<30 g/dL)	Normal (31–36 g/dL)	Normal (31–36 g/dL)
Examples	Iron deficiency anemia, thalassemia	During blood loss, anemia of chronic diseases	Deficiency of vitamin B_{12} and folic acid
Morphology of RBC			

Spurious anemia is the term used when RBC concentration decreases due to hemodilution as seen in third semester of pregnancy.

Q. Etiological classification of anemia.

BOX 11.2: Etiological classification of anemia (according to underlying mechanism).

1. **Blood Loss**
   - **Acute:** Trauma
   - **Chronic:** Lesions of gastrointestinal tract (e.g., carcinoma colon), gynecological disorders
2. **Impaired Red Cell Production**
   - **Nutritional deficiencies**
     - ***Deficiencies affecting hemoglobin synthesis:*** Iron deficiency
     - ***Deficiencies affecting DNA synthesis:*** Megaloblastic anemias due to deficiency or impaired utilization of vitamin B_{12} and folic acid
     - ***Vitamin C deficiency***
   - **Inherited genetic defects**
     - ***Defects affecting erythroblast maturation:*** Thalassemia syndromes
     - ***Defects leading to stem cell depletion:*** Fanconi anemia, telomerase defect
   - **Erythropoietin deficiency:** Renal failure, anemia of chronic disease
   - **Immune-mediated injury of progenitors:** Aplastic anemia, pure red cell aplasia
   - **Inflammation-mediated iron sequestration:** Anemia of chronic disease
   - **Primary hematopoietic neoplasms:** Acute leukemia, myelodysplastic syndromes, myeloproliferative disorders
   - **Space-occupying marrow lesions:** Metastatic tumors, granulomatous disease
   - **Infections of red cell progenitors:** Parvovirus B_{19} infection
   - **Unknown mechanisms:** Endocrine disorders, liver disease
3. **Increased Red Cell Destruction (Hemolytic Anemias)**
   - **Inherited genetic defects**
     - ***Red cell membrane disorders:*** Hereditary spherocytosis, hereditary elliptocytosis
     - ***Enzyme deficiencies***
       - ◊ **Hexose monophosphate shunt enzyme deficiencies:** G6PD deficiency
       - ◊ **Glycolytic enzyme deficiencies:** Pyruvate kinase deficiency, hexokinase deficiency
     - ***Hemoglobin abnormalities***
       - ◊ Deficient globin synthesis: Thalassemia syndromes
       - ◊ Structurally abnormal globins (hemoglobinopathies): Sickle cell disease
   - **Acquired genetic defects**
     - ***Deficiency of phosphatidylinositol-linked glycoproteins:*** Paroxysmal nocturnal hemoglobinuria
   - **Antibody-mediated destruction**
     - Hemolytic disease (Rh disease) of the newborn, transfusion reactions, drug-associated, autoimmune disorders (e.g., systemic lupus erythematosus)
   - **Mechanical trauma**
     - ***Microangiopathic hemolytic anemias:*** Hemolytic uremic syndrome, disseminated intravascular coagulation, thrombotic thrombocytopenia purpura
     - ***Cardiac traumatic hemolysis:*** Defective cardiac valves
   - **Infections of red cells:** Malaria, babesiosis
   - **Toxic or chemical injury:** Clostridial sepsis, snake venom, lead poisoning
   - **Sequestration:** Hypersplenism

(G6PD: glucose-6-phosphate dehydrogenase; PK: pyruvate kinase)

- It is a better indicator of hypochromasia than MCH.

- Normal range: 31–35 g/dL.

MCHC = Hb (in g/dL)/PCV = 15/0.45 = 33 g/dL

4. **Red cell distribution width (RDW)**

Q. What is RDW? What is its significance?

- Red cell distribution (RDW) is a quantitative measure of anisocytosis.
- Normal RDW is 11.5–14.5%.
- A normal RDW indicates that RBCs are relatively uniform in size. A raised RDW indicates that red cells are heterogeneous in size and/or shape. In early iron deficiency anemia, RDW increases along with low MCV while in thalassemia trait, RDW is normal with low MCV.

RDW = (Standard deviation ÷ mean cell volume) × 100

Clinical Features of Anemia

Irrespective of the cause, anemia when severe, presents with certain clinical features. The symptoms depend on four main factors:

1. **Speed of onset of anemia:** Rapidly progressive anemia causes more symptoms than that of gradual onset. This is because there is less time for adaptation in the cardiovascular system.
2. **Severity of anemia:** Mild anemia produces no symptoms compared to significant symptoms in severe anemia.
3. **Age of the patient:** Young patients can tolerate anemia better than elderly.
4. **Underlying illness:** The disease which caused the anemia.

In anemia, the lowered oxygen content of the circulating blood leads to tissue hypoxia. General clinical features are either due to tissue hypoxia or compensatory mechanisms **(Fig. 11.3)**.

1. **Due to tissue hypoxia:**
   - *Nonspecific symptoms:* Weakness, malaise and easy fatigability due to hypoxia of muscles.
   - *Dyspnea on mild exertion:* It is due to the lowered oxygen content of the circulating blood.
   - *Pallor:* Patients appear pale due to deficiency of red colored hemoglobin which is better appreciated in the conjunctiva, mucous

membrane of tongue and nail beds. Pallor associated with icterus is suggestive of a hemolytic anemia.

- *CNS:* Patients of severe anemia may complain of headache, vertigo, tinnitus and lack of concentration.

2. **Due to compensatory mechanisms:**
   - *Cardiac features:* Dyspnea on mild exertion, palpitation, tachycardia and cardiac murmur occur due to compensatory mechanisms. The resulting increase in the cardiac output may cause congestive cardiac failure.

General scheme of investigation of anemia are listed in **Box 11.3** and classification of severity of anemia is presented in **Box 11.4**.

CC: Enumerate describe the investigation of anemia.

BOX 11.3: General scheme of investigation of anemia.

- Estimation of hemoglobin and packed cell volume (hematocrit)
- Blood cell counts: RBC, WBC and platelet counts
- Peripheral smear examination
- Red cell indices
- Reticulocyte count
- Erythrocyte sedimentation rate
- Bone marrow examination

BOX 11.4: Severity of anemia.

- Mild anemia: Hb 9.1 to 10.5 g/dL
- Moderate anemia: Hb 6.0 to 9.0 g/dL
- Severe anemia: Hb less than 6.0 g/dL

Anemia is characterized by decreased oxygen carrying capacity of blood. Shows decreased Hb and PCV.

Red cell indices: MCV, MCH, MCHC and RDW.

Microcytic anemia have MCV <80 fL and macrocytic anemia have MCV >100 fL.

MCH <26 pg is seen in microcytic anemia and MCH >33 pg is seen in macrocytic anemia.

MCHC <31 g/dL is seen in hypochromic RBC such as iron deficiency anemia (IDA) and thalassemia. MCHC >36 g/dL is an indication of hyperchromic RBCs.

RDW is useful for differentiating anemia due to iron deficiency and thalassemia.

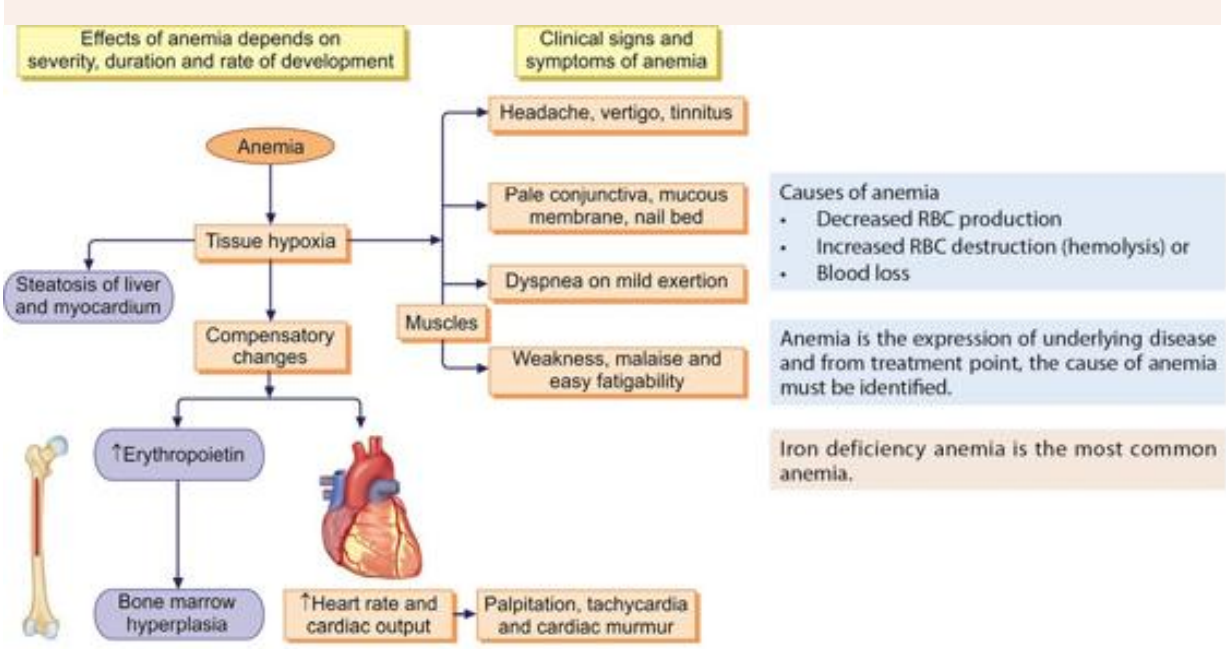


Fig. 11.3: Clinical features and effects of anemia.

IRON DEFICIENCY ANEMIA

Q. Define anemia. Give the etiological classification of anemia. Describe the peripheral blood smear and bone marrow findings and list the laboratory investigations in iron deficiency anemia.

Q. Define and classify anemia. Discuss the causes and laboratory diagnosis of iron deficiency anemia.

Q. Describe the etiology, clinical features and the laboratory diagnosis of iron deficiency anemia.

Q. Discuss the etiopathogenesis of iron deficiency anemia.

Iron is required for synthesis of normal heme of hemoglobin. Its deficiency leads to decreased erythropoiesis and anemia. Iron deficiency anemia (IDA) is the most common nutritional disorder in the world.

Iron Metabolism

CC: Describe iron metabolism.

Distribution of Iron

Iron is an essential metal present in the human body. The total body iron content is divided into functional and storage compartments **(Table 11.5)**. Iron in the body is extensively recycled between the functional and storage pools.

- **Functional:** Approximately 80% of the functional iron is present in hemoglobin. The remaining functional iron is found in myoglobin and iron-containing enzymes (catalase, cytochromes and peroxidases).
- **Storage**: The storage pool contains 15–20% of total body iron. Free iron is highly toxic, because it can result in tissue damage due to its capacity to form free radicals. Therefore, iron is bound to protein and stored in the body in two forms namely—**ferritin** and **hemosiderin**. The storage iron can be readily mobilized whenever there is increase in the requirements

of iron, as may occur after blood loss. Two-thirds of the iron is stored as ferritin and one-thirds as hemosiderin.

- Ferritin is a protein-iron complex (apoferritin + iron) found in all tissues but particularly in liver, spleen, bone marrow and skeletal muscles. Very small amounts of ferritin circulate in the serum, the value normally being 15–300 µg/L. Serum ferritin levels reflect the iron stores. Serum ferritin level is usually below 12 µg/L in iron deficiency and it is very high (as high as 5000 µg/L may be observed) in conditions associated with iron overload. Ferritin is water-soluble and not visible by light microscopy.
- Hemosiderin is an aggregate of iron and protein which is found in the reticuloendothelial cells of bone marrow, spleen and liver. Hemosiderin appears as golden yellow granules in the cytoplasm of the cells when stained with routine Hematoxylin and Eosin. When treated with Prussian blue (Perl's) stain, hemosiderin takes up blue-black color due to the iron reacting with potassium ferrocyanide of Perl's stain. In iron-overload, most of the iron is stored in the cells as hemosiderin.

Daily requirements: The daily requirement in adult males is 5–10 mg/day and in females 20 mg/day.

Dietary sources: The diet contains iron either in the form of **heme** contained in animal products and/or **nonheme** iron in vegetables.

Iron Absorption

Site of absorption: Iron is absorbed from the **duodenum** and **proximal jejunum**. Iron balance is maintained mainly by regulating the dietary absorption of iron.

Molecular Aspect of Iron Absorption (Fig. 11.4)

Nonheme iron: The absorption of heme iron is better than nonheme. Iron in nonheme form is mostly in the Fe^{3+} (ferric) state and is reduced to Fe^{2+} (ferrous) iron by ferrireductases, such as duodenal cytochrome b.

Fe^{2+} iron is then transported across the brush border of the cytoplasmic membrane of the enterocyte (duodenal or jejunal epithelial cells) by **divalent metal transporter 1 (DMT1).**

Heme iron is moved across the brush border of the cytoplasmic membrane of the enterocyte through heme transporters into the cytoplasm.

Iron (heme or nonheme) that enters the cytoplasm of the enterocyte may be either transported to the blood or stored as mucosal iron. Fe^{2+} iron destined for transport from cytoplasm to the circulation is carried out by **ferroportin-1**. This process is coupled by the iron oxidase like hephaestin (which is expressed predominantly in the enterocyte) which oxidizes Fe^{2+} iron to Fe^{3+} form.

Note: Both DMT1 and ferroportin are also involved in iron transport in other tissues and are distributed widely in the body.

TABLE 11.5: Iron distribution in healthy young adults (in mg).

Pool	***Men***	***Women***
Total	3450	2450
Functional Hemoglobin Myoglobin and enzymes	2000–2500 350–400	1750 300
Storage Ferritin, hemosiderin	500–1000	400

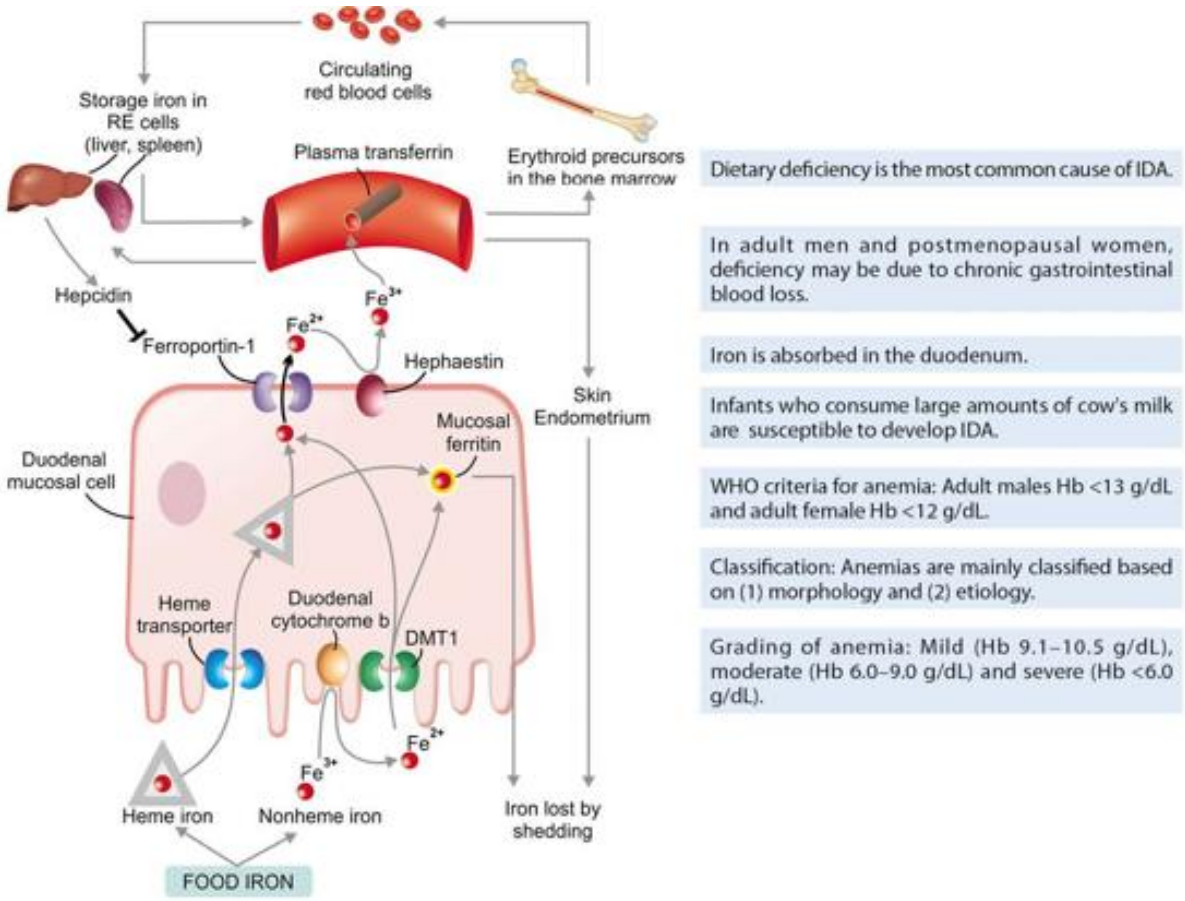


Fig. 11.4: Metabolism and molecular aspects of iron absorption.
(DMT: divatent metal transporter 1; RE: reticuloendothelial)

Transport of Iron

Iron is transported in plasma by the iron transport protein **transferrin**, which is synthesized in the liver. The major function of plasma transferrin is to deliver iron to erythroid precursors for the synthesis of hemoglobin. In normal individuals, transferrin is about 33% saturated with iron, with an average serum iron levels of 120 µg/dL in men and 100 µg/dL in women. Thus, the total iron-binding capacity of serum is in the range of 310–340 µg/dL.

Iron Excretion

Iron metabolism is unique as it is very efficiently utilized and reutilized by the body. There is no physiological regulated mechanism for iron excretion and 1–2 mg per day is lost by shedding of epithelial cells of GI tract, skin epithelial cells (by sweat) and renal tubules.

Regulation of Iron Balance

Iron is essential for cellular metabolism; at the same time excess of it is highly toxic. Therefore, the total body iron stores must be properly regulated. Iron balance is mainly achieved by regulating the absorption of iron in the diet. As body stores decreases, the absorption of iron rises and vice versa. **Hepcidin** synthesized in the liver is the **central regulator of iron homeostasis** and it **controls absorption and storage**. Hepcidin inhibits iron transfer from the enterocyte to plasma by binding to ferroportin. When hepcidin levels rise, iron gets stored within enterocytes forming mucosal ferritin and is shed with the cells **(Fig. 11.4)**.

Etiology of Iron Deficiency Anemia (Box 11.5)

CC: Describe the etiology, investigations and differential diagnosis of microcytic hypochromic anemia.

BOX 11.5: Causes of iron deficiency anemia.

- **Dietary deficiency/lack**
  - Milk-fed infants
  - Elderly with improper diet and poor dentition
  - Low socioeconomical sections
  - Vegetarians (contains poorly absorbable inorganic iron)
- **Impaired absorption**
  - Total/partial gastrectomy
  - Intestinal absorption is impaired in sprue, other causes of intestinal steatorrhea and chronic diarrhea
  - Specific items in the diet, like phytates of cereals, tannates, carbonates, oxalates, phosphates and drugs can impair iron absorption
- **Increased demand/requirement**
  - Growing infants, children and adolescents
  - Pregnancy and lactation
- **Chronic blood loss: due to bleeding from the**
  - Gastrointestinal tract (e.g., peptic ulcers, gastric carcinoma, colonic carcinoma, hemorrhoids, hookworm infestation or nonsteroidal anti-inflammatory drugs)
  - Urinary tract (e.g., renal or bladder tumors)
  - Genital tract (e.g., menorrhagia, uterine cancer)
  - Respiratory tract (e.g., hemoptysis)

Iron deficiency anemia (IDA) is due to deficiency of iron causing **defective heme synthesis**.

Pathogenesis of Iron Deficiency Anemia

It is due to decreased synthesis of heme and can be divided into three stages:

- **Stage 1 (Iron depletion):** Iron adequate to maintain normal hemoglobin level and **only serum ferritin decreased.**
- **Stage 2 (Iron deficient erythropoiesis): Lowering of serum iron and transferrin saturation levels** without anemia (Hb, MCV and MCH within normal range). Bone marrow shows iron deficient erythropoiesis.
- **Stage 3 (Iron deficiency anemia):** Low serum iron, serum ferritin and transferrin saturation. Impaired hemoglobin production. Morphologically, **first reduction in the size (microcytic) and later increase in the central pallor (hypochromia) of RBCs.**

Laboratory Findings

CC: Describe the etiology, investigations and differential diagnosis of microcytic hypochromic anemia.

Q. Discuss the laboratory findings in iron deficiency anemia.

Q. Describe the peripheral blood picture and bone marrow finding in iron deficiency anemia.

Peripheral Blood

- **Hemoglobin and hematocrit (PCV): Decreased**
- **Red cell indices:**
- **MCV:** <80 fL (normal 82–98 fL)
- **MCH:** <25 pg (normal 27–32 pg)

- **MCHC:** <27 g/dL (31–36 g/dL)
- **RDW: Increased** and >15%. It is **earliest sign of iron deficiency** (normal 11.5–14.5%).

Q. Write short note on peripheral smear findings in iron deficiency anemia.

CC (Show How): Identify and describe the peripheral smear in microcytic anemia
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)**.**

PERIPHERAL SMEAR (FIGS. 11.5 AND 11.6)

- RBCs: Microcytic (small) and hypochromic (pale). Severe anemia shows ring/pessary cells. Moderate anisocytosis and poikilocytosis with pencil/cigar-shaped cells.
- WBCs: Normal; eosinophilia in hookworm infestation.
- Platelets: Normal.

- **Reticulocyte count:** Low for the degree of anemia.

BONE MARROW

- Cellularity: Moderately hypercellular.
- M:E ratio: varies from 2:1 to 1:2 (normal 2:1 to 4:1).
- Erythropoiesis: Hyperplasia and micronormoblastic maturation.
- Myelopoiesis: Normal.
- Megakaryopoiesis: Normal.
- Absence of bone marrow iron: "Gold standard" test, demonstrated by negative Prussian blue reaction.

Serum Iron Profile

Table 11.6 shows the serum iron profile in iron deficiency anemia.

Reticulocyte Hemoglobin

It is **decreased** and is **an early feature of IDA**.

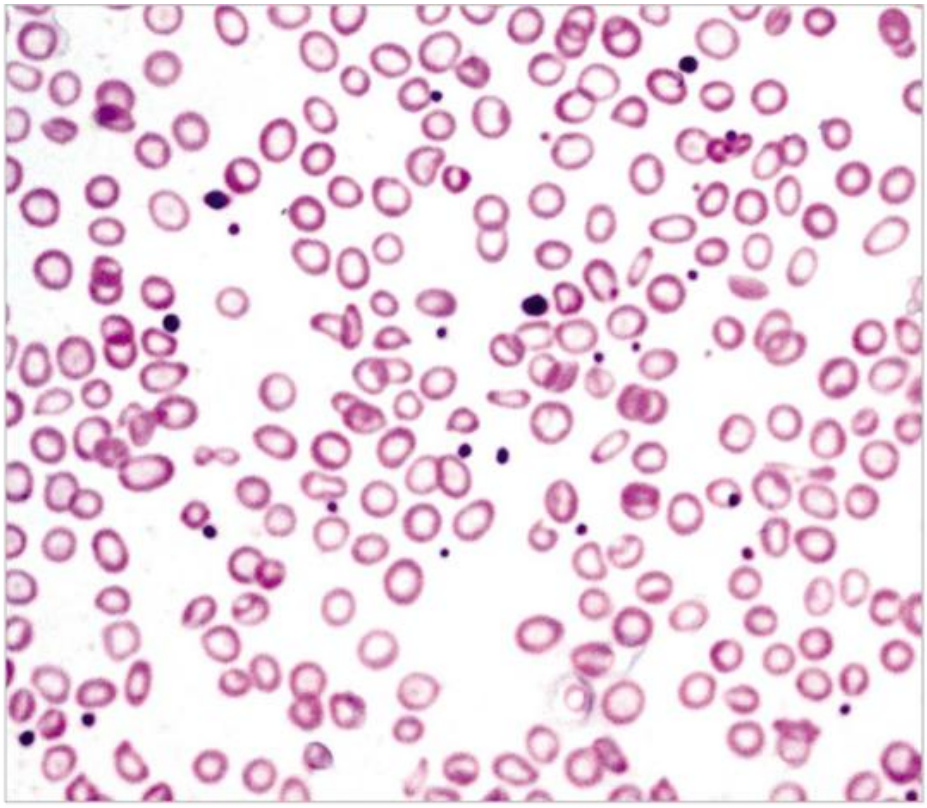


Fig. 11.5: Peripheral blood smear showing microcytic hypochromic red blood cells.

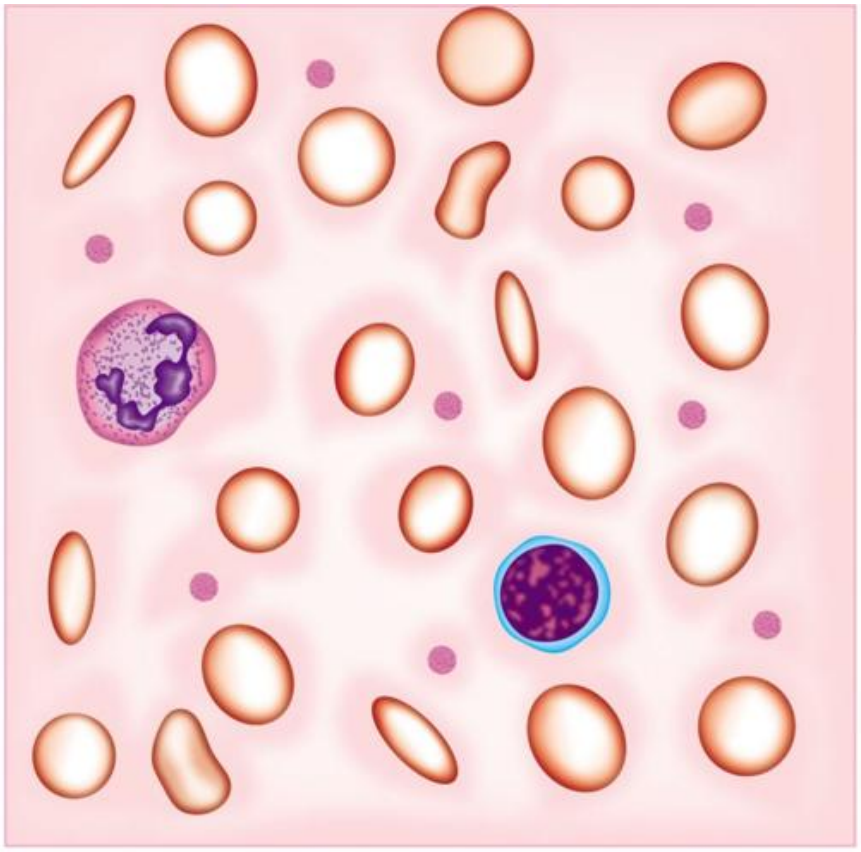


Fig. 11.6: Diagrammatic appearance of peripheral blood smear with microcytic hypochromic red blood cells.

Clinical Features of IDA

Q. Mention the various clinical features of iron deficiency anemia.

Nonspecific and related to both severity and the cause of the anemia (e.g., gastrointestinal disease)

- **Onset:** Insidious.
- **Nonspecific symptoms: Fatigue**, **palpitations**, **breathlessness**, weakness and irritability.
- **Pharyngeal/esophageal webs** formed cause dysphagia.
- **Patterson-Kelly or Plummer-Vinson syndrome:**
  - Microcytic hypochromic anemia
  - Atrophic glossitis

- Esophageal webs

- **Congestive heart failure in severe** anemia.
- **Central nervous system: Pica**-unusual craving for substances with no nutritional value such as clay or chalk. **Craving for ice** (pagophagia) **specific to iron deficiency**. Pica may be the cause rather than effect of IDA.

Physical Findings

Diminished tissue enzymes cause characteristic epithelial changes of iron deficiency anemia.

- Angular stomatitis and glossitis
- Chronic atrophic gastritis
- Koilonychia (spoon nails)

Causes of Microcytic Hypochromic Anemia

CC: Differential diagnosis of microcytic hypochromic anemia.

Q. Enumerate the causes of microcytic hypochromic anemia.

- **Iron deficiency anemia**
- **Thalassemia major and minor**
- **Anemia of chronic disorders**
- **Others:** Alcohol, lead poisoning and drugs
- Sideroblastic anemia (rare cause)

Various differentiating features of microcytic hypochromic anemias are presented in **Table 11.7**.

Hepcidin is the master regulator of iron metabolism.

Stages of IDA in sequence: absent of iron stores→decreased serum ferritin→decreased serum iron→increased TIBC → decreased iron saturation→ microcytic hypochromic anemia.

MCV, MCH and MCHC are reduced. RDW is raised.

IDA: Peripheral smear shows microcytic hypochromic RBCs.

Bone marrow in IDA shows micronormoblastic erythroid hyperplasia. Marrow iron is absent. Prussian blue reaction negative.

Reduced: Serum iron, ferritin, % transferrin saturation.
Increased: TIBC, TFR and red cell protoporphyrin.

The earliest laboratory indicator of IDA is reduced reticulocyte hemoglobin.

Patterson-Kelly or Plummer-Vinson syndrome: Microcytic hypochromic anemia, atrophic glossitis and esophageal webs.

Koilonychia (spoon nails) is a physical finding seen in iron deficiency. First fingernails become thin and flat-platonychia, then brittle and finally spoon-shaped.

MEGALOBLASTIC ANEMIA

TABLE 11.6: Serum iron profile in iron deficiency anemia.

	Normal range	*Value in IDA*	*Observation*
Serum ferritin	15–300 µg/L	<15 µg/L	
Serum iron	50–150 µg/dL	10–15 µg/dL	
Serum transferrin saturation	30–40%	<15%	
Total plasma iron-binding capacity (TIBC)	310–340 µg/dL	350–450 µg/dL	
Serum transferrin receptor (TFR)	0.57–2.8 µg/L	3.5–7.1 µg/L	
Red cell protoporphyrin	30–50 µg/dL	>200 µg/dL	

TABLE 11.7: Various differentiating features of microcytic hypochromic anemias.

	Iron deficiency anemia	***Thalassemia trait (minor)***	***Anemia of chronic disease***	***Sideroblastic anemia***
Mean corpuscular volume (MCV)	Reduced	Very low for degree of anemia	Low normal or normal	Low in inherited type but often raised in acquired type
Serum iron (normal 60–170 µg/dL)	Reduced (<30 µg/dL)	Normal to high	Reduced (<50 µg/dL)	Raised
Serum TIBC (total iron binding capacity) (normal 300–350 µg/dL)	Raised (>350 µg/dL)	Normal	Reduced (<300 µg/dL)	Normal
Serum ferritin (normal 15–300 µg/dL)	Reduced (<15 µg/dL)	Normal(50–300 µg/dL)	Normal or raised (30–200 µg/L)	Raised
Serum soluble transferrin receptors	Increased	Normal or raised	Normal	Normal or raised
Iron in marrow	Absent	Present	Present	Present

TABLE 11.7: Various differentiating features of microcytic hypochromic anemias.

	Iron deficiency anemia	***Thalassemia trait (minor)***	***Anemia of chronic disease***	***Sideroblastic anemia***
Iron in erythroblasts	Absent	Present	Absent or reduced	Ring forms
Hemoglobin A_2 (normal <3%)	Reduced	Increased	Normal	Reduced

CC: Describe the metabolism of vitamin B_{12} and the etiology and pathogenesis of B_{12} deficiency.

Megaloblastic anemias are characterized by **defective**/impaired **DNA synthesis** and **distinct megaloblasts in the bone marrow.** Megaloblastic anemias are common among anemias due to impaired red cell production.

METABOLISM OF VITAMIN B_{12} AND FOLIC ACID

Metabolism of vitamin B_{12} and folic acid are closely related and both are essential for normal DNA synthesis and nuclear maturation. Other disorders may be associated with macrocytosis but megaloblastic hematopoiesis is most commonly due to deficiency of vitamin B_{12} or folic acid.

Vitamin B_{12} Metabolism

Human beings are totally dependent on animal products in the diet for vitamin B_{12} requirement. Vitamin B_{12} is not present in food from vegetable sources. Therefore, strict vegetarians do not get an adequate quantity of vitamin B_{12}. A balanced diet (not rigid vegetarian!) contains significantly large amounts of vitamin B_{12}, which accumulates in the body (liver) and is enough for several years. Due to this adequate storage, if there is any dietary deficiency or malabsorption of vitamin B_{12}, its clinical manifestations appear only after about 2 to 4 years. Vitamin B_{12} is a complex compound known as cobalamin and daily requirement is about 2 to 3 µg.

Absorption, Transport and Storage (Fig. 11.7)

- Vitamin B_{12} in food is usually in coenzyme form (as deoxyadenosylcobalamin and methylcobalamin) and bound to binding proteins in the diet.
- In the stomach, peptic digestion at low pH is required for release of vitamin B_{12} from binding protein in the food. The released vitamin B_{12} binds with salivary protein called **haptocorrin**, which is secreted in salivary juices.
- These haptocorrin-B_{12} complexes leave the stomach along with unbound special protein called **intrinsic factor** (IF), which is produced by gastric (fundus and cardia) parietal (oxyntic) cells (intrinsic factor is also called as Castle intrinsic factor).
- As the haptocorrin-B_{12} complexes pass into the second part of the duodenum, pancreatic proteases release vitamin B_{12} from haptocorrin. Vitamin B_{12} then associates with the intrinsic factor and forms IF-B_{12} complex.
- This stable IF-vitamin B_{12} complex is transported to the ileum, where it is endocytosed by ileal enterocytes. These ileal enterocytes express a receptor on their surfaces for the intrinsic factor. These receptors are called **cubilin**.
- In the ileal epithelium, vitamin B_{12} combines with a major carrier protein, transcobalamin II, and is actively transported into the mucosal cells and then into the blood.
- Transcobalamin II-vitamin B_{12} complex delivers vitamin B_{12} to the liver and other cells of the body, particularly rapidly proliferating cells in the bone marrow and mucosal lining of the gastrointestinal tract.

Role of Vitamin B_{12}

Vitamin B_{12} is indirectly required for DNA synthesis in various metabolic steps and its deficiency impairs DNA synthesis.

- Methylcobalamin is the main form of vitamin B_{12} in plasma, and is an essential coenzyme for conversion of homocysteine to methionine and formation of tetrahydrofolate (THF) from methyl THF **(Fig. 11.8).** During the former reaction, vitamin B_{12} loses its methyl group and this is replaced from methyl THF, the principal form of folic acid in plasma.

Tetrahydrofolate is essential for the generation of a precursor of DNA known as deoxythymidine monophosphate (dTMP).

In vitamin B_{12} deficiency, the main cause of impaired DNA synthesis is that methyl THF is not converted into THF. Methyl THF accumulates in the cell and is known as **methyl THF trap.**

- Vitamin B_{12} is also required for conversion of methylmalonyl CoA to succinyl malonyl CoA **(Fig. 11.9)**. Deficiency of vitamin B_{12} causes increased levels of methylmalonic acid in plasma and urine. This results in the formation of abnormal fatty acids which get incorporated into neuronal lipids. Consequently, this predisposes to myelin breakdown and is probably responsible for neurologic complications of vitamin B_{12} deficiency.

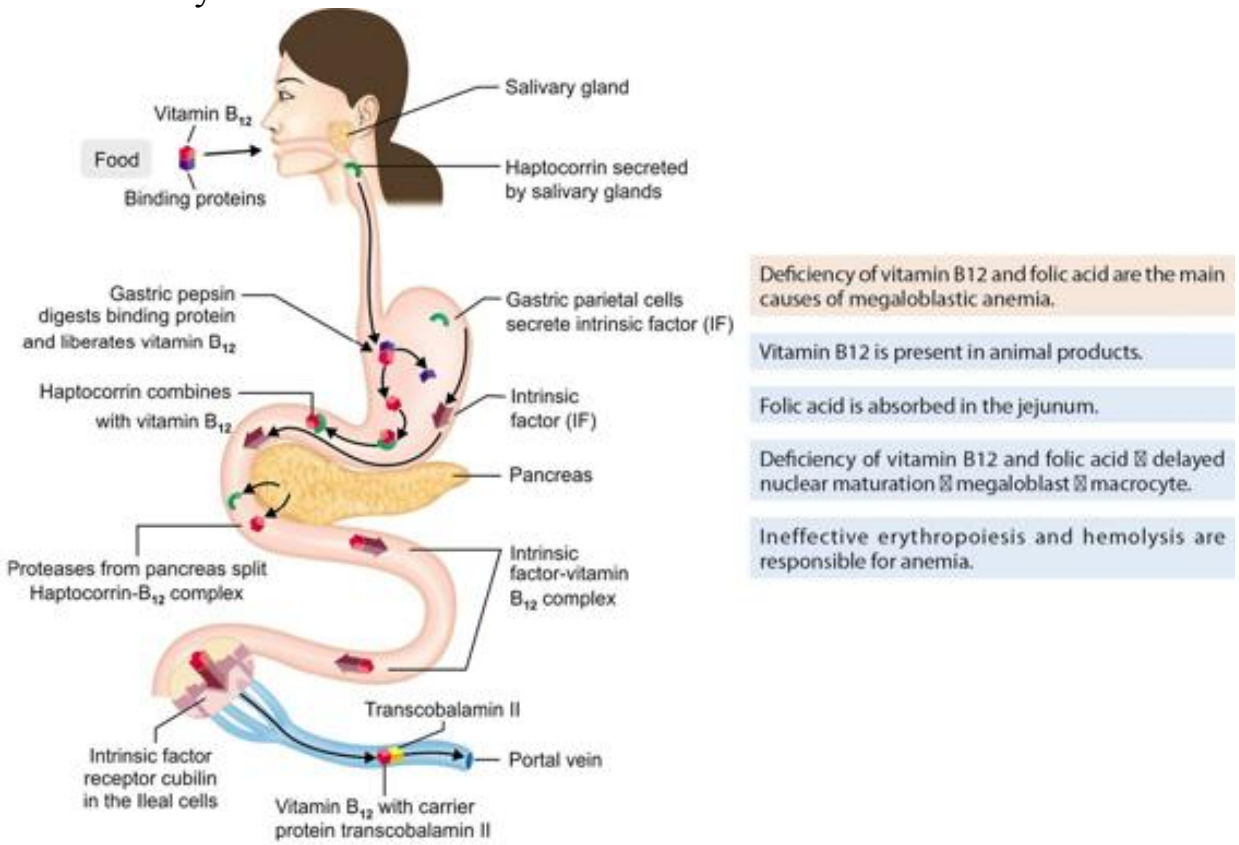


Fig. 11.7: Mechanism of vitamin B_{12} absorption.

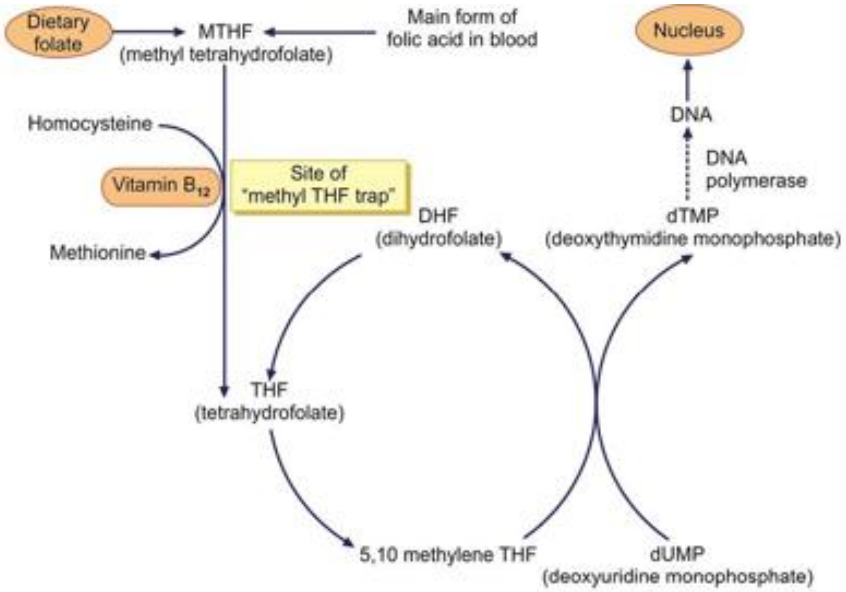


Fig. 11.8: Interrelation and role of vitamin B_{12} and folic acid in DNA synthesis.

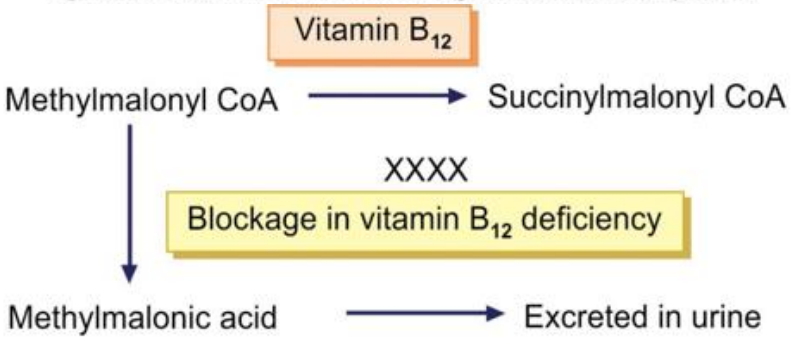


Fig. 11.9: Role of vitamin B_{12} in methylmalonyl CoA metabolism. Blocked in folic acid deficiency

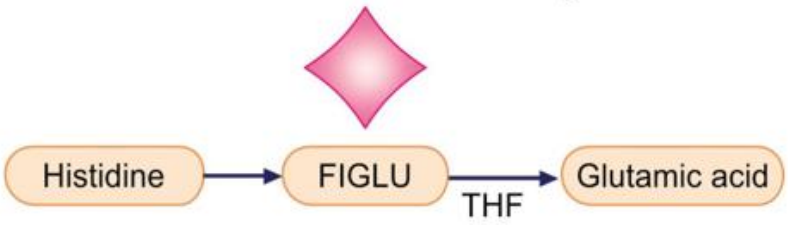


Fig. 11.10: Role of folic acid in metabolism of histidine. (FIGLU: formiminoglutamate).

Folic Acid Metabolism

Humans are entirely dependent on dietary sources for their folic acid requirement. The daily requirement is 50–200 mg. Green vegetables, yeast, legumes, fruits and animal proteins are the richest sources and most normal diets contain sufficient amounts of folic acid. The folic acid in these foods is largely in the form of polyglutamates. Polyglutamates are sensitive to heat

(thermolabile); boiling, steaming or frying and cooking destroys most of the folic acid. Intestinal conjugases split the polyglutamates into monoglutamates that are readily absorbed in the proximal jejunum. During intestinal absorption, they are modified to 5 methyltetrahydrofolate, the normal transport form of folic acid.

Role of Folic Acid (FA)

The active form of folic acid is tetrahydrofolate (THF) which is the biologic "middleman" involved in metabolic processes which synthesize DNA. The various reactions in which folic acid plays a main role are:

- Synthesis of purine (required for DNA and RNA).
- Conversion of homocysteine to methionine, a reaction also requiring vitamin B_{12}.
- Conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP): 5,10-methylene THF polyglutamate is required for conversion of dUMP to dTMP and DNA, a rate limiting step in pyrimidine synthesis **(Fig. 11.8)**.
- Metabolism of histidine: Histidine is metabolized to formiminoglutamic acid (FIGLU) which combines with THF to form glutamic acid **(Fig. 11.10).** In FA deficiency, this reaction cannot take place and therefore FIGLU accumulates and is excreted as such in urine. This is used as a test to measure folic acid deficiency.

Q. Discuss the causes and pathogenesis of megaloblastic anemia.

Causes of Megaloblastic Anemia

Box 11.6 shows the causes of megaloblastic anemia.

Pathogenesis of Megaloblastic Change

- **Impaired DNA synthesis:** Megaloblastic anemia is commonly due to deficiency of **vitamin B_{12} (cyanocobalamin) or folic acid.** Both are required for the synthesis of DNA.

- **Delayed maturation of nucleus**. The **nuclear maturation lags behind the cytoplasmic maturation** and results in **abnormally large** nucleated **erythroid precursors** named as **megaloblasts.**
- Cytoplasm matures normally**.** RBCs are larger than normal → **macrocytes**.
- Affects all rapidly dividing cells of the body (including skin, gastrointestinal tract, and bone marrow).

- **Ineffective erythropoiesis:** Megaloblast precursors undergo intramedullary destruction.

BOX 11.6: Causes of megaloblastic anemia.

Vitamin B_{12} Deficiency

1. **Decreased intake:** Inadequate diet, "pure vegetarians" (vegans)
2. **Impaired absorption**
   - **Gastric:** Deficiency of gastric acid or pepsin or intrinsic factor
     - Pernicious anemia
     - Post-gastrectomy
   - **Intestinal**
     - Loss of absorptive surface
       - Malabsorption syndromes
       - Diffuse intestinal disease, e.g., lymphoma, systemic sclerosis
       - Ileal resection, Crohn disease
     - Bacterial or parasitic competition for vitamin B_{12}
       - Bacterial overgrowth in blind loops and diverticula of bowel
       - Fish tapeworm infestation (*Diphyllobothrium latum*)
3. **Increased demand:** Pregnancy, hyperthyroidism, disseminated cancer

Folic Acid Deficiency

1. **Decreased intake:** Inadequate diet—alcoholism, malnutrition
2. **Impaired absorption**
   - Malabsorption states: Nontropical and tropical sprue
   - Diffuse infiltrative diseases of the small intestine (e.g., lymphoma)
   - Drugs: anticonvulsant phenytoin and oral contraceptives
3. **Increased loss:** Hemodialysis
4. **Increased demand:** Pregnancy, infancy, disseminated cancer, markedly increased hematopoiesis
5. **Impaired utilization:** Folic acid antagonists, such as methotrexate

Laboratory Findings of Megaloblastic Anemia

CC: Describe laboratory investigations of macrocytic anemia.

Q. Write short note on the laboratory findings/peripheral smear findings in megaloblastic anemia.

Blood findings in vitamin B_{12} and/or folic acid deficiency are similar.

Peripheral Blood

- **Hemoglobin and hematocrit (PCV): Reduced**
- **Red cell indices**
- **MCV:** Above 100 fL (normal 82–98 fL)
- **MCH** (normal 27–32 pg)
- **Normal MCHC** (31–36 g/dL)

Q. Write short note on macropolycyte.

Q. Write short note on oval macrocyte.

CC (Show How): Identify and describe the peripheral blood picture of macrocytic anemia.
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

PERIPHERAL SMEAR

Pancytopenia (decreased RBCs, WBCs and platelets).

- RBCs:
  - Macrocytic and oval (**egg-shaped macro-ovalocytes**)—**diagnostic**.
  - Most macrocytes **lack the central pallor (Figs. 11.11 and 11.12)**.
  - **Marked** variation in the size and shape of red cells **(anisopoikilocytosis**).
  - Evidence of dyserythropoiesis: Basophilic stippling, Cabot ring and Howell Jolly bodies.
- WBCs:
  - **Decreased** WBC count (leukopenia).
  - **Hypersegmented neutrophils** (more than five nuclear lobes): First and specific morphological sign of megaloblastic anemia. These neutrophils are also larger than normal (**macropolys**).
- Platelets: Decreased.

- **Reticulocyte count:** Normal or low.

Dimorphic Anemia

- Combined vitamin B_{12}/folic acid and iron deficiency.
- Peripheral smear shows two populations of RBCs namely—macro-ovalocytes and microcytic hypochromic **(Fig. 11.13).**

BONE MARROW

- Cellularity: Moderately to markedly hypercellular.
- M:E ratio: Due to marked erythroid hyperplasia, M:E ratio is reversed ranging from 1:1 to 1:6 (normal 2:1 to 4:1).

Q. Write short note on megaloblast.

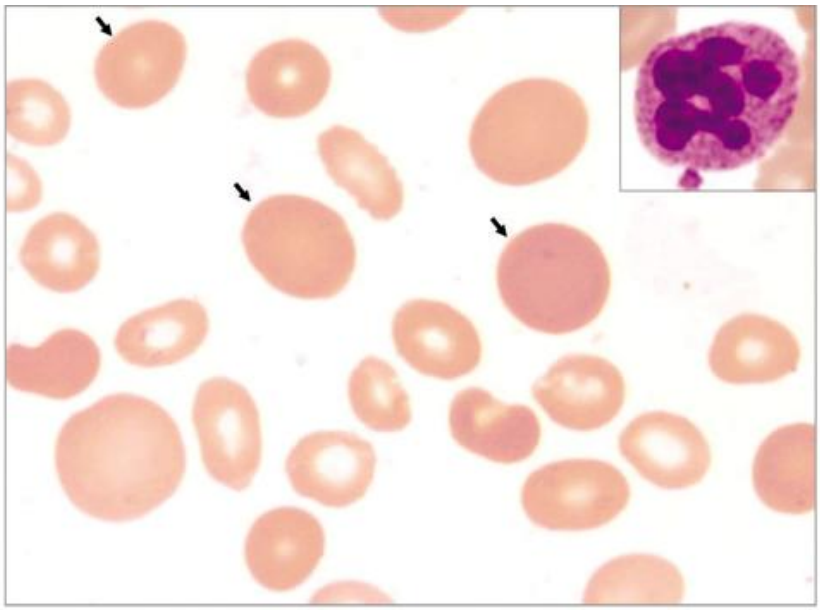


Fig. 11.11: Peripheral blood smear showing macro-ovalocytes (arrows) and hypersegmented neutrophil (inset).

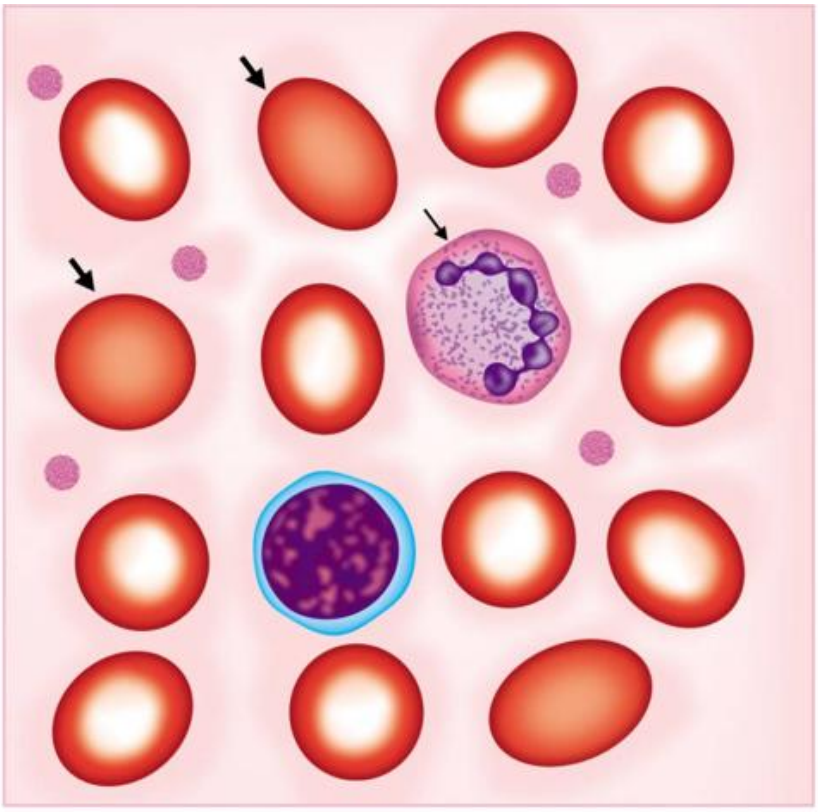


Fig. 11.12: Diagrammatic peripheral blood smear showing macro-ovalocytes (thick arrows) and hypersegmented neutrophil (thin arrow).

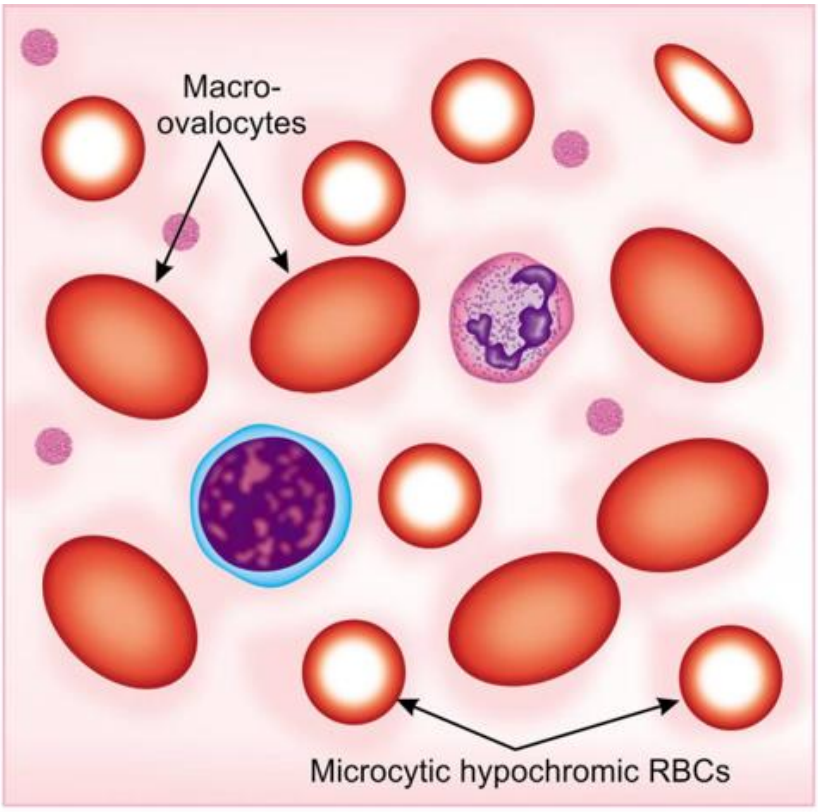


Fig. 11.13: Diagrammatic peripheral blood smear of dimorphic anemia showing macro-ovalocytes and microcytes.

- Erythropoiesis: Megaloblastic type **(Figs. 11.14 and 11.15)**
  - **Megaloblasts: Large, abnormal counterparts of normal normoblasts**. Megaloblast shows **asynchrony of nuclear and cytoplasmic maturation.** The cytoplasm shows normal hemoglobinization.
  - **Ineffective erythropoiesis:** Developing megaloblasts die in marrow (intramedullary hemolysis).
- Myelopoiesis:
  - Myeloid cells adequate in number.
  - Granulocytic precursors display nuclear-cytoplasmic asynchrony in the form of **giant metamyelocytes and band forms**.
- Megakaryopoiesis: Normal or increased in number.
- Bone marrow iron: Moderately increased.

The differences between normoblasts and megaloblasts are shown in **Table 11.8**.

Biochemical Tests for Megaloblastic Anemia

Common for Both Vitamin B_{12} andFolic Acid Deficiency

- Serum homocysteine
- Serum bilirubin: Mild increase causes mild jaundice
- Serum iron and ferritin
- Plasma lactate dehydrogenase (LDH)
- Serum vitamin B_{12}/folate decreased.

Diagnostic Tests for Vitamin B_{12} Deficiency

- Serum vitamin B_{12} levels: decreased
- Serum methylmalonic acid
- Urinary excretion of methylmalonic acid
- **Schilling test** for vitamin B_{12} absorption (*refer* page 302).

Deoxyuridine suppression test: It is a sensitive measure of deficiency of 5, 10-methylene THF, which occurs in both folic acid and vitamin B_{12} deficiency.

Specific Tests for Folic Acid Deficiency

- **Serum folic acid levels:** Decreased
- **FIGLU in urine: Excessively excreted.**

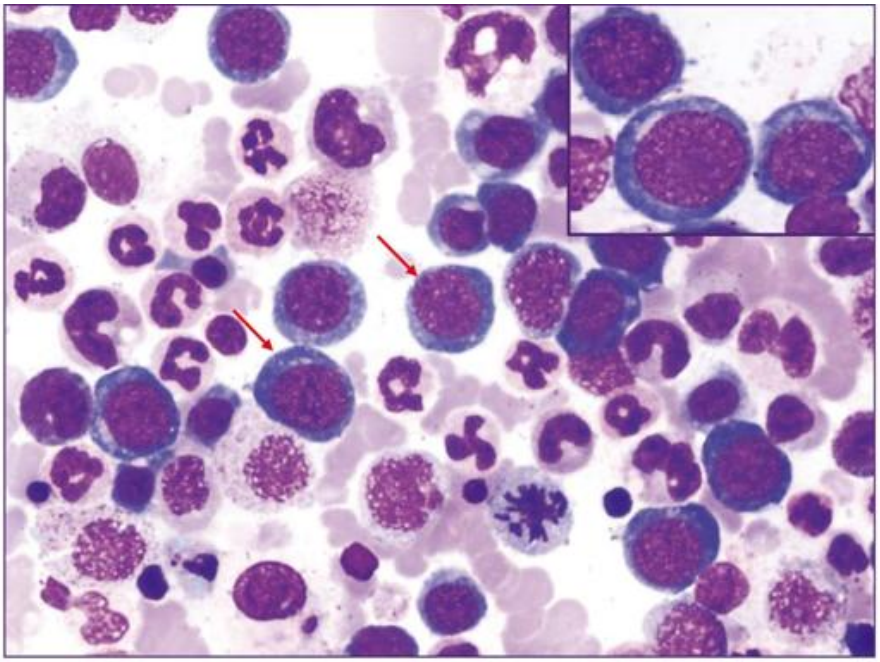


Fig. 11.14: Bone marrow aspirate showing megaloblastic precursors (arrows) in varying stages of maturation (inset shows early megaloblast).

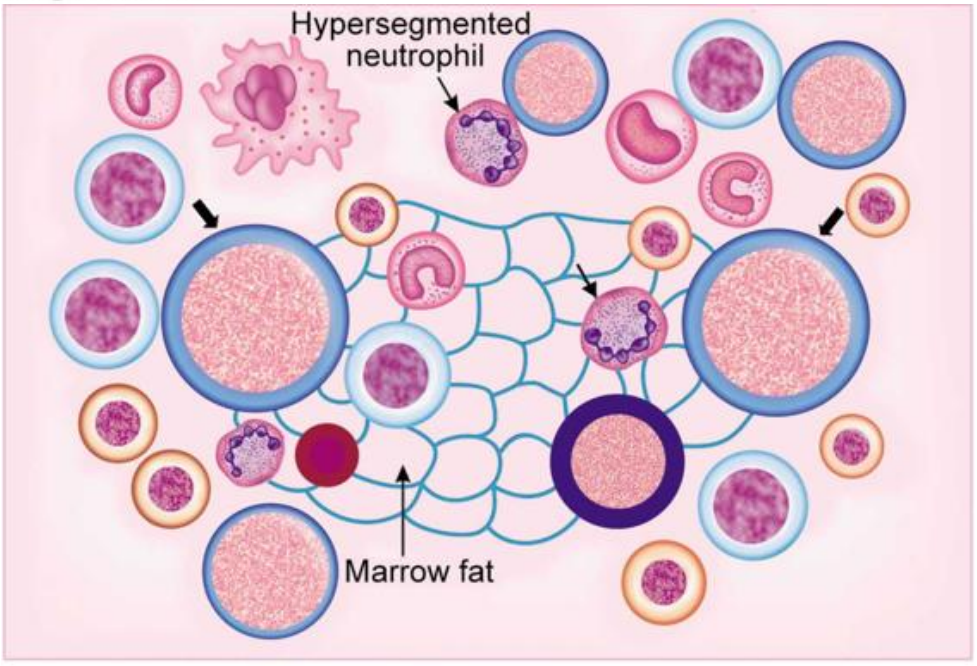


Fig. 11.15: Diagrammatic picture of bone marrow aspirate showing megaloblastic precursors (thick arrows) in varying stages of maturation.

Q. List the differences between normoblasts and megaloblasts.

TABLE 11.8: Differences between normoblasts and megaloblasts.

Characteristics	*Normoblast*	*Megaloblast*
Cell size	Normal	Larger than corresponding normoblast
Nuclear chromatin	Normal	Open sieve-like
Nuclear maturation	Normal	Lags behind cytoplasmic maturation
Mitosis	Normal	Increased and abnormal
Maturation in bone marrow	Normal (Late > intermediate > early normoblast)	Increased proportion of more primitive erythroid cells (Late < intermediate < early megaloblast)
Evidence of dyserythropoiesis	Absent	Present (irregular nuclei, Howell Jolly bodies)
Myelopoiesis	Normal	Shows giant metamyelocytes
Found in	Normal bone marrow	Bone marrow of megaloblastic anemia

Megaloblasts:
- Nuclear maturation lags behind cytoplasmic maturation.
- Nuclei have open sieve-like chromatin.

Megaloblastic anemia- bone marrow:
- Megaloblasts
- Giant metamyelocytes.

PERNICIOUS ANEMIA

Q. Define macrocytic anemia. Describe the laboratory diagnosis of pernicious anemia.

Q. Discuss the etiopathogenesis and morphology of pernicious anemia.

Definition: Pernicious anemia (PA) is an autoimmune disease due to **deficiency of intrinsic factor** causing **impaired absorption of vitamin B_{12}** and megaloblastic anemia.

Rare in India. A genetic predisposition is suspected.
Age: older age—**fifth to eighth decades** of life.
Sex: females are more involved than males (F:M is 1.5:1).

Etiopathogenesis

- An **autoimmune** disease due to destruction of gastric mucosa.
- Stomach shows **damage to parietal cells**, dense infiltration by lymphocytes and plasma cells → **chronic atrophic gastritis → failure of production of intrinsic factor**.

- **Presence of autoantibodies: Two major types** of autoantibodies:
  - **Anti-intrinsic factor (IF) antibody**
    - **Type I (blocking): antibody:** Blocks the binding of vitamin B_{12} to IF. Present in 50–75% of the cases.
    - **Type II (binding) antibody:** attaches to the IF–vitamin B_{12} complex and prevent its binding to receptors in the ileum. Present in about 40% of patients.
  - **Parietal cell (Type III) antibody:** Neither specific for PA nor other autoimmune disorders. It is found in 90% of patients.

MORPHOLOGY

Alimentary System

- Atrophic glossitis: Tongue shiny, glazed and beefy.
- Stomach:
  - **Diffuse chronic atrophic gastritis** and impaired secretion of hydrochloric acid, pepsin and intrinsic factor.
    - Histologically, atrophy of the glands, with loss of both chief cells and parietal cells.
    - Nuclei of mucosal cells look similar to that of megaloblasts.
    - Dense infiltration by lymphocytes and plasma cells.
  - Intestinal metaplasia.

Central Nervous System

Found in 75% of cases.

- Demyelination in the dorsal and lateral tracts: Subacute combined degeneration.
- Peripheral neuropathy.

Laboratory Findings (Fig. 11.16)

Q. Write short note on laboratory findings in pernicious anemia.

Blood, bone marrow and biochemical test findings are similar to those described earlier for megaloblastic anemias (*refer* pages 300-2).

Specific Diagnostic Tests for Pernicious Anemia

Q. What is Schilling test?

- **Schilling test for vitamin B_{12} absorption: abnormal**
  - Radioactive vitamin B_{12} is used to assess the status of intrinsic factor (IF) and vitamin B_{12}.
  - Helps in distinguishing megaloblastic anemia due to IF deficiency (pernicious anemia) from other causes of vitamin B_{12} deficiency.
- **Serum antibodies to intrinsic factor a**re highly specific for pernicious anemia
- **Achlorhydria** with histamine/pentagastrin stimulation.
- Severe **deficiency of intrinsic factor**.

Clinical Features of Megaloblastic Anemia

Q. Mention the various clinical features of megaloblastic anemia.

The clinical features of vitamin B_{12} deficiency anemia and pernicious anemia are:

- **Onset: insidious** and progresses slowly.
- **Classic triad of presentation:** weakness, sore throat and paresthesias.
- **Tongue:** Painful red **"beefy"** tongue.
- **Neurological manifestations.**

Q. Write short note on effects of vitamin B_{12} deficiency on the nervous system.

- **Bilateral peripheral neuropathy:** Glove and sock distribution of **numbness or paresthesia.**

- **Demyelination of spinal cord: Subacute combined demyelination/degeneration of dorsal and lateral tracts—ataxia**, uncoordinated gait, impairment of vibration and position sense.

- **Atherosclerosis:** Serum homocysteine level is raised and is a **risk factor for atherosclerosis** and thrombosis.

Macrocytic Anemia

NC: Enumerate the differences and describe the etiology and distinguishing features of megaloblastic and non-megaloblastic macrocytic anemia.

Macrocytic anemia is characterized by the presence of abnormally large RBCs in the peripheral blood. This is usually recognized by the automated blood cell counter and confirmed on examination of the peripheral blood smear. Macrocytic anemia can be divided into two categories, megaloblastic and nonmegaloblastic, based on the examination of the bone marrow. This division is important and frequently helps in determining the etiology of the anemia. Causes of megaloblastic anemia is presented in **Box 11.6.**

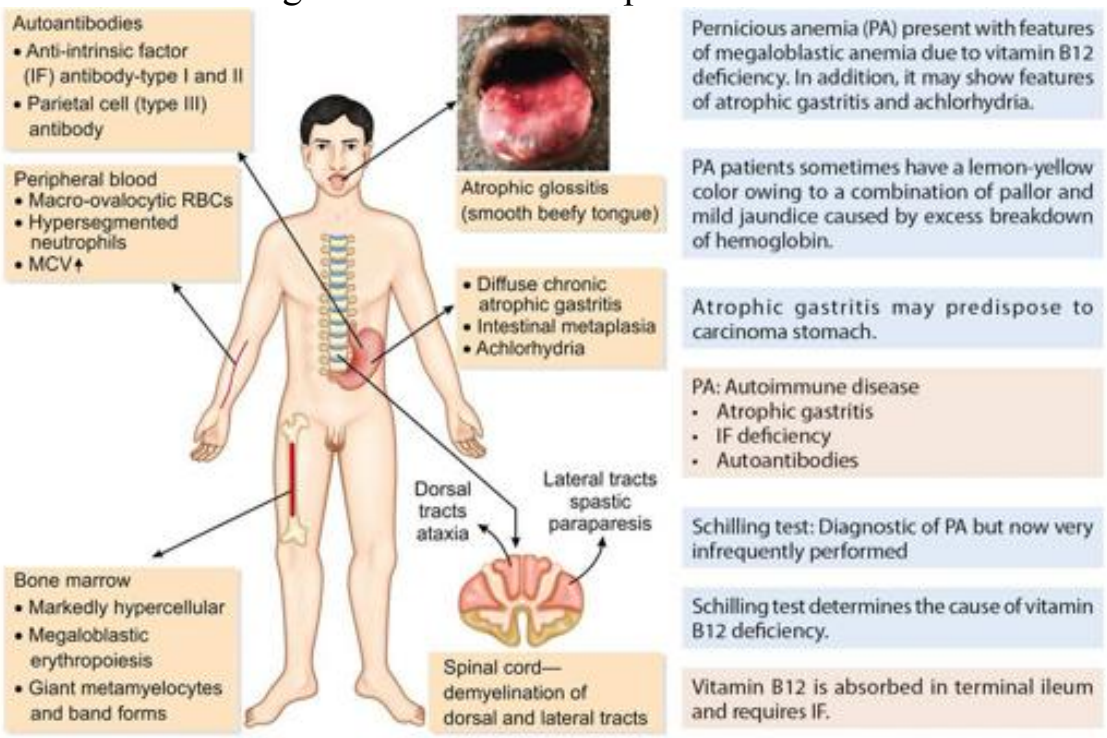


Fig. 11.16: Clinical features and laboratory findings in pernicious anemia.

Nonmegaloblastic Causes of Macrocytic Anemia

Box 11.7 shows the etiology of nonmegaloblastic macrocytic anemia.

Investigation of Macrocytosis

The evaluation of macrocytosis needs a systemic approach and consists of comprehensive history and physical examination followed by appropriate laboratory investigations. Laboratory studies include a complete blood count, a peripheral blood smear and reticulocyte count. In certain cases, it may be necessary to perform bone marrow examination.

Peripheral blood smear: Examination of the peripheral smear is important for determination of the etiology of macrocytosis.

- The presence of **macro-ovalocytes having an MCV >115 fL,**anisocytosis, poikilocytosis and **hypersegmented neutrophils** suggests a **megaloblastic anemia** due to a nutritional deficiency (i.e., vitamin B_{12} or folate deficiency).
- Round macrocytes are commonly seen in many chronic disorders.
- Round target-appearing macrocytes are characteristic of liver disease such as hepatitis, obstructive jaundice, and acute and chronic alcoholism with liver disease.

Reticulocyte count: The presence of increased polychromasia of the macrocytes on the peripheral smear and a reticulocyte count of >10% should raise suspicion of hemolysis or an acute bleed. The large polychromatophilic RBCs on the peripheral smear represent reticulocytes, immature RBCs that are larger than mature RBCs. Their presence indicates increased erythropoiesis or RBC production and, if present in increased number, can raise MCV.

Bone marrow examination: Macrocytosis associated with a megaloblastic marrow is usually associated with anemia due to ineffective erythropoiesis (*refer* bone marrow findings in megaloblastic anemia on pages 300-2). Nonmegaloblastic macrocytic anemia does not show any megaloblasts in the marrow.

Other investigations: They depend on the probable cause of nonmegaloblastic macrocytic anemia.

BOX 11.7: Etiology of nonmegaloblastic macrocytic anemia.

- Physiological causes (macrocytosis without anemia)
  - Pregnancy
  - Newborn
- Pathological causes (macrocytosis with associated anemia)

- Alcohol excess
- Chronic liver disease
- Reticulocytosis
- Hypothyroidism
- Post-splenectomy
- Myeloproliferative disorders
- Hematological disorders
  - Aplastic anemia
  - Sideroblastic anemia
  - Pure red cell aplasia
- Drugs
  - Azathioprine
  - Hydroxycarbamide

Megaloblastic anemia
- Pancytopenia
- Macro-ovalocytes
- Hypersegmented neutrophils
- Macropolys

In megaloblastic anemia due to vitamin B12 deficiency, reticulocyte count may be normal or low and high reticulocyte count is seen on 7th day following vitamin B12 therapy.

A mixture of microcytic hypochromic and macrocytic RBCs is termed as dimorphic picture and occurs in mixed deficiency of iron and folic acid or vitamin B12.

Megaloblasts are large, abnormal precursors of RBCs seen in the bone marrow of patients with megaloblastic anemia.

Deoxyuridine suppression test is abnormal even before the morphological changes.

Folate deficiency anemia presents with features of megaloblastic anemia due to vitamin B12. Unlike with vitamin B12 deficiency, neurological symptoms does not occur.

Q. Write short note on causes of macrocytic anemia.

Megalobalstic anemia: Vitamin B12 and folic acid

Nonmegaloblastic causes of macrocytic anemia:
- Alcohol
- Liver disease
- Myxedema
- Cytotoxic drugs
- Myeloma
- Aplastic anemia
- Reticulocytosis
- Red cell aplasia

APLASTIC ANEMIA

NC: Enumerate the etiology, pathogenesis and findings in aplastic anemia.

Q. Write short note on aplastic anemia.

Definition: Chronic primary hematopoietic stem cell (HSC) disorder characterized by:

- Pancytopenia (anemia, neutropenia and thrombocytopenia).
- With markedly hypocellular bone marrow (less than 30% cellularity).

Etiology

Q. Write short note on causes of aplastic anemia.

The most common causes associated with aplastic anemia are shown in **Box 11.8.**

Pathogenesis (Fig. 11.17)

- Direct damage to the hematopoietic stem cells and progenitor cells.
- Immune-mediated destruction
- Primary stem cell abnormality—inherited defect in the stem cells.

Clinical Features

- Any age of both sexes
- Insidious
- Progressive weakness, pallor and dyspnea due to anemia.
- Frequent (mucocutaneous bacterial infections) or fatal infections due to neutropenia.
- Bleeding manifestations in the form of petechiae, bruises and ecchymoses due to thrombocytopenia.

Laboratory Findings

Peripheral Blood

- **Hemoglobin**
- **Packed cell volume (PCV)**

BOX 11.8: Common causes of aplastic anemia.

1. **Acquired**

   Idiopathic
   - Acquired defects in stem cell
   - Immune mediated

   Secondary

   Chemical Agents
   - Cytotoxic drugs: Alkylating agents, antimetabolites
   - Inorganic arsenicals
   - Benzene
   - Chloramphenicol

   Idiosyncratic
   - Chloramphenicol
   - Penicillamine
   - Gold salts
   - Methylphenylethyl hydantoin
   - Phenylbutazone
   - Carbamazepine
   - Organic arsenicals

   Physical Agents: Whole-body irradiation

   Viral Infections: Hepatitis virus, Epstein-Barr virus, cytomegalovirus, herpes zoster (Varicella-zoster), HIV

2. **Inherited:** Fanconi anemia, telomerase defects

6 "I"s of the causes of aplastic anemia:
1. Idiopathic
2. Ingestion of drugs and chemicals
3. Idiosyncratic
4. Irradiation
5. Infections
6. Inherited

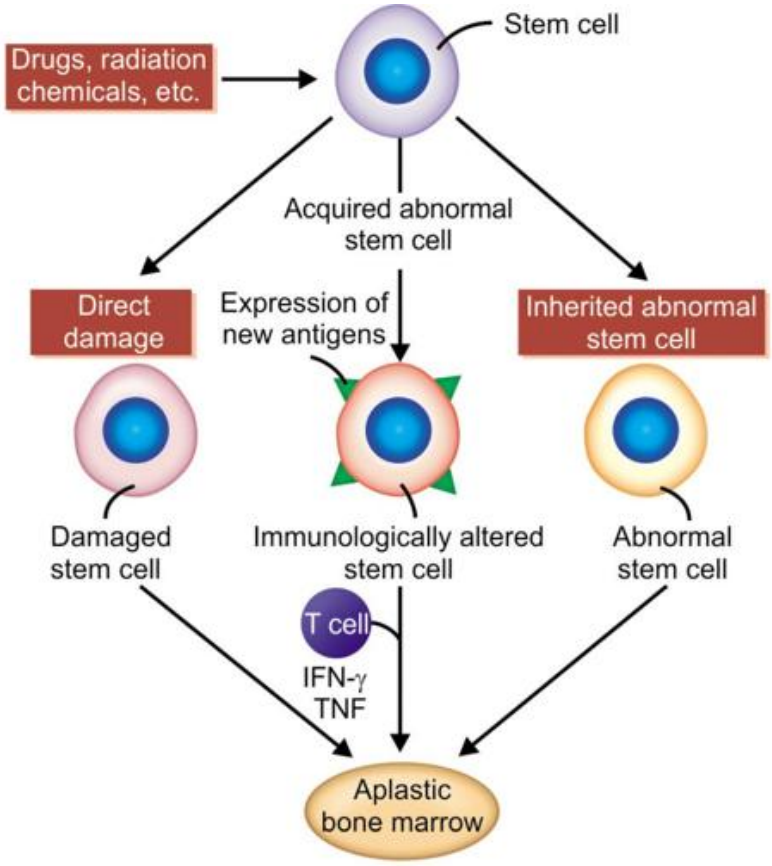


Fig. 11.17: Pathogenesis of aplastic anemia.

- **Reticulocyte count:** Markedly decreased.

Q. Write short note on peripheral smear in aplastic anemia.

PERIPHERAL SMEAR

Pancytopenia, i.e., decreased red cells, neutrophils and platelets.

- RBCs: Normocytic normochromic anemia
- WBCs: Total leukocyte count decreased. Neutrophils markedly diminished and neutropenia is a reflection of the severity of aplasia. Initial stages, lymphocytes normal in number as the disease progresses their count decreases.
- Platelets: Count is decreased.

BONE MARROW

Marrow aplasia—best appreciated in a **bone marrow (trephine) biopsy**

- Cellularity: Marked hypocellularity.
- Hematopoiesis: Paucity of all erythroid, myeloid and megakaryocytic precursors.
- Other cells: Lymphocytes and plasma cells are prominent.

No Splenomegaly

Diagnosis: Diagnosis is made with **peripheral blood** and **bone marrow biopsy findings.**

Differential Diagnosis

Pancytopenia

Definition: Pancytopenia is defined as a condition in which there is a reduction in the number of red and white blood cells, as well as platelets.

Q. Write short note on pancytopenia.

- Should be distinguished from other causes of pancytopenia **(Box 11.9).**

BOX 11.9: Causes of pancytopenia.

Decreased bone marrow function

- Aplastic anemia
  - Idiopathic
  - Secondary
  - Inherited
- Myelodysplastic syndromes
- Bone marrow infiltration with:
  - Leukemia
  - Lymphoma
  - Myeloma
  - Tumors (carcinoma)
  - Granulomatous diseases (e.g., tuberculosis, sarcoidosis)
- Nutritional deficiencies:
  - Megaloblastic anemia (vitamin B_{12} and folic acid deficiency)
- Paroxysmal nocturnal hemoglobinuria
- Myelofibrosis (rare)
- Hemophagocytic syndrome

Increased peripheral destruction

- Hypersplenism

Prognosis: Unpredictable.

Aplastic anemia: Bone marrow elements are replaced by fat and aspiration usually yields dry tap. Reticulocyte count is markedly low in aplastic anemia and is characteristic feature. Absence of splenomegaly and in its presence the diagnosis of aplastic anemia should not be made.

HEMOLYTIC ANEMIAS

CC: Define and classify hemolytic anemia.

Q. Define and enumerate the causes/classify hemolytic anemia.

Definition

Hemolytic anemias are due to **increase in the rate of red cell destruction** (hemolysis).

Classification of Hemolytic Anemias

Box 11.10 shows the classification and causes of hemolytic anemia.

Q. Enumerate the causes of hemolytic anemia. Discuss the laboratory diagnosis of hemolytic anemia in general.

Depending on:

- **Location of hemolysis: Intravascular and extravascular**
- **Source of defect causing hemolysis: Intracorpuscular defect** and **extracorpuscular defect**
- **Mode of onset: Hereditary and acquired disorders.**
- **Underlying mechanisms of hemolysis (Box 11.10).**

Location of Hemolysis

It may be intravascular and/or extravascular. The differences between these two types are listed in **Table 11.9**.

BOX 11.10: Classification and causes of hemolytic anemia.

Inherited Genetic Defects
- *Red cell membrane disorders:* Hereditary spherocytosis, hereditary elliptocytosis
- *Enzyme deficiencies*
  - **Hexose monophosphate shunt enzyme deficiencies:** G6PD deficiency
  - **Glycolytic enzyme deficiencies:** Pyruvate kinase deficiency, hexokinase deficiency
- **Hemoglobin abnormalities**
  - **Deficient globin synthesis:** Thalassemia syndromes
  - **Structurally abnormal globins (hemoglobinopathies):** Sickle cell disease

Acquired Genetic Defects
- *Deficiency of phosphatidylinositol-linked glycoproteins:* Paroxysmal nocturnal hemoglobinuria

Antibody-mediated Destruction (immunohemolytic anemias)
- *Isohemagglutinins:* Hemolytic disease (Rh disease) of the newborn, transfusion reactions
- *Autoantibodies:* Idiopathic (primary), drug-associated, systemic lupus erythematosus

Mechanical Trauma to RBCs (Fragmentation syndrome)
- *Microangiopathic hemolytic anemias:* Hemolytic uremic syndrome, disseminated intravascular coagulation, thrombotic thrombocytopenia purpura
- *Cardiac traumatic hemolysis:* Defective cardiac valves

Infections of Red Cells: Malaria, babesiosis
Toxic or Chemical Injury: Clostridial sepsis, snake venom, lead poisoning
Sequestration: Hypersplenism

(G6PD: glucose-6-phosphate dehydrogenase).

Decreased red cell survival does not always cause anemia as there is a compensatory increase in red cell production by the bone marrow.

Laboratory Findings in Hemolytic Anemias

CC: Describe the pathogenesis and clinical features and hematologic indices of hemolytic anemia.

Q. Write short essay/note on laboratory findings in hemolytic anemias.

Q. Write short essay/note on peripheral blood picture of hemolytic anemias.

Peripheral Blood

CC: Describe the peripheral blood picture in different hemolytic anemias.

Hemoglobin: It is decreased and varies with the type and duration of hemolytic anemia.

Reticulocyte count: Increased.

CC: Perform and show how to prepare a peripheral blood smear (*refer* Appendix 3) and identify hemolytic anemia from it. Skill assessment: Procedure require certification.

Q. List the differences between extravascular hemolysis and intravascular hemolysis.

TABLE 11.9: Differences between extravascular and intravascular hemolysis.

Characteristics	*Extravascular hemolysis*	*Intravascular hemolysis*
Site of hemolysis	RE system (spleen, bone marrow)	Within circulation
Splenomegaly	Usual	Uncommon
Laboratory findings		
• Serum bilirubin-unconjugated	Moderately raised	Mildly raised
• Serum haptoglobin	Normal	Decreased
• Hemoglobinemia	Not seen	Positive
Urine		
• Hemoglobinuria	Absent	Present
• Hemosiderinuria	Absent	Present
Examples	Thalassemia, sickle cell anemia	G6PD deficiency, PNH

(RE: reticuloendothelial; G6PD: glucose-6-phosphate dehydrogenase; PNH: paroxysmal nocturnal hemoglobinuria).

In most hemolytic anemias red cell destruction is extravascular.

PERIPHERAL SMEAR

It is the most important investigation in hemolytic anemia. The following findings alone or in combination suggest hemolysis:

- **Red blood cells (RBCs):** They show markedly increased **reticulocyte count,** which appear as large **polychromatophilic** red blood cells in the peripheral blood. Moderate to marked hemolysis results in appearance of **nucleated red cells**, mostly late normoblasts. Red cell morphology provides a clue to the underlying hemolytic disorder like: spherocyte, sickle cell, target cell, acanthocyte, schistocyte, malarial parasite, etc.
- **White blood cells: Neutrophilia** with shift to left (increase in the percentage of immature/young neutrophils in the circulating blood) and presence of metamyelocytes and myelocytes is seen in active hemolysis.
- **Platelets:** In acute hemolysis, there is **thrombocytosis** with numerous large platelets.

BONE MARROW

Bone marrow examination is usually not necessary for the diagnosis of hemolytic anemia.

- **Cellularity:** Due to erythroid hyperplasia, the overall cellularity of the bone marrow is increased.
- **Erythropoiesis:** Anemia with tissue hypoxia stimulates increased production of erythropoietin, which causes erythroid hyperplasia in bone marrow. Erythroid hyperplasia is the morphological hallmark of various hemolytic anemias and is characterized by **increased number of erythroid precursors (normoblasts) in the marrow.**
- **M:E ratio:** The myeloid-erythroid ratio is decreased with a reversal ranging from 1:1 to 1:6.

Extramedullary Hematopoiesis

- Extramedullary hematopoiesis develops when marrow erythroid hyperplasia is not able to ameliorate moderate to severe anemia because of **"ineffective erythropoiesis".** It can appear in the liver, spleen and lymph nodes.
- Mostly found in hereditary hemolytic anemias like thalassemia and sickle cell anemia. X-ray of the bone show expansion of marrow space especially in tubular bones and skull.

Features of Increased Red Cell Destruction

- Increased unconjugated bilirubin in blood: **Jaundice.**
- **Increased stercobilinogen** in stool causing dark-colored stool.
- **Increased urobilinogen in urine** leading to **high-colored urine.**
- **Increased iron stores**-iron released from heme is stored in bone marrow.
- **Characteristic findings of anemia due to intravascular hemolysis**.
  - **Hemoglobinemia**

- **Decreased serum haptoglobin:** It is **characteristic of intravascular hemolysis**
- **Hemoglobinuria**
- **Hemosiderinuria**
- **Plasma lactate dehydrogenase (LDH):** Increased.

Features of Increased Red Cell Production

- Anemia and resultant tissue hypoxia causes increased erythropoietin production by kidney.
- **Peripheral blood** (mentioned above)
- **Bone marrow:** Increased erythropoietin stimulates bone marrow and produces **compensatory erythroid hyperplasia**. Bone marrow hyperplasia leads to **increased reticulocytes** in the peripheral blood.

Features of Damaged Red Cells

- **Morphological features:** These include presence of **microspherocytes, elliptocytes**, **red cell fragments**, etc. in the peripheral blood.
- **Lifespan of red cells:** Red cell survival is **shortened** and can be detected by 51Cr-labeled method.
- Other tests include: Osmotic fragility test, autohemolysis test, antiglobulin tests, electrophoresis for abnormal hemoglobins, estimation of HbA_2, HbF, sickling test and screening test for G6PD deficiency.

Laboratory features of hemolytic anemia are summarized in **Box 11.11**.

HEREDITARY SPHEROCYTOSIS

Q. Classify hemolytic anemia. Discuss the etiology and diagnosis of hereditary spherocytosis. Mention conditions in which spherocytes are found in peripheral smear.

Definition: Hereditary spherocytosis (HS) is a rare **inherited hemolytic anemia** resulting from the **defect in the red cell membrane**.

Normal structure of RBC membrane is depicted in **Figure 11.18**.

BOX 11.11: Summary of laboratory features of hemolytic anemia.

1. **Features/consequences of increased RBC destruction/ breakdown**
   - Anemia
   - Unconjugated hyperbilirubinemia (jaundice)
   - Increased urobilinogen in urine (resulting from bilirubin breakdown in the intestine) leading to high colored urine
   - Shortened red cell lifespan (demonstrated by 51Cr-labeled red blood cells).
   - Other features:
     - In extravascular hemolysis: Splenomegaly is usual
     - In intravascular hemolysis:
       - Decreased plasma haptoglobin and hemopexin
       - Increased plasma lactic dehydrogenase (LDH), hemoglobinemia, hemoglobinuria, hemosiderinuria and methemoglobinemia (in some).
2. **Consequences of increased RBC production**
   - Peripheral smear: Reticulocytosis
   - Bone marrow: Erythroid hyperplasia
3. **Morphological features of damaged red cells**
   - Peripheral smear (e.g., microspherocytes, elliptocytes, red cell fragments)

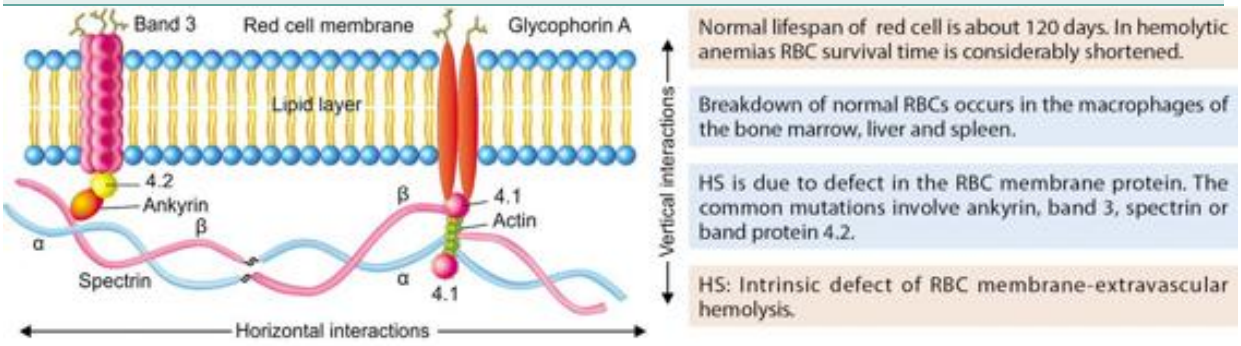


Fig. 11.18: Structure of the red cell membrane.

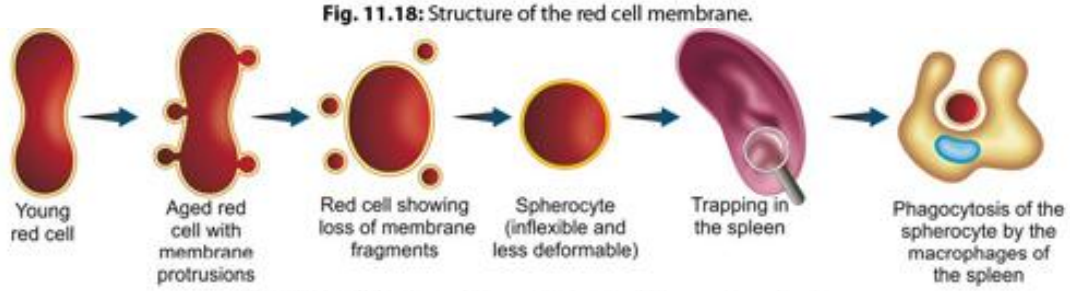


Fig. 11.19: Mechanism of hemolysis in hereditary spherocytosis.

Etiopathogenesis

Q. Describe the etiopathogenesis/molecular pathology of hereditary spherocytosis.

- **Autosomal dominant** disorder
- RBC membrane protein defect caused by various **mutations**. Most common mutations involve **ankyrin, band 3, spectrin, or band protein 4.2**.

Mechanism of Hemolysis in HS (Fig. 11.19)

- Young HS RBCs are normal in shape. But as they age, they undergo **loss of membrane fragments in the circulation.** These small **RBCs assume a spherical shape (spherocytes).**
- **Spherocytes are rigid, inflexible and less deformable.** They get trapped in the spleen leading to **premature destruction** of spherocytes.

Laboratory Findings

Q. Write short notes on laboratory findings in hereditary spherocytosis.

Peripheral Blood

- **Hemoglobin: Decreased** and level depends on degree of hemolysis.
- **Red cell indices**
  - **MCV: Reduced** (normal 82–98 fL)
  - **MCHC: Raised and >35 g/dL** (normal 31–36 g/dL).

Q. Write short note on spherocyte.

PERIPHERAL SMEAR

Peripheral smear examination is very important for diagnosis **(Figs. 11.20 and 11.21).**

- RBCs:
  - **Spherocytes** are **most distinctive** but **not pathognomonic**. Spherocytes are **small, dark-staining** (hyperchromic) **RBCs without any central pallor.**
  - Polychromatophilia due to reticulocytosis.
- WBCs: Total leukocyte count (TLC) increased.
- Platelets: Normal.

- **Reticulocyte count: Increased (Fig. 11.22)**.

BONE MARROW

- Cellularity: Markedly hypercellular
- Erythropoiesis: Erythroid hyperplasia
- Myelopoiesis: Normal
- Megakaryopoiesis: Normal.

Autohemolysis Test

In this test, blood is incubated at 37°C for 48 hours and amount of hemolysis noted. Hereditary spherocytes shows marked increase in spontaneous autohemolysis (10–15% of red cells compared to normal which is less than 4%) and may be partially corrected by addition of glucose.

Direct Antiglobulin (Coomb's) Test

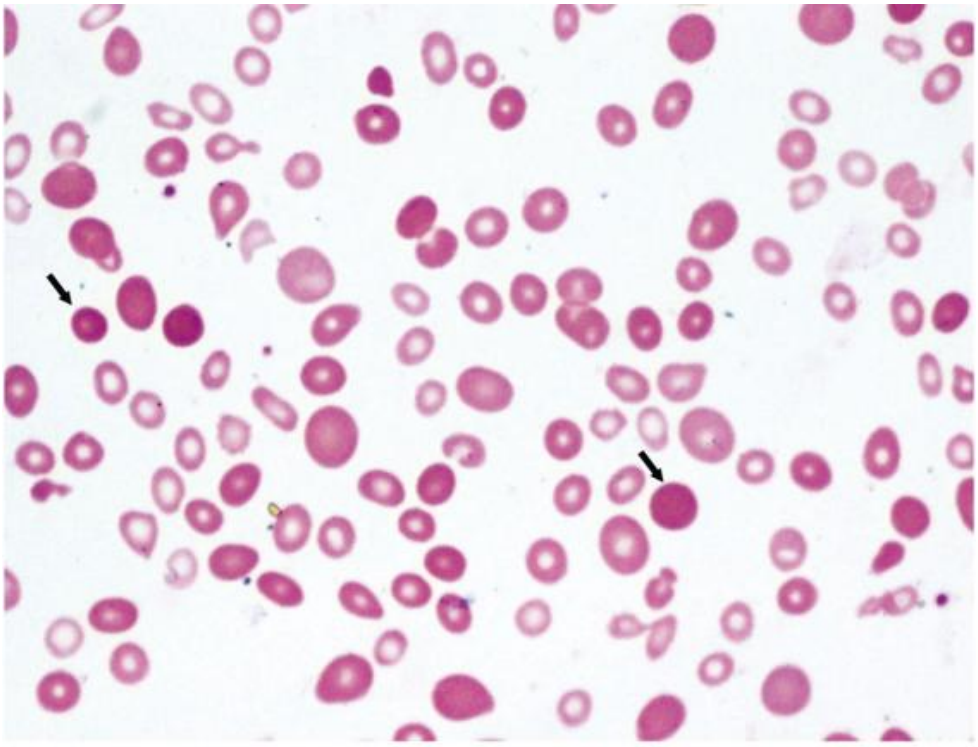


Fig. 11.20: Peripheral blood smear with numerous spherocytes (arrows).

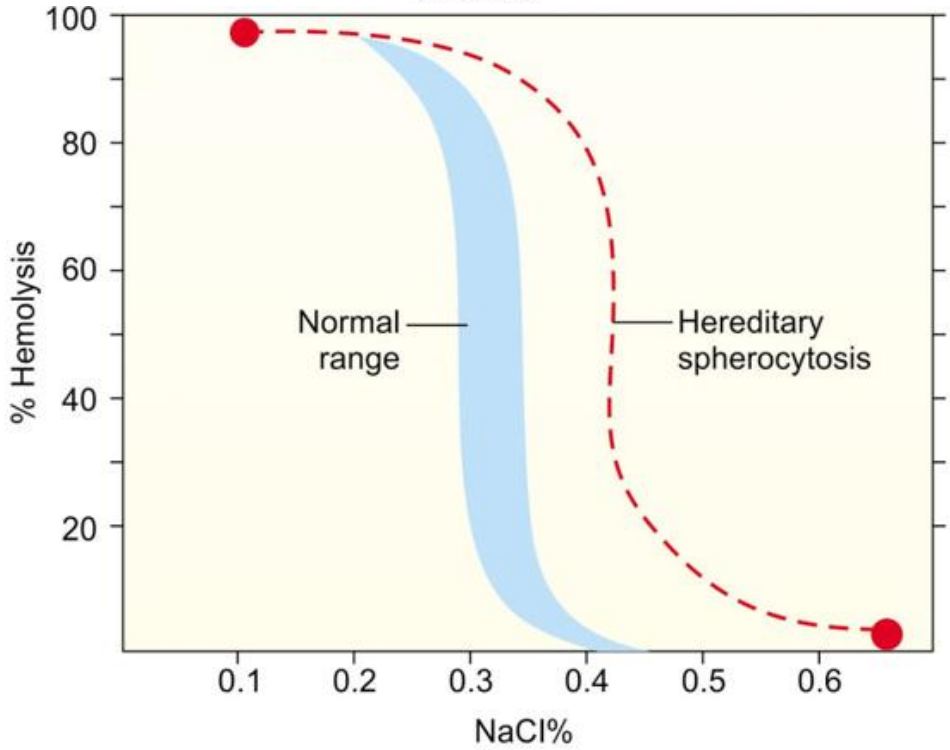


Fig. 11.23: Osmotic fragility test. Normal curve (blue) and increased osmotic fragility in hereditary spherocytosis.

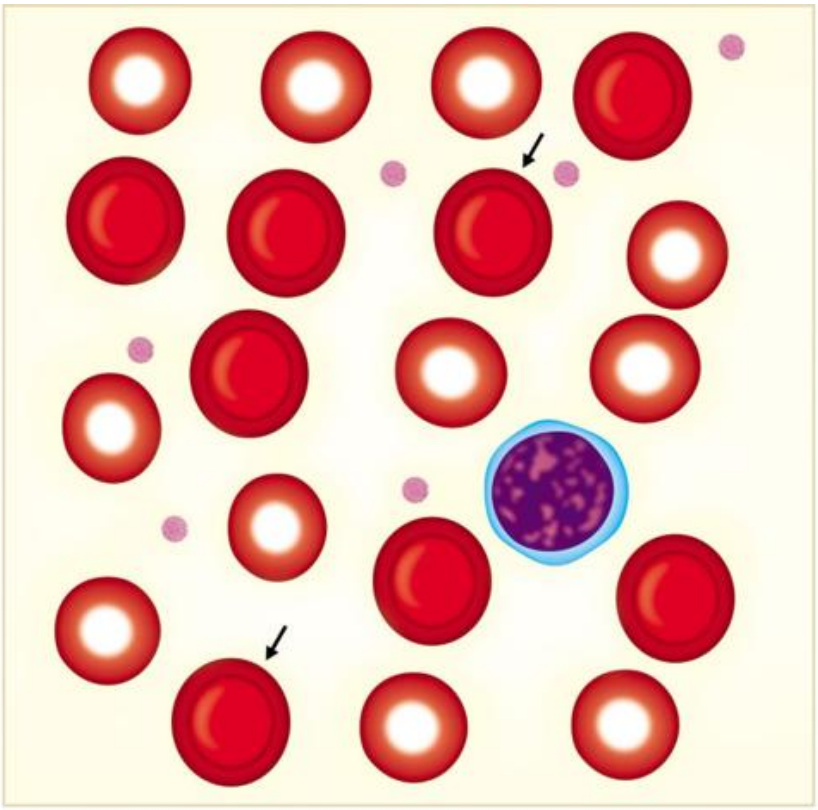


Fig. 11.21: Diagrammatic peripheral blood smear with numerous spherocytes (arrows).

It is negative and helps to distinguish from acquired immunohemolytic anemia where it is positive (for details, *refer* pages 322-3).

Biochemical Findings

- **Serum bilirubin: Mildly raised**
- **Urine urobilinogen: Increased**
- **Serum haptoglobin: Decreased**

Osmotic Fragility Test

Osmotic fragility (for details, *refer* pages 390-1) **is increased and there is shift of the curve to the right (Fig. 11.23)**.

Clinical Features

- **Age:** Anytime from the **neonatal** period **to adulthood**.
- **Family history:** Most (75%) are inherited as **autosomal dominant trait**.

- **Anemia:** Mild to moderate
- **Jaundice: Intermittent attacks**, precipitated by pregnancy, fatigue, or infection.
- **Splenomegaly: Moderate** (500 to 1000 g)
- **Gallstones: Pigment** gallstones
- **Aplastic crises:** May be triggered by an acute **parvovirus** infection.

In hereditary spherocytosis MCHC is >35 g/dL.

Q. Causes of spherocytes in peripheral smears.

Spherocytes and reticulocytosis are observed in the peripheral blood.

Spherocytes are seen in hereditary spherocytosis. They may also be seen in autoimmune hemolytic anemia, ABO hemolytic disease of newborn, hypersplenism, hemolytic transfusion reaction and burns.

Bone marrow shows erythroid hyperplasia.

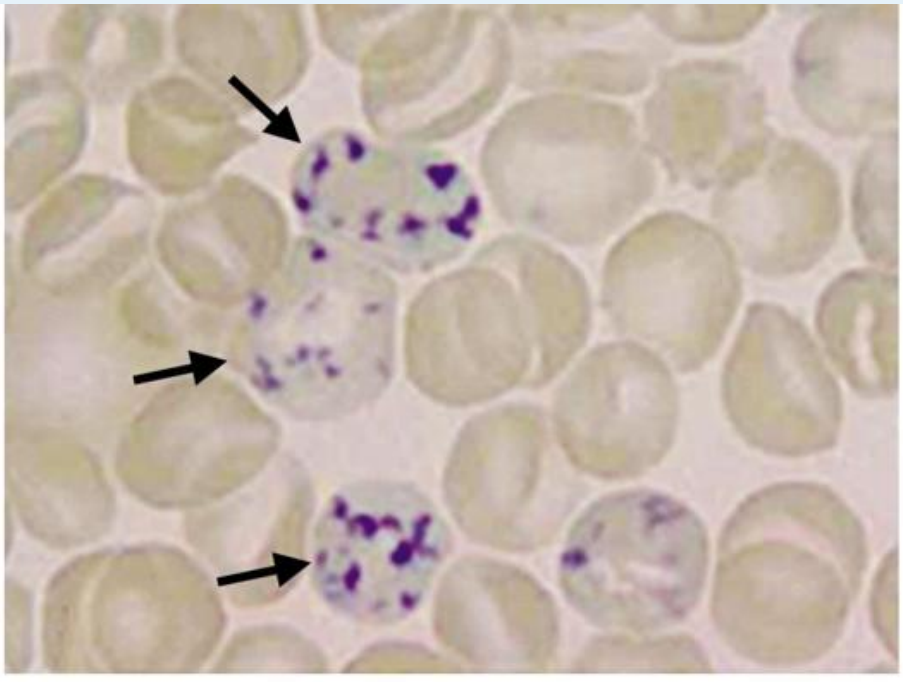


Fig. 11.22: Smear shows reticulocyte (arrows) with blue filamentous/granular material (new methylene blue stain).

Clinical features of intermittent jaundice, splenomegaly and spherocytes in the peripheral smear is highly suggestive of HS.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY

Q. Write short note on G6PD deficiency.

- Hemolytic disease due to **red cell enzyme defects.**
- In G6PD deficiency, **RBCs are susceptible to oxidative injury** by free radicals.
- It is an **X-linked recessive disorder** and its **full expression is seen only in males**.
- There are different subtypes.

Role of G6PD (Fig. 11.24)

Reduced glutathione (GSH) in the normal RBCs protects them against oxidant injury by breakdown of compounds such as H_2O_2 to H_2O. The **housekeeping enzyme, G6PD is required for normal GSH**.

Sequence of Events in G6PD Deficiency

In G6PD deficiency, oxidants can cause **both intravascular and extravascular hemolysis.**

- In G6PD deficiency, there is decreased synthesis of **reduced glutathione**.
- **RBCs when exposed to oxidant stress (**during infections, exposure to drugs or chemical, fava beans) **accumulate H_2O_2.** It damages RBC membrane causing **hemolysis**.
- Hemolyzed red cells **liberate hemoglobin**.
- **The hemoglobin is oxidized** by oxidants leading to formation of **methemoglobin, which** forms **Heinz bodies (Fig. 11.25)** in the cytoplasm of RBCs.
- Heinz bodies are removed from RBC membrane by macrophages in the spleen and **bite cells** are produced**.** These bite cells are removed via **erythrophagocytosis in the spleen.**

Clinical Presentation

G6PD deficiency manifests in several distinct clinical patterns. Usually present as self-limited **acute intravascular hemolytic anemia** following

exposure to oxidative stress.

Laboratory Findings

Peripheral Blood

- **Hemoglobin: decreased**
- **Reticulocyte count: increased**

PERIPHERAL SMEAR

- RBCs: Moderate anisopoikilocytosis with polychromatophilia, microspherocytes and bite cells (Fig. 11.25). Heinz bodies identified with a supravital stain and are best seen during active hemolysis.
- WBCs: Mild leukocytosis.
- Platelets: Normal.

- **Self-limited hemolysis:** Primarily, the **old red cells are hemolyzed**, hence hemolysis is self-limited.

Urine

Hemoglobinuria will be found **during hemolysis** and may last for about 1–6 days.

RBC Enzyme Analysis

***Tests for* G6PD deficiency** are positive and should be assessed a few weeks after the acute hemolytic episode.

G6PD deficiency is an intrinsic defect and hemolysis is primarily intravascular.

In G6PD, RBCs exposed to oxidant stress, the hemoglobin is oxidized to methemoglobin which forms Heinz bodies in the cytoplasm of RBCs.

G6PD deficiency has a protective effect against Plasmodium falciparum malaria.

G6PD deficiency–oxidant damage to RBC
• Bite cells
• Heinz bodies

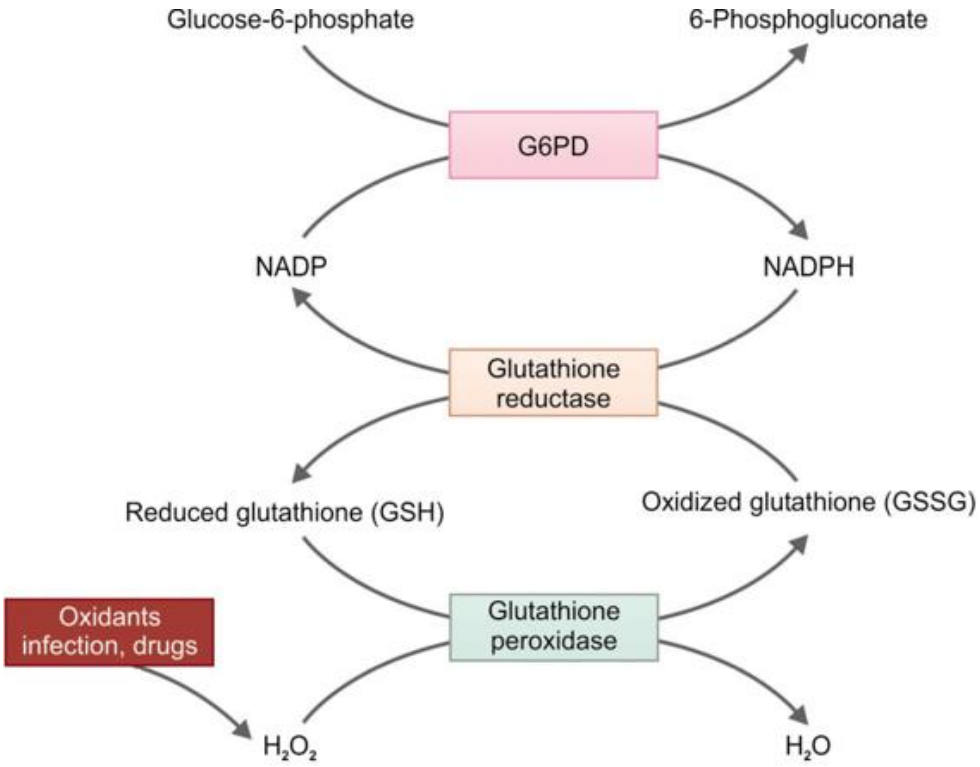


Fig. 11.24: Role of G6PD against injury by oxidants.

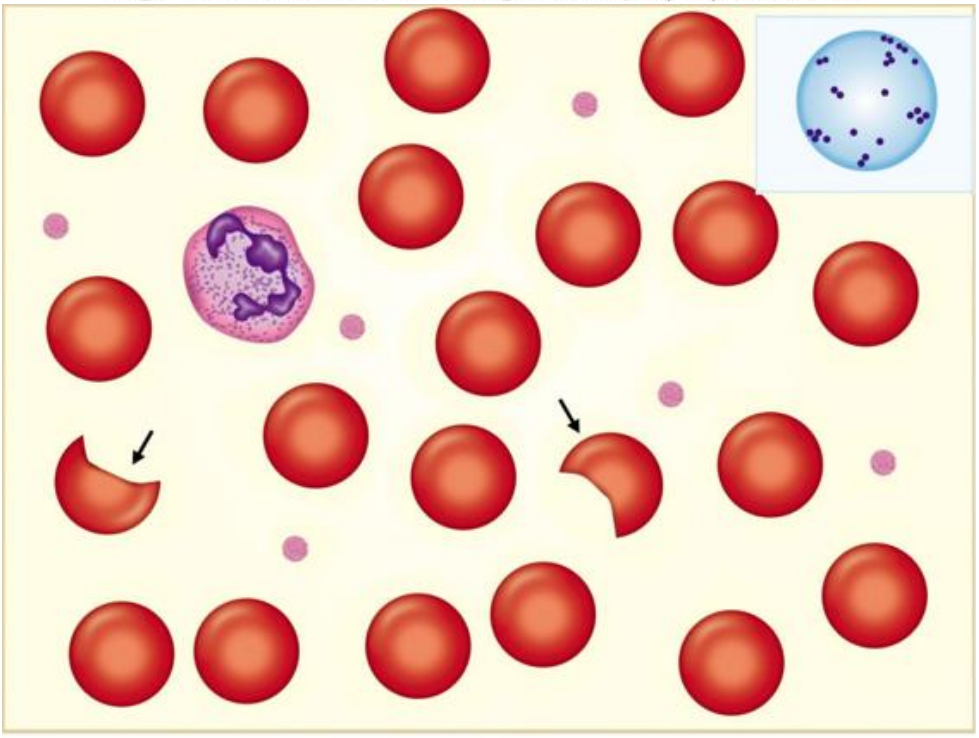


Fig. 11.25: Peripheral blood smear in G6PD deficiency with "bite cells" (arrows). Inset shows Heinz bodies (supravital stain).

G6PD: enzyme analysis–confirmatory test.

Conditions providing protection against malaria: (1) G6PD deficiency, (2) thalassemia, (3) sickle cell disease, (4) Pyruvate kinase deficiency, (5) HbC and (6) absence of Duffy antigen.

HEREDITARY DEFECTS IN HEMOGLOBIN

Classification of Hereditary Defects in Hemoglobin

Q. Classify hereditary disorders of hemoglobin.

Hemoglobin defects may be quantitative (reduced production of normal hemoglobin) or qualitative (production of abnormal hemoglobin).

- ***Quantitative defect:*** Genetic mutations in the globin loci (e.g., thalassemia) may quantitatively reduce the synthesis of a-globin or b-globin chain. It leads to net reduction of hemoglobin.
- ***Qualitative defect:*** Genetic mutations in the a-globin or b-globin locus may produce abnormal hemoglobin (e.g., sickle cell anemia). The abnormal hemoglobin may be functionally normal, but its physical or physiologic properties differ from normal hemoglobin.

THALASSEMIA SYNDROME

CC: Describe the pathogenesis, features, hematologic indices and peripheral blood picture of thalassemia.

Q. Classify thalassemia syndromes. Write a note on molecular pathogenesis of β-thalassemia. Describe the laboratory findings in β-thalassemia major.

Q. Classify hemolytic anemia. Write in detail about β-thalassemia. Discuss the laboratory diagnosis of thalassemia.

Q. Classify thalassemia syndromes.

- These are group of inherited disorders due to **abnormality of globin production**.
- It is characterized by **decreased or absence of synthesis** of either **a or b-globin** chain of adult hemoglobin, HbA (a_2b_2).

Classification

Thalassemia syndromes are mainly classified as:

- b-**Thalassemia syndromes: Impaired synthesis of b-chains** of globin.
- a-**Thalassemia syndromes: Impaired synthesis of a-chains** of globin.
- **Miscellaneous thalassemia syndromes**.

b-Thalassemia

Q. Write in detail about β-thalassemia.

- **Autosomal recessive** hereditary disorder
- **Diminished synthesis** of b-**globin chains** and normal synthesis of a-chains.

Molecular Pathology

- b-globin chains are encoded by a single gene.
- The molecular errors having over 200 genetic defects leading to b-thalassemia have been identified.
- Different types of **mutations in** b-**globin gene** can occur but mainly point mutations rather than gene deletions (unlike in a-thalassemia). The mutations result in defects in transcription, RNA splicing and

modification, translation via frame shifts and nonsense codons. **Mutations leading to aberrant RNA splicing are the most common cause.**

Clinical and Genetic Classification

Table 11.10 shows the clinical and genetic classification of b-thalassemias.

b-THALASSEMIA MAJOR

- It is a **hereditary hemolytic anemia** due to **absence of synthesis of** b-**globin** chain of hemoglobin. The **synthesis of** a-**globin** chain **is not affected**.
- Homozygous form of b^0/b^0 or b^+/b^+ or double heterozygous b^0/b^+ **(Table 11.10)**.
- Most common in Mediterranean countries, parts of Africa and South East Asia.
- **Hemolytic anemia** is of **severe degree**.

Pathophysiology of b-thalassemiaMajor (Fig. 11.26)

TABLE 11.10: Clinical and genetic classification of β-thalassemias.

Clinical syndromes	*Genotype*	*Clinical features*
β-thalassemia major	Homozygous (β^0/β^0, β^+/β^+) or double heterozygous (β^0/β^+)	Severe form, severe anemia and transfusion dependent High level of HbF in the blood
β-thalassemia intermedia	Variable (β^0/β^+, β^+/β^+, β^0/β, β^+/β)	Moderately severe and not transfusion dependent
β-thalassemia minor/ β-thalassemia trait	Heterozygous (β°/β, β^+/β)	Mild anemia and asymptomatic

- β^0 = Total absence of β-globin synthesis
- β^+ = Markedly reduced or diminished β-globin synthesis
- β = Normal β-globin synthesis.

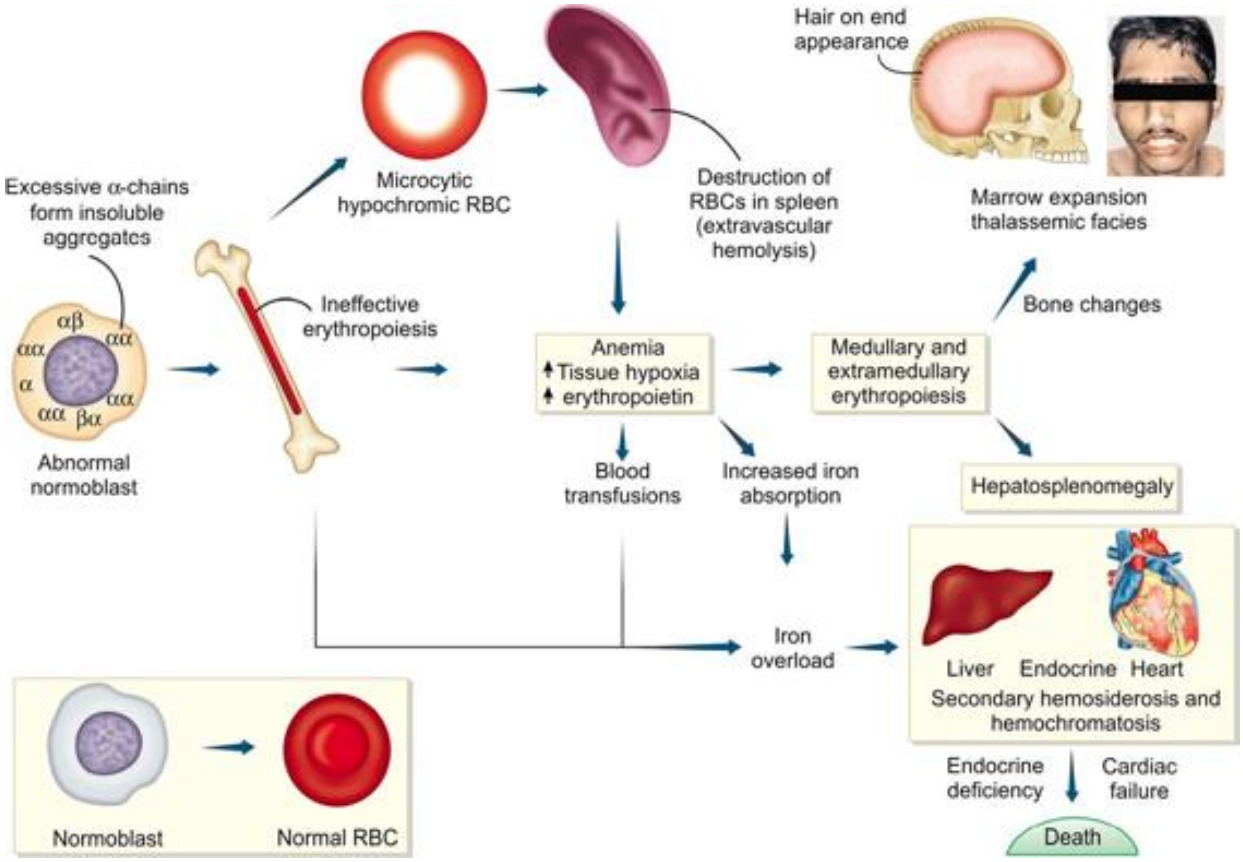


Fig. 11.26: Pathogenesis of β-thalassemia major and its consequence.

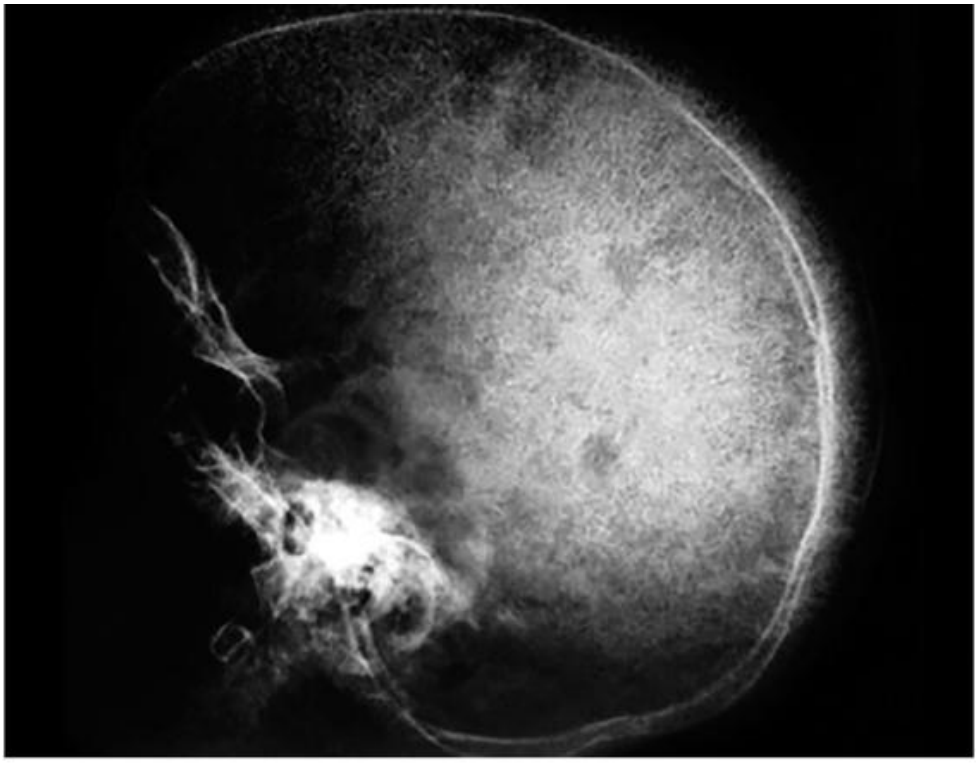


Fig. 11.27: X-ray appearance of skull in β-thalassemia showing hair-on-end (crew-cut) appearance due to new bone formation.

Q. Describe the pathophysiology/pathogenesis of β-thalassemia major.

Consequences of Defective or Absent b*-chains*

- **Severe hemolytic anemia** due to:
  1. **Absence of** b**-globin chain:** Results in **absence of synthesis of HbA** (a_2b_2). This produces RBCs that are poorly hemoglobinized (hypochromic) and small in size (microcytic).
  2. **Ineffective erythropoiesis: Unpaired and excess** a**-chains aggregate** into insoluble precipitates, which bind to and **damage the membrane of erythroid precursors**. These erythroid precursors fail to mature and **undergo apoptosis** in the marrow.
  3. **Extravascular hemolysis:** RBCs with a-chain inclusions are removed by macrophages of spleen (extravascular hemolysis).

- **Synthesis of fetal hemoglobin (HbF):** The ϒ-globin chain synthesis continues even 6 months after birth and combines with a-globin leading to **increased levels of HbF** ($a_2ϒ_2$). The level of HbF varies from 30–90%.

Consequences of Ineffective Erythropoiesis

- Changes in bone marrow: Marked erythroid hyperplasia.
- **Changes in bone:**
  - **Skull X-ray: Hair-on-end (crew-cut) appearance (Fig. 11.27)**
  - **Typical facies: Thalassemic (chipmunk face) face (Fig. 11.28)**—prominent forehead, cheek bones and upper jaw.
- **Extramedullary hematopoiesis:** in **liver and spleen** → consequent hepatosplenomegaly.
- **Cachexia:** Develops in untreated patients.

Iron Overload and its Consequences

- Causes of iron overload:



  - **Increased absorption of dietary iron** from duodenum.
  - **Hemolysis**
  - **Repeated transfusions** (usual mode of treatment).

- **Consequences:** Iron overload produces **hemosiderosis** and **secondary hemochromatosis** and damages to parenchyma of organs (e.g., **heart**, **liver** and **pancreas).**

Clinical Features

- **Age:** Infants develop **moderate to severe anemia,** 6–9 months after birth.
- **Growth and development:** Untreated/untransfused children **fail to thrive** and die within 4–5 years of age.
- **Bone changes:** Those who survive longer develop distortion of skull and facial bones. X-ray **skull** shows **hair-on-end appearance (Fig. 11.27)** and face shows a characteristic **thalassemic (Chipmunk) face (Fig. 11.28)**.
- **Marked splenomegaly:** Up to 1500 grams due to hyperplasia and extramedullary hematopoiesis.
- **Extramedullary hemopoiesis: Liver and lymph nodes** may show **extramedullary hematopoiesis.**
- **Iron overload:** Multiple blood transfusions may lead to iron overload and result in **hemosiderosis and secondary hemochromatosis** (heart, liver and pancreas).

Laboratory Findings

Q. Mention the laboratory findings in b-thalassemia major.

Peripheral Blood

- **Hemoglobin** (ranges from 3–8 g/dL) **and hematocrit** (ranges from 8–23%)**: Markedly reduced.**
- **RBC count increased/normal** (in contrast to iron deficiency anemia).
- **Reticulocyte count increased** and in the range of 5–15%.
- **Red cell indices:**
  - **MCV decreased** and in the range of 45–70 fL (normal range 82–98 fL).
  - **MCHC decreased** and in the range of 22–30 g/dL (normal range 31–35 g/dL).

- **MCH decreased** and in the range of 20–28 pg (normal range 27–32 pg).

PERIPHERAL SMEAR

Q. Write short note on peripheral smear findings in b-thalassemia major.

Q. Write about target cell and its appearance.

- RBCs:
  - **Microcytic hypochromic anemia**
  - Moderate to marked anisocytosis and poikilocytosis
  - Many **target cells (Figs. 11.29 and 11.30).** Target cell is morphologically abnormal RBC. In this hemoglobin is redistributed in such way that only the periphery and central region of RBC appear hemoglobinized and it resembles a target. These target cells are found in thalassemia major, sickle cell anemia, HbC, post-splenectomy, liver disease and obstructive jaundice
  - Basophilic stippling
  - **Nucleated red cell precursors** (normoblasts) in variable numbers (5–40%).
- WBCs: Leukocytosis with mild left shift.
- Platelets: Normal.

BONE MARROW

- Cellularity: Markedly hypercellular.
- M:E ratio: reversed to 1:1 to 1:5 depending upon the degree of erythroid hyperplasia.
- Erythropoiesis: Normoblastic with marked erythroid hyperplasia.
- Myelopoiesis: Normal.
- Megakaryopoiesis: Normal.
- Bone marrow iron: Markedly increased due to increased dietary absorption and hemolysis.

Fig. 11.28: Appearance of typical thalassemic face:Chipmunk face.

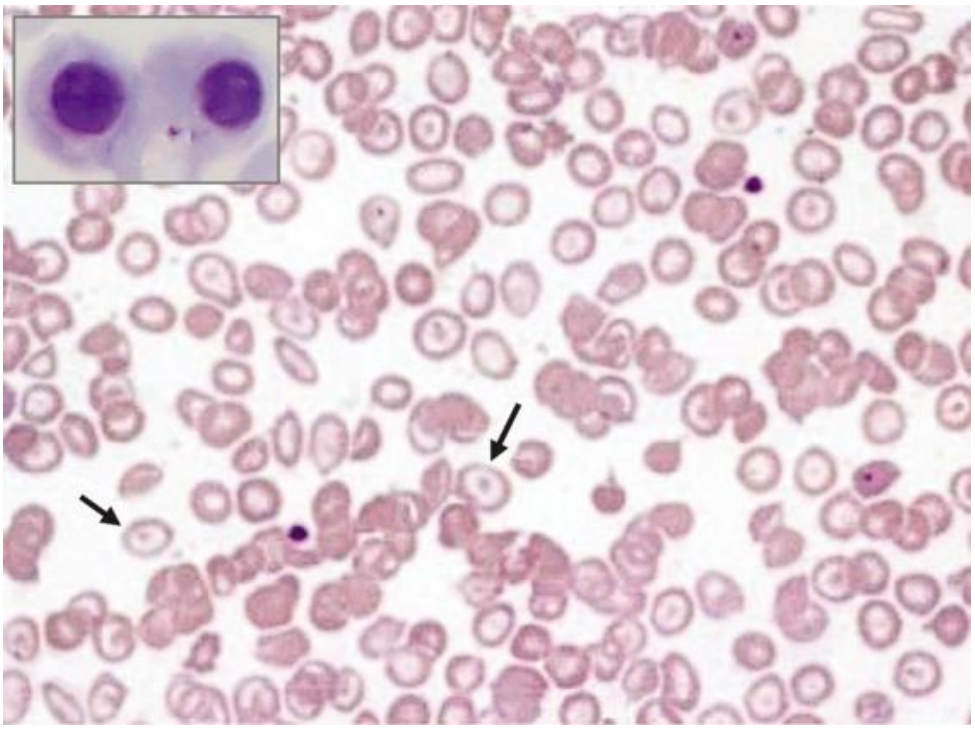
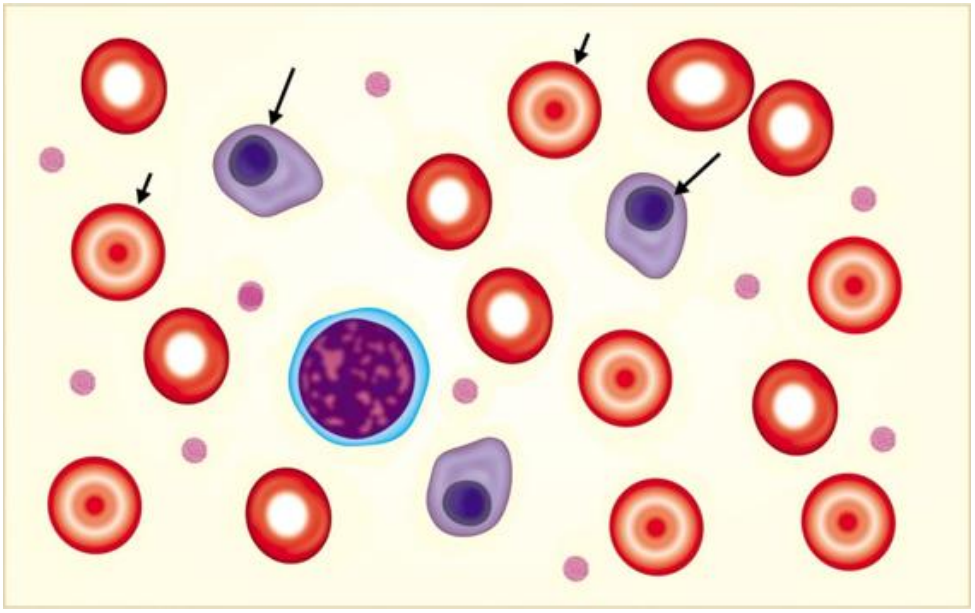


Fig. 11.29: Peripheral blood smear in β-thalassemia showing target cells (arrows). Inset shows 2 nucleated RBCs.



Biochemical Findings

- **Bilirubin: increased**—mainly of unconjugated type.
- **Urine urobilinogen: Increased**
- **Serum haptoglobin: Markedly reduced.**
- **Serum iron status:**

- Serum iron, serum ferritin and transferrin saturation are markedly increased
- Total iron-binding capacity (TIBC): Reduced.

Special Tests

- **Fetal hemoglobin (HbF):** increased to 30–90% (normal range 0–1%).
- **Hemoglobin electrophoresis (Table 11.11)**:
  - b^+ thalassemia (b^+/b^+ or b^0/b^+ genotypes): demonstrates bands of **both HbA and HbF**.
  - b° thalassemia (b^0/b^0 genotype): Since no b-chains are formed, there is no HbA. Major hemoglobin is HbF with normal or low HbA_2.
- **High performance liquid chromatography (HPLC):** HbF is increased (30–90%). HPLC measures various fractions of hemoglobin (Hb) and is used **for confirmation of diagnosis**.
- Prenatal diagnosis by **molecular analysis of DNA**.
- **Estimation of globin chains:** Normally, a: b ratio is 1:1. Lack of b-chain alter this ratio to 5–30:1.

Differences between iron deficiency anemia and b-thalassemia major are presented in **Table 11.12.**

b-THALASSEMIA MINOR/TRAIT

- More common than b-thalassemia major.
- Most patients are heterozygous for thalassemic gene.
- **Usually asymptomatic** and anemia is mild.

Laboratory Findings in b-Thalassemia Minor

- **Peripheral blood: Microcytosis, hypochromia,** basophilic stippling and target cells.
- **Bone marrow: Mild erythroid hyperplasia**.
- **Hemoglobin electrophoresis:** Increase in HbA_2 ($a_2\delta_2$) to 4–8% of the total hemoglobin (normal 2.5 ± 0.3%). HbF levels may be normal or slightly increased.
- **NESTROF test** (Naked eye single tube red cell osmotic fragility test): **positive**.

- In this test, 0.02 mL of patient's blood is added to 5 mL of 0.35% saline in a test tube.
- After half an hour white paper with a dark black line is held behind the tube.
- The microcytic hypochromic RBCs of thalassemia minor are resistant to lysis than normocytic normochromic RBCs.
- Hence, the black line on the paper is not clearly visible through the test tube compared to normal cells.
- This test is used as screening test for thalassemia. However, this test is also positive in α-thalassemia trait, sickle cell trait and iron deficiency anemia.
- **Estimation of HbA_2:** HPLC is used for accurate estimation. HbA_2 estimation is diagnostic and level ranges from 4–8%.

a-THALASSEMIA

β-thalassemia trait/minor should be differentiated from iron deficiency anemia **(Table 11.13)**.

- Inherited disorders characterized by **reduced or absent synthesis of** a-**globin chains.**
- Autosomal recessive disorder.

Molecular Pathology

In contrast to a single gene coding b-globin chain, each a-globin chain are encoded by two genes. **Deletion of** a-**gene is the most common cause** of reduced a-chain synthesis.

Clinical Syndromes

Four genes control a-chain synthesis. Severity of a-thalassemia varies greatly depending on the number of a-globin genes deleted **(Table 11.14)**. Each of the four a-globin genes normally contributes 25% of the total a-globin chains.

The term hemoglobinopathy is usually used for a qualitative hereditary disorder of hemoglobin.

In α-Thalassemia, there is reduced/absence of synthesis of α-chains of globin.

In β-Thalassemia, there is decreased/absence of synthesis of β-chains.

Fig. 11.30: Diagrammatic appearance of peripheral blood smear in β-thalassemia showing target cells (short arrows) and nucleated red cells (long arrows).

TABLE 11.11: Hemoglobin F and A_2 percentage in thalassemia syndromes.

Type	*HbF*	*HbA_2*
β -Thalassemia major (homozygous)	30–90%	<3.5%
β -Thalassemia intermedia (double heterozygous)	10–30%	<3.5%
β -Thalassemia minor/trait (heterozygous)	0–5%	3.6–8%

Note: Normal adult cell contains 96% HbA (α2β2), 3% HbA22(α2d2) and 1% HbF(α2γ2).

TABLE 11.12: Differences between iron deficiency anemia and β-thalassemia major.

Character	*Iron deficiency anemia*	*β-thalassemia major*
Etiology	Deficiency of iron	Reduced synthesis of β-chain
Laboratory findings		
• **Peripheral smear**		
– Type of RBCs	Microcytic hypochromic	Microcytic hypochromic
– Anisopoikilocytosis	Mild to moderate	Severe
– Target cells	Absent	Present
• **Bone marrow iron**	Absent	Markedly increased
• **Serum iron profile**		
– Serum ferritin	Reduced <15 µg/L	Increased (300–1000 µg/L)
– Serum iron	Reduced	Increased
– TIBC	Increased	Normal
• **Fetal hemoglobin (HbF)**	Normal (0–1%)	Markedly increased (30–90%)
Clinical features		
• Age	Any age	Presented <2 years of age
• Growth and development	Normal	Retarded
• Hepatosplenomegaly	Absent	Present
X-ray findings	Nil	Hair-on-end appearance

β-thalassemia should be differentiated from iron deficiency anemia. Treatment with iron in β-thalassemia major worsens the iron load and its consequences.

β-thalassemia intermedia: It is a clinical entity intermediate between thalassemia trait and thalassemia major.

b-thalassemia major: MCV, MCH and MCHC decreased.

NESTROF test positive because the microcytic hypochromic RBCs of β-thalassemia minor are resistant to lysis than normocytic normochromic RBCs.

TABLE 11.13: Differences between iron deficiency anemia and β-thalassemia minor/trait.

Character	*Iron deficiency anemia*	*β-thalassemia minor*
Etiology	Deficiency of iron	Reduced synthesis of β-chain
Laboratory findings		
Peripheral smear—RBCs	Microcytic hypochromic	Microcytic hypochromic
• **Serum iron profile** – Serum ferritin – Serum iron – TIBC – Transferrin saturation %	 Reduced <15 µg/L Reduced Increased <15%	 Normal/slightly increased Normal Normal 30–40%
• **HbA_2 level**	Normal or decreased (2.5 ± 0.3%)	Increased (4–8%)
• **RBC count**	<5 million/cu mm	>5 million/cu mm
• **RDW**	Increased	Normal

(RDW: red cell distribution width; TIBC: total iron-binding capacity).

TABLE 11.14: Clinical syndromes associated with a-thalassemia disorders.

Clinical syndrome	***No. of* a-*globin deleted***	***Clinicopathological features***
Silent carrier state	1	Asymptomatic
a-**Thalassemia trait**	2	Usually asymptomatic. Normal hemoglobin level or minimal anemia
Hemoglobin H disease	3	Moderate microcytic hypochromic anemia
Hydrops fetalis(Hb Barts)	4	Severe form, fatal and usually results in intrauterine death

Point mutations leading to aberrant RNA splicing is the most common cause of β-thalassemia.

β-thalassemia is the commonest quantitative disorder of hemoglobin.

β-thalassemia major also called Mediterranean or Cooley's anemia.

β-thalassemia major
- Absence of synthesis of HbA produces severe microcytic hypochromic anemia
- Increased synthesis of HbF

β-thalassemia major
- Thalassemic facies
- Crew-cut appearance on skull X-ray
- Splenomegaly

β-thalassemia major: Iron overload damages parenchymal organs due to hemosiderosis and

secondary hemochromatosis.

Failure to thrive, retarded growth, Chipmunk faces, and hepatosplenomegaly are clinical features of β-thalassemia major.

Bone marrow in β- thalassemia major shows marked normoblastic erythroid hyperplasia. Marrow iron is markedly increased.

β-thalassemia major: The peripheral blood smear shows microcytic hypochromic anemia, target cells and anisopoikilocytosis.

Reduced/absence of synthesis of β-chains; the excess α-chains combine with γ-chains leading to increased HbF.

α-Thalassemia: Anemia due to—
- Lack of adequate hemoglobin
- Effect of excess unpaired non-α-chains (β, γ, δ)

α-thalassemia is one of the cause of non-immune hydrops fetalis.

Immune hydrops fetalis is a hemolytic disease caused by blood group incompatibility between mother and fetus.

Sickle Cell Disease

Q. Give the etiological classification of anemias. Discuss the pathogenesis and laboratory diagnosis of sickle cell disease.

Definition: Sickle cell disease (SCD) is a group of **hereditary disorders** of hemoglobin characterized by **production of defective hemoglobin** called **sickle hemoglobin (HbS).** On low oxygen tension or deoxygenation, HbS imparts **sickle shape to RBCs**. HbS is produced due to **qualitative defect** in hemoglobin production caused by **mutation in β-globin gene**.

Classification of sickle cell disease is presented in **Table 11.15**.

SICKLE CELL ANEMIA

CC: Describe the pathogenesis, features, hematologic indices and peripheral blood picture of

Q. Define and classify hemolytic anemia. Describe the laboratory diagnosis of sickle cell anemia.

Characteristic Features

- Autosomal recessive disorder manifests early in life.
- Homozygous state (SS) caused by a mutation in the β-globin gene.
- HbS constitutes more than 70% of hemoglobin in their RBCs with no HbA.

Etiopathogenesis

Q. Discuss the etiopathogenesis of sickle cell anemia.

- Production of abnormal hemoglobin called sickle hemoglobin (HbS).
- **Missense point mutation:** In HbS, there is **substitution of glutamic acid by valine in the 6th position, the β-globin chain of hemoglobin (Fig. 11.31)**. It alters the solubility or stability of the hemoglobin and produces hemolytic anemia.
- HbS is responsible for the characteristics of the disease.

Molecular Basis of Sickling (Fig. 11.32)

Q. Write short note on irreversible sickle cells.

- During **low O_2 tension or deoxygenation, HbS** molecules **undergo aggregation and polymerization**.
- If deoxygenation continues, the **aggregated HbS** molecules **form long needle-like fibers** (or pseudocrystalline structures known as **tactoids**) within RBCs.
- The **tactoids grow** in length beyond the diameter of RBCs and **distort RBC shape**.

- **RBC** become elongated and assumes a **shape** such as **sickle** (or crescent moon or holly-leaf or boat) and **predisposes to stasis and vascular occlusion**.
- **When** the **oxygen tension returns** to normal, the **sickled red cell returns to normal shape**.
- **Recurrent sickling** causes red cell membrane damage and these RBCs become **irreversibly sickled cells (ISC)**.

Factors Affecting Sickling

Table 11.16 shows the factors affecting sicking.

Mechanism of Red Cell Damage

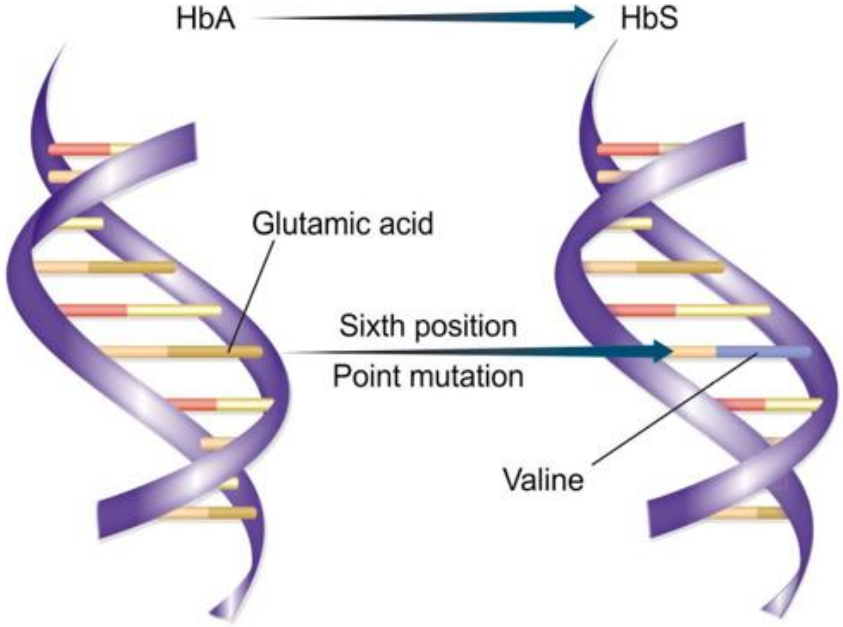


Fig. 11.31: Replacement of glutamic acid with valine in the sixth position of β-globin.

TABLE 11.15: Classification of sickle cell disease.

Sickle cell anemia (SS)	*Sickle cell trait (AS)*
• Homozygous state—both the β-globin chains are abnormal/defective	• Heterozygous state—one gene is defective (for HbS) and while the other gene is normal (for HbA)
Other sickling syndromes (Compound heterozygous)	

TABLE 11.15: Classification of sickle cell disease.

- If both the β-globin chains have different abnormalities, (e.g., Hb SC, Hb S-β-thalassemia)—termed as **compound heterozygous**

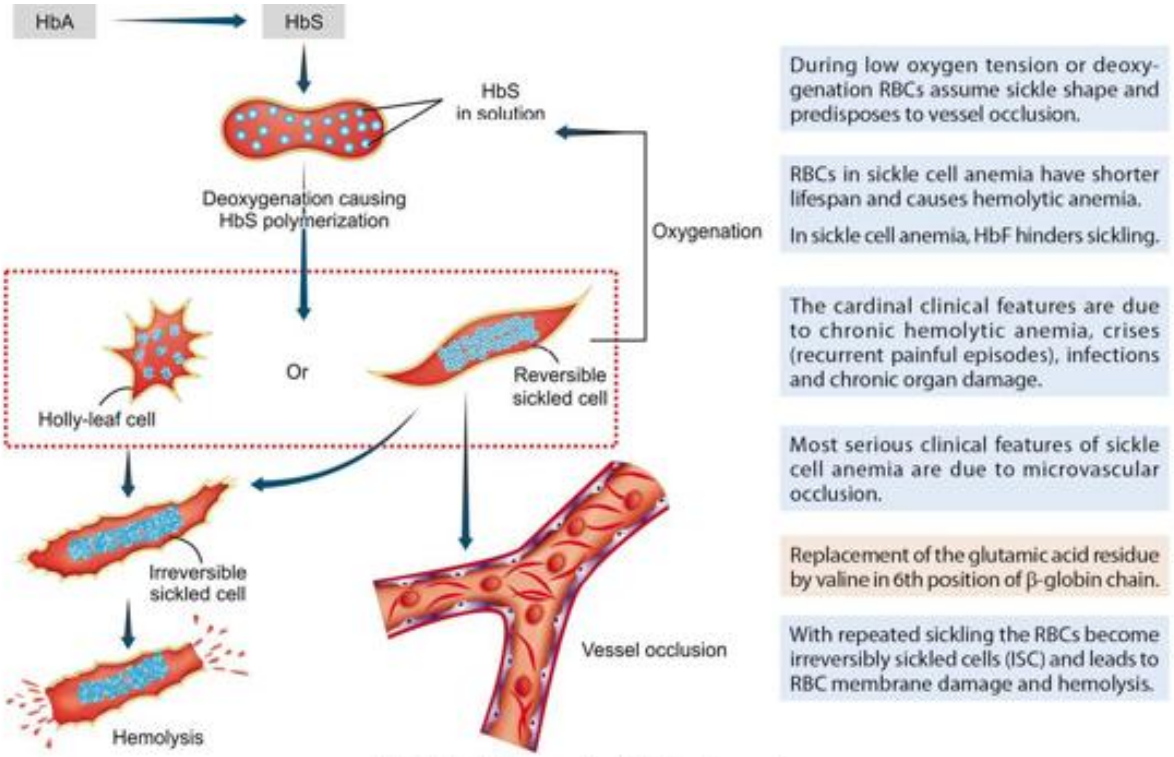


Fig. 11.32: Pathogenesis of sickle cell anemia.

- **HbS polymerization:** When HbS polymerizes, it grows beyond the RBC membrane and project through it.
- **Dehydration:** Repeated episodes of sickling leads to increased dehydration of RBCs. These **RBCs become more rigid and nondeformable** (irreversible sickled cells).
- **Percentage of ISC:** Degree of the hemolysis correlates with the percentage of irreversibly sickled cells.
- **Impaired cation homeostasis:** Structural changes in the RBC membrane causes the influx of Ca^{2+} ions, which activate an ion channel resulting in the efflux of K^+ and H_2O.

Pathogenesis of the Microvascular Occlusions

Most serious clinical features are due to occlusion of microvasculature.

- **Deformability:** Sickle cells are **rigid and tend to aggregate**. The aggregated sickle cells **block** the small blood **vessels**.

- **Factors that slow the blood flow:** RBC **cytoskeletal damage** slow the movement of RBCs through microvascular beds.
- **Higher expression of adhesion molecules: Sickle cells** express higher levels of adhesion molecules and thus **become abnormally sticky to the endothelium**.
- **Inactivation of nitric oxide: Lysed sickle cells** liberate free hemoglobin, which binds and **inactivates nitric oxide (NO)**. This **narrows the vessels** and produces microvascular stasis and sickling.

Clinical Features (Fig. 11.33)

- Presence of **HbF in the first 6 months** of life has a **protective** role.
- **Symptoms appear after 6 months** of age as the HbF disappears.
- Infants and children present with acute problems like severe infection, acute chest syndrome, splenic sequestration and stroke.
- Chronic hypoxia in children is responsible for generalized impairment of growth and development. Adults manifest with chronic organ damage.

Chronic Hemolytic Anemia

Q. Complications of sickle cell anemia.

TABLE 11.16: Factors affecting sickling.

Factors	*Favors sickling*	*Hinders sickling*
Type of other associated hemoglobins	-	HbA
	-	HbF
	HbC	-
Transit time in microvasculature	Slowing of bloodstream	-
MCHC	Increased MCHC	Decreased MCHC
Intracellular pH	Decreased pH	-
Other factors	Temperature above 37°C	-
	Infections	-

(MCHC: mean corpuscular hemoglobin concentration)

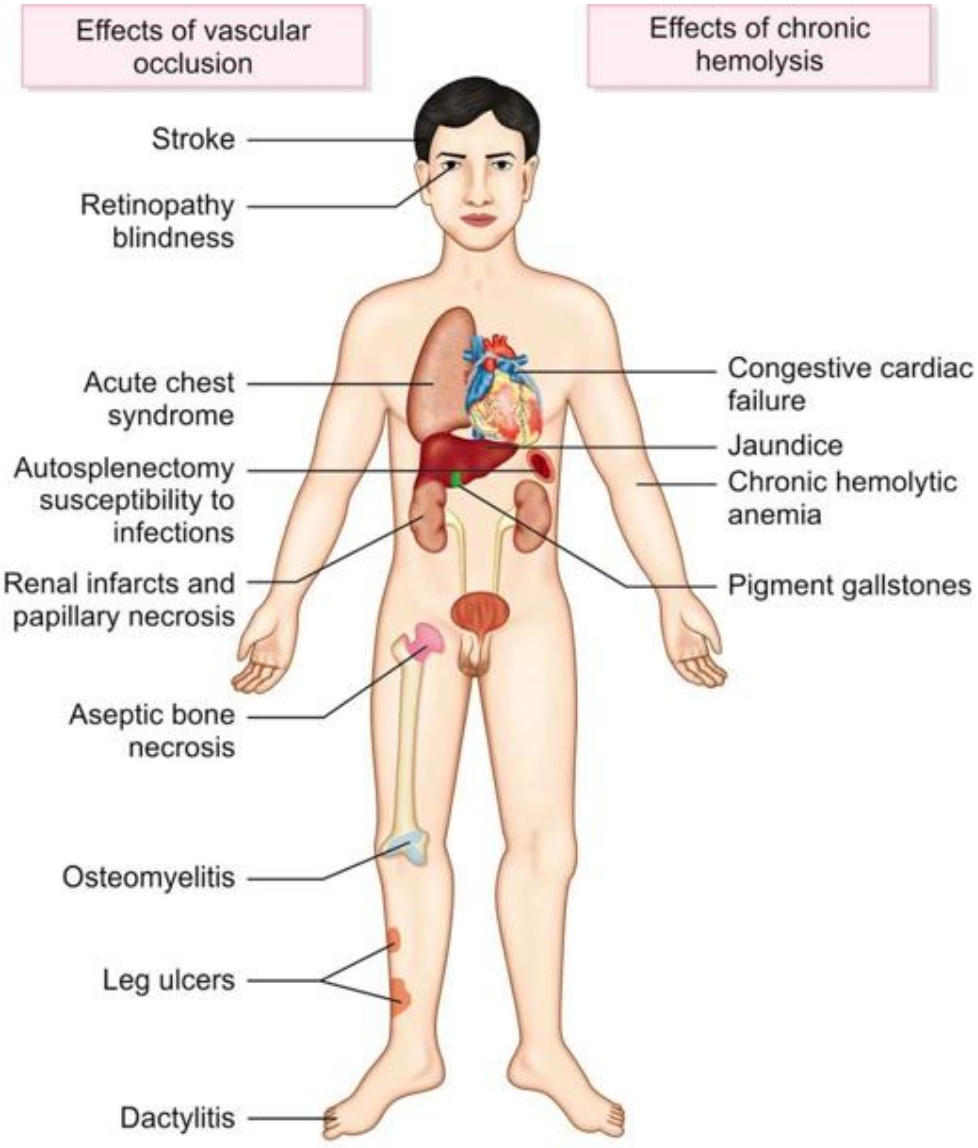


Fig. 11.33: Various effects of vascular occlusion and hemolysis in sickle cell anemia.

Infants most commonly present with dactylitis.

Most common cause of death in adults is acute chest syndrome.

Lifelong hemolysis (mainly extravascular) and causes chronic hemolytic anemia, which is of moderate degree. This produces raised unconjugated (indirect) bilirubin, and predisposes to pigment bilirubin gallstones (cholelithiasis) and cholecystitis.

Crises

Q. Write short essay/note on crises in sickle cell anemia.

Four types of crises are encountered. These are:

1. Sickling Crisis (vaso-occlusive/pain/painful/infarctive crisis)

- Most common
- Blockage of microcirculation by sickled red cells produces hypoxic injury and infarction.
- Bone: Manifest as the hand-foot syndrome, dactylitis of the bones of the hands or feet or both.
- Lung: Acute chest syndrome (dangerous).
- Spleen: Acute abdominal pain due to infarcts of abdominal viscera caused by occlusion of vessels. Recurrent splenic infarction results in autosplenectomy.

2. Hemolytic Crisis

Rare type and presents with marked increase in **hemolysis.**

3. Aplastic Crisis

- Associated with parvovirus B_{19}
- Reticulocytopenia

4. Sequestration Crisis

- Usually occurs in **children.**
- **Sudden trapping** of blood in spleen or liver causes rapid enlargement of the organ and drop in hematocrit leading to hypovolemic shock.

Other crises encountered rarely are hypoplastic crisis and megaloblastic crisis (due to inadequate folate).

Increased Susceptibility to Infections

- Common infections are pneumonia due to *Pneumococcus meningitis* due to *Streptococcus pneumoniae* and osteomyelitis due to *Salmonella*. Increased frequency of osteomyelitis is due to bone infarcts, which act as a nidus for infection.
- Septicemia and meningitis are the most common causes of death in children.

Causes of Susceptibility to Infections

- Hypofunction of spleen:
  - In children: due to congestion and poor blood flow.
  - In adults: due to **multiple infarcts** and resultant **autosplenectomy**.
- Defects in the alternative complement pathway.

Impairs opsonization of encapsulated bacteria such as pneumococci and *Haemophilus influenzae.*

Chronic Organ Damage

Particularly seen in the spleen, bones, kidneys, heart, lungs, brain and skin.

- **Spleen:**
  - Children after 6 months of life present with splenomegaly (up to 500 g).
  - After 5–6 years of age, the spleen gets fibrosed and **gradually reduces in the size** due to **multiple infarcts**.
  - Gradual **loss of splenic function** secondary to infarcts results in **autosplenectomy**.
- **Bone:** Osteomyelitis, particularly with *Salmonella typhimurium*.
- **Extremities:** Skin ulcers over the lower extremities.

Laboratory Findings in Sickle Cell Anemia

Q. Mention laboratory findings/diagnosis of sickle cell anemia.

Peripheral Blood

- **Hemoglobin:** Decreased

- **Hematocrit (PCV):** Decreased
- **ESR:** Reduced
- **Reticulocyte count:** Increased and range from 3–10%.

PERIPHERAL SMEAR

Q. Write short note on peripheral smear findings in sickle cell anemia.

- RBCs:
  - Normocytic normochromic to mildly hypochromic.
  - Moderate to severe degree of **anisopoikilocytosis**.
  - **Characteristic cell is** the **sickle cell—**appear as long, curved cells with pointed ends **(Figs. 11.34 and 11.35)**; may also show **target cells (**due to red cell dehydration) **and ovalocytes**.
  - **Polychromatophilia due to reticulocytosis**.
- WBCs: Mildly increased with shift to left.
- Platelets: Mildly increased.

BONE MARROW

- Cellularity: Hypercellular.
- Erythropoiesis: Compensatory normoblastic erythroid hyperplasia, which expands the marrow and causes resorption of bone and secondary new bone formation.
- Myelopoiesis: Normal.
- Megakaryopoiesis: Normal.
- Iron stores: Usually increased.

Serum Findings

- **Serum bilirubin:** Raised and predisposes to pigment gallstones.
- **Iron status:** Raised serum iron, serum ferritin and transferrin saturation.
- **Serum haptoglobin: Reduced.**
- **Urine urobilinogen: Increased.**

Diagnostic/Confirmatory Tests

Q. Enumerate tests of sickling in sickle cell anemia.

Q. Principle of sickling test.

- Sickling test:
  - **Principle:** Sickling is induced by adding a reducing (oxygen-consuming) agent like 2% sodium metabisulfite or sodium dithionite to blood sample.
  - **Sickling** is **induced** by adding a **reducing** (oxygen-consuming) **agent** like 2% sodium metabisulfite or sodium dithionite to blood sample.
  - Red cells with HbS show **sickled (Fig. 11.36) and holly leaf** appearance.
  - It is **diagnostic** of sickle cell anemia.
- **Solubility test for hemoglobin S:** This is performed by adding the anticoagulated blood to the reducing reagent solution (consisting of phosphate buffer, saponin and sodium dithionate). RBCs are hemolyzed and if HbS is present, it will be reduced by dithionate. HbS forms tactoids and refract light. The solution appears turbid whereas normal Hb gives a clear solution.
- **Hemoglobin electrophoresis:** HbS is a slow moving compared to HbA and HbF.
- **Estimation of HbF:** In homozygous state constitutes about 10–30% of hemoglobin.

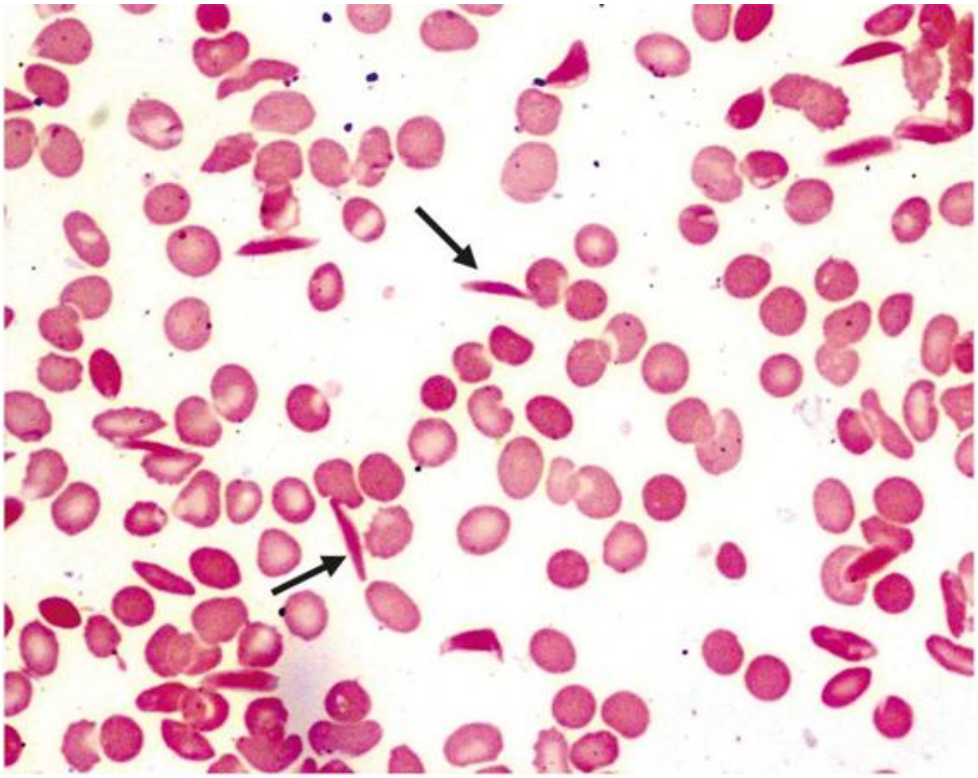


Fig. 11.34: Peripheral blood smear with sickle cells (arrows).

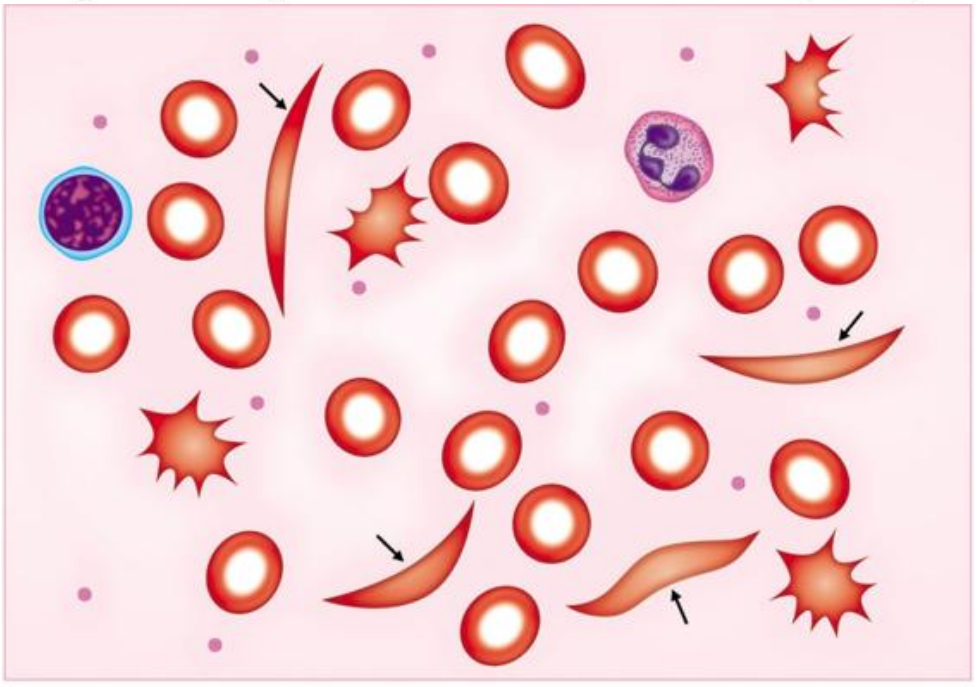


Fig. 11.35: Diagrammatic peripheral blood smear with sickle cells (arrows).

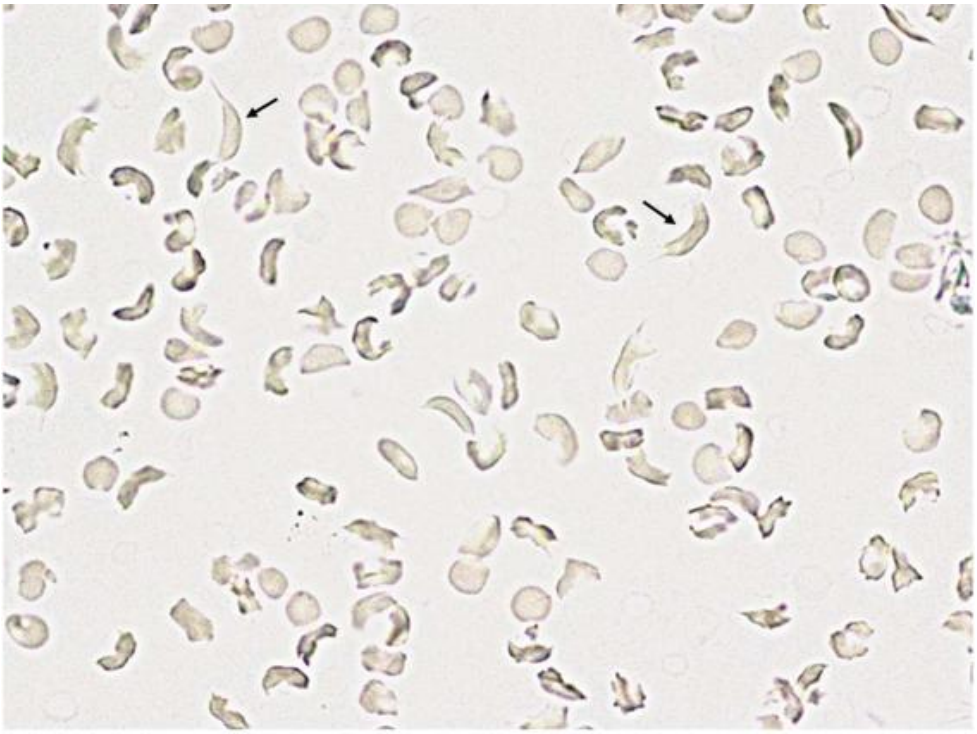


Fig. 11.36: Sickling test. Sickled red cells (arrows) induced by reducing agent (2% sodium metabisulfite).

- **HPLC:** Useful for confirmation of diagnosis.

Prevention of Sickle Cell Anemia

Prenatal diagnosis: By analysis of fetal DNA obtained by amniocentesis or chorionic villous biopsy, to detect the point mutations.

SICKLE CELL TRAIT

Heterozygous state for the hemoglobin S mutation and shows both HbA and HbS (HbAS). One defective gene (from one parent with HbS) and while the other gene is normal.

Pathogenesis

In sickle cell trait, the hemoglobin A in RBCs prevents hemoglobin S polymerization. However, RBCs may sickle under extreme conditions (e.g., flight at high altitude in unpressurized aircraft, deep sea diving).

Clinical Features

Usually asymptomatic. Normal growth and development, lifespan and life expectancy.

Laboratory Findings

Peripheral Blood

- Hemoglobin: Normal or mildly decreased.

PERIPHERAL SMEAR

- RBCs: Normocytic normochromic picture with very few target cells and mild degree of anisopoikilocytosis.
- WBCs: Normal.
- Platelets: Normal.

BONE MARROW

Hypercellular because of a compensatory normoblastic erythroid hyperplasia.

Sickle cell trait:
- Usually no anemia
- No significant clinical features
- Amount of HbS varies from 25% to 40%
- HbA in RBCs prevents polymerization of HbS

Diagnostic Tests

In sickle cell trait: HbS 40–45% and HbA 55–60%.

- **Hb elec**trophoresis: Demonstrates two bands of HbS and HbA.
- Sickling test: Sickling test is positive.
- **High-performance liquid chromatography:** Useful for confirmation of diagnosis.

Abnormal Hemoglobins

Q. List the abnormal hemoglobins.

These include HbS, HbC, HbD-Punjab, HbE, Hb Barts, HbH, Hb Portland, HbO-Arab, etc.

Sickle cell diseases are hemoglobinopathies characterized by qualitative defect in hemoglobin synthesis.

Sickle cell anemia is a homozygous state in which both β-globin chains are abnormal.

Sickle cell anemia: Autosomal recessive disorder with extravascular hemolysis.

HbS provides protection against falciparum malaria.

Sickle cell trait: One β-globin chain is abnormal and other β-globin chain is normal.

Four crises encountered in sickle cell anemia: Sickling crisis, hemolytic crisis, aplastic crisis and sequestration crisis.

Recurrent splenic infarction due to sickling crisis lead to autosplenectomy.

Reticulocytopenia is seen in aplastic crisis and reticulocytosis in sequestration crisis.

Susceptible to acute infections with encapsulated organisms.

Common pathogens: S. pneumoniae, Salmonella and Pneumococcus.

SCA: Severe hemolytic anemia; Sickling crisis; autosplenectomy.

In severe cases, skull bone shows crew-cut appearance in roentgenograms.

Extramedullary hematopoiesis can also develop as a compensatory mechanism.

Sickle cell anemia: ESR is reduced because sickle cells do not form rouleaux.

Peripheral smear shows characteristic sickle cells number of which varies.

Sickle cell anemia: HbS 70–90%, HbF 10–30%, no HbA.

Sickling test is a diagnostic test for sickle cell anemia.

Other Anemias

CC: Describe the etiology pathogenesis, hematologic indices and peripheral blood picture of acquired hemolytic anemia.

IMMUNOHEMOLYTIC ANEMIAS

BOX 11.12: Classification of immunohemolytic anemias.

Alloimmune hemolytic anemia
- Hemolytic disease of the newborn
- Hemolytic transfusion reactions: Mismatched blood transfusion

Autoimmune hemolytic anemia
- **Warm antibody type (IgG antibodies active at 37°C)**
  - Primary (Idiopathic)
  - Secondary: Autoimmune disorders (systemic lupus erythematosus), drugs, lymphomas
- **Cold agglutinin type (IgM antibodies active at 4°C–18°C)**
  - Acute: mycoplasmal infection, infectious mononucleosis
  - Chronic: Idiopathic, lymphomas
- **Cold hemolysin type (Donath–Landsteiner antibodies)**

Anemias due to **premature RBC destruction** (hemolysis) mediated **by antibodies** that bind to RBCs. The antibodies may be either allo or auto type.

Classification of Immunohemolytic Anemias

Box 11.12 shows the classification of immunohemolytic anemias.

Alloimmune Hemolytic Anemia

- Production of antibody against foreign antigen not present on individual's red blood cell.
- Alloantibodies are present either in the serum or bound to red cells.

HEMOLYTIC DISEASE OF THE NEWBORN

Q. Write short note on hemolytic disease of newborn.

- It is an alloimmune hemolytic anemia developing in the fetus and newborn baby.
- Hemolysis is extravascular.
- HDN develops when the IgG antibodies against blood group of fetus passes from mother to fetus through the placenta.
- Occurs in two forms:

- **Rh incompatibility** in which mother is Rh negative and fetus is Rh positive. The anti-D antibodies are responsible for the hemolytic anemia.
- **ABO incompatibility** in which mother's blood group is O and fetus is either of A or B blood group. Either anti-A or anti-B antibodies cause hemolysis.

Rh Hemolytic Disease of the Newborn (Fig. 11.37)

Q. Write short note on Rh hemolytic disease of the newborn.

Rh hemolytic disease of the newborn is more important than due to ABO incompatibility.

Pathogenesis

- Occurs when **mother is Rh (D antigen) negative and fetus is Rh positive.**
- **Sensitization** occurs when fetal Rh positive RBCs enter into Rh negative mothers. Rh negative mother develops **anti-Rh antibodies.**
- **Sensitization occurs** only at the time of **delivery or during miscarriage**. So, it **does not manifest in the first pregnancy.**
- **In subsequent pregnancy, anti-Rh antibodies from mother cross placenta and coat the Rh positive fetal red cells.** These antibodies cause immune **destruction of fetal red cells and results in severe hemolytic anemia** leading to **jaundice** of the newborn.
- Fetus may develop cardiac failure—**hydrops fetalis** (immune type).

Clinicopathological Features

- Infants may have jaundice at birth.
- When the disease is severe, the levels of **unconjugated bilirubin** in the blood are **high** and bilirubin can **pass the blood-brain barrier**.
- Bilirubin is deposited in the **central nervous system** (especially the basal ganglia) producing **neurological damage** and is known as **kernicterus**

(yellow coloration of cerebellum and basal ganglia due to bilirubin deposition). It can cause **death** of the infant.

Prevention of Rh HDN: By the prophylactic removal of fetal cells entering the maternal circulation before sensitization develops, by **injecting anti-D** into the **Rh D negative mother**.

Laboratory Findings

Peripheral Blood

- Hemoglobin: Decreased
- Reticulocyte count: Increased

PERIPHERAL SMEAR

- RBCs: Normocytic normochromic anemia with numerous nucleated RBCs, polychromatophils and occasional spherocytes.
- WBCs: Normal.
- Platelet: Normal.

- **Antiglobulin test (Coombs test):** Antibodies in the mother and baby are detected by indirect and direct Coombs test respectively **(Fig. 11.38)**.

Serum Findings

- Serum bilirubin: Increased
- Lactate hydrogenase (LDH): Increased
- Haptoglobin: Decreased

ABO Hemolytic Disease of the Newborn

- It is less severe.
- The fetus may be affected in the first pregnancy of a **mother with blood group O**.
- The IgG antibodies to A or B from maternal blood cross placenta and enter the fetal circulation. These **anti-A or anti-B antibodies react** with A and B antigenic determinants present in **fetal fluids and tissues**.

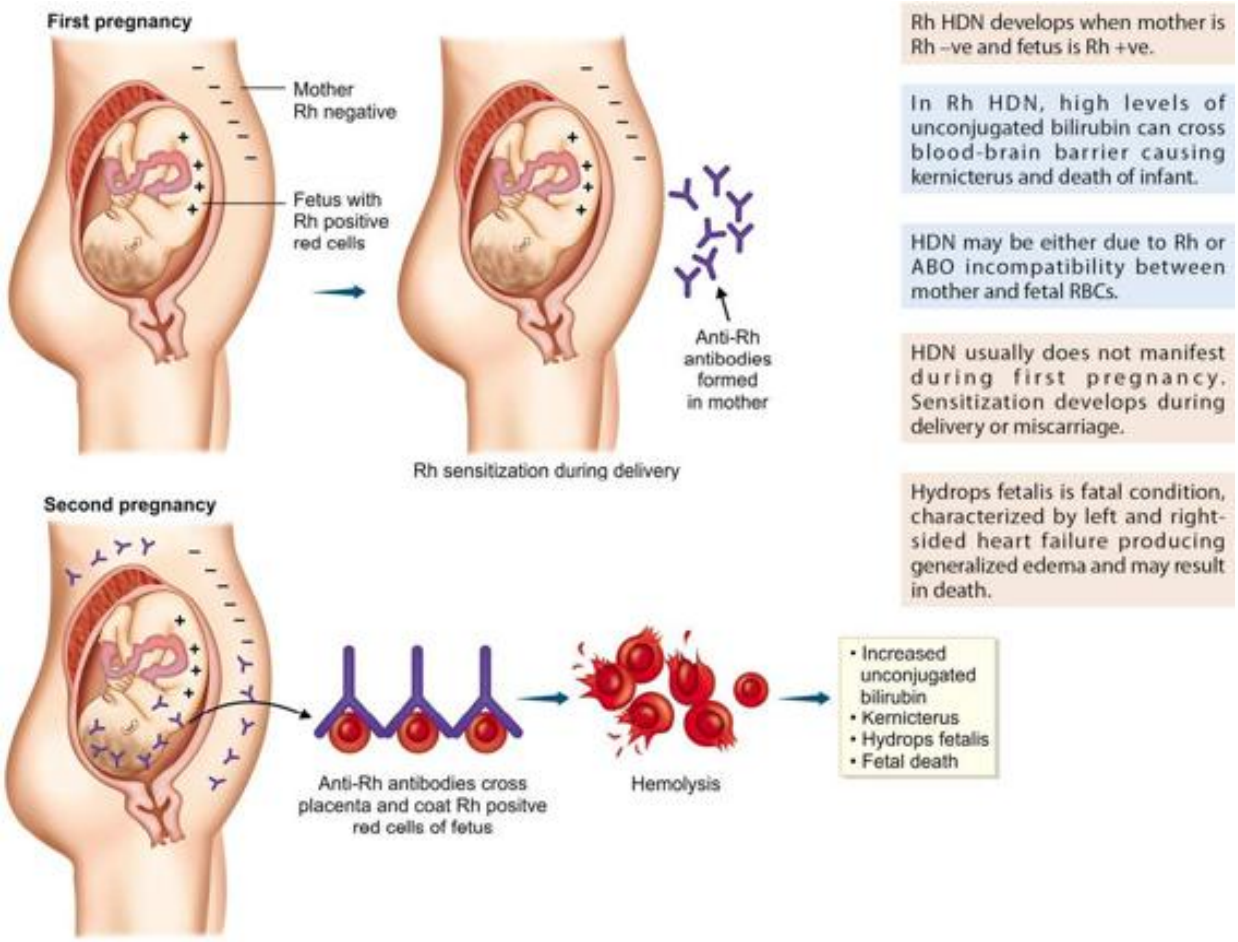


Fig. 11.37: Pathogenesis of Rh hemolytic disease of the newborn.

- This results in consumption of major portion of the maternal IgG and the small portion which is left combines with fetal red cells causing **only mild hemolysis.**

ANTIGLOBULIN (COOMBS) TEST

Q. Write short note on Coombs (antiglobulin) test.

It is useful to detect the presence of incomplete antibody (IgG) and/or complement on the RBC membrane.

Principle

- **RBCs coated with incomplete antibody (IgG) or C3 complement does not cause agglutination of RBCs.**

- Coombs reagent contains antibodies (antiglobulins) against human IgG/IgM/complement.
- If the RBCs coated by incomplete antibody or complement, are **treated with Coombs reagent**, the antiglobulins in the reagent will **induce agglutination** of such RBCs.

Types of Antiglobulin Test (Fig. 11.38)

- **Direct** (Coombs) **antiglobulin test (DAT)**
- **Indirect** (Coombs) **antiglobulin test (IAT)**

Direct Antiglobulin Test (Fig. 11.38)

Q. Write short note on direct Coombs (antiglobulin) test, its procedure and conditions in which it is positive.

Direct antiglobulin test (DAT) or direct Coombs test **detects antibodies** (IgG) and/or complement coated **on the surface of patient's RBC** membrane.

- Patient's RBCs are taken in a test tube and washed three times in normal saline.
- Coombs (antiglobulin) reagent is added and observed for agglutination.
- Agglutination indicates the presence of antibody on the RBC membrane and interprets as positive DAT.

Uses of Direct Antiglobulin Test

- **Hemolytic disease of the newborn** (HDN), in which direct Coombs test is performed on the newborn baby's red cells from the cord blood. This test will be positive.
- **Autoimmune hemolytic anemia:** To demonstrate in vivo attachment of antibodies to red cells.

- **Drug-induced red cell sensitization.**

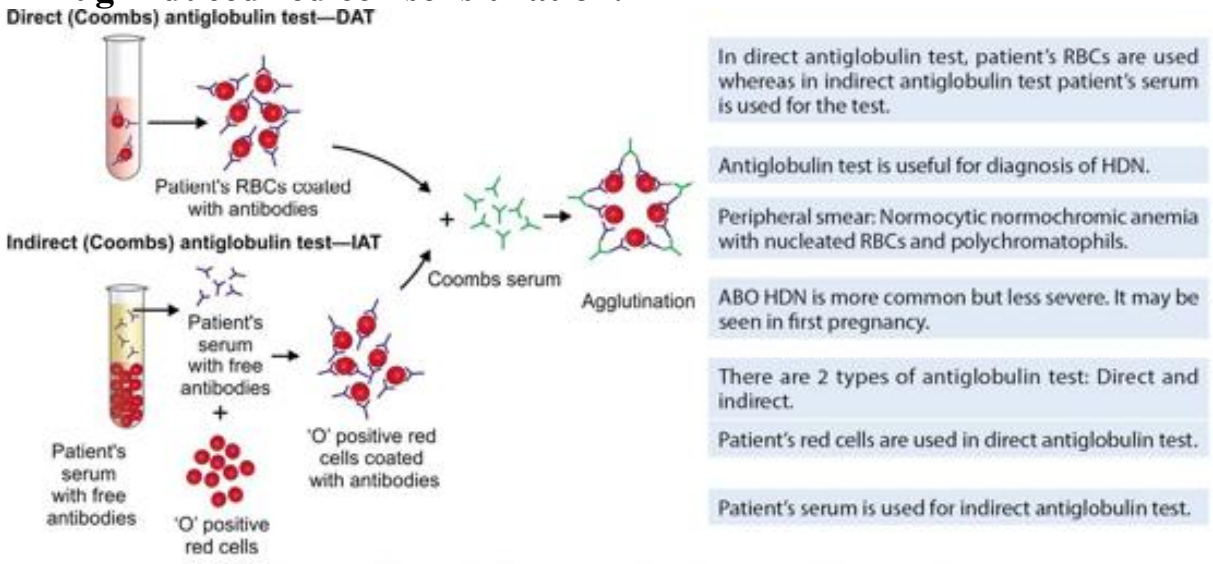


Fig. 11.38: Direct and indirect methods of antiglobulin test (Coombs test).

- **Investigation of hemolytic transfusion reaction.**

Indirect Antiglobulin Test (Fig. 11.38)

Indirect antiglobulin test (IAT) or indirect Coombs test **detects the presence of incomplete antibodies (IgG) and/or complement in the patient's serum.**

- In this test, patient's serum is taken and "O" Rh positive cell suspension of any normal individual is added.
- "O" Rh positive RBCs are coated with (lgG) anti-Rh antibodies (if present) in the patient's serum.
- Add Coombs (antiglobulin) reagent and examine for agglutination.
- Agglutination of RBCs indicates the presence of antibodies in the patient's serum and test is reported as positive for indirect antiglobulin test.

Patient's serum + O Rh positive RBC suspension + Coombs reagent → Agglutination (test positive).

Uses of Indirect Antiglobulin Test

- **Hemolytic disease of newborn:** Mother's serum is tested to detect anti-Rh antibody.
- **Cross-matching for blood transfusion:** To detect incompatibility of recipient's serum with donor's cells.

AUTOIMMUNE HEMOLYTIC ANEMIA

- Antibodies against self-antigens on the RBC membrane cause premature destruction of RBCs.
- Anti-RBC antibodies can be divided into three general categories **(Box 11.12)**. Interaction of the autoantibody with the red cell antigen is **dependent on the temperature, i.e., warm or cold type.**

Warm Antibody Type

- Most common type (50–70%).
- **Idiopathic** (primary) or **secondary** to drug exposure or predisposing disease.
- **IgG** type antibodies combine with RBC antigen at 37°C—**warm antibody.**
- **Direct antiglobulin test:** DAT (Coombs test) **positive** in 90–95% cases.
- **LE cell test: Positive** in SLE with secondary autoimmune hemolytic anemia (AIHA).

Cold Agglutinin Type

- Caused by **cold agglutinins.**
- Mediated by IgM antibodies optimally active below 30°C.
- Occurs as a complication of infections (e.g., infectious mononucleosis, *Mycoplasma* infections) and lymphoid neoplasms.

Cold Hemolysins Type (Donath–Landsteiner Antibodies)

- Autoantibodies directed against the P antigen system on red cells.
- Responsible for a rare disorder known as **paroxysmal cold hemoglobinuria**.
- **Direct antiglobulin test** is usually **negative.**

Immunohemolytic anemias are characterized by the destruction of RBCs by either allo or auto antibodies.

Immunohemolytic anemias are mainly classified as:

1. Alloimmune and
2. Autoimmune hemolytic anemia.

Hemolytic transfusion reactions are due to ABO mismatch. The antibodies present in the recipient's serum coat donor's RBCs and lead to intravascular hemolysis.

The type of antibody causing autoimmune hemolytic anemia may be warm antibody or cold agglutinin or cold hemolysin.

Warm AIHA: Mediated by IgG autoantibody-optimally active at 37°C.

FRAGMENTATION SYNDROME

The **RBCs subjected to trauma** (physical or mechanical) in the circulation can undergo **fragmentation** and result in **intravascular hemolysis** leading to hemolytic anemias. These are known as fragmentation syndrome.

Classification

According to the site of hemolysis it is classified as:

Macroangiopathic (large vessels) **hemolytic anemia:** They are also known as cardiac hemolytic anemias the red cell trauma is from an abnormal vascular surface (e.g., prosthetic heart valve, synthetic vascular graft).

Q. Write short note on microangiopathic hemolytic anemia.

Microangiopathic hemolytic anemia (MAHA) occurs in capillaries due to abnormal narrowing of the lumen (e.g., disseminated intravascular coagulation).

Laboratory Findings

Q. Write short note on peripheral blood picture in microangiopathic hemolytic anemia.

They are similar in both macro- and microangiopathic hemolytic anemias.

- **Hemoglobin:** Decreased. Anemia is mild to moderate.

PERIPHERAL SMEAR

- RBCs: Show **fragmented red blood cells (known as schistocytes), polychromasia,** with or without nucleated red blood cells and microspherocytes. The **fragmented red cells** assume different shapes like **"triangular cells", "burr cells", "helmet cells"and are the hallmark of diagnosis** of this group of hemolytic anemias.
- WBCs: Count within normal limits or increased.
- Platelets: Thrombocytopenia is often seen.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

Q. Write short note on paroxysmal nocturnal hemoglobinuria.

It is a **rare** and is the only hemolytic anemia **acquired** mutation in the hematopoietic stem cell.

Etiology and Pathogenesis

- **Acquired mutations in the phosphatidylinositol glycan-group A** (PIGA) gene in **the hematopoietic stem cell.**
- PIGA gene mutation causes **deficient synthesis of GPI-linked proteins** in blood cells and loss of anchor for decay-accelerating factor (DAF). Normally, DAF is responsible for complement degradation.
- RBCs are abnormally sensitive to **complement-mediated intravascular hemolysis**.

Clinical Features

- **Intravascular hemolysis:** Hemoglobin in acidic urine is converted into acid hematin and **results in dark brown urine**.
- **Thrombosis:** In the hepatic, portal or cerebral veins.

Laboratory Findings

- **Ham's acidified serum test and sucrose hemolysis test:** Patient's RBCs undergo lysis when incubated with acidified serum (Ham test) or sugar (sucrose hemolysis test).
- **Flow cytometry:** Detects RBC deficient in GPI-linked proteins (CD55 and CD59) and is useful for diagnosis of PNH.

PNH is an acquired disorder in which there is deficiency of GPI- linked proteins, which normally protect the red cells against complement mediated lysis.

In PNH, RBCs are very sensitive to complement-mediated hemolysis.

PNH: Ham's acidified serum test and sucrose hemolysis test positive.

During recovery phase of acute blood loss, peripheral smear show reticulocytosis.

ANEMIAS OF BLOOD LOSS

Acute Blood Loss (Hemorrhage)

- Causes **loss of intravascular volume** and if massive can lead to **hypovolemic shock** and death.
- **Bleeding** may be **external** (e.g., open fracture, knife wound) or **internal** (e.g., ruptured spleen, ruptured abdominal aneurysm).

PERIPHERAL SMEAR

- RBCs: Normocytic normochromic anemia. Polychromasia during the recovery phase due to increased reticulocytes.
- WBCs: Leukocytosis.
- Platelets: Increased in number (thrombocytosis) during recovery phase.

Chronic Blood Loss

Produces anemia when the rate of blood loss exceeds the regenerative capacity of the bone marrow or when iron reserves are depleted and results in **iron deficiency anemia**.

SIDEROBLASTIC ANEMIAS

Q. Write short note on sideroblastic anemia.

Rare heterogeneous group of refractory anemias characterized by:

- **Ring sideroblasts** in the bone marrow aspirate **(Fig. 11.39)**.
- **Dimorphic peripheral blood picture:** Microcytic hypochromic red cells in hereditary form and macrocytic in the acquired form of the disease mixed with normochromic cells.
- Iron-containing inclusions **(Pappenheimer bodies)** in the RBCs.
- **Increased serum-iron** concentration and markedly increased storage iron.
- Ineffective erythropoiesis

It is classified as:

1. Hereditary sideroblastic anemia
2. Acquired sideroblastic anemia: idiopathic or secondary.

Sideroblastic anemias are rare refractory anemias which may be hereditary or acquired.

APPROACH TO THE DIAGNOSIS OF HEMOLYTIC ANEMIA

It is a three step process:

1. Diagnosis of anemia as hemolytic **(Flowchart 11.1)**.
2. Determine the cause of the hemolysis **(Flowchart 11.2)**
   - Acquired
   - Hereditary
3. Confirmation of the disease by specific test **(Table 11.17)**.

MISCELLANEOUS

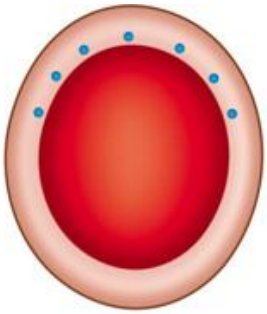
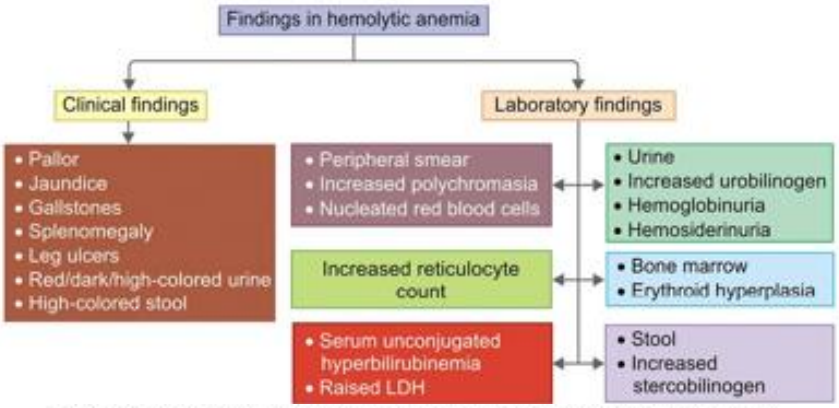


Fig. 11.39: Ring sideroblasts with partial perinuclear ring of iron granules.

Flowchart 11.1: Steps involved in the diagnosis of hemolytic anemia.



Flowchart 11.2: Steps involved in identifying the causes of hemolytic anemias.

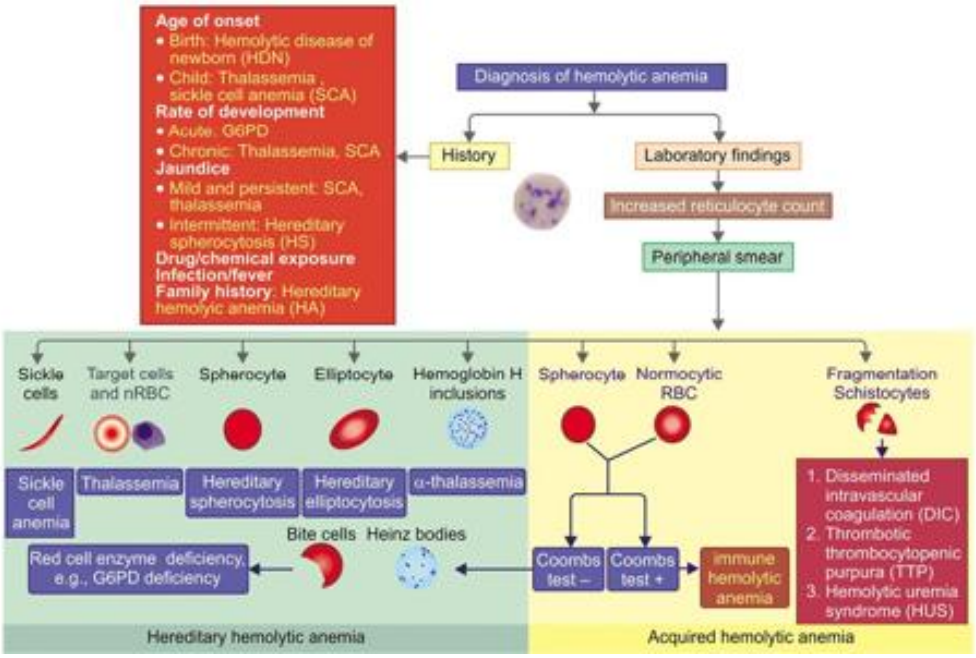


TABLE 11.17: Confirmation of hemolytic anemia by special tests.

Osmotic fragility test	***Sickling test***
Autohemolysis test	Screening test for G6PD

Hemoglobin electrophoresis	High-performance liquid chromatography (HPLC)
Estimation of HbF, HbA_2	

Erythropoietin (EPO)

Q. Write short note on erythropoietin.

It is a glycosylated protein synthesized mainly by kidney and minor part from liver. It is produced in response to hypoxia. EPO acts on the erythroid precursors through EPO receptors. This stimulates proerythroblast to proliferate and differentiate to produce RBCs.

Parasites Causing Anemia

Q. Write short note on parasites causing anemia.

- Parasites causing anemia
- Plasmodium: falciparum, vivax, ovale, malariae
- Diphyllobothrium latum (megaloblastic)
- Leishmania donovani
- Ancylostoma duodenale (iron deficiency)
- Necator americanus
- Taenia solium

CLINICAL SCENARIO

Iron Deficiency Anemia

1. History strongly indicative of **iron deficiency (IDA):** A pale patient with tiredness, weakness, malaise, a sore tongue (glossitis), pica **(craving for ice)** and **spoon-shaped nails** (koilonychia). **Signs and symptoms are** usually **due** to both **anemia** and the **underlying cause** of anemia.
2. Diagnosis of **IDA** would be strengthened in a patient who has underlying cause of anemia such as gastrointestinal (e.g., carcinoma colon,

hemorrhoids) or gynecologic disease (e.g., menorrhagia/excessive menstrual blood flow), malnutrition, pregnancy, and malabsorption.

3. IDA: Diagnosis is typically based on laboratory results. **Microcytic hypochromic anemia, low MCV, MCH, MCHC, reduced serum ferritin** and other serum profile observed in IDA (**Table 11.6**).

Case 1

History: A 28-year-old **pregnant** lady comes to hospital with complains of weakness, easy fatigability and breathlessness of 4 months duration. She is a **laborer** of low economic status and used to **eat clay**. On examination, she is pale and had **spoon shaped (koilonychia) nails.**

Case 2

History: A 20-year-old girl complains of weakness, easy fatigability and breathlessness of 6 months duration. She also complains of **heavy menstrual bleeding** every month. On examination, she is pale and had **spoon shaped (flattened) brittle nails** and **cheilosis.**

Case 3

History: A 73-year-old male complains of increasing weakness, malaise, and easy fatigability for the past 10 months. On examination, he is pale without hepatosplenomegaly. He also complains of **black tarry stool** (stool for occult blood was positive).

Laboratory findings for cases 1, 2, 3: Hb 9.1 g/dL, hematocrit 27.3%, RBC count 3 million/cu mm, WBC count 6,700/cu mm and platelet count 4,20,000/cu mm. **MCV 70 fL**, **MCH 26 pg**, **MCHC 28 g/dL** and **RDW 18**.

Cause of IDA: The causes for iron deficiency:

- Case 1 is probably due to **inadequate intake** (due to low socioeconomic status) or increased demand of iron during pregnancy.
- Case 2 is caused by **chronic blood loss**, due to **excessive menstrual flow.**
- Case 3 is due to **chronic blood loss** through **gastrointestinal tract**. The black tarry stool indicates that the patient has occult bleeding probably from GI tract malignancy.

Megaloblastic Anemia

- History strongly indicative of **megaloblastic anemia** (vitamin B_{12} deficiency and/or pernicious anemia): Middle-aged, pale patient with prematurely gray hair, **severe glossitis** and stomatitis and a smooth painful tongue, **significant neuropathy** and difficulty in walking,

uncoordinated gait, **impairment of vibration sense and position sense** with delirium/dementia.

- Patients with megaloblastic anemia due to folic acid/folate deficiency present with symptoms of anemia or of the underlying cause.
- Peripheral smear with **macro-ovalocytes, hypersegmented neutrophils, raised MCV, bone marrow with megaloblasts** are features of **megaloblastic anemia**.

Case 4

History: A 34-year-old male, **pure vegetarian**, executive complains of anorexia, premature graying of hair, weight loss and **tingling and numbness** in fingers and toes. On examination, he is pale and anemic. The **tongue** is **shiny** and has **glazed** appearance (glossitis).

Laboratory findings: Hb 9 g/dL, WBC count 3,800/cu mm, platelet count 60,000/cu mm, RBC count 2.5 million/cu mm. **MCV is 104 fL**, MCH 32 pg and MCHC is 32 g/dL.

Case 5

History: A 45-year-old female complains of tiredness, mild breathlessness while climbing steps and **tingling and numbness**. On examination, she is pale, has **raspberry-red tongue**, and shows numbness and paresthesia. Biochemical investigation reveals **normal serum iron profile**.

Laboratory findings: Hb 8.2 g/dL, WBC count 4,800/cu mm, platelet count 1,20,000/cu mm, **MCV is 108 fL**, MCH 33 pg and MCHC is 32 g/dL. Peripheral smear and bone marrow examination confirms the clinical diagnosis. Patient is further subjected to gastric biopsy and shows **atrophic gastritis**. Other test including serological tests is done.

Case 6

History: A 50-year-old male complains of loss of appetite, increasing fatigue tingling and **numbness of both lower limbs** and difficulty in walking for the past 10 months. On physical examination, he is pale and tongue was beefy red.

Laboratory findings: Hb 9.2 g/dL, hematocrit 27.9%, **MCV 132 fL**, platelet count 242,000/cu mm, and WBC count 7590/cu mm. Peripheral smear shows **macro-ovalocytes** and **hypersegmented neutrophils.**

Hereditary Spherocytosis

History strongly indicative of chronic extravascular hemolysis, usually but not always **hereditary spherocytosis**: Normal patient presenting with **pallor, mild jaundice, an enlarged spleen (classic triad**) and a family history of a relative who had been ‘‘cured’’ of the same problem by splenectomy. Also family history of **pigment gallstones** in a young person.

Peripheral smear with **spherocytes and raised osmotic fragility** test are features of HS.

Case 7

History: A 24-year-old women complains of chronic fatigue since childhood and mild icterus. On examination she is **anemic, jaundiced and has moderate splenomegaly**. Her mother had similar complaints and has multiple **pigment gallstones**.

Case 8

History: A 15-year-old girl presents with weakness and fatigue. On examination, her sclera shows mild jaundice and mild splenomegaly. **Family history** indicated about her father having recurrent gallstones.

Laboratory findings for cases 7 and 8: Hb 11.1 g/dL, RBC count 3.6 million/cu mm. WBC count 6,800/cu mm and platelet count 1,75,000/cu mm. MCV is 78 fL, MCH is 32 pg and MCHC is 38 g/dL. In a laboratory test, her **RBCs lyse at a higher concentration of saline (osmotic fragility test)** compared to normal patients.

Thalassemia Major

- History strongly indicative of **thalassemia major: Short stature, failure to thrive, severe pallor, jaundice,** maxillary over-growth (bossing), **frontal bossing**, sallow complexion and splenomegaly.
- Laboratory finding of **microcytic hypochromic anemia** with **many target cells, reduced MCV, MCH and MCHC, increased serum iron** are features of thalassemia major.

Case 9

History: An 11-month-old male child was brought to the pediatric outpatient by his parents and complained that the child was **failing to thrive**. On examination, jaundice, **pallor** and a **palpable spleen** was detected.

Laboratory findings: Hb 7.8 g/dL, hematocrit 23.4%, **MCV 66 fL,** platelet count 1,75,000/cu mm, and WBC count 8,200/cu mm. His **serum ferritin was 3250 ng/mL**.

Peripheral examination showed **microcytic hypochromic anemia** with many **target cells.** A bone marrow aspiration performed and reveals a myeloid: erythroid ratio of 1:4, and increased iron stores.

Sickle Cell Anemia

- History strongly indicative of **sickle cell anemia: Recurrent episodes of vaso-occlusion** in connective and musculoskeletal structures anywhere in the body producing ischemia and manifesting as **acute pain**, **tenderness and fever**. Child may show generalized impairment of growth and development. **Adults** manifest with **chronic organ damage**.
- Presence of **sickle cells** in the peripheral smear, **positive sickling test**, along with demonstration of HbS by **hemoglobin electrophoresis** and **HPLC** is diagnostic of **sickle cell anemia**.

Case 10

History: A 10-year-old boy complains of **severe pain** in the **chest, abdomen and bones**. On enquiry, his mother reveals that he had **several episodes** of severe abdominal and back pain since early childhood. Physical examination shows **anemia, jaundice and leg ulcer.**

Laboratory findings: Hb 11.0 g/dL, RBC count 3.2 million/cu mm, WBC count 8,800/cu mm and platelet count 1,95,000/cu mm. Peripheral smear examination, **hemoglobin electrophoresis and HPLC** confirms the diagnosis.

CHAPTER 12

Disorders of White Blood Cell, Lymph Node, and Spleen

QUANTITATIVE AND QUALITATIVE DISORDERS OF LEUKOCYTES

NORMAL DIFFERENTIAL LEUKOCYTE COUNT

The normal range of differential leukocyte count (DLC) in an adult is presented in **Table 12.1**.

TABLE 12.1: Normal range of different leukocytes in an adult.

Type of white blood cell	*Normal range*
Neutrophils	40–70% (2.0–7.0 × 10^9/L)
Lymphocytes	20–40% (1.0–3.0 × 10^9/L)
Monocytes	2–10% (0.2–1.0 × 10^9/L)
Eosinophils	1–6% (0.02–0.5 × 10^9/L)
Basophils	<1% (0.02–0.1 × 10^9/L)

QUANTITATIVE DISORDERS OF LEUKOCYTES

CC: Enumerate and describe the causes of leukocytosis leukopenia lymphocytosis and leukemoid reactions.

Leukocytosis

Q. Define leukocytosis and list its causes.

Definition: An increase in the **total** number of **leukocytes** in the blood **>11,000/cu mm** (11×10^9/L).

Causes: Common causes of leukocytosis are shown in **Box 12.1**.

Leukopenia

Q. Write short note on leukopenia.

Definition: Total leukocyte count is <4,000/cu mm (4×10^9/L).

Causes: Common causes of leukopenia are shown in **Box 12.2**.

Disorders of Neutrophils

Q. Define neutrophilia and mention its causes.

Neutrophilia (Fig. 12.1)

Definition: An absolute neutrophil count of >8000/cu mm (8×10^9/L). Differential count shows >70% neutrophils and is usually accompanied by leukocytosis ($15–30 \times 10^9$/L).

BOX 12.2: Common causes of leukopenia.

- Typhoid and paratyphoid
- Anemia
  - Aplastic anemia
  - Megaloblastic anemia
- Hypersplenism
- Drugs including cytotoxic drugs
- Radiation
- Rarely leukemia

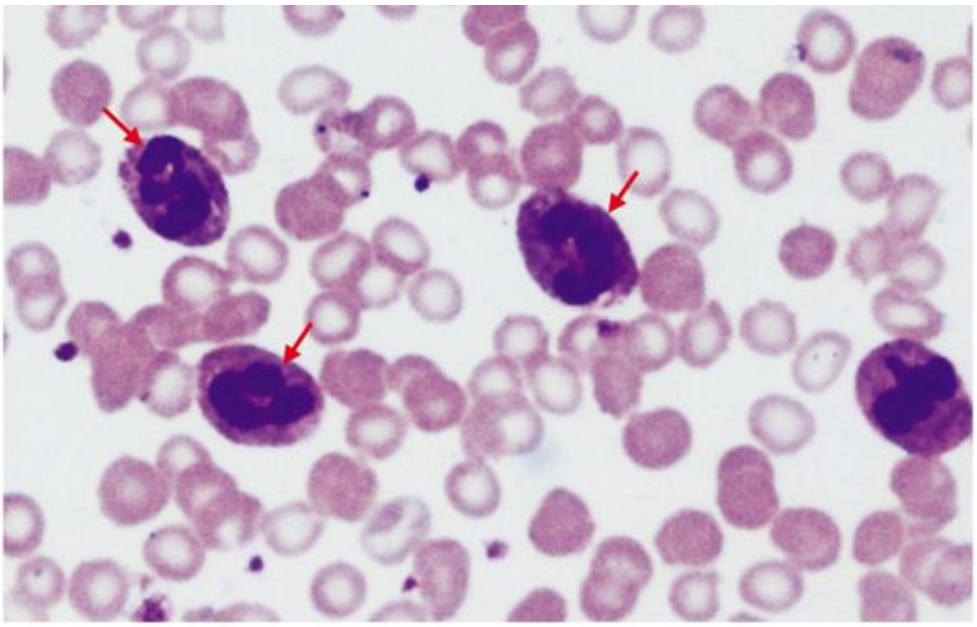


Fig. 12.1: Peripheral smear showing neutrophilia (arrows).

BOX 12.1: Common causes of leukocytosis.

- Infections
  - Bacterial
  - Viral infections (e.g., infectious mononucleosis)
- Leukemia
  - Acute
  - Chronic: Chronic lymphocytic leukemia and chronic myeloid leukemia
- Leukemoid reactions
- Physiological
  - Pregnancy
  - Exercise

Causes of Neutrophilia (Box 12.3)

BOX 12.3: Major causes of neutrophilia.

1. **Pathological:**
   - Acute bacterial and fungal infections:
     - Localized: Pyogenic microorganisms causing infections, e.g., pneumonias, pyogenic meningitis, cellulitis, diphtheria, abscess, tonsillitis, etc.
     - Generalized: Septicemia, acute rheumatic fever
   - Acute inflammatory processes: Inflammatory conditions (acute appendicitis), vasculitis
   - Tissue necrosis: Burns, myocardial infarction, gangrene, neoplasms (tumor necrosis)
   - Acute stress or hypoxic states: Following hemorrhage, hemolysis and surgery
   - Myeloproliferative neoplasms: Chronic myeloid leukemia, polycythemia vera

- Metabolic: Uremia, acidosis, gout
- Miscellaneous: Eclampsia, steroid therapy

2. **Physiological:**
   - Exercise (shift from marginating pool to circulating pool), newborns, extremes of temperature, pain, emotional stress and during obstetric labor

Leukemoid Reaction

Q. Write a short note on leukemoid reaction.

Definition: Benign leukocytic proliferation characterized by a moderately increased **total leukocyte count that rarely exceeds 50 × 10^9/L** with few immature white cells (like band forms, metamyelocytes and myelocytes).

It is characterized by a reactive increase in the white blood cell and the blood picture closely resembles various types of leukemia.

Types of leukemoid reactions

1. **Myeloid leukemoid reaction:** Various causes include—
   - **Severe bacterial infections** (e.g., staphylococcal pneumonia, endocarditis, meningitis, septicemia).
   - **Intoxications** (e.g., eclampsia, severe burns, mercury poisoning).
   - **Malignancy** (e.g., multiple myeloma, Hodgkin lymphoma, bone marrow metastasis).
   - **Severe hemorrhage and hemolysis.**

It should to be differentiated from chronic myelocytic/myeloid leukemia **(Table 12.2)**.

2. **Lymphoid leukemoid reaction:** Various causes include—
   - **Infections:**
     - **Viral** (e.g., infectious mononucleosis, cytomegaloviral infections)
     - **Bacterial:** Tuberculosis, whooping cough.
   - **Malignancy.**

Blood picture of lymphoid leukemoid reaction: Shows leukocytosis (not exceeding 100,000/μL) and mostly mature lymphocytes resembling the blood picture of chronic lymphocytic leukemia.

Neutropenia (Agranulocytosis)

Q. Write short note on agranulocytosis.

Definition: Reduction in the absolute neutrophil count (total WBC × % segmented neutrophils and band forms) below 1.5 × 10^9/L (1500/cu mm).

Etiology

Causes of neutropenia are described in **Box 12.4**.

Eosinophilia (Fig. 12.2)

Q. Write short essay/note on eosinophilia—definition and its causes.

Definition: Eosinophil count of >450/cu mm (0.45 × 10^9/L).

Causes of eosinophilia are presented in **Table 12.3**.

Q. Tabulate the differences between leukemia and leukemoid reaction.

TABLE 12.2: Differences between leukemoid reaction and chronic myeloid leukemia.

	Leukemoid reaction	***Chronic myeloid leukemia***
Clinical features	Features of causative disease	Splenomegaly, and bone pain are common
Peripheral blood findings		
WBC		
Total WBC count	Moderately increased, rarely exceeds 50 × 10^9/L	Markedly increased and usually> 50 × 10^9/L
Differential leukocyte count	Shift to the left with few immature forms. Toxic granulation seen	Shift to the left with numerous immature forms. Myelocyte and neutrophil peak
Eosinophilia and basophilia	Variable	Present
Leukocyte alkaline phosphatase (LAP)	Increased	Decreased
RBC		

TABLE 12.2: Differences between leukemoid reaction and chronic myeloid leukemia.

	Leukemoid reaction	***Chronic myeloid leukemia***
Anemia	Usually minimal or absent	Severe and progressive
Platelets		
Number	Variable	Normal or increased
Extramedullary myeloid tumors	Absent	Present
Philadelphia chromosome	Absent	Present

In leukemoid reaction, LAP score is raised and neutrophils may show toxic granulation.

BOX 12.4: Causes of neutropenia.

1. **Inadequate production:**
   - ***Suppression of stem cells****:* In these disorders granulocytopenia represents a component of pancytopenia
     - Aplastic anemia
     - Marrow infiltration
     - Metastatic tumors
     - Granulomatous disorders
   - Suppression of committed granulocytic precursors
     - Drugs and chemicals (e.g., sulfonamides, analgesics, arsenicals)
     - Ionizing radiation
   - Diseases associated with ineffective hematopoiesis
     - Megaloblastic anemias: Vitamin B_{12} or folate deficiency
     - Myelodysplastic syndromes
     - ***Congenital:*** Kostmann syndrome (rare)
     - ***Severe infections:***
       - Bacterial (e.g., typhoid, paratyphoid, septicemia)
       - Viral (e.g., influenza, infectious mononucleosis, hepatitis, measles)
       - Rickettsial (e.g., scrub typhus)
       - Protozoal (e.g., malaria, kala-azar)
2. **Increased destruction of neutrophils:**
   - Immunologically mediated destruction
     - Idiopathic
     - Secondary
       - Drugs
       - Autoimmune disorders, e.g., systemic lupus erythematosus
     - ***Splenic sequestration*** may be associated with pancytopenia
3. **Shift from the circulating pool to marginating pool:**
   - Hemodialysis and cardiopulmonary bypass
4. **Idiopathic: Mechanism not known**
   - Hodgkin and non-Hodgkin lymphoma

- Chronic lymphocytic leukemia
- Viral infections (HIV, hepatitis)
- Cyclic neutropenia

TABLE 12.3: Causes of eosinophilia.

1. Allergic/atopic conditions
- Asthma
- Hay fever
- Allergic rhinitis
- Urticaria
- Drug reactions

2. Parasitic infestations (with tissue invasion)
- Roundworm infestation
- Hookworm infestation
- Filariasis

3. Fungal infections (e.g., coccidioidomycosis)

4. Skin diseases
- Dermatitis (eczema)
- Scabies
- Pemphigus
- Dermatitis herpetiformis

5. Hematological diseases
- Chronic myeloid leukemia
- Hodgkin lymphoma
- Eosinophilic leukemia
- Polycythemia
- Acute myelomonocytic leukemia

6. Miscellaneous
- Tropical eosinophilia
- *Löeffler's* syndrome
- Eosinophilic granuloma
- Pulmonary eosinophilia
- Hypereosinophilic syndrome

Eosinophilia is seen in allergic reactions and parasitic infestations with tissue invasion.

Basophilia

Q. Write short essay/note on basophilia and its causes.

Normally basophils **(Fig. 12.3A)** are <1% of WBCs in peripheral blood.

Causes of basophilia include chronic myeloid leukemia, immediate hypersensitivity reactions, mastocytosis, viral infections (e.g., smallpox, chickenpox), etc.

Monocytosis (Box 12.5)

Q. Write short essay/note on monocytosis and its causes.

Definition: More than 10% of differential count or an absolute monocyte **(Fig. 12.3B)** count exceeding 500/cu mm (0.5×10^9/L).

Lymphocytosis

Q. Write short essay/note on lymphocytosis and its causes.

Definition: Lymphocyte (Fig. 12.3C) count >4,000/cu mm (4×10^9/L) in adults and >8,000/cu mm (8×10^9/L) in child.

Common causes of lymphocytosis **(Box 12.6)**.

Lymphocytopenia

Definition: Lymphocyte count below 1,500/cu mm (1.5×10^9/L) in adults and below 3000/cu mm (3×10^9/L) in children.

Some of the important causes of lymphocytopenia are listed in the **Box 12.7**.

Agranulocytosis: Neutrophil count below 0.5 × 109/L. The patients are highly susceptible to bacterial and fungal infections.

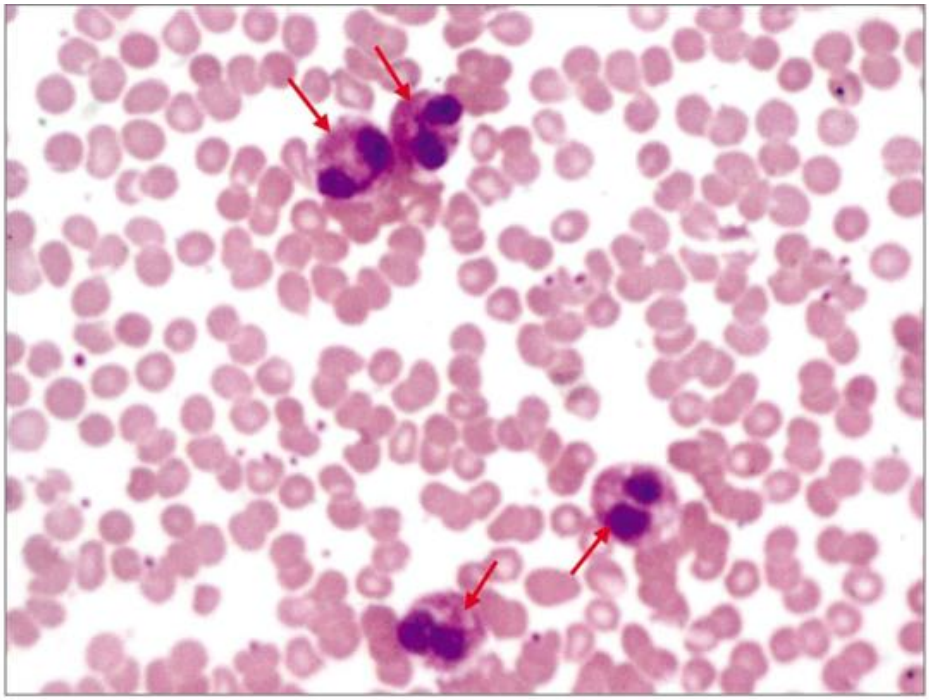
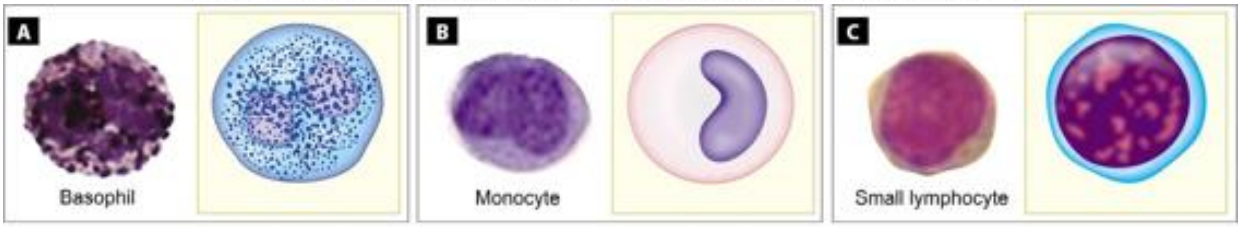


Fig. 12.2: Peripheral smear showing eosinophilia (arrow).



Figs. 12.3A to C (right diagrammatic): (A) Basophil; (B) Monocyte; (C) Lymphocyte.

BOX 12.5: Causes of monocytosis.

1. **Infections**
   - Bacterial: Tuberculosis, bacterial endocarditis, brucellosis
   - Protozoal: Malaria, kala-azar
   - Spirochetal: Syphilis
   - Rickettsial: Typhus, Rocky Mountain fever
   - Recovery phase of neutropenia and acute infections
2. **Inflammatory diseases**
   - Inflammatory bowel disease: Ulcerative colitis, Crohn disease
   - Autoimmune diseases: Systemic lupus erythematous, rheumatoid arthritis
   - Sarcoidosis
3. **Hematologic malignancies**
   - Acute monocytic, myelomonocytic and myelocytic leukemias
   - Chronic myelomonocytic leukemia
   - Hodgkin lymphoma
   - Multiple myeloma

BOX 12.7: Causes of lymphocytopenia.

1. **Increased destruction**
   - Corticosteroids
   - Cytotoxic drugs
   - Radiation
2. **Decreased production**
   - Aplastic anemia
   - Advanced malignancy: Hodgkin lymphoma
   - Infections: AIDS, miliary tuberculosis
3. **Increased loss via GI tract**
   - Obstruction to intestinal lymphatic drainage (e.g., tumor)
   - Congestive heart failure

Leukoerythroblastic Reaction/Blood Picture

Q. Write short note on leukoerythroblastic reaction/blood picture and its causes.

Definition: It is the presence of immature white blood cells (myelocytes, metamyelocytes and promyelocytes) as well as nucleated RBCs in the peripheral blood.

Causes:

- **Infectious diseases:** Miliary tuberculosis
- **Bone metastasis** (e.g., carcinoma of lung, breast, prostate, GI tract)
- **Hematological conditions:** Myelofibrosis, severe hemolysis (e.g., erythroblastosis fetalis), multiple myeloma, lymphoma
- **Storage disorders:** Gaucher's disease, Niemann–Pick disease.

Neutrophils in bacterial infections show toxic granules.

Dohle bodies are small round to oval structures seen in the cytoplasm, can also be observed in bacterial infections.

Leukemoid reaction: Benign exaggerated leukocyte proliferation to be differentiated from leukemia.

Neutropenia: Absolute neutrophil count below 1500 cells/cu mm.

Eosinophilia: Eosinophil count >450 cells/cu mm.

Lymphocytosis: Lymphocyte count >4,000/cu mm in adults and >8,000/cu mm (8 × 109/L) in child.

BOX 12.6: Causes of lymphocytosis.

1. **Acute infections**
   - Viral infections: Infectious mononucleosis, mumps, measles, chickenpox, infectious hepatitis
   - Toxoplasmosis
2. **Chronic infections/inflammatory diseases**
   - Tuberculosis
   - Syphilis
   - Brucellosis
   - Inflammatory bowel disease: Crohn disease and ulcerative colitis
3. **Hematologic malignancies**
   - Acute lymphoblastic leukemia
   - Chronic lymphocytic leukemia
   - Non-Hodgkin lymphoma with spill over
   - Adult T-cell leukemia/lymphoma
   - Hairy cell leukemia

Dengue fever is caused by flavi virus transmitted by freshwater mosquito (Aedes aegypti). Peripheral smear shows transformed lymphocytes and thrombocytopenia.

QUALITATIVE DISORDERS OF LEUKOCYTES

Q. Write short note on qualitative/functional disorders of leukocytes.

Qualitative disorders of leukocytes are rare familial disorders that manifest as morphologic changes in the leukocytes **(Fig. 12.4)**.

INFECTIOUS MONONUCLEOSIS (GLANDULAR FEVER)

Q . Write short essay/note on infectious mononucleosis.

Definition: Acute, benign, self-limiting lymphoproliferative disorder **caused by Epstein-Barr virus** (EBV).

- ***Incubation period:*** 4–8 weeks.
- ***Mode of transmission:*** Oropharyngeal secretions (kissing), hence the nickname kissing disease.

Pathogenesis

- **EBV infects B lymphocytes** by binding to **CD21 (CR2) receptor.**
- Viral infection begins in the submucosal lymphoid tissues of nasopharynx and oropharynx.
- Virus remains dormant inside the B-cells.
- B-cells are **"immortalized"** and are capable of **proliferation indefinitely.**

Clinical Features

- ***Age:*** Young adults among upper socioeconomic classes in developed nations and children of low socioeconomic status.
- ***Signs and symptoms:*** **Classical triad**
  - **Fever**
  - **Pharyngitis (sore throat)**
  - **Lymphadenopathy.**

Laboratory Finding

Q. Mention the laboratory findings in infectious mononucleosis.

- **Total leukocytes count increased** (12,000–25,000 cells/cu mm): **Absolute lymphocytosis.**
- **Atypical lymphocytosis (mononuclear cells): These are $CD8^+$ subset (cytotoxic) of T-cells** and not the virus-infected B-cells.

Q. Write short note on qualitative disorders of leukocytes.

Qualitative abnormality	Mode of inheritance	Characteristic morphology	Functional abnormalities	Appearance of leukocyte
Pelger–Huet anomaly	Autosomal dominant	Lack of neutrophil nuclear segmentation beyond 2 lobes	Normal	
Alder–Reilly anomaly	Autosomal recessive	**Large lilac inclusions** in cytoplasm of all leukocytes	Normal	
May–Hegglin anomaly	Autosomal dominant	**Large basophilic inclusions** in all leukocytes. Giant platelets and thrombocytopenia	Abnormal bleeding due to thrombocytopenia	
Chediak–Higashi anomaly	Autosomal recessive	• **Large gray blue granules in cytoplasm** of monocytes and granulocytes • Defective lysosomal granules	• Poor chemotaxis • Increased susceptibility to pyogenic infections • Bleeding tendency	
Chronic granulomatous disease of childhood (CGD)	X-linked autosomal recessive	Normal appearance but defective function	• Deficient NADPH oxidase, with absent H_2O_2 production • Phagocytosis and killing of organisms is impaired	

Fig. 12.4: Various quantitative disorders of leukocytes.

Chediak–Higashi anomaly is associated with increased susceptibility to pyogenic infections.

CGD is associated with impaired phagocytosis and killing of organisms.

Differential leukocyte count (DLC) is one of the routine, useful and important investigations.

Leukocytosis is usually due to increase in the neutrophils, but may also be due to increased lymphocytes (or rarely monocytes and eosinophils).

Leukopenia is the decrease in the WBC count below 4,000/cu mm.

The causes of leukopenia include typhoid and paratyphoid fever and aplastic anemia.

Neutrophilia: Absolute neutrophil count >8000 cells/mm.

Common causes of neutrophilia are infections, inflammatory conditions and tissue necrosis.

- Serological tests:
  - **Demonstration of heterophile antibodies:**
    - Paul Bunnell test is characteristically positive.
    - Monospot test is a sensitive slide test.
  - **Demonstration of specific antibodies against EBV antigens:**
    - Antibody against viral capsid antigens (anti-VCA).
    - Antibodies to Epstein–Barr nuclear antigen (EBNA).

EBV infects B-cells but the peripheral blood shows CD8+ T-cells, which appear as atypical lymphocytes.

Lesions caused by EBV:

- Infectious mononucleosis
- Burkitt lymphoma
- Nasopharyngeal carcinoma
- Hodgkin lymphoma
- X-linked lymphoproliferataive disorders
- Body cavity lymphoma

Demonstration of specific antibodies to EBV is the most specific test for infectious mononucleosis.

Acute Leukemia

CC: Describe the etiology, genetics, pathogenesis classification, features, hematologic features of acute leukemia.

Q. Define leukemia.

Definition: Acute leukemia is a malignant disease of the bone marrow stem cell and its characteristic features are:

BONE MARROW

Diffuse replacement with proliferating neoplastic blast cells that fail to mature. Blast cells >20% (WHO criteria) of the nucleated cells in the marrow.

PERIPHERAL BLOOD

Abnormal numbers and forms of immature white blood cells.

Aleukemic/subleukemic leukemia is characterized by very few/no blasts in the peripheral blood.

Acute leukemia are mainly divided into two groups, namely (1) acute lymphoblastic leukemia (ALL) and (2) acute myeloblastic leukemia (AML).

Etiology and Pathogenesis

Q . Write short essay/note on etiology of leukemias.

Risk factors **(Box 12.8)** may cause mutations in the proto-oncogenes and tumor suppressor genes.

Classification

Q. Classify acute leukemia.

Traditional classification depending on microscopic appearance of the involved cell and the course of leukemias is presented in **Box 12.9**.

FAB Classification of Acute Leukemias

- **First French, American and British (FAB)** classification (1976) was **based on the (1) morphological and (2) cytochemical characteristics of** blast cells.
- **Revised FAB classification (Box 12.10)**. It includes:
  1. Morphology and cytochemistry of blast cells
  2. Immunophenotyping
  3. Cytogenetics
  4. Molecular genetics.

WHO Classification (2016) of Acute Leukemia (Box 12.11)

Q. Classify acute leukemia as per WHO guidelines.

Differences between Myeloblast and Lymphoblast (Table 12.4)

Q. List the differences between myeloblast and lymphoblast.

BOX 12.8: Risk factors for acute leukemia.

ENVIRONMENTAL FACTORS

- **Ionizing radiation**
- **Drugs:**
  - Alkylating agents—nitrogen mustard, chlorambucil, etc.
  - AML occurs in myeloma patients treated with melphalan
  - Leukemia follows chemotherapy of lung and ovarian cancer
- **Chemicals:** Benzene (used in paint industry, plastic glues, etc.)

GENETIC DISORDERS

Example: Down syndrome (ALL or AML), Fanconi anemia (AML), ataxia telangiectasia (ALL, NHL)

ACQUIRED DISORDERS

- PNH and aplastic anemia may transform into acute leukemia
- AML may develop de novo or secondary to myelodysplastic syndrome (MDS)

BOX 12.9: Traditional classification of leukemia.

- **Acute leukemia**
  - Acute myelogenous/myeloblastic/myeloid leukemia (AML)
  - Acute lymphoblastic leukemia (ALL)
- **Chronic leukemia**
  - Chronic myeloid leukemia (CML)
  - Chronic lymphocytic leukemia (CLL)

BOX 12.10: Revised French, American and British (FAB) classification of acute leukemias.

Acute Lymphoid Leukemia

L1 Small homogenous cells with inconspicuous nucleoli

L2 Large cells with variable size and 1–2 nucleoli

L3 Large, homogeneous cells with finely stippled chromatin and prominent nucleoli. Cytoplasm is basophilic and vacuolated

Acute Myeloid Leukemia

M0 Minimally differentiated AML

M1 AML without maturation

M2 AML with maturation

M3 Promyelocytic leukemia

M4 Myelomonocytic leukemia

M5 Monocytic leukemia

M6 Erythroleukemia

M7 Megakaryocytic leukemia

AML M2 is the most common type of AML. AML M3 is associated with disseminated intravascular coagulation (DIC). AML M7 is the most common type of AML in Down syndrome.

BOX 12.11: WHO classification (2016) of acute lymphoid and myeloid leukemia.

A. Acute lymphoid leukemia

I. **B lymphoblastic leukemia/lymphoma**

- B lymphoblastic leukemia/lymphoma, NOS
- B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
  - B lymphoblastic leukemia/lymphoma with t(9;22)(q34; q 11.2); *BCR-ABL*1
  - B lymphoblastic leukemia/lymphoma with t(v;11q23.3); *KMT2A* rearranged
  - B lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
  - B lymphoblastic leukemia/lymphoma with hyperdiploidy
  - B lymphoblastic leukemia/lymphoma with hypodiploidy
  - B lymphoblastic leukemia/lymphoma with t(5; 14) (q31;q32); *IL3-IGH*

- B lymphoblastic leukemia/lymphoma with t(1;19) (q23:p13.3); *TCF3-PBXI*

II. **T lymphoblastic leukemia/lymphoma**

B. Acute myeloid leukemia

I. **AML with recurrent genetic abnormalities**

- AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
- AML with inv(16)(p13;q22); *CBFB-MYH11*
- APL with t(15;17)(q22;q12); *PML-RARA*
- AML with t(9;11)(p22;q23); *MLLT3-MLL*
- AML with t(6;9)(p23;q34); *DEK-NUP214*
- AML with inv(3)(q21q26.2) *RPN1-EVI1*
- AML(megakaryoblastic) with t(1;22) (p13;q13); *RBM15-MKL1*
- AML with mutated *NPM1*
- AML with mutated *CEBPA*

II. **AML with MDS-related changes**

III. **Therapy-related myeloid neoplasms**

IV. **AML not otherwise specified**

- AML minimally differentiated (M0)
- AML without maturation (M1)
- AML with maturation (M2)
- Acute myelomonocytic leukemia (M4)
- Acute monoblastic and monocytic leukemia (M5)
- Acute erythroid leukemia (M6)
- Acute megakaryoblastic leukemia (M7)

V. **Myeloid sarcoma**

VI. **Myeloid proliferation related to Down syndrome**

(AML: acute myeloid leukemia; APL: acute promyelocytic leukemia; MDS: myelodysplastic syndrome)

Cytochemistry in Leukemia

Q. Write short essay/note on cytochemistry in acute leukemia.

Cytochemistry is the study of chemical elements found in the cytoplasm of the cells. The chemical element in the cell may be an enzyme (e.g., myeloperoxidase) or non-enzymatic substance (e.g., lipids and glycogen).

Uses

- To characterize the blast cells as myeloid or lymphoid in acute leukemias. Sometimes, differentiating AML from ALL is not possible only on morphological grounds and in such cases cytochemical stains will be of help.

- To identify granulocytic and monocytic components of acute myeloid leukemia.
- To detect cytoplasmic abnormalities and enzyme deficiencies in myeloid disorders.

Myeloperoxidase

- Myeloperoxidase (MPO) is an enzyme present in the primary granules of **mature and immature** cells of **myeloid series (Fig. 12.8)**.
- **Principle:** When hydrogen peroxide is present, the myeloperoxidase within the cytoplasm oxidizes the dye substrate creating brown granules in the cytoplasm and the intensity of positivity increases with maturity of the cell.
- **Interpretation:** (1) Leukemic myeloblasts are positive (AML M1, M2, M3 and M4 blasts and Auer rods). (2) Lymphoblasts and normoblasts are negative. Thus, useful in differentiating AML from ALL.

Sudan Black B

- **Principle:** Sudan Black B (SBB) stains lipids and phospholipids present in the granules of the cells of **myeloid series (Figs. 12.9A and B)**. The granules take up black color. Staining pattern in MPO and SBB are similar.
- **Interpretation:** Useful in differentiation of AML from ALL.

TABLE 12.4: Differences between myeloblast and lymphoblast based on morphology and cytochemistry.

	Lymphoblast (Figs. 12.5, 12.10 and 12.11)	***Myeloblast (Figs. 12.6, 12.12 and 12.13)***
Size	2–3 times the size of lymphocyte	3–5 times the size of lymphocyte
Cytoplasmic characters		
Amount	Scanty (less cytoplasm than myeloblast)	Scanty to moderate (more cytoplasm than lymphoblast)
Color	Blue	Gray
Cytoplasmic granules	Agranular	May have cytoplasmic granules
Auer rod	Negative	Positive

TABLE 12.4: Differences between myeloblast and lymphoblast based on morphology and cytochemistry.

	Lymphoblast (Figs. 12.5, 12.10 and 12.11)	***Myeloblast (Figs. 12.6, 12.12 and 12.13)***
Nuclear characters		
Nuclear chromatin	Uniform, coarse	Uniform, fine
Nucleoli	Inconspicuous or 1–2	3–5, prominent
N:C (nuclear cytoplasmic) ratio	High	High
Accompanying cells	Lymphocytes	Promyelocytes, myelocytes, meta- myelocytes, band forms and neutrophils
Cytochemistry		
Myeloperoxidase	Negative	Positive
Sudan Black	Negative	Positive
PAS	Block positivity	Negative
Nonspecific esterase	Negative	Positive in M4 and M5

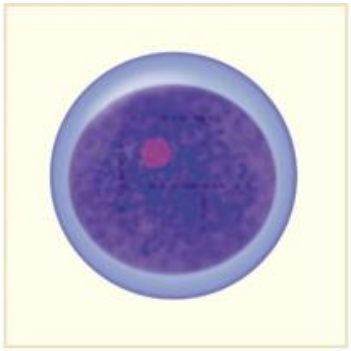


Fig. 12.5: Diagrammatic appearance of lymphoblast.

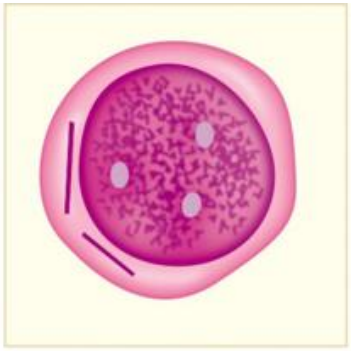


Fig. 12.6: Diagrammatic appearance of myeloblast.



Fig. 12.7: Periodic acid Schiff (PAS) stain showing lymphoblast with block positivity.

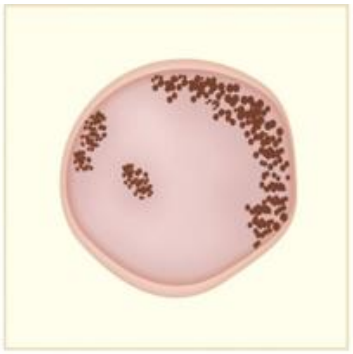
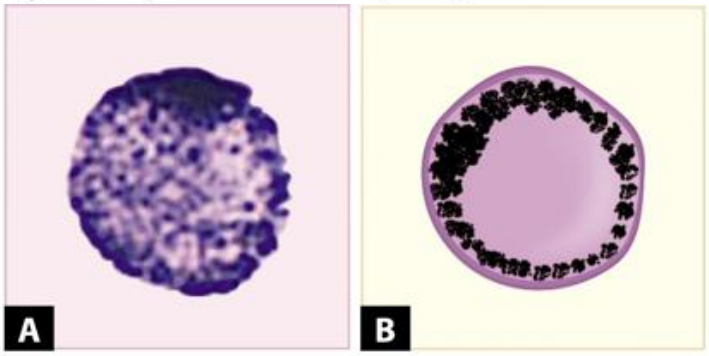


Fig. 12.8: Myeloblast stained positively with myeloperoxidase (MOP).



Figs. 12.9A and B: (A) Myeloblast stained positively with Sudan Black; (B) Diagrammatic.

Nonspecific Esterase

- Esterase is used to differentiate **myeloblast, neutrophilic precursors and neutrophilic granulocytes** from monocytic cells.
- **Principle:** Esterases are enzymes in cells of monocytic series and are used to differentiate them from myeloblasts and lymphoblasts.
- **Interpretation:** Esterase is useful for identification and confirmation of the monocytic component in AML M4 and M5. Monocytic cells show dark red cytoplasmic granules.

Periodic Acid-Schiff Reaction

- Periodic acid-Schiff (PAS) stain is useful for the diagnosis of ALLs, and erythroid and megakaryocytic (M6 and M7) type of acute myeloid leukemia.

- **Principle:** Many cells contain glycogen in their cytoplasm. The periodic acid oxidizes glycogen, mucoproteins and other high molecular weight (HMW) carbohydrates to aldehydes. These aldehydes react with colorless Schiff reagent and take bright-red pink (*magenta*) color. The staining pattern may be fine and diffuse, coarse and granular (block positivity), or a mixture of both patterns.
- **Interpretation:** Useful for differentiating AML from ALL.
  - **Myeloblasts are negative**. Erythroid type of acute myeloid leukemia may show PAS—positive erythroblasts.
  - **Lymphoblasts show blocks of PAS positive (Figs. 12.7** and **12.10** inset**)** material in the cytoplasm. Block positivity in lymphoblasts is observed in most of the ALL L1 cases.

Myeloblast: Comparison with lymphoblast has four M's
M: More in size
M: More nucleoli (3–5)
M: Moderate cytoplasm
M: Myeloperoxidase positive
Auer rod: Positive

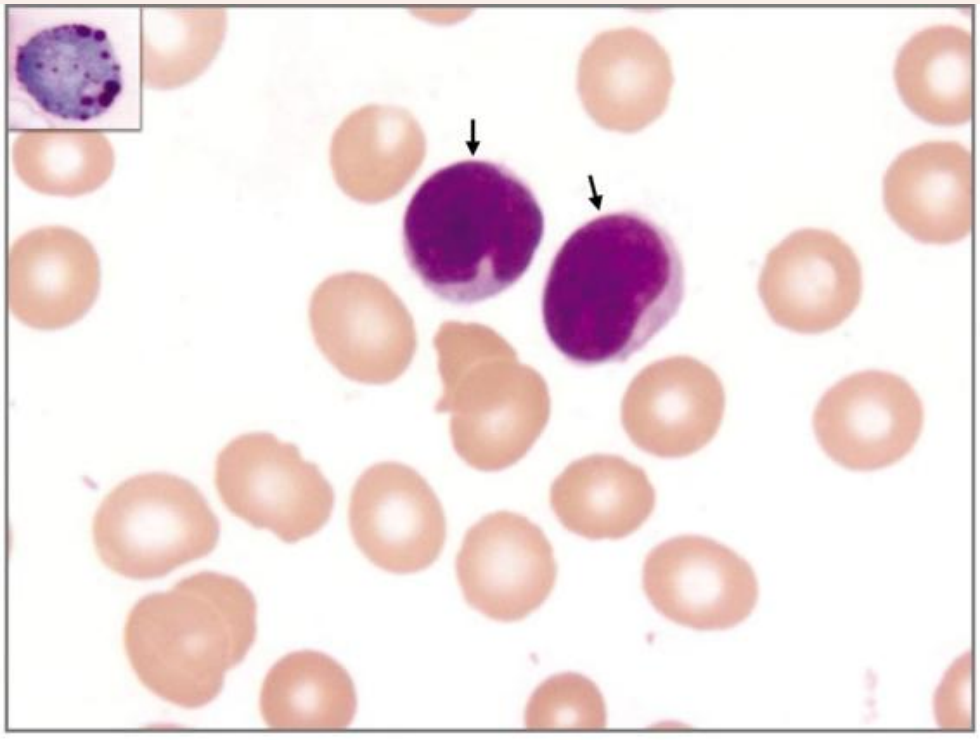


Fig. 12.10: Peripheral blood smears in acute lymphoblastic leukemia showing lymphoblasts (arrows). Inset shows lymphoblast with block positivity with PAS stain.

Neutrophil Alkaline Phosphatase

- **Principle:** Alkaline phosphatase is an enzyme located in the specific (secondary) or tertiary granules present in the cytoplasm of mature neutrophils, with some activity in metamyelocytes. The positive reaction gives rise to blue and granular appearance to the cytoplasmic granules.
- Normal NAP/LAP score: 40–100.
- **LAP increased** in (1) hematological disorders such as **leukemoid reaction, myelofibrosis, polycythemia rubra vera, idiopathic thrombocythemia,** and (2) non-hematological disorders such as pregnancy and Down syndrome.
- **LAP decreased** in **paroxysmal nocturnal hemoglobinuria and chronic myeloid leukemia**.

ACUTE LYMPHOBLASTIC LEUKEMIA/LYMPHOMA

Q. Define and classify leukemias. Describe the laboratory diagnosis (including peripheral smear, bone marrow finding and special stains) of acute lymphoblastic leukemia (ALL).

Definition: Acute lymphoblastic leukemia/lymphoma (ALL) is a group of neoplasms consisting of **lymphoblasts.**

- Lymphoblast is immature, precursor B (pre-B) or T (pre-T) lymphocyte.
- WHO classification **(Box 12.11)**:
  - **B lymphoblastic leukemia/lymphoma** (about 85%) are seen in **childhood** and present as **acute leukemias.**
  - **T lymphoblastic leukemia/lymphoma** (15%) present in adolescent males as **lymphomas**, often with involvement of mediastinum (thymus).

Molecular Pathogenesis

- Chromosomal abnormalities are found in about 90% of ALLs.

- **Numerical abnormality:** Hyperploidy (>50 chromosomes) and hypoploidy.
- **Structural abnormality:** Balanced chromosomal translocations (e.g., Philadelphia chromosome).
  - Most T-ALLs have mutations in *NOTCH1* gene.
  - Most B-ALLs have mutations in genes *PAX5*, *E2A* and EBF or a balanced translocation t(12; 21) involving the genes *TEL* and *AML1*.

Classification of Acute Lymphoblastic Leukemia (Boxes 12.10, 12.11 and Table 12.5)

Clinical Features

Age: Most common hematological malignancy of **children**. Most common between **1 and 5 years** of age and between **30 and 40 years.**

Sex: Slight male preponderance.

Onset: **Abrupt**.

Symptoms:

- **Bone marrow failure:**
  - Anemia: Causes **fatigue, weakness.**
  - Neutropenia: **Infections** by bacteria or opportunistic fungi. Develop sore throat and respiratory infections.
  - Thrombocytopenia: **Bleeding** into the skin and mucosa in the form of purpura or ecchymoses.
  - **Bone pain** and **sternal tenderness.**
- **Extramedullary infiltration:**
  - **Lymphadenopathy:** 75% of patients, usually involve **cervical lymph nodes.**
  - **Bone pain and tenderness.**
  - **Hepatosplenomegaly:** Splenomegaly is more common than hepatomegaly.
  - **Mediastinal thymic mass:** More common in T-ALL.
- **CNS involvement:** Spread into the meninges causes leukemic meningitis ALL (pre-B).

- Testicular involvement (ALL).

Laboratory Findings

Q. Write short note on laboratory/peripheral smear findings in acute lymphoblastic leukemia.

Peripheral Blood

- **Total WBC count: Markedly raised** ranging from 20 × 10^9/L to 200 × 10^9/L
- **Platelet count:** Reduced (thrombocytopenia).
- **Hemoglobin: Decreased** and may be as low as 3 g/dL.

TABLE 12.5: Characteristics of FAB subtypes of acute lymphoid leukemias (ALL).

FAB type	L_1	L_2	L_3
Cell size	Small cell size	Large heterogeneous cell population	Large, homogeneous cell population
Nuclear characteristics			
Shape	Regular	Irregular, clefting and indentation common	Regular, oval or round
Chromatin	Condensed	Dispersed chromatin	Finely stippled
Nucleolus	Small and inconspicuous	Visible, 1–2 in number	Usually prominent
Cytoplasmic characteristics			
Amount	Scanty	Variable, often abundant	Moderately abundant
Cytoplasmic basophilia	Slight to moderate	Variable	Strong
Cytoplasmic vacuolation	Absent	Variable	Prominent and oil red O stain positive

ALL-L1 has better prognosis than ALL-L3.

Morphologically, as per the FAB classification lymphoblast are classified as L1, L2 and L3.

ALL-L3 is a leukemic counterpart of Burkitt lymphoma.

PERIPHERAL SMEAR (FIGS. 12.11 AND 12.12)

- *RBCs:* Normocytic normochromic anemia.
- *WBCs:* Total count **markedly increased** and **20% or more lymphoblasts**.
  - **Morphology of lymphoblasts:**
    - **Larger than small lymphocyte**
    - **High N:C (nuclear cytoplasmic) ratio**
    - Nucleus with **condensed chromatin and nucleoli are either absent or inconspicuous**
    - **Scant to moderate agranular basophilic cytoplasm**.
- *Platelets:* Thrombocytopenia.

Cytochemistry of Lymphoblasts

- **PAS:** Cytoplasmic aggregates of **PAS positive (Figs. 12.7 and 12.10)** material **(block positivity).**
- **Myeloperoxidase (MPO) negative.**
- **Sudan Black B negative.**

BONE MARROW

- *Cellularity:* Markedly **hypercellular** due to proliferation of blasts.
- *Erythropoiesis and myelopoiesis:* Reduced.
- *Megakaryopoiesis:* Megakaryocytes gradually decrease.
- ***Blasts:*** **Constitute 20–100% of the marrow cells.**

Immunophenotyping

Terminal-deoxynucleotidyl-transferase (TdT) + in pre-B and **pre-T** lymphoblasts.

- Immature B-cells + positive for pan B cell marker CD19 and CD10 (CALLA—common ALL antigen).
- Precursor T ALL cells are positive for CD2, CD5 and CD8.

Biochemical Findings

- **Serum uric acid: Raised** due to destruction of leukemic cells **during chemotherapy** leading to hyperuricemia.
- **LDH: Raised**, because of increased turnover of leukemic cells.

CSF Examination

To know/rule out CNS involvement.

Prognosis: Prognostic features of ALL are presented in **Table 12.6**.

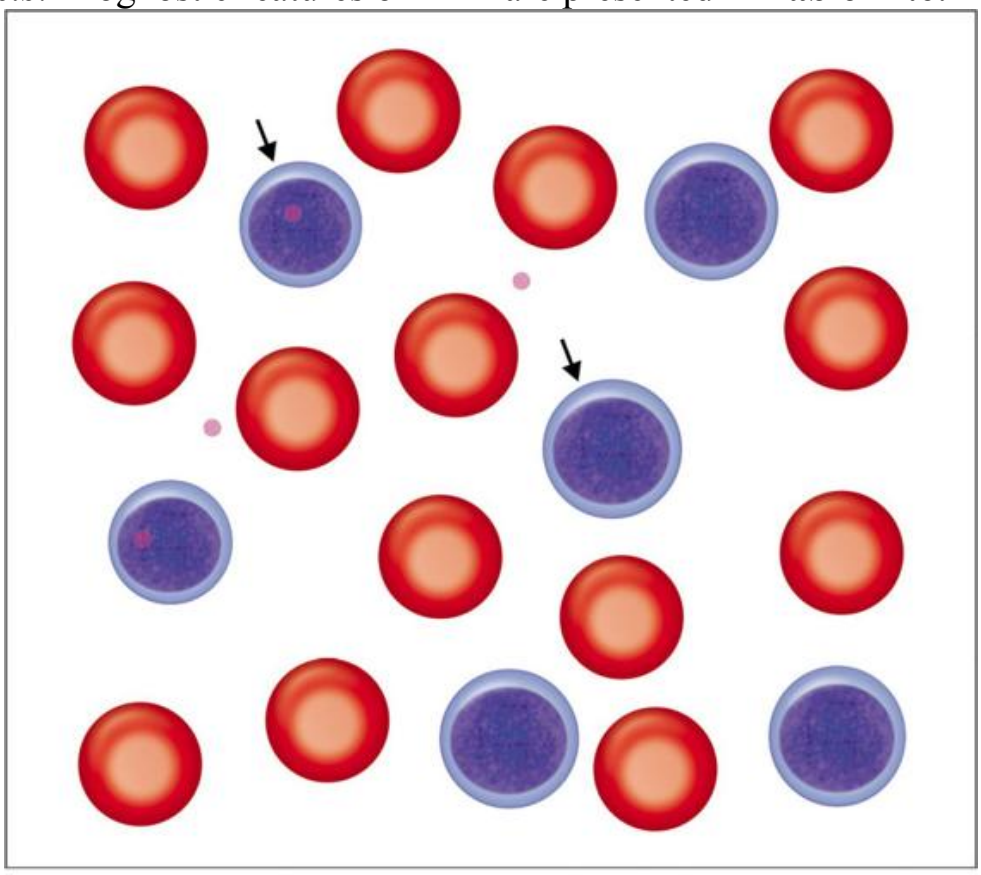


Fig. 12.11: Diagrammatic peripheral blood smear in acute lymphoblastic leukemia showing lymphoblasts (arrows).

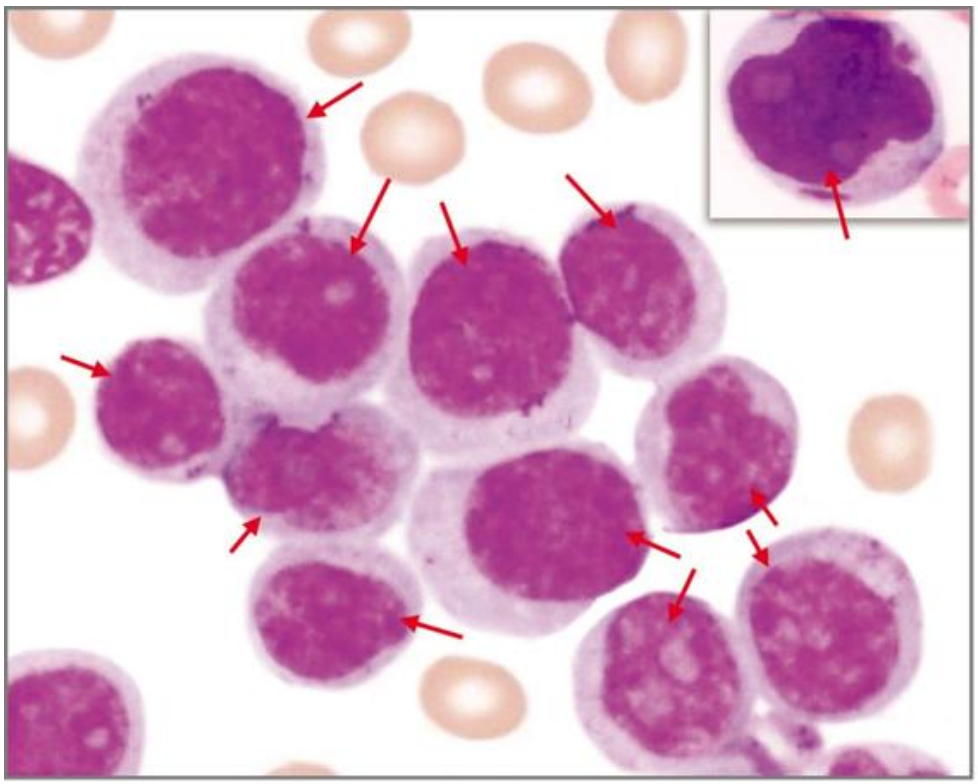


Fig. 12.12: Peripheral smear in AML with myeloblasts (arrows). Inset shows myeloblast with Auer rod (black arrow).

ACUTE MYELOGENOUS LEUKEMIA

Q. Define and classify leukemias. Describe the laboratory diagnosis of acute myeloid leukemia (AML). Describe the bone marrow findings in AML including special stains.

Q. Define leukemia. Classify acute myeloid leukemia (AML) as per WHO guidelines. Add a note on laboratory findings in a case of acute promyelocytic leukemia.

Definition: Neoplasm of hematopoietic progenitors characterized by proliferation resulting in **accumulation of immature myeloblasts in the marrow**.

Classification of acute myelogenous leukemia (AML): ***Refer*** **Boxes 12.10 and 12.11.**

Molecular Pathogenesis

- Many recurrent genetic abnormalities can disrupt genes encoding transcription factors involved in normal myeloid differentiation.
- **Mutated tyrosine kinase activation** is common.

Clinical Features

Age: AML may develop at **any age**, but is more **common in adults**.
Onset: Acute leukemias are **abrupt in onset**.
Symptoms: Related to depressed marrow function.

- ***Bone marrow failure*****:**
  - Anemia: **Fatigue and weakness**.
  - Neutropenia: **Life-threatening infections** by bacteria or opportunistic fungi.
  - Thrombocytopenia: **Bleeding**, patient may also develop disseminated intravascular coagulation (DIC) in AML M3 and primary fibrinolysis.
  - **Bone pain and tenderness**.
- ***Extramedullary infiltration:***
  - **Gingival hypertrophy (M4 and M5)** and infiltration of skin (leukemia cutis).
  - **Hepatosplenomegaly: Usually more than in ALL**.

Laboratory Findings

Q. Write short note on laboratory/peripheral smear findings in AML/ promyelocytic leukemia.

Peripheral Blood

- **Total WBC count:** Markedly raised, ranging from 20 × 10^9/L to 100 × 10^9/L.
- **Hemoglobin:** Decreased and ranges from 5 g/dL to 9 g/dL.

Q. Write short note on Auer rods.

PERIPHERAL SMEAR (FIGS. 12.12 AND 12.13)

- *RBCs:* Normocytic normochromic type of anemia.
- *WBCs:* Total WBC **count markedly increased.**
  - Differential count: **>20% myeloid blasts.** May show more than one type of blast or blasts with hybrid features.
  - **Morphology of myeloblasts:**
    - **3 to 5 times larger than** the diameter of a **small lymphocyte**.
    - High N:C (nuclear cytoplasmic) ratio.
    - **Fine nuclear chromatin with 2–4** variably prominent **nucleoli**.
    - **More cytoplasm than lymphoblasts**—azurophilic, peroxidase-positive granules.
    - Presence of Auer rods is definitive evidence of myeloid differentiation.
  - **Auer rods are azurophilic needle-like peroxidase-positive structures** in the cytosol of myeloblasts (M2 and M3 subtype).
- *Platelets:* Moderate to severe thrombocytopenia and causes bleeding from skin and mucosa.

TABLE 12.6: Prognostic factors in ALL.

	Unfavorable prognosis	*Favorable prognosis*
Age	Below 2 years and above 10 years (adolescence or adulthood)	Between 2 to 10 years
Sex	Males	Females
Total WBC count	High (>50,000 cells/cu mm)	Low
Blast count in peripheral blood	Greater than 100,000 cells/cu mm	< 100,000 cells/cu mm
Meningeal involvement	Present	Absent
Cytogenetic abnormalities	t (9;22) (the Philadelphia chromosome)	Hyperploidy, trisomy of chromosomes 4, 7 and 10 and t (12;21)
Time required for clearing blasts from blood	>1 week	<1 week

Prognosis is far better in ALL than AML.

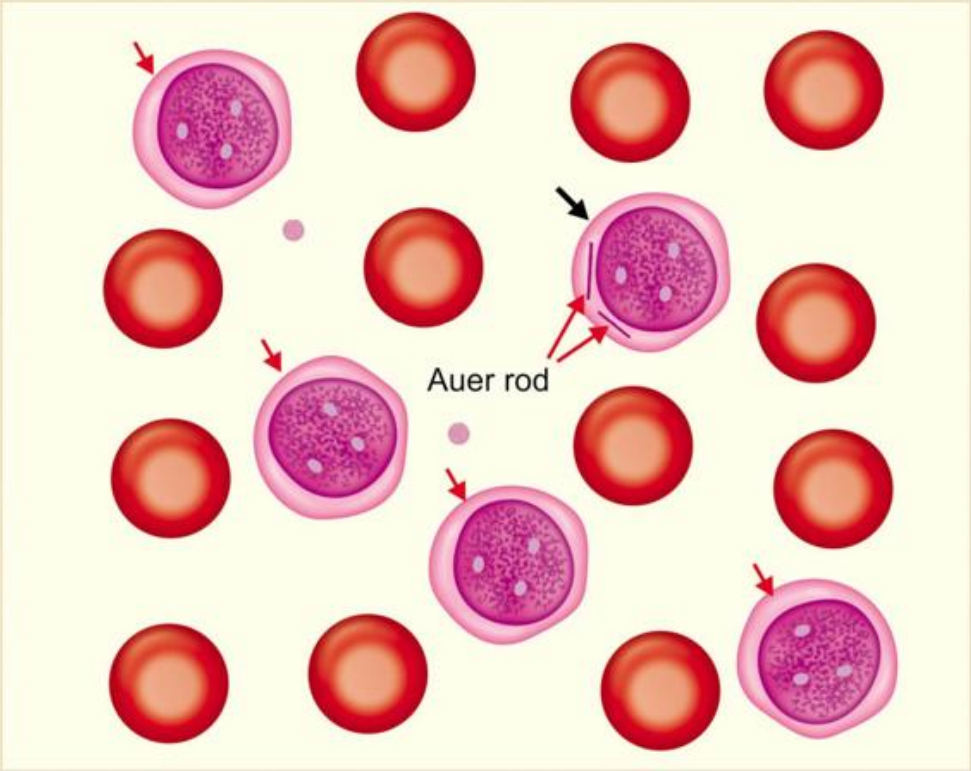


Fig. 12.13: Diagrammatic peripheral blood smear in AML with myeloblasts (arrows). One myeloblast with two Auer rods (arrow).

Cytochemistry of Myeloblasts (Figs. 12.8 and 12.9)

Q. Write short note on special stains in AML.

- **Stain positively** with **myeloperoxidase (MPO) and Sudan Black B.**
- Monoblasts stain with nonspecific esterases.

BONE MARROW

- *Cellularity:* Markedly **hypercellular**.
- *Erythropoiesis:* **Markedly suppressed.**
- *Myelopoiesis:* Suppression of myeloid maturation and **myeloblasts constitute >20% of marrow cells.**
- *Megakaryopoiesis:* **Gradually decreased.**

Immunophenotyping

Diagnosis of AML is confirmed by using stains for myeloid specific antigens.

Cytogenetics

Very important in the WHO classification of AML (**Box 12.11**).

MYELOID SARCOMA

Tumor mass consisting of myeloid blasts with or without maturation occurring at extramedullary sites.

- **On sectioning:** Tumor is **green** (hence the term **chloroma**)
- **Microscopically show myeloblasts** with or without features of promyelocytic or neutrophilic maturation.

Normally blast cells are <5% of nucleated cells in the marrow.

Leukemia: Malignant disease of bone marrow stem cell, arises in the marrow and spreads.

FAB criteria for the diagnosis of acute leukemia: Bone marrow should show a blast count of 30% or more.

WHO classification of AML: Based on clinical, morphological, immunophenotypic and genetic features.

Minimum blast cells in bone marrow should be >20%.

It is important to differentiate between lymphoblast and myeloblast because of difference in treatment and prognosis of AML and ALL.

Differentiating malignant pre-B and pre-T lymphoblasts on morphology is difficult.

Requires immunophenotyping for subclassification of ALL.

T-ALL has worse prognosis compared to B-ALL.

Symptoms in acute leukemia are due to bone marrow infiltration by blasts.

Bone marrow failure:
(1) Anemia, (2) Neutropenia, and (3) Thrombocytopenia.

ALL is the most common leukemia in children and is usually associated with lymphadenopathy.

Subleukemic leukemia: Total WBC count <4 × 109/L and peripheral blood shows very few blasts.

Aleukemic leukemia: Total white cell count is low (<4 × 109/L) with no blasts in the peripheral blood.

Both in subleukemic and aleukemic leukemia bone marrow contains blasts >20%.

Lymphoblasts should be differentiated from myeloblasts (Table 12.4).

Lymphoblast: Cytoplasm shows block positivity with PAS stain.

Distinction between precursor B- and T-cell ALL requires lineage-specific markers.

Presence of Philadelphia chromosome in ALL: Prognosis unfavorable.

AML synonyms: Acute myeloid/myeloblastic/myelocytic leukemia.

AML: Develop at any age. Usually 15–60 years of age.

Symptoms in acute leukemia are due to anemia, neutropenia and thrombocytopenia.

Acute promyelocytic leukemia (AML-M3) may be associated with widespread bleeding due to DIC.

AML: Auer rods in the cytoplasm of myeloblasts, seen in AML; not in CML.

Myeloblasts stain positively with myeloperoxidase and Sudan Black B.

AML prognosis:
- Fulminant course and has worse prognosis than ALL.
- Cytogenetic markers are major determinants of prognosis

Biphenotypic acute leukemia is acute leukemia with a single blast cell populations showing markers of two different lineage. Bilinear acute leukemia is acute leukemia with two different blast cell population.

Myeloid sarcoma synonym: Extramedullary myeloid tumor/granulocytic sarcoma/chloroma.

Myeloid sarcoma is frequent in skin, lymph node, GI tract, bone, soft tissue and testis.

Myelodysplastic Syndromes

INTRODUCTION

Q. Write short essay/note on myelodysplastic syndrome.

Definition: Myelodysplastic syndromes (MDS) are a heterogeneous group of **acquired clonal stem cell disorders affecting stem cells**.

MDS is characterized by:

- **Progressive cytopenias**
- **Dysplasia** in one or more cell lines
- **Ineffective hematopoiesis**
- **Risk of** development of **AML**.

Classification

- **Idiopathic or primary MDS**
- **Secondary/therapy-related MDS (t-MDS):** Complication of previous cytotoxic drug or radiation therapy.

WHO classification of myelodysplastic syndromes is presented in **Box 12.12**.

Clinical Features

- Elderly above 60 years
- Slightly more common in males
- Symptoms are due to cytopenias
- About 10–40% of MDS patients progress to AML.

Laboratory Findings

PERIPHERAL SMEAR

Cytopenias in the peripheral blood

- *RBCs:* Mild to moderate degree of macrocytic or dimorphic anemia.
- *WBCs:* Normal or low total leukocyte count.
- *Platelets:* Variable thrombocytopenia, large hypogranular or giant platelets.

BONE MARROW

Dysplasia of all nonlymphoid lineages (erythroid, granulocytic, monocytic and megakaryocytic) associated with cytopenias.

- *Cellularity:* **Hypercellular**.
- *Erythropoiesis:* Dysplastic changes in erythroid precursors with **megaloblastoid** change and presence of **ringed sideroblasts** in iron stain.
- *Myelopoiesis:* **Hyperplasia with dysgranulopoiesis.**
- *Megakaryopoiesis:* **Dysmegakaryopoiesis—pawn ball megakaryocytes.**
- *Iron stores:* **Increased with ring sideroblasts**.

Ineffective hematopoiesis

Bone Marrow Trephine Biopsy

Abnormal localization of immature precursors (ALIP) in refractory anemia with excess blasts (RAEB).

MDS: Cytopenias with hypercellular bone marrow.
About 30% progress to AML.

Bone marrow in MDS: Pawn ball megakaryocytes, dysgranulopoiesis, erythroid precursors with megaloblastoid change and presence of ringed sideroblasts.

Myeloproliferative Neoplasms

INTRODUCTION

Q. Write short note on myeloproliferative neoplasms.

Definition: Clonal hematopoietic stem cell disorders characterized by **proliferation of one or more of the myeloid lineages** (erythroid, granulocytic, megakaryocytic and mast cells).

BOX 12.12: WHO (2016) classification of myelodysplastic syndromes.

- MDS with single lineage dysplasia
- MDS with ring sideroblasts
  - MDS with ring sideroblasts and single lineage dysplasia
  - MDS with ring sideroblasts and multilineage dysplasia
- MDS with multilineage dysplasia
- MDS with excess blasts
- MDS with isolated del (5q)
- MDS, unclassifiable

Splenomegaly and **hepatomegaly** due to sequestration of excess hematopoietic cells or proliferation of abnormal hematopoietic cells.

WHO Classification of MPN

It is presented in **Box 12.13**.

BOX 12.13: WHO (2016) classification of myeloproliferative neoplasm (MPN).

Chronic myeloid myelogenous leukemia, BCR-ABL-1 positive

Chronic neutrophilic leukemia

Polycythemia vera—JAK2 V617F or exon 12 mutation

Primary myelofibrosis—JAK2 or MPL mutation

Essential thrombocythemia
- Platelet count >450 × 10^9/L
- *JAK2* mutation

Chronic eosinophilic leukemia, NOS
- No *BCR-ABL1*, PDGFRA, PDGFRB or FGFR1 translocation

Myeloproliferative neoplasm, unclassifiable

Note: Mastocytosis is no longer listed under the broad heading of MPN in WHO (2016) classification.

Pathogenesis

Presence of mutated, constitutively activated tyrosine kinases leads to proliferation of hematopoietic stem cells and results in hypercellular marrow.

All MPN show splenomegaly.

MPN peaks in the 5th to 7th decade.

POLYCYTHEMIA OR ERYTHROCYTOSIS

Polycythemia is characterized by **increase in the RBC mass**, usually with a corresponding increase in hemoglobin level.

Pathophysiologic classification of polycythemia is given in **Box 12.14**.

POLYCYTHEMIA VERA

Q. Write short note on polycythemia vera.

Definition: Polycythemia vera (PV) is an **acquired myeloproliferative neoplasm** arising from malignant transformation of **hematopoietic stem cell**.

- It is characterized by **trilineage** (erythroid, granulocytic, and megakaryocytic) **hyperplasia** in the bone marrow.
- It leads to **uncontrolled production of red cells, granulocytes and platelets** (panmyelosis) and leads to **erythrocytosis** (polycythemia) and or granulocytosis and thrombocytosis.

Molecular Pathogenesis (Figs. 12.14A and B)

- Normally, a tyrosine kinase protein called JAK2 (Janus 2 kinase gene), is activated following binding of the growth hormone erythropoietin.
- *JAK2* then activates a signaling pathway causing cells to replicate.
- This process is strictly regulated by various feedback pathways.
- Polycythemia vera (PV) is due to **mutation in** tyrosine kinase ***JAK2 V617F***, which causes proliferation of not only erythroid lineage but also granulocytic and megakaryocytic lineage.

Clinical Features

- Insidious.
- Late middle age (median age at onset is 60 years).
- Plethora and cyanosis, headache, dizziness and visual problems result from vascular disturbances in the brain and retina.
- Thrombotic episodes [e.g., deep venous thrombosis, myocardial infarction, thrombosis of hepatic veins (producing Budd-Chiari syndrome)].

Phases

There are three phases of polycythemia vera:

1. **Proliferative phase:** Erythroid proliferation and increased red cell mass.
2. **Spent phase:** In 10%, excessive proliferation of erythroid cells ceases with stable or decreased RBC mass.
3. **Myelofibrosis: About** 10% progress to myelofibrosis.

BOX 12.14: Pathophysiologic classification of polycythemia.

ABSOLUTE

Primary (low erythropoietin level)

- Polycythemia vera

Secondary (high erythropoietin level)

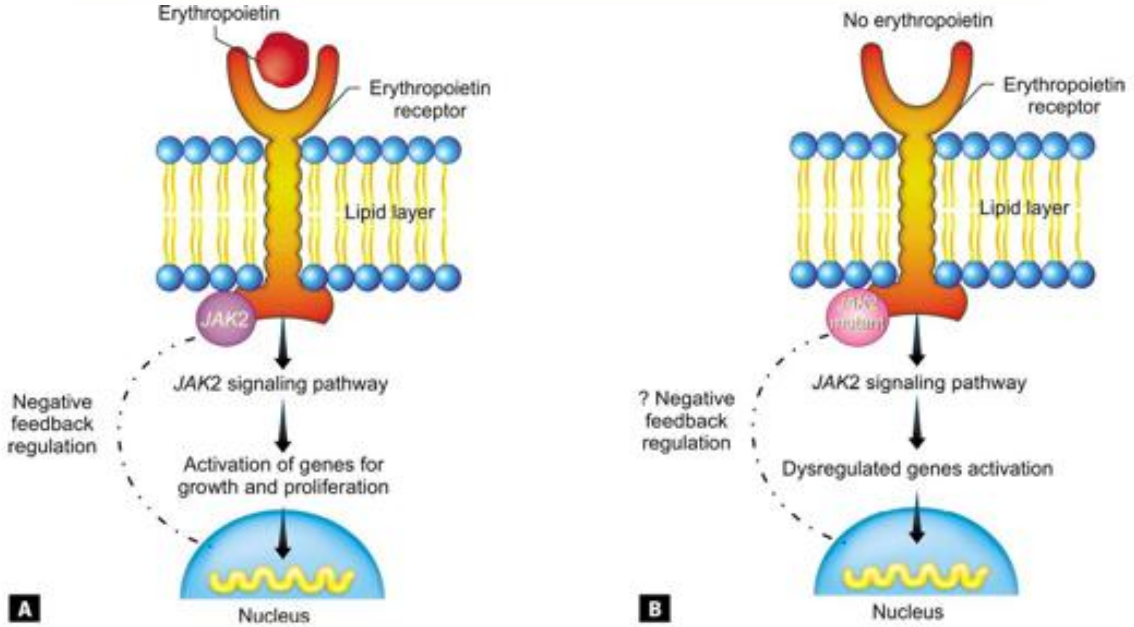
- Physiologically appropriate
  - Compensatory
  - Lung disease
  - Living in high-altitude
  - Cyanotic heart disease (tetralogy of Fallot)
- Physiologically inappropriate (with increased erythropoietin)
  - Paraneoplastic: Erythropoietin-secreting tumors (e.g., renal cell carcinoma, uterine leiomyoma, hepatocellular carcinoma)

RELATIVE

Reduced plasma volume

- Hemoconcentration (dehydration due to diarrhea, vomiting)
- Gaisböck's syndrome (spurious polycythemia)

Polycythemia: Increase in red cells can be absolute or relative.



Figs. 12.14A and B: (A) Normal signaling by *JAK2*; (B) In polycythemia vera, the presence of a mutant version of *JAK2* results in dysregulated downstream signaling in the absence of erythropoietin.

Laboratory Findings

Peripheral Blood

- **Hemoglobin: Increased** and are <18.5 g/dL in men and 16.5 g/dL in women.
- **Hematocrit: Increased** and about 60% **(Fig. 12.15)**.
- **Red cell count: Increased** and usually about **6 million/cu mm (6 × 10^{12}**/L).
- **White cell count:** Normal or increased.
- **Platelet count:** Normal or increased.

PERIPHERAL SMEAR

- *RBCs:* Normocytic normochromic picture
- *WBCs:*
  - **Mild to moderate leukocytosis**
  - Neutrophils are morphologically normal
  - **Basophils** often **increased**
  - **NAP** (LAP) score is **increased** to 150–300 (Normal 40–100).
- *Platelets:* **Abnormally large** and **functionally defective**.

BONE MARROW

Hypercellular due to hyperplasia of all elements (trilineage hyperplasia/panmyelosis) namely erythroid, myeloid and megakaryocytic series with prominence of erythroid precursors in the bone marrow.

Bone Marrow Biopsy

Shows increased reticulin fibers and fibrosis as the disease progresses.

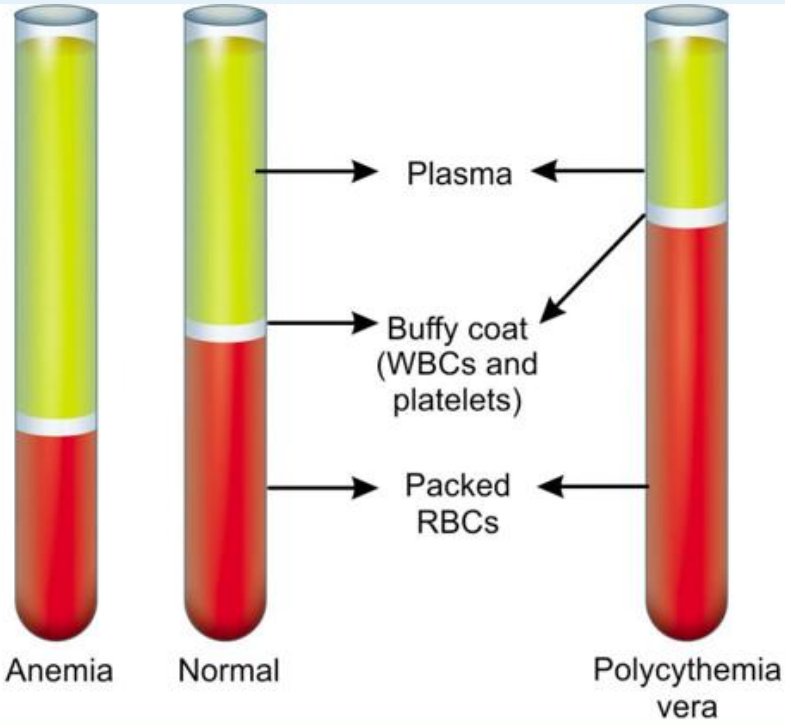
Other Findings

- **Extramedullary hematopoiesis** in the **liver and spleen** that causes **hepatosplenomegaly**.
- **Arterial oxygen saturation (pO_2):** Normal (75–100 mm Hg)and is useful for **differentiating it from secondary** polycythemia.
- **Erythropoietin levels: Decreased** in contrast to secondary polycythemia.
- **Serum vitamin B_{12} and uric acid: Increased** indicating increased cell turnover.

- ***JAK2 V617F* mutation:** Can be demonstrated.

JAK2 mutation is diagnostic of polycythemia vera.

PV: Erythropoietin is decreased.



PV: Hematocrit is increased and >60%.

Fig. 12.15: Normal hematocrit in comparison with anemia and polycythemia vera.

PV: Most symptoms are due to the increased red cell mass and hematocrit.

PV develops into acute myelogenous leukemia in 2–5%.

Polycythemia vera is a chronic myeloproliferative neoplasm with RBC count of >6 million/cu mm.

In PV, arterial oxygen saturation (pO2) is normal (>92%) whereas in secondary polycythemia, it is <90%.

ESSENTIAL THROMBOCYTHEMIA

Definition: Chronic myeloproliferative neoplasm (MPN) primarily of **megakaryocytic lineage**. It is characterized by **increased megakaryopoiesis** and **thrombocytosis** (>450 × 10^9/L).

Etiology

Most due to point **mutations in *JAK2* gene** and constitutive activation of *JAK2*, and thrombopoietin-independent proliferation of megakaryocytes.

Clinical Features

- **Age:** 50–60 years
- **Thrombosis and hemorrhage**
- **Erythromelalgia:** One of the characteristic features.

Laboratory Findings

PERIPHERAL SMEAR

- *RBCs:* Normocytic normochromic
- *WBCs:* Mild **leukocytosis**
- *Platelets:*
  - **Increased** number (thrombocytosis) >600,000/cu mm
  - Variation in size and shape—abnormally large platelets are common.

BONE MARROW

- *Cellularity:* Mild to marked hypercellularity.
- *Erythropoiesis:* Normal or mild hyperplasia.
- *Myelopoiesis:* Normal or mild hyperplasia.
- *Megakaryopoiesis:* **Markedly increased** in number with **abnormally large megakaryocytes (giant megakaryocytes).**

Extramedullary hematopoiesis: Mild hepatosplenomegaly.

Essential thrombocythemia (ET) synonym: Primary (essential/idiopathic) thrombocytosis.

ET: Mutation of JAK2 gene
Thrombocytosis with a count of >450 × 109/L.

ET: Throbbing and burning sensation of hands and feet due to blocking of arterioles by aggregates of platelets is known as erythromelalgia.

Megakaryocytic hyperplasia and abnormal (giant) platelets are characteristic features.

ET course: Indolent.

PRIMARY MYELOFIBROSIS

Q. Write short essay/note on myelofibrosis.

Definition: Clonal MPN characterized by a **proliferation of** predominantly **megakaryocytes and granulocytes** in the bone marrow.

Fully developed disease results in **reactive marrow fibrosis** and replaces hematopoietic cells leading to cytopenias and extensive extramedullary hematopoiesis.

Molecular Pathogenesis

Most show *JAK2* mutations.

Clinical Features

- **Age:** Above 60 years of age.
- Progressive anemia.
- Splenomegaly.

Laboratory Findings

PERIPHERAL SMEAR

- *RBCs:* Moderate to severe degree of **normochromic normocytic anemia** accompanied by **leukoerythroblastosis**. **Teardrop-shaped red cells** (dacryocytes), probably due to damage in the fibrotic marrow can also be found.
- *WBCs:* Total white cell count is usually normal or reduced, but can be markedly elevated 80–100 × 10^9/L in early stages of the disease.
- *Platelets:* They may be **abnormally large**. The **platelet count is usually normal** or elevated, but as the disease progresses the count decreases.

BONE MARROW

- *Cellularity:* In early stages, it is often hypercellular due to increase in maturing cells of all lineages. In later stages, it is replaced by fibrosis and becomes hypocellular and diffusely fibrotic resulting in a **dry tap.**
- *Erythroid and granulocytic precursors:* These are morphologically **normal.**

- *Megakaryocytes:* **These are large, dysplastic and abnormally clustered**.

Bone Marrow Biopsy

Stages: Two stages have been recognized.

1. **Prefibrotic (cellular) stage:** Hypercellular bone marrow. Megakaryocytes increased and markedly abnormal.
2. **Fibrotic stage:** Fibrosis distorts the marrow and prematurely releases nucleated erythroid and early granulocyte progenitors **(leukoerythroblastosis).**

Reticulin stain demonstrates the increase in reticulin fibers (fibrosis).

Extramedullary hematopoiesis in spleen and liver **produces hepatosplenomegaly.**

Course: Variable.

Bone marrow biopsy is essential for the diagnosis of myelofibrosis as aspirate results in a dry tap late in the course of the disease.

Myelofibrosis: Mutation in JAK2 gene.

Massive splenomegaly due to extramedullary hemopoiesis.

Primary myelofibrosis: Peripheral smear shows leukoerythroblastosis and tear drop cells.

Primary myelofibrosis: Bone marrow fibrosis leads to cytopenias.

CHRONIC MYELOGENOUS LEUKEMIA

CC: Describe the etiology, genetics, pathogenesis classification, features, hematologic features of chronic leukemia.

Q. Discuss peripheral blood smear, bone marrow, biochemical findings and the characteristic chromosomal abnormality associated with chronic myeloid leukemia.

Definition: Chronic myelogenous leukemia (CML) is one of the myeloproliferative neoplasm (MPN) of **pluripotent hematopoietic stem cell** characterized by overproduction of cells of the

myeloid series which results in marked splenomegaly and leukocytosis.

Distinguished from other myeloproliferative neoplasms by the presence of:
1. Chimeric fusion *BCR-ABL1* gene.
2. Philadelphia (Ph) chromosome in more than 90% of cases.

Etiology and Pathogenesis

Risk factor: Exposure to ionizing radiation and benzene.

Molecular Pathogenesis

Q. Write short note on Philadelphia chromosome.

Philadelphia (Ph) Chromosome (Fig. 12.16)

- Acquired chromosomal abnormality **in all proliferating hematopoietic stem cells** (erythroid, myeloid, monocytic and megakaryocytic precursors).
- **Balanced reciprocal translocation** between long arm of chromosome 9 and 22, i.e., **t(9; 22) (q 34; q 12.2)**. It increases the length of chromosome 9 and shortening of 22. This **shortened chromosome 22** is known as Philadelphia chromosome **(Fig. 12.16)**.

BCR-ABL1 Fusion Gene

- **ABL1** proto-oncogene from chromosome 9 **joins** the **BCR** on chromosome 22.
- It produces a new **chimeric (fusion) gene called BCR-ABL1, thus converting ABL1 proto-oncogene into oncogene.** The product of the fusion gene plays a central role in the development of CML. **In CML, the tumor cells are dependent on BCR-ABL1 fusion gene tyrosine kinase activity**. This is called **oncogene addiction**.

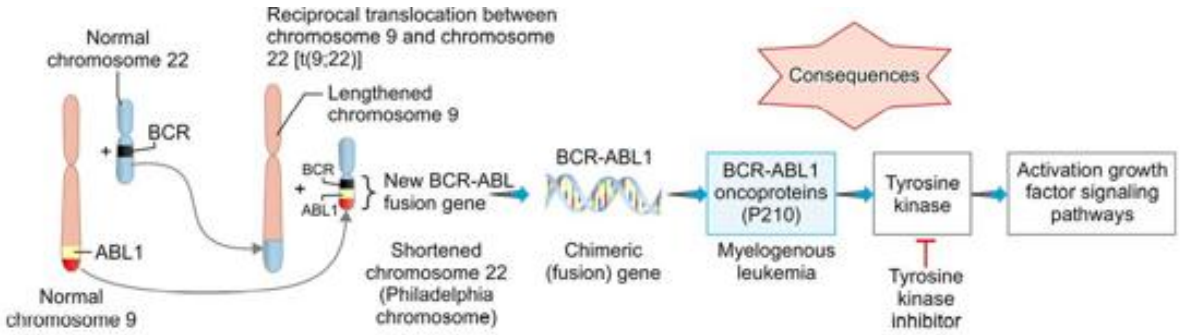


Fig. 12.16: Balanced reciprocal translocation between long arm of chromosome 9 and chromosome 22 resulting in shortened chromosome 22 known as Philadelphia chromosome.

CML: Translocation results in the head-to-tail fusion of the breakpoint cluster region (BCR) gene on chromosome 22 with the ABL (named after the Abelson murine leukemia virus) gene located on chromosome 9.

CML synonyms: Chronic myelocytic/myeloid/granulocytic leukemia.

CML is an acquired MPN of pluripotent hematopoietic stem cell.

- The product of this oncogene, i.e., **oncoprotein** (e.g., **p210) causes cell division** and **inhibition of apoptosis**.

Clinical Features

- ***Age*: Usually occurs between 40 and 60 years** of age.
- ***Sex:*** Males slightly more affected than females.
- ***Onset:*** **Insidious**.

***Symptoms*:**

- **Nonspecific symptoms:** Fatigue, weakness, weight loss, anorexia.
- **Fullness of abdomen due to splenomegaly** (caused by leukemic infiltration and extramedullary hematopoiesis). Splenomegaly is moderate to severe and is characteristic feature in majority (80–90%) of patients.
- **Hepatomegaly:** Mild or moderate seen in 60–70% of cases.

Natural History of Chronic Myeloid Leukemia

Three different phases: (1) chronic phase, (2) accelerated phase, and (3) blastic phase.

Chronic/Stable/Indolent Phase (CP)

- **Most are diagnosed in this phase.**
- Lasts for 2–6 years.
- If not treated, progresses gradually to accelerated phase or abruptly to blastic phase.

Laboratory Findings

Q. Write short note on laboratory findings/peripheral smear in chronic myeloid leukemia (CML).

Peripheral blood

Hemoglobin: Usually <11 g/dL.

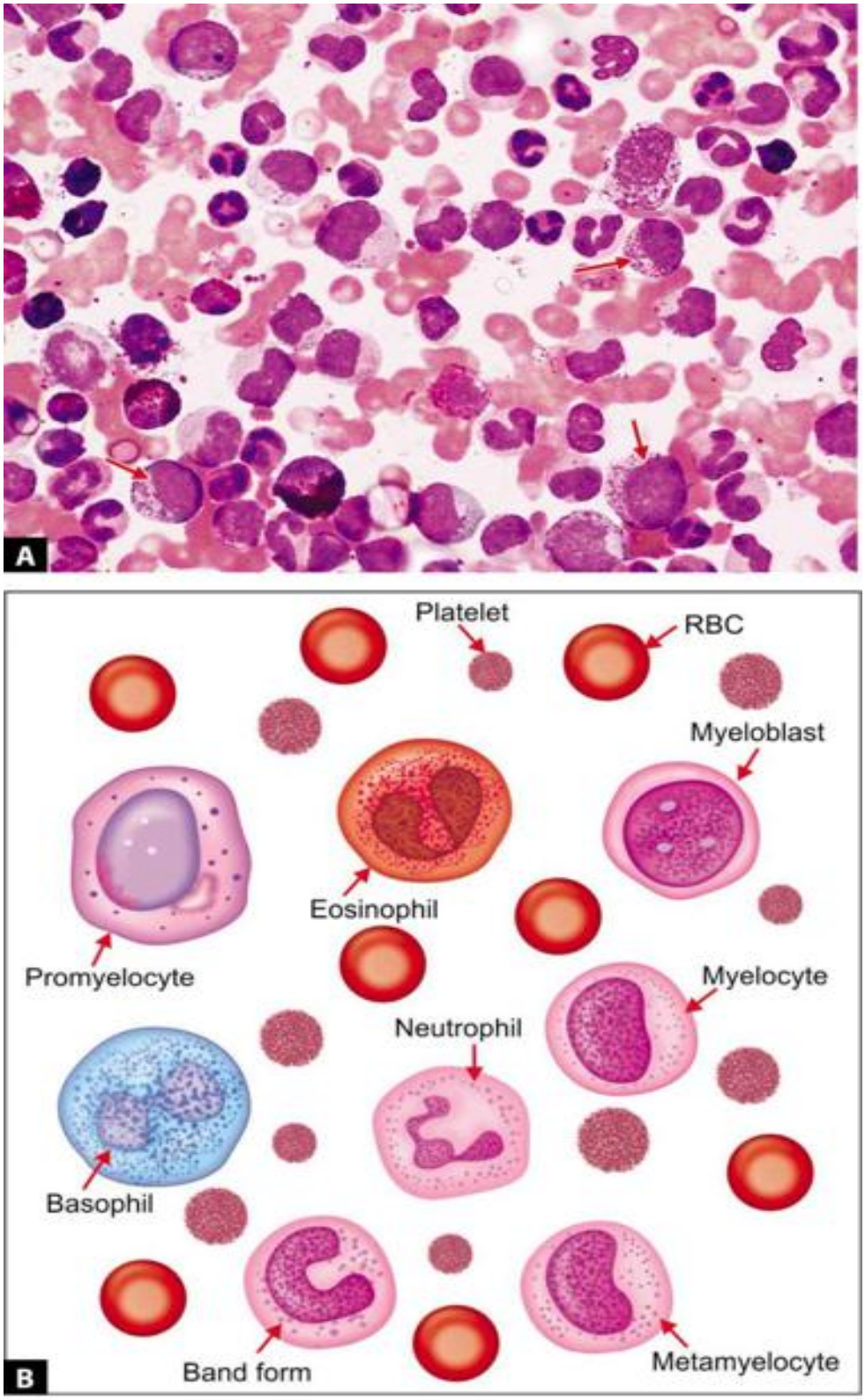
PERIPHERAL SMEAR

- *RBCs:* **Normocytic normochromic anemia**
- *WBCs:*
  - **Marked leukocytosis** (12–600 × 10^9/L) total **leukocyte count** usually **exceeds 100 × 10^9/L (1,00,000/cu mm).**
  - **Shift to left (shift to immaturity)—**granulocytes at all stages of development (neutrophils, metamyelocytes, myelocytes, promyelocytes and an occasional myeloblasts).
  - **Predominant cells** are **neutrophils and myelocytes.**
  - **Blasts** are usually **<10%** of the circulating WBCs **(Figs. 12.17A and B)**.
  - **Basophilia and eosinophilia.**
  - **Decreased NAP/LAP score:** NAP score in CML is decreased below 20 (normal score range is 40–100). Helpful in differentiating CML from leukemoid reaction **(Table 12.2)**.
- *Platelets:* Platelets range from normal (150–450 × 10^9/L) to> 1000 × 10^9/L. Up to 50% have **thrombocytosis.**

BONE MARROW

- *Cellularity:* Markedly **hypercellular** due to **myeloid hyperplasia**.
- *M: E ratio:* Often exceeds **20:1**.
- *Erythropoiesis:* **Diminished erythropoiesis** as disease progresses.

- *Myelopoiesis:* **Marked hyperplasia. Blast** cells usually **less than 10%.** Basophils, eosinophils and their precursors are usually found.
- *Megakaryopoiesis:* Megakaryocytes are either normal or increased. **Dwarf megakaryocytes**.
- *Sea-blue histiocytes* **(Gaucher-like cells/pseudo Gaucher cells)** are seen.



Figs. 12.17A and B: Peripheral blood picture in chronic/stable phase of chronic myeloid leukemia: (A) Leishman stain (myelocytes with arrows); (B) Diagrammatic.

Biochemical Findings

- Serum uric acid raised
- Serum LDH raised.

Philadelphia chromosome and ***BCR-ABL1 fusion gene demonstrated*** either by chromosomal analysis or **fluorescent in situ hybridization (FISH)** or PCR based tests.

Accelerated Phase (AP)

- **More aggressive** and lasts for few months.
- **Myeloblasts: 10–19%** in the blood or bone marrow.
- **Striking basophilia** (20% or more).
- **Persistent thrombocytopenia** (<100 × 10^9/L) unrelated to therapy or persistent thrombocytosis (>1000 × 10^9/L) uncontrolled by therapy.
- **Megakaryocyte proliferation in sheets** or clusters in association with fibrosis.
- **Persistent or increasing splenomegaly** unresponsive to therapy.

Blast Phase/Crisis (BP)

Q. Write a short answer on blast crisis in chronic myeloid leukemia (CML).

Blood picture resembles acute leukemia and has **poor prognosis.**

PERIPHERAL SMEAR

- **Blasts 20% or more.** May be **either myeloblast (70% cases) or lymphoblast (30% cases).** Myeloblast does not contain Auer rods.
- **Thrombocytopenia** causes bleeding episodes.

Philadelphia (Ph) chromosome is a shortened chromosome 22 and is due to balanced reciprocal translocation between chromosome 9 and 22-t(9; 22).

Translocation results in a BCR-ABL1 fusion gene, which produces neoplastic proliferation.

CML: Usually occurs between 40 and 60 years of age.

CML: Moderate to massive splenomegaly.

CML has three phases: Chronic stable, accelerated and blast phase.

CML: Neutrophilia with the whole spectrum of mature myeloid precursors.

CML is characterized by anemia, extreme leukocytosis, granulocytic immaturity, basophilia, thrombocytosis.

The preponderance of myelocyte in CML is called as myelocyte bulge.

In CML, LAP (NAP) is markedly reduced.

CML: Accelerated phase is more aggressive and myeloblasts range from 10% to 19%.

CML blast crisis: Blasts 20% or more, myeloblast (no Auer rods) or lymphoblasts.

Prognosis of CML: Poor with accelerated phase or blast crisis.

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

CHRONIC LYMPHOCYTIC LEUKEMIA

Q. Discuss the laboratory findings/diagnosis and clinical manifestations of chronic lymphocytic leukemia.

Q. Write short note on chronic lymphocytic leukemia.

Definition: Chronic lymphocytic leukemia **(CLL)**/small lymphocytic lymphoma **(SLL)** is a **tumor composed of monomorphic small B lymphocytes** in the peripheral blood, bone marrow and lymphoid organs (spleen and lymph nodes).

- Both CLL and SLL is a **single entity with different presentations**.
- **Small lymphocytic lymphoma** is tissue equivalent of chronic lymphocytic leukemia.
- CLL/SLL tumor cells coexpress CD5 and CD23.

Etiology and Pathogenesis

- **Environmental factors:** Suggested but none proved.
- **Hereditary factors:** Families with higher risk of CLL or other lymphoid neoplasms.

Cytogenetic Abnormalities

Common mutations are deletions of 13q14.3, 11q22-23, and 17p11. About 20% of CLL show trisomy 12.

Clinical Features

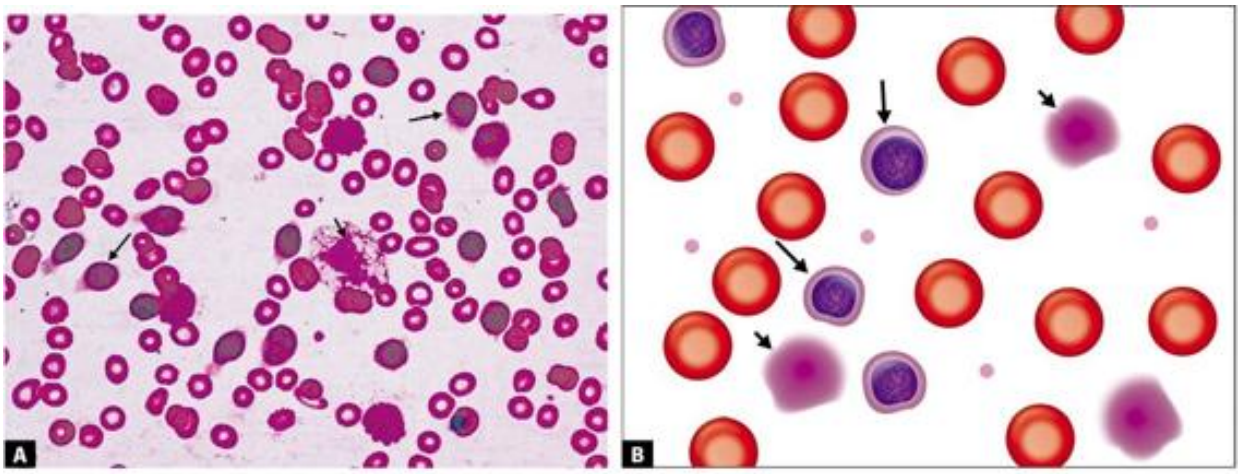
- ***Age:*** Between **50–60 years** of age.
- ***Sex:*** More in males than in females (2:1).
- ***Symptoms:***
  - **Asymptomatic** in about 25–30%
  - **Nonspecific symptoms*:* Fatigue,** loss of weight and anorexia
  - **Generalized lymphadenopathy**
  - **Immunological defects** either as i**mmune deficiency or autoimmunity.**

Laboratory Findings

Peripheral Blood

Q. Write short essay/note on peripheral blood picture in chronic lymphocytic leukemia.

- **Hemoglobin: Decreased** and usually below 13 g/dL.
- **Total leukocyte count is increased (20–50 × 10^9/L).**



Figs. 12.18A and B: Peripheral blood smear (A, Leishman stain and B, diagrammatic) in chronic lymphocytic leukemia showing numerous small lymphocytes (long arrows) and few smudge cells (short arrow).

PERIPHERAL SMEAR (FIGS. 12.18A AND B)

- *RBCs:* Normocytic normochromic anemia.
- *WBCs:*
  - Differential leukocyte count shows **lymphocytosis** and constitutes **more than 50%** of the white cells.
  - **Lymphocytes mature type**—small with scant cytoplasm, **nuclei** round with clumped coarse chromatin **("soccer ball"/block-type chromatin).** Nucleoli absent.
  - **Smudge cells** or basket cells (fragile leukemic cells).
- *Platelets:* Initially **normal** count and later may be decreased.

BONE MARROW

- *Cellularity:* **Hypercellular** marrow due to infiltration by mature lymphocytes.
- *Erythropoiesis:* Normal.
- *Myelopoiesis:* Normal.
- *Megakaryopoiesis:* Normal.
- *Lymphocytic infiltrate:* As the disease advances, neoplastic lymphocytes replace the normal erythroid, myeloid and megakaryocytic series in the bone marrow resulting in anemia, neutropenia and thrombocytopenia.

Immunophenotype

Tumor cells express the pan-B cell markers CD19 and CD20. CD5+ and CD23+ are distinctly positive in CLL.

Lymph Node

- Show loss of normal architecture.
- Diffuse infiltration by monomorphic, small, round lymphocytes.
- Lymphocytes have nuclei with coarse chromatin and scanty cytoplasm.
- Small, nodular aggregates of medium to large-sized lymphocytes known as **proliferation centers or pseudo-follicles or growth centers** and when found are **pathognomonic for CLL/SLL**.

Monoclonal B-cell lymphocytosis (MBL): WHO criteria for monoclonal B-cell lymphocytosis (MBL) is the presence of monoclonal B-cell populations in the peripheral blood (PB) of up to 5×10^9/L either with the phenotype of chronic lymphocytic leukemia (CLL), atypical CLL, or non-CLL (CD52) B-cells in the absence of other lymphomatous features. It has been found that MBL precedes almost all cases of CLL/SLL. In 2018 WHO subdivided MBL into "low count" MBL (characterized by PB CLL count of $<0.5 \times 10^9$/L) and "high count" MBL. The distinction is important because low count MBL does not require routine follow-up whereas, high count MBL requires routine/yearly follow-up.

Course and prognosis: Median survival rate is 4 to 6 years. They may progress to B-cell prolymphocytic transformation or into diffuse large B-cell lymphoma (Richter syndrome).

CLL/SLL are tumors derived from B lymphocytes.

CLL patients may be asymptomatic or present with generalized lymphadenopathy.

CLL: Absolute lymphocyte count is more than 5 × 109/L. It is the characteristic feature.

CLL: Lymphocytosis with smudge cells in the peripheral smear. Smudge cells are fragile leukemic cells produced due to rupture while making the peripheral smear.

Lymphocytes constitute more than 30% of the nucleated cells of the bone marrow cells—diagnostic feature of CLL.

CLL/SLL: Lymph node with proliferation centers are pathognomonic.

HAIRY CELL LEUKEMIA

Q. Write short essay/note on hairy cell leukemia.

Definition: Uncommon neoplasm of small mature B cells having abundant cytoplasm with fine **hair-like cytoplasmic projections** (hence the name **hairy cell leukemia**) when viewed under the **phase-contrast microscope**.

Laboratory Findings

Peripheral Blood

- **Hemoglobin: Decreased**.
- **Total leukocyte count: Decreased (leukopenia).**
- **Platelet count: Decreased** (<50 × 10^9/L).

PERIPHERAL SMEAR

Pancytopenia

- *RBCs:* Normocytic normochromic
- *WBCs:* Leukopenia with few **hairy cells (Fig. 12.19)**
- *Platelets:* Reduced

BONE MARROW ASPIRATION

- **Dry tap**
- **Hairy cells** may be seen in the marrow
- Moderate to marked **reduction in myeloid, erythroid and megakaryocytic** cell lines.

Bone Marrow Trephine Biopsy

Neoplastic cells have **"fried egg" or "honeycomb"** appearance. Reticulin stain shows marked increase of thin reticulin fibers surrounding neoplastic cells.

Spleen

- **Enlarged** due to leukemic infiltrate.
- **Sinuses lined by hairy cells** and **grossly** impart a **beefy red** appearance.

Immunophenotype and Molecular Characteristics

Express the **CD20, CD22, CD11c and CD25** (the IL-2 receptor α-chain) **positivity**. Annexin A1 is the most specific marker of hairy cell leukemia.

Clinical Features

- Affects **middle-aged to elderly men**.
- Male-to-female ratio of 5:1.
- **Massive splenomegaly**.
- **Pancytopenia**.

Hairy cells have hair-like cytoplasmic projections.

HCL: Is B cell neoplasm and involves peripheral blood, bone marrow, spleen and liver and usually seen in old age.

HCL: Bone marrow biopsy—hairy cells have fried egg appearance.

Tartrate resistant acid phosphatase (TRAP) positivity in the cytoplasm is a characteristic feature of HCL.

HCL: Only leukemia without lymphadenopathy.

HCL prognosis: Indolent course and prognosis is excellent.

Plasma Cell Neoplasms

CC (Show How): Describe the features of plasma cell myeloma. (DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Definition: Plasma cell neoplasms are group of **B-cell neoplasms** associated with the **proliferation of single clone** (monoclonal) **of immunoglobulin-secreting plasma cells** (also known as dyscrasias).

Characteristics of Plasma Cell Neoplasms

Q. Write short note on M proteins.

Monoclonal neoplastic plasma cells **secrete complete single type of immunoglobulin** (Ig) **or Ig fragment.** Hence, are known as **monoclonal gammopathies.**

- **Serum:** Single Ig proteins detected as **monoclonal spike** [M protein (M for myeloma)] **on electrophoresis.**
- **Urine: Excess of free light chains** is excreted in the urine as **Bence–Jones (BJ) proteins.**

Classification of Plasma Cell Neoplasms (Box 12.15)

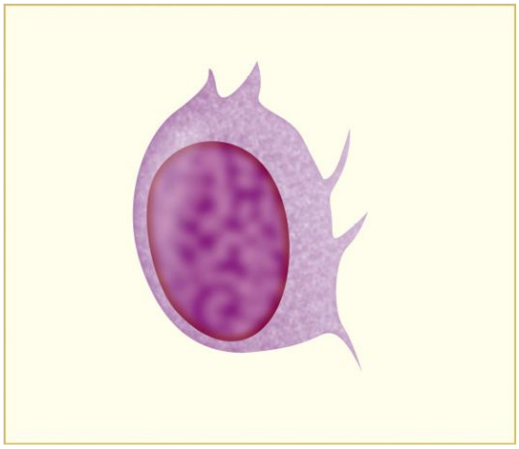


Fig. 12.19: Hairy cell.

BOX 12.15: Classification of plasma cell neoplasms(WHO 2016).

- Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extraosseous plasmacytoma
- Immunoglobulin deposition diseases
- Monoclonal gammopathy of undetermined significance (MGUS)
- Osteosclerotic myeloma (POEMS syndrome)

PLASMA CELL MYELOMA (MULTIPLE MYELOMA)

CC: Describe the features of plasma cell myeloma.

Definition: Plasma cell myeloma is a **malignant, multifocal** plasma cell **neoplasm of the bone marrow** associated with **M-protein in the serum and/or urine**.

- **Most common monoclonal gammopathy**.
- Presents as **multiple tumor masses** throughout the **skeletal system**.

Etiology

Risk Factors

- **Genetic predisposition.**
- **Exposure to ionizing radiation.**
- **Chronic antigenic stimulation** associated with chronic infections (HIV and chronic osteomyelitis) and chronic inflammatory disorders (e.g., rheumatoid arthritis).
- **Exposure to chemicals** like benzene, herbicides and insecticides.

Laboratory Findings

Q. Write short note on the laboratory diagnosis of multiple myeloma.

Peripheral Blood

- **Hemoglobin: Decreased** and ranges from 6 g/dL to10 g/dL.

PERIPHERAL SMEAR

- *RBCs:* Normocytic normochromic anemia, red blood cells show r**ouleaux formation** due to increased immunoglobulins.
- *WBCs:* **Normal.**
- *Platelets:* **Normal**.

- **ESR: High** and is due to high gamma globulin (immunoglobulin) and rouleaux formation.
- **Bleeding time: Increased.**

BONE MARROW

Q. Write short note on morphology of plasma cells in multiple myeloma.

Q. Write short note on bone marrow findings in multiple myeloma.

- *Cellularity:* **Hypercellular** due to **myeloma plasma (myeloma) cells** (neoplastic plasma cells) **(Figs. 12.20A and B)**.
- **Myeloma plasma cells: More than 30%** are **diagnostic**.
  - Myeloma plasma cells are neoplastic plasma cells **(Fig. 12.21)**, which are **large oval cells** having **abundant pale blue cytoplasm**.
  - The **nucleus** is round to oval, **eccentric** and shows **perinuclear clearing**/hof.
  - The nuclear **chromatin** appears like a **clock-face/spoke wheel**.
  - These cells are usually uninucleated or may show binucleation.
  - Other cells can also be seen in myeloma **(Fig. 12.21)**.

- *Erythropoiesis:* Diminished and is normoblastic.
- *Myelopoiesis:* Normal.
- *Megakaryopoiesis:* Normal.

Serum Findings

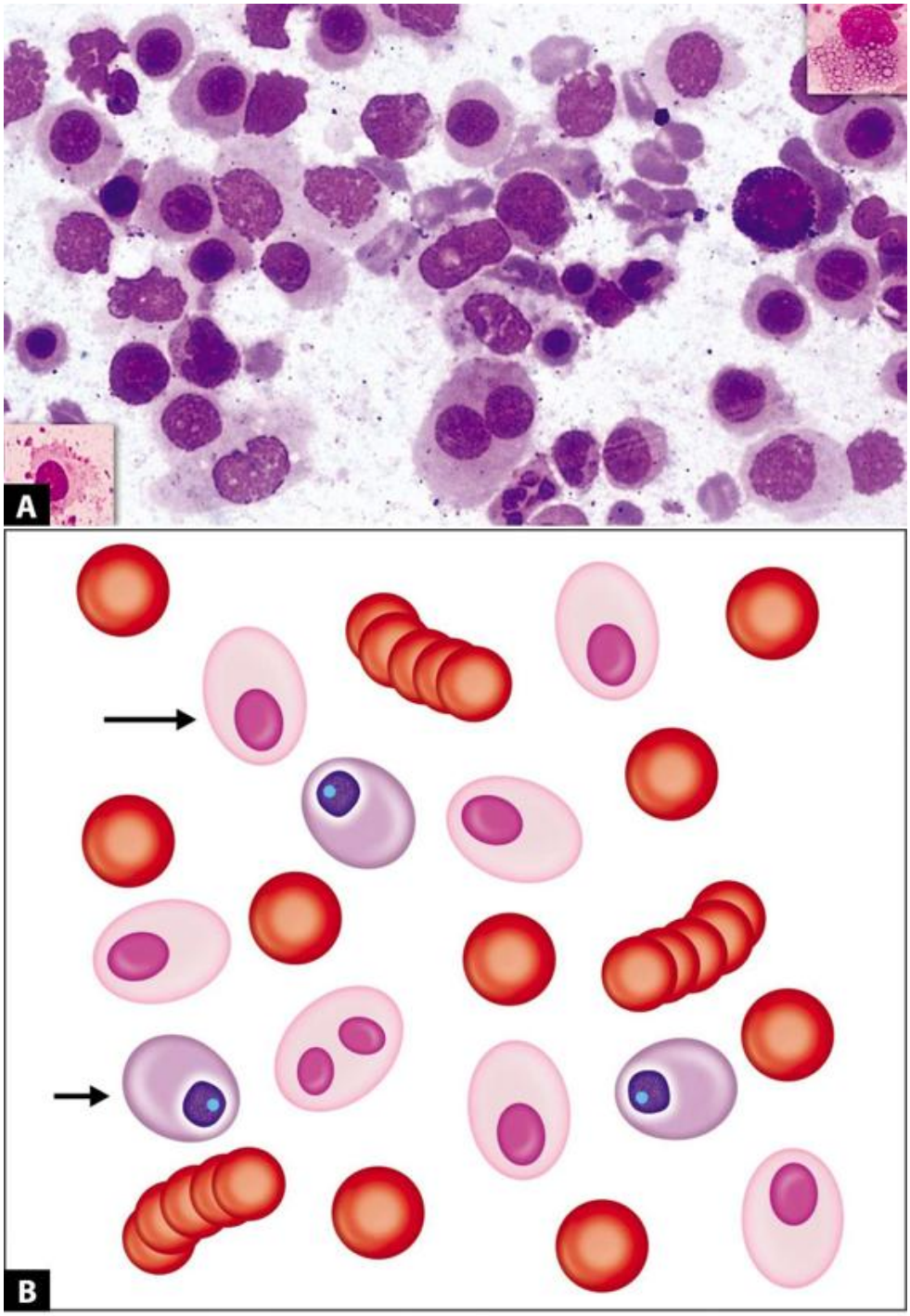
- **Serum β_2 microglobulin: Prognostic marker** and **high values signify poor prognosis.**
- **Hypercalcemia**
- **Renal function tests: Blood urea, serum creatinine and uric acid levels** are **raised** with renal involvement.
- **Serum albumin:** Decreases in advance stages of the disease.

Electrophoretic Studies on Serum and Urine (Figs. 12.22A and B)

Q. Write short note on urinary findings in multiple myeloma.

Q. Write short note on Bence–Jones protein and its demonstration.

- **Monoclonal spikes** in 80–90% of cases.



Figs. 12.20A and B: Bone marrow aspirate in multiple myeloma: (A) Leishman stain shows numerous myeloma plasma cells. Inset shows flame cell (left lower corner) and mott cell (right upper corner); (B) Diagrammatic appearance showing plasmablasts (short arrow) and plasma cells (long arrow).

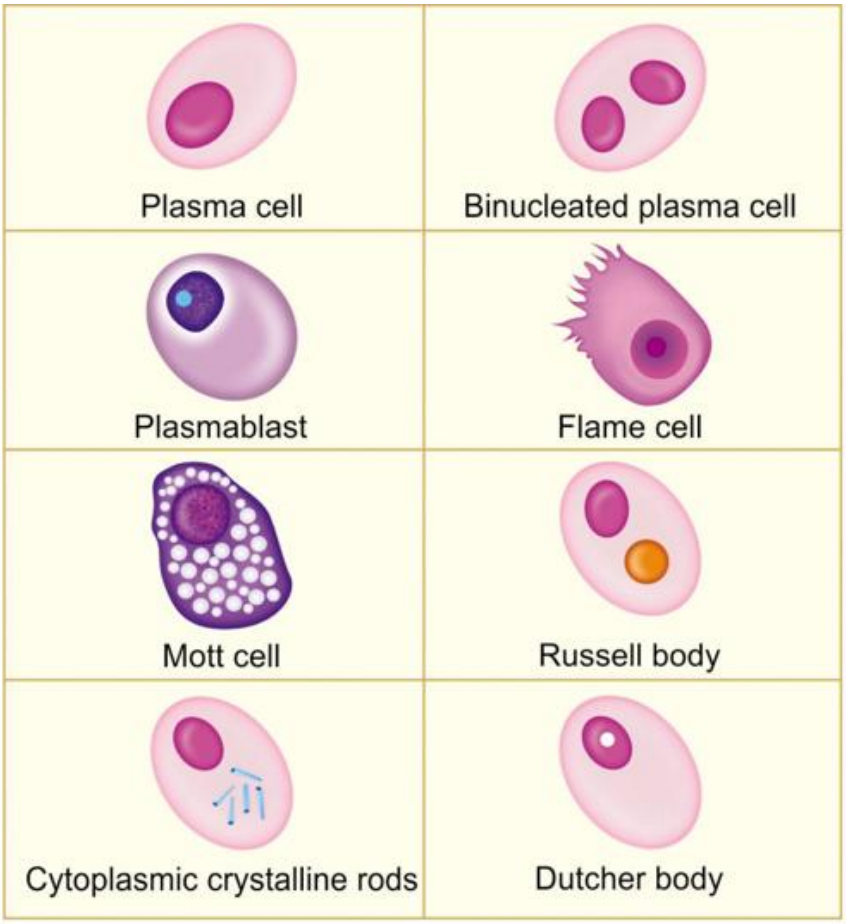


Fig. 12.21: Diagrammatic appearance of the various cells that can be seen in bone marrow in multiple myeloma.

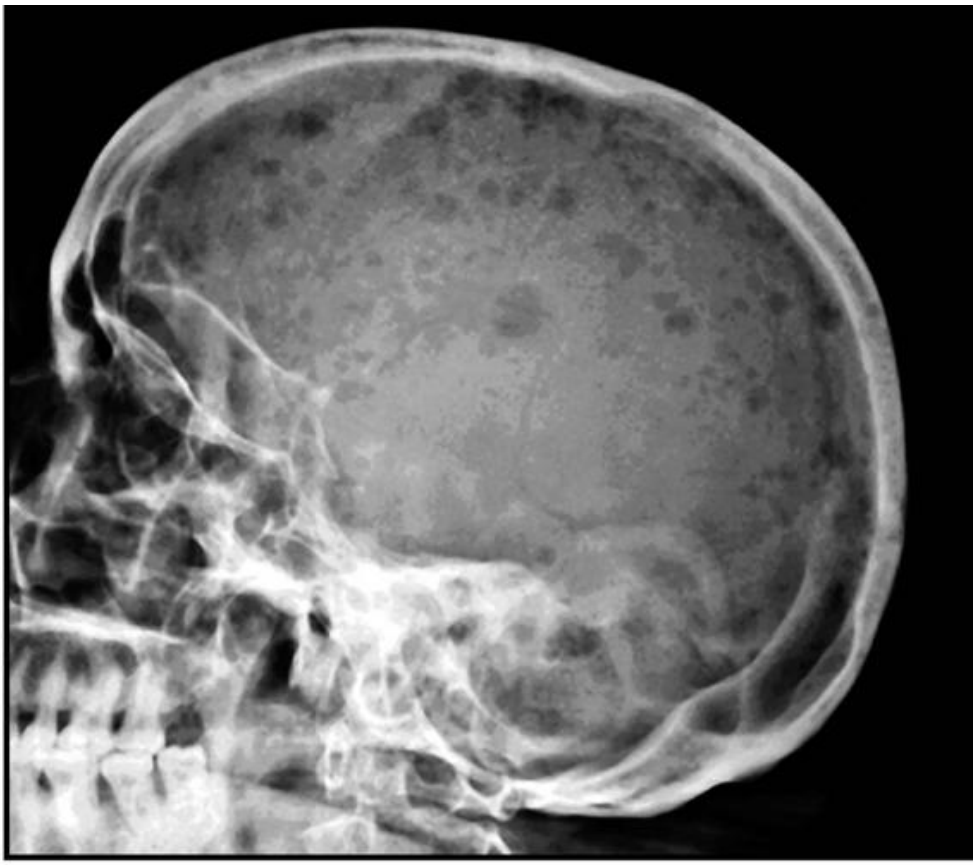


Fig. 12.23: Skull X-ray showing multiple punched-out lytic lesions.

- Raised **monoclonal immunoglobulins** in the blood. Immunoglobulin may be **IgG** (most common)/IgD/IgA/IgE type.
- **Light chains** or **Bence–Jones (BJ) proteins** in the urine may be seen in 60–80% of cases. BJ protein may be of κ or λ type of light chain.

Morphology of Organs Involved

- **Bone: Destructive punched-out** lytic lesions **(Fig. 12.23)**.
- **Renal lesions:**
  - **Myeloma kidney:** Light-chain cast of BJ protein **damages renal tubules**.
  - **Amyloidosis** of the AL type and leads to **nephrotic syndrome.**
    - Hypercalcemia leads to nephrocalcinosis.
    - Prone to acute and chronic pyelonephritis.
    - Renal failure.

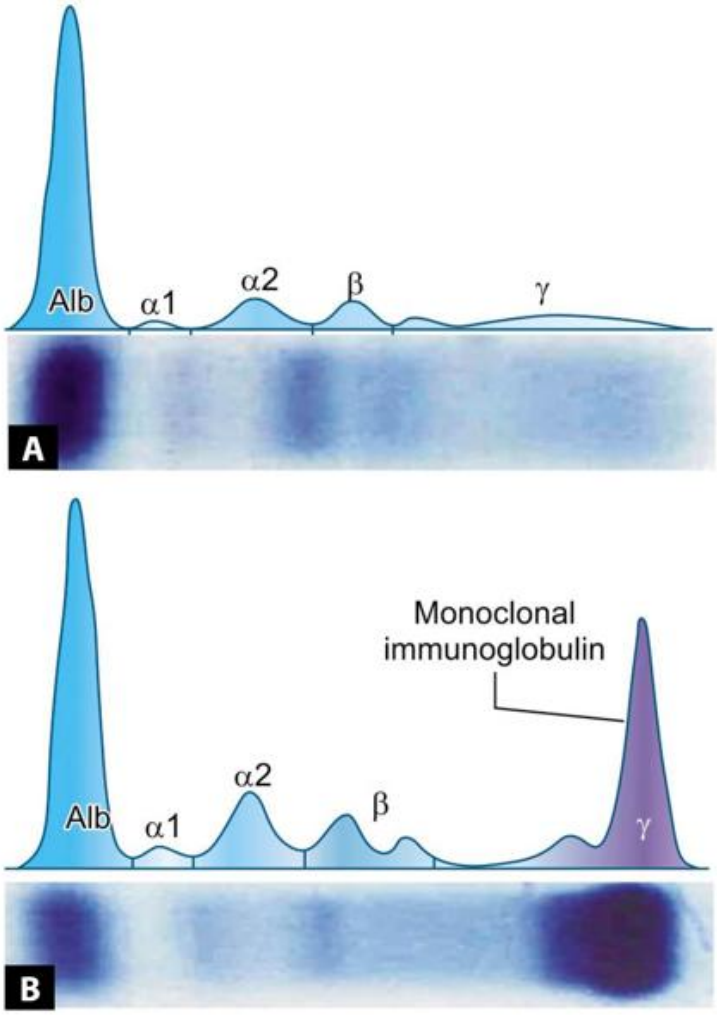
Clinical Manifestations (Flowchart 12.1)

Onset: Insidious.

Age and sex: Affects old age between 50 and 60 years with slight male preponderance.

The **clinical features** of multiple myeloma are:

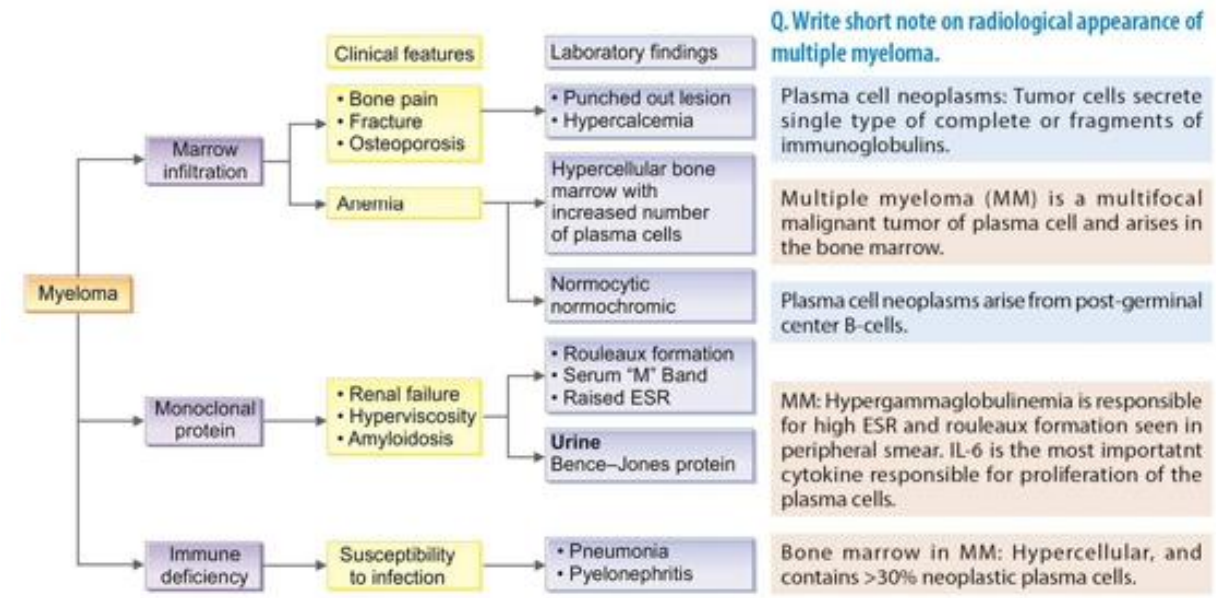
- ***Due to tumor cells causing bone lesions:***
  - **Resorption of bone:** This results in pathologic fractures, chronic bone pain and tenderness.
  - **Compression:** Lesion in the vertebra may compress the spinal cord nerve root.
  - **Hypercalcemia**.
  - **Pallor:** Due to anemia and result in weakness and fatigue.
- ***Production of M-proteins (increased immunoglobulins):***
  - **Bleeding tendency.**
  - **Coagulation abnormalities.**
  - **Amyloidosis** of the AL type.
- ***Humoral immune deficiency:*** Predisposes to recurrent bacterial infections.
- ***Renal disease:*** Renal insufficiency, infections or nephrotic syndrome.



Figs. 12.22A and B: Serum electrophoresis (A) shows normal pattern and (B) shows monoclonal immunoglobulin ("M Band") in multiple myeloma.

MM: involved bone shows multiple punched out lytic lesions.

Flowchart 12.1: Clinical features and laboratory findings in myeloma.



(ESR: erythrocyte sedimentation rate).

Diagnostic Criteria for Plasma Cell (Multiple) Myeloma (Table 12.7)

Q. Write short essay/note on diagnostic criteria for multiple myeloma.

Myeloma plasma cells are commonly called as myeloma cells.

MM: IgG is the most common immunoglobulin secreted.

MM:
- Monoclonal gammopathy peak 50–60 years.
- Multiple lytic lesions in bones.
- Hypercalcemia.

MM: Renal failure and sepsis are common causes of death.

MM: Higher levels of serum β2 microglobulin are associated with poor prognosis.

MM prognosis: Progressive course with poor prognosis.

Extraosseous plasmacytoma is usually found in the upper respiratory tract, especially in the nasal cavity and sinuses, nasopharynx and larynx.

MGUS prognosis: Most of the patients remain stable.

DISORDERS OF THE LYMPH NODE

NORMAL STRUCTURE

The lymph nodes are bean-shaped or oval structures and are the part of lymphatic network throughout the body. Each lymph node is covered by a connective tissue called capsule. Afferent lymphatics carrying lymph from the region drained by the lymph node enter the convex surface of the capsule. They drain into the peripheral subcapsular sinus and travel the lymph node. They form a single efferent lymphatic vessel and leave the lymph node at the concavity (hilum).

TABLE 12.7: Diagnostic criteria for plasma cell myeloma (PCM) adapted from the International Myeloma Working Group (IMWG) updated criteria for the diagnosis of multiple myeloma.

Clonal bone **marrow plasma cell percentage ≥10%** or tissue biopsy-proven plasmacytoma and ≥1 of the following myeloma-defining events:

- Evidence of myeloma-related end-organ damage or tissue impairment
  - **Hyperclcemia:** Serum calcium >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11 mg/dL)
  - **Renal insufficiency:** Creatinine clearance <40 mL/minute or serum creatinine >177 μmol/L (>2 mg/dL)
  - **Anemia:** A hemoglobin value of >20 g/L below the lower limit of normal or a hemoglobin value <100 g/L
  - **Bone lesions:** ≥1 osteolytic lesion on skeletal radiography, CT, or PET/CT
- ≥1 of the following biomarkers of malignancy:
  - Clonal bone marrow plasma cell percentage ≥60%
  - Free serum light chain ratio between involved to uninvolved light chains ≥100
  - Presence of >1 focal bonelesion on MRI

The parenchyma of the lymph node is divided into a peripheral (outer) cortex and central medulla. The cortex consists of many rounded aggregates of lymphocytes called lymphoid follicles. Each follicle consists of a pale-staining germinal center surrounded by small dark-staining lymphocytes called the mantle zone. The deeper region of the cortex or paracortex consists of zone between the peripheral cortex and the inner medulla.

The medulla mainly composed of cords of plasma cells and some lymphocytes.

Lymphadenopathy

CC: Enumerate the causes and describe the differentiating features of lymphadenopathy.

Definition: Enlargement of the lymph nodes is called as lymphadenopathy.

Significance: It is **common clinical finding**. It **may be an incidental finding** during an examination of patient for various reasons, or it **may be a presenting sign or symptom of the patient's illness**. Hence, it is necessary to differentiate whether the lymphadenopathy is a normal finding or needs further study (e.g., fine needle aspiration, biopsy). It is common to observe soft, flat, palpable submandibular nodes (<1 cm) in healthy children and young adults. Palpable inguinal nodes of up to 2 cm usually are considered as normal in healthy adults. Hence, these normal nodes do not require further evaluation. In contrast, if the node(s) appear to be abnormal, it requires a more precise diagnosis.

Approach to Lymphadenopathy

Lymphadenopathy may develop in many primary or secondary disorders **(Box 12.16)**. Most of these disorders are infrequent causes of lymphadenopathy. In more than two-thirds of cases cause of lymphadenopathy is nonspecific or is due to upper respiratory diseases (viral or bacterial) and less than 1% has a malignancy. Thus, majority of patients with lymphadenopathy have nonspecific etiology and require only few diagnostic tests.

Clinical Assessment

The examining physician should obtain a careful medical history, followed by physical examination, selected laboratory tests, and may need an excisional biopsy of lymph node.

Medical History

Symptoms such as sore throat, cough, fever, night sweats, fatigue, weight loss, or pain in the nodes should be asked for.

Personal details: It is necessary to know patient's age, sex, occupation, exposure to pets, sexual behavior, and use of drugs (e.g., diphenylhydantoin), etc.

- **Children and young adults** usually have **benign** (i.e., nonmalignant) disorders such as viral or bacterial upper respiratory infections; infectious mononucleosis; tuberculosis and toxoplasmosis.
- In contrast, **after age 50**, the incidence of malignant disorders is more than benign disorders.

Physical Examination

Systemic and local examination: It will help to know several important features. First it is necessary to know the extent of lymphadenopathy (localized or generalized).

Localized or regional lymphadenopathy indicates involvement of a single anatomic region. The site of localized or regional lymphadenopathy may provide a useful clue about the likely cause. A search should be made for a source of inflammation or primary malignancy in the appropriate drainage area, e.g., scalp, ear, mouth and throat, face, teeth or thyroid for neck nodes; the breast for axillary nodes and the perineum or external genitalia for inguinal nodes.

- **Non-neoplastic causes:** The location of the nodes involved lymphadenopathy often provides a clue to its cause.
  - **Head and neck:** Occipital lymphadenopathy usually due to an infection of the scalp, preauricular lymphadenopathy due to conjunctival infections and cat-scratch disease, posterior auricular lymph nodes in rubella infection, posterior cervical lymph nodes in toxoplasmosis. The most common site of regional adenopathy is the neck, and most of the causes are benign (e.g., upper respiratory infections, oral and dental lesions, infectious mononucleosis). A thorough ear, nose, and throat (ENT) examination should be done in adult patients with cervical adenopathy and a history of tobacco use. Tuberculosis, sarcoidosis, and toxoplasmosis can cause supraclavicular lymphadenopathy.

BOX 12.16: Causes of lymphadenopathy.

- **Infectious**
  - **Bacterial:** Streptococci, staphylococci, cat-scratch disease, chancroid, tuberculosis, primary and secondary syphilis, leprosy
  - **Viral:** Infectious mononucleosis syndromes* [Epstein-Barr virus (EBV), cytomegalovirus (CMV)], human immunodeficiencyvirus (HIV)*

- **Fungal:** Histoplasmosis, coccidioidomycosis
- **Chlamydial:** Lymphogranuloma venereum, trachoma
- **Parasitic**: Filariasis, toxoplasmosis, leishmaniasis, trypanosomiasis
- **Rickettsial:** Scrub typhus, Q fever

• **Malignancy (neoplastic)**
- **Primary hematological:** Hodgkin's lymphoma, non-Hodgkin's lymphomas*, acute or chronic lymphocytic leukemia*
- **Metastatic (secondary):** From numerous primary sites (e.g., lung, breast, thyroid, stomach, melanoma)

• **Immunological**
- **Autoimmune diseases:** Rheumatoid arthritis*, systemic lupus erythematosus (SLE)*
- Drug hypersensitivity*: Diphenyl hydantoin, hydralazine

• **Others**
- Castleman's disease (giant lymph node hyperplasia)*
- Sarcoidosis*, amyloidosis*
- Dermatopathic lymphadenitis
- Histiocytic necrotizing lymphadenitis (Kikuchi's disease)*

* Most likely associated with generalized lymphadenopathy.

- **Axilla:** Axillary adenopathy may be due to **injuries or localized infections** of the ipsilateral **upper extremity** (arms or chest wall).
- **Inguinal:** Inguinal lymphadenopathy is usually **secondary to infections or trauma of the lower extremities** (legs). It may also occur in sexually transmitted diseases (e.g., lymphogranuloma venereum, primary syphilis, genital herpes, or chancroid).

v **Malignant causes**: Main malignant causes include **metastatic cancer from head and neck region, breast, lung, and thyroid primaries**.
- If enlarged **supraclavicular and scalene nodes** are found, they are **always abnormal**. Since these lymph nodes drain regions of the lung and retroperitoneal space, their enlargement may be due to **lymphomas, other cancers**, or infectious processes arising in these areas.
- **Virchow's node is an enlarged left supraclavicular node** with **metastatic cancer from a gastrointestinal primary** (usually stomach). Metastases to supraclavicular nodes can also occur from primary cancers in the lung, breast, testis, or ovary.
- Malignant causes of **axillary lymphadenopathy** include **melanoma** or **lymphoma** and, in females **breast cancer**.
- **Inguinal lymphadenopathy** may occur with lymphomas and metastatic cancer from primary lesions of the rectum, genitalia, or

lower extremities (melanoma).

Generalized lymphadenopathy is defined as involvement of three or more noncontiguous lymph node areas. Many of the causes of lymphadenopathy **(Box 12.16)** can produce localized or generalized adenopathy. So this distinction is of limited usefulness in the differential diagnosis. Generalized lymphadenopathy may be secondary to systemic infection (e.g., viral), autoimmune disease, drug hypersensitivity reactions but is more likely to signify underlying hematological malignancy.

- **Nonmalignant disorders** with generalized lymphadenopathy: It is frequently found in infectious mononucleosis [Epstein-Barr virus (EBV) or cytomegalovirus (CMV)], toxoplasmosis, AIDS, other viral infections, systemic lupus erythematosus (SLE), and mixed connective tissue disease.
- **Malignant disorders:** Acute and chronic lymphocytic leukemias and malignant lymphomas also produce generalized adenopathy in adults.

Other features to be examined: These include the size of nodes, texture, presence or absence of nodal tenderness, signs of inflammation over the node, skin lesions, and splenomegaly.

Size and texture of the lymph node(s) and the presence of pain are useful in evaluating a patient with lymphadenopathy.

- **Size of lymph nodes:** Lymph nodes <1.0 cm^2 in area (1.0 cm × 1.0 cm or less) are almost always due to benign, nonspecific reactive causes. If the node(s) ≤1.0 cm^2, patient should be observed after excluding infectious mononucleosis and/or toxoplasmosis unless there are symptoms and signs of an underlying systemic illness. If the node is larger than 2 cm, FNAC or a biopsy should be performed.
- **Texture of lymph nodes:** Various descriptive terms used for texture include soft, firm, rubbery, hard, discrete, matted, tender, movable, or fixed.
  - Tenderness develops when the capsule of node is stretched during rapid enlargement, usually secondary to an inflammatory process. Lymphadenopathy in response to local infection or inflammation (reactive nodes) usually expands rapidly and is painful. Some malignant diseases (e.g., acute leukemia in which it is more frequently painless) may produce rapid enlargement and pain in the nodes.

- **Nodes involved by lymphoma are usually large, discrete, symmetric, rubbery, firm, mobile, and nontender**.
- **Nodes with metastatic cancer are usually hard, nontender, and nonmovable** (because of fixation to surrounding tissues).
- The presence of splenomegaly in a patient with lymphadenopathy usually indicates presence of a systemic illness (e.g., infectious mononucleosis, lymphoma, acute or chronic leukemia, SLE, sarcoidosis).
- Nonsuperficial lymphadenopathy (thoracic or abdominal) is usually detected during diagnostic workup of a patient with symptoms.

Thoracic (mediastinal and hilar) adenopathy may be detected by routine chest radiography. It may be due to primary lung disorders and systemic illnesses that characteristically involve mediastinal or hilar nodes. In older patients, it may be due to primary lung cancer (especially among smokers), lymphomas, metastatic carcinoma (usually lung), tuberculosis, fungal infection, and sarcoidosis.

Enlarged intra-abdominal or retroperitoneal nodes are usually malignant (lymphomas or germ cell tumors in young men).

Laboratory Investigation

The laboratory investigation for lymphadenopathy depends on the etiology suspected from the patient's history and physical findings. About 50% of patients do not require laboratory studies.

Complete blood count: Initial investigation required is a complete blood count (CBC) [to detect neutrophilia in pyogenic infection, EBV or CMV mononucleosis, immune cytopenias in illnesses such as SLE, evidence of hematological disease (acute or chronic leukemias, lymphoma with a leukemic component)].

Other laboratory tests: These include measurement of **erythrocyte sedimentation rate** (ESR), throat culture (if suspected infection of throat) and **serologic studies** (to demonstrate antibodies specific to components of EBV, CMV, HIV, etc.). If SLE is suspected, antinuclear and anti-DNA antibody studies.

Radiological: A **chest X-ray** to detect the presence of a pulmonary infiltrate or mediastinal lymphadenopathy. It suggests tuberculosis, lymphoma, primary lung cancer, or metastatic cancer and requires further investigation.

Imaging techniques (CT, MRI, ultrasound, color Doppler ultrasonography) may be used to differentiate benign from malignant lymph nodes (especially in patients with cancer in the head and neck region).

FNAC of lymph node: Fine-needle aspiration of lymph node should not be performed as the first diagnostic procedure unless for the diagnosis of certain infections (e.g., tuberculosis) or metastatic tumors (e.g., secondaries from carcinoma of upper respiratory tract). Most diagnoses (e.g., lymphoma) need more tissue than obtained by aspiration and may delay a definitive diagnosis.

Biopsy of lymph node: Most lymphadenopathy does not require a biopsy. However, it is a valuable diagnostic tool. If the patient's history and physical findings suggest a benign condition, careful follow-up for 2- to 4-weeks is suggested. If the patient's history and physical findings suggest a malignancy, a cutting needle or excision biopsy of a representative node is indicated for a histological diagnosis. Examples include presence of a solitary, hard, nontender cervical node in an older patient who uses tobacco; supraclavicular lymphadenopathy; and solitary or generalized firm, movable adenopathy suggestive of lymphoma.

LYMPHADENITIS

Inflammation of lymph nodes is called as lymphadenitis. It may be acute or chronic.

Acute Nonspecific Lymphadenitis

Acute lymphadenitis occurs in lymph nodes that drain sites of acute bacterial or fungal infections. These lymph nodes rapidly become enlarged and are usually tender (painful) because of the distension of their capsule. When there is abscess formation, the nodes become fluctuant and the overlying skin appears red.

Lymphadenitis in the cervical region is usually due to drainage of microbes or microbial products from infections of the teeth or tonsils, whereas in the axillary or inguinal regions it is usually due to infections in the extremities. Acute lymphadenitis can occur in mesenteric lymph nodes draining acute

appendicitis. Systemic viral infections (especially in children) and bacteremia often produce acute generalized lymphadenopathy.

MORPHOLOGY

Gross

The involved lymph nodes are swollen enlarged, and gray-red.

Microscopy

The germinal centers appear large and prominent. They show numerous mitotic figures. Macrophages may show particulate debris derived from dead bacteria or necrotic cells. In lymphadenitis accompanying pyogenic organisms, neutrophils are prominent and the centers of the follicles may show necrosis. Few neutrophils may be found around the follicles within the lymphoid sinuses.

Chronic Nonspecific Lymphadenitis

Chronic nonspecific lymphadenitis may show different morphological patterns and three important patterns are (i) follicular hyperplasia, (ii) paracortical hyperplasia and (iii) sinus histiocytosis.

Follicular Hyperplasia

Definition: It is characterized by the presence of large oblong germinal centers (secondary follicles), which are surrounded by a collar of small resting naive B cells (the mantle zone). In nonspecific reactive follicular hyperplasia, hyperplastic follicles are mainly found in the cortex of the lymph node.

Causes: The cause of nonspecific reactive follicular hyperplasia is usually unknown and caused by stimuli that activate humoral immune responses. It may occur due to viral infection, drug or inflammatory process. Other causes include rheumatoid arthritis, toxoplasmosis, and early stages of infection with HIV.

MORPHOLOGY

Gross

Grossly, the affected lymph nodes are usually enlarged, firm and non-tender in any type of chronic nonspecific lymphadenitis.

Microscopy

Follicular hyperplasia shows marked enlargement and prominence of the germinal centers of lymphoid follicles. Follicles are round or irregularly shaped. These germinal centers have normal polarization. Each germinal center consists of two distinct regions: (1) a dark zone containing proliferating blast-like B cells (centroblasts) and (2) a light zone composed of B cells with irregular or cleaved nuclear contours (centrocytes). Interspersed between the germinal B centers are scattered benign macrophages (often called as tingible-body macrophages), with abundant pale cytoplasm containing pyknotic nuclear and cytoplasmic debris of B cells. A well-defined mantle of normal small B cells surrounds the follicles, sharply separating them from interfollicular region.

Differentiation from follicular lymphoma: Follicular hyperplasia appears morphologically similar to follicular lymphoma.

Features favoring a reactive (non-neoplastic) hyperplasia are:

- Preservation of the lymph node architecture
- Marked variation in the shape and size of the follicles
- Presence of frequent mitotic figures, phagocytic macrophages, and recognizable light and dark zones.

Course: It undergoes rapid and complete resolution after the causative stimulus disappears.

Paracortical Hyperplasia

Definition: Paracortical hyperplasia is due to hyperplasia of T-cell-dependent area (paracortical region) of the lymph node.

Causes: It is caused by stimuli that trigger T-cell–mediated immune responses such as acute viral infections (e.g., infectious mononucleosis, measles and CMV lymphadenitis) and autoimmune disorders.

MORPHOLOGY

Gross

It is similar to follicular hyperplasia mentioned above.

Microscopy

It shows expansion of the paracortex (T-cell area) with increased number of immunoblasts and activated T cells. These cells are three to four times the size of resting lymphocytes. They contain round nuclei, open chromatin, several prominent nucleoli, and moderate

amounts of pale cytoplasm. The enlarged paracortical region may encroach on the lymphoid follicles. There may be hyperplasia of the mononuclear phagocytic cells in the lymphatic sinuses.

Course: It usually resolves promptly.

Sinus Histiocytosis

Definition: It is characterized by an increase in the number and size of the cells that line lymphatic sinusoids. It is also called reticular hyperplasia.

Causes: Although nonspecific, sinus histiocytosis is common in lymph nodes draining cancers (e.g., carcinoma of the breast) and, less often, inflammatory and infectious foci.

MORPHOLOGY

Gross

It is similar to follicular hyperplasia mentioned above.

Microscopy

In sinus histiocytosis, there is expansion and distension of the subcapsular and trabecular sinuses of the lymph node. The lining lymphatic endothelial cells are markedly hypertrophied and there is marked increase in number of macrophages (histiocytes) within the sinuses. Sinus histiocytes derive from blood monocytes and they contain phagocytosed material.

Tuberculous Lymphadenitis

CC: Describe the pathogenesis and pathology of tuberculous lymphadenitis.

Lymph nodes are the most common extrapulmonary site of tuberculosis For pathogenesis of tuberculosis *refer* pages 512-3, of Chapter 17.

Region involved: Most commonly it involves the lymph nodes of cervical and mediastinal region, followed by axillary and inguinal. Sometimes, more than one region may be involved.

Type of infection: Tuberculous lymphadenitis may occur as a part of primary infection or spread from contiguous sites or reactivation. Primary

focus in the pharynx and tonsil will produce enlargement of cervical lymph node. Supraclavicular lymphadenopathy is usually due to the spread from mediastinal disease.

Clinical presentation: Lymphadenitis is the most frequent presentation of extrapulmonary tuberculosis, usually occurring in the cervical region. The involved nodes are usually painless and initially mobile. Later they become matted together due to periadenitis. When caseation and liquefaction occur, the swelling becomes fluctuant. The caseous material may be discharge through the skin with the formation of a 'collar-stud' abscess and sinus formation ("scrofula"). About 50% of patients do not show constitutional features, such as fevers or night sweats.

Tuberculin test: It is usually strongly positive.

MORPHOLOGY

CC (Show How): Identify and describe the features of tuberculous lymphadenitis in a gross and microscopic specimen.

DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform). Show the gross and microscopic features of tuberculoud lymphadenitis.

Gross (Fig. 12.24A)

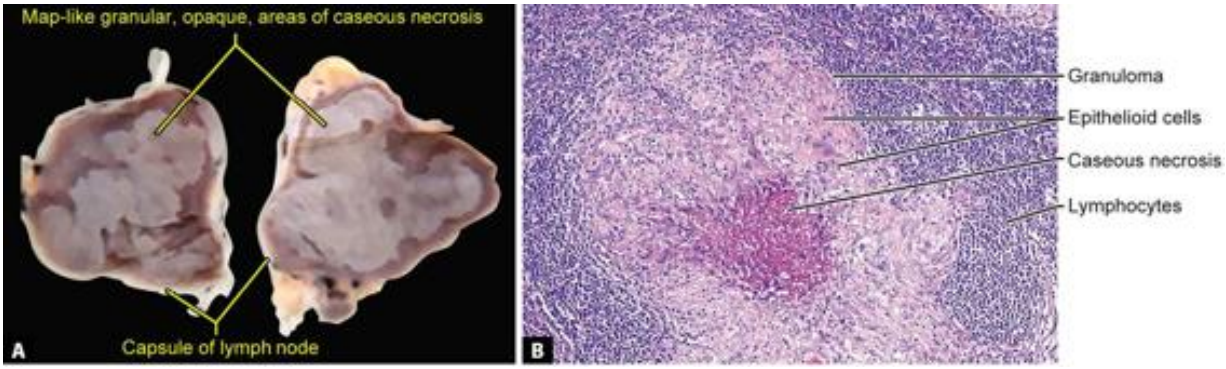
The lymph nodes are enlarged and matted together because of periadenitis (inflammation of the capsule of lymph node and surrounding structures). The cut surface in the tuberculous areas shows characteristic map-like areas which are yellowish, granular, opaque, soft necrotic material that resembles dry cheese.

Microscopy (Fig. 12.24B and *refer* Fig. 2.17)

It shows tuberculous granuloma. Granuloma in tuberculosis is called as tubercle. Tubercle may show central area of caseous necrosis (caseating granuloma/soft tubercle) or may not show caseation (noncaseating tubercles/hard tubercle). Individual tubercles are identified only by microscopic examination. When multiple granulomas coalesce, they may be grossly identified.

Caseating granuloma consists of:

- Central area of caseous necrosis
- Surrounded by epithelioid cells (modified macrophages), some of which may fuse to form multinucleate giant cells. The giant cells may be Langhans type (nuclei arranged in horse-shoe pattern) or foreign body type (nuclei in the center).
- The epithelioid cells are surrounded by rim of lymphocytes.
- These granulomas are usually enclosed within fibroblasts.



Figs. 12.24A and B: Tuberculous lymphadenitis: (A) Cut section of lymph node shows opaque areas of caseous necrosis;(B) Microscopy shows granuloma with caseous necrosis.

LYMPHOID NEOPLASMS

CC: Describe and discuss the pathogenesis, pathology and the differentiating features of Hodgkin's and non-Hodgkin's lymphoma.

Classification of Lymphoid Neoplasms (Box 12.17)

Q. Write short essay/note on B-cell lymphoma.

WHO classification of lymphoid neoplasms depends on clinicopathological and immunological profile **(Table 12.8)** and has clinical and therapeutic importance.

Majority (80–85%) of lymphoid neoplasms are of B-cell origin and remaining of T-cell/NK cell type.

T-cell lymphoblastic lymphoma or Burkitt lymphoma usually seen in childhood.

Lymphoid neoplasms: Most resemble some stage of B- or T-cell differentiation.

Lymphoid neoplasms: Second most common malignant tumor in HIV.

Lymphoid neoplasms: About 1/3rd arise from extranodal sites.

FOLLICULAR LYMPHOMA

Q. Write short note on follicular lymphoma.

Composed of follicle center (germinal center) B cells of lymphoid follicles (centrocytes and centroblasts).

Molecular Pathogenesis

Q. Molecular pathogenesis of follicular lymphoma.

Follicular lymphoma is strongly associated with chromosomal translocations involving *BCL2*. It is characterized by a (14;18) translocation that juxtaposes the *IgH* locus on chromosome 14 and the *BCL2* locus on chromosome 18. This causes overexpression of *BCL2* which acts as antiapoptotic factor and promotes the survival of follicular lymphoma cells. Normal germinal centers contain numerous B cells which undergo apoptosis. In follicular lymphoma, there are no apoptotic cells. In about 90% of cases there are mutations in the *MLL2* gene (codes histone methyltransferase involved in epigenetics).

Q. Name peripheral/mature B-cell neoplasms.

BOX 12.17: WHO classification of the lymphoid neoplasms (2016).

I. PRECURSOR LYMPHOID NEOPLASMS
- B lymphoblastic leukemia/lymphoma
- T lymphoblastic leukemia/lymphoma

II. MATURE B-CELL NEOPLASMS
- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- B-cell prolymphocytic leukemia
- Monoclonal B-cell lymphocytosis*
- Splenic B-cell marginal zone lymphoma
- Hairy cell leukemia
- Lymphoplasmacytic lymphoma
- Heavy chain disease
- Plasma cell neoplasm
- Follicular lymphoma
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma
- Burkitt lymphoma

III. MATURE T AND NK CELL NEOPLASMS
- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- Mycosis fungoides
- Sézary syndrome
- Peripheral T-cell lymphoma, NOS
- Angioimmunoblastic T-cell lymphoma
- Anaplastic large cell lymphoma
- Adult T-cell leukemia/lymphoma
- Extranodal NK/T cell lymphoma, nasal type

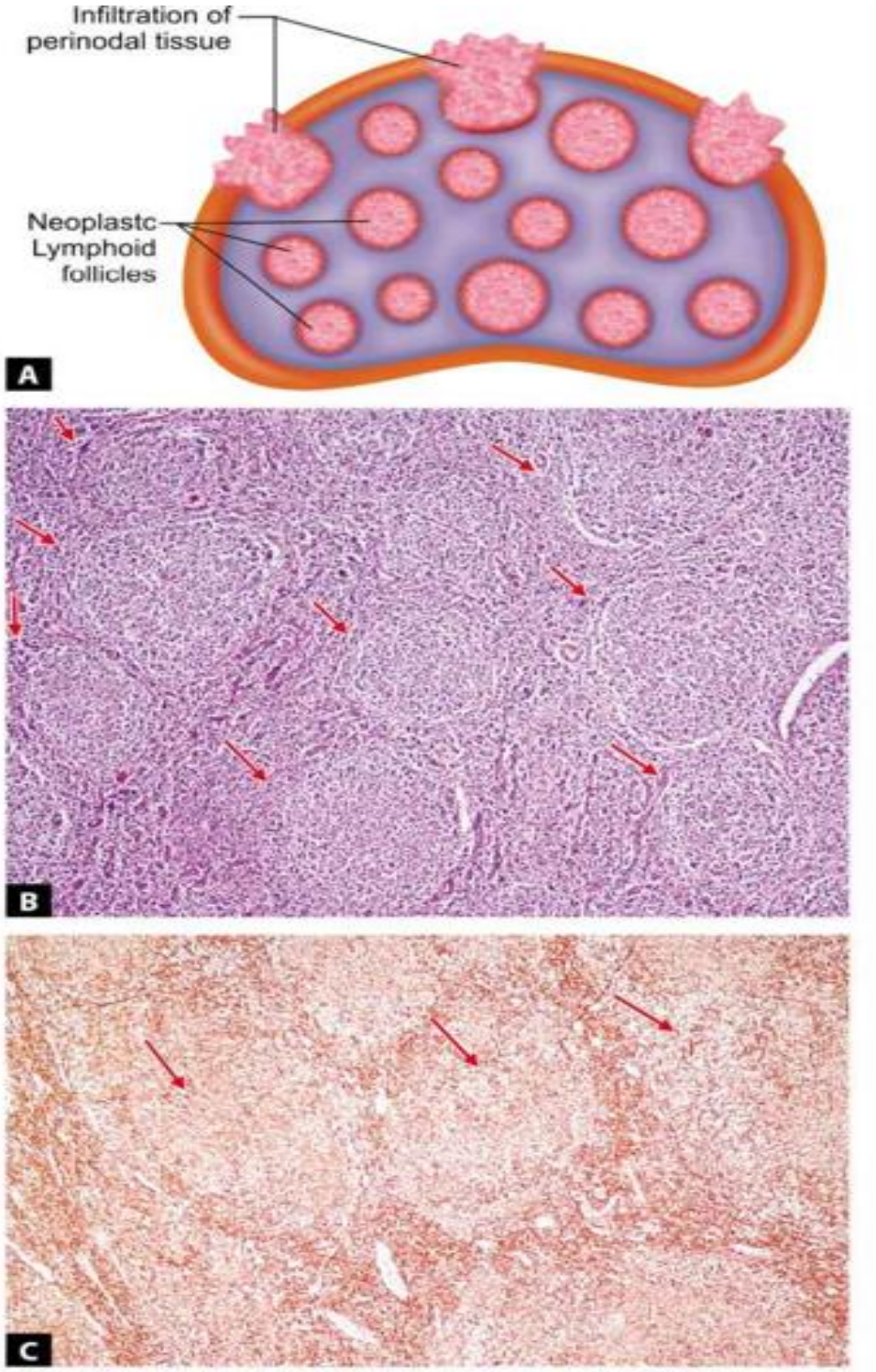
IV. HODGKIN LYMPHOMA
- **Classical Hodgkin lymphoma**
  - Nodular sclerosis classical Hodgkin lymphoma
  - Mixed cellularity classical Hodgkin lymphoma
  - Lymphocyte-rich classical Hodgkin lymphoma
  - Lymphocyte depleted classical Hodgkin lymphoma
- Nodular lymphocyte predominance Hodgkin lymphoma

*Changes from the 2008 classification.

TABLE 12.8: Cell type and its antigens detected by monoclonal antibodies.

Cell type	*Antigen detected*
T-cell	CD1, CD3, CD4, CD5, CD8
B-cell	CD10, CD19, CD20, CD21, CD23, CD79a
Monocyte or macrophage	CD11c, CD13, CD14, CD15, CD33, CD64
NK cell	CD16, CD56
Stem cell and progenitor cell	CD34
All leukocytes	CD45 (LCA)

(CD: cluster designation; NK: natural killer; LCA: leukocyte common antigen)



Figs. 12.25A to C: Follicular lymphoma: (A) Diagrammatic. Neoplastic follicles are seen in both the cortex, medulla of lymph node and infiltration of the perinodal tissue; (B) Follicular lymphoma shows nodular aggregates of malignant lymphoid cells (arrows); (C) *BCL2* expression (arrows) in neoplastic lymphoid cells of follicles.

Morphology

Q. Write short essay on morphology of follicular center cell lymphoma.

Gross

- Involves **lymph nodes**, spleen and bone marrow.
- **Architecture** of lymph node is **lost**; frequently infiltrate the perinodal tissue **(Fig. 12.25A)**.

Microscopy

- **Follicular** (nodular) growth pattern, **neoplastic follicles** are **poorly defined (Fig. 12.25B)**.
- **Two types of B cells.**
  - **Centrocytes** (small cleaved cells)
    - Cleaved nuclei
    - Inconspicuous nucleoli.
  - **Centroblasts** (large noncleaved cells)
    - Round or oval nuclei with open nuclear (vesicular) chromatin.
    - Multiple (1 to 3) nucleoli.
    - Usually 3 times the size of lymphocyte.

Immunophenotype

Expresses CD19, CD20 (pan-B cell markers), CD10 (CALLA), surface immunoglobulin and *BCL2* protein.

Cytogenetics and Molecular Genetics

t(14; 18) (q32:q21), with *IgH* and *BCL2* as partner genes and leads to constitutive overexpression of *BCL2* protein.

Clinical Features

- Peak in **sixth and seventh decades**.
- **Generalized lymphadenopathy**.

FL: Arises from follicle center B cells.

FL: Centrocytes and centroblasts form poorly defined follicles.

FL: Grade ranges from 1 to 3. Grade 1 with <5 centroblasts/hpf and grade 3 with >15/hpf.

FL: Peripheral blood smear may show lymphocytosis [<20 × 109/L (20,000/cu mm)].

FL: Bone marrow involved in 85%.

FL: Prognosis indolent.

DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

Heterogeneous group of **aggressive** neoplasm of large B-cell with **diffuse** growth pattern.
Constitutes about 20 to 30% of NHL and 60 to 70% of aggressive lymphoid neoplasms.

Microscopy

- **Loss of** lymph node **architecture** with **diffuse growth pattern.**
- **Neoplastic cells:**
  - **Large** round or oval cells, **4 to 5 times** of a **small lymphocyte.**
  - Moderate pale or basophilic cytoplasm.
  - **Nucleus** equal or larger than the **nucleus of a macrophage** with IG gene by *IgH* gene different appearances.

Immunophenotype

- Express pan-B cell markers such as **CD19, CD20, CD22 and CD79a**.
- Also express **germinal center markers** like CD10 and *BCL6*.
- **Negative for TdT.**

Cytogenetics and Molecular Profile

- **Translocation of *BCL2* gene:** t(14; 18) translocation
- **Mutations of the *BCL6* gene.**

Clinical Features

- More common between **65 and 70 years** of age.
- Slight **male** preponderance.
- **Rapidly enlarging** mass at a single or multiple **nodal or extranodal sites.**

DLBCL: Aggressive, diffuse large B-cell neoplasm.

DLBCL may involve lymph nodes or extranodal sites.

DLBCL: Prognosis aggressive and rapidly fatal if untreated.

BURKITT LYMPHOMA

Q. Write short note on Burkitt lymphoma.

- **Highly aggressive B-cell** neoplasm, often presents as **extranodal** lymphoma or as an acute leukemia.
- Composed of **medium-sized, monomorphic lymphoid cells** with basophilic **vacuolated cytoplasm.**

Clinical Variants

- **Endemic (African) Burkitt lymphoma (BL):**
  - Occurs in Africa, affects **children and adolescents.**
  - **Associated with Epstein-Barr virus infection and malaria**.
  - Usually involves the **jaw** and present as a mandibular mass.
- **Sporadic (nonendemic) BL:**
  - Occurs in **children or young adults.**
  - **Abdominal mass** and involves **ileocecum** and **peritoneum**.
- **Immunodeficiency-associated (HIV) BL:** Involves lymph nodes and bone marrow.

Microscopy

Q. Write short note on microscopy of Burkitt lymphoma.

- Burkitt lymphomas, irrespective of the categories, are **histologically similar**.
- Lymph node shows **loss of architecture**.

- Involved tissues show **diffuse** infiltrate of **monotonous medium-sized lymphoid cells (Figs. 12.26A and B)**.
- **Appearance of neoplastic lymphoid cells:**
  - **Medium-sized cells**.
  - **Round or oval nuclei** having clumped **coarse chromatin** with **several (2–5) nucleoli.**
  - **Moderate** amount of deeply **basophilic cytoplasm, multiple, small**, **round lipoid** (clear) **vacuoles** which stain positive with oil red O.
  - **Numerous mitotic figures**.
- **Starry sky pattern:** Tumor cells undergo **apoptosis** and nuclear remnants of these apoptotic cells are phagocytosed and cleared by benign macrophages. These macrophages in the background of lymphoid cells creates **"starry sky"** appearance **(Figs. 12.26A and B)**.

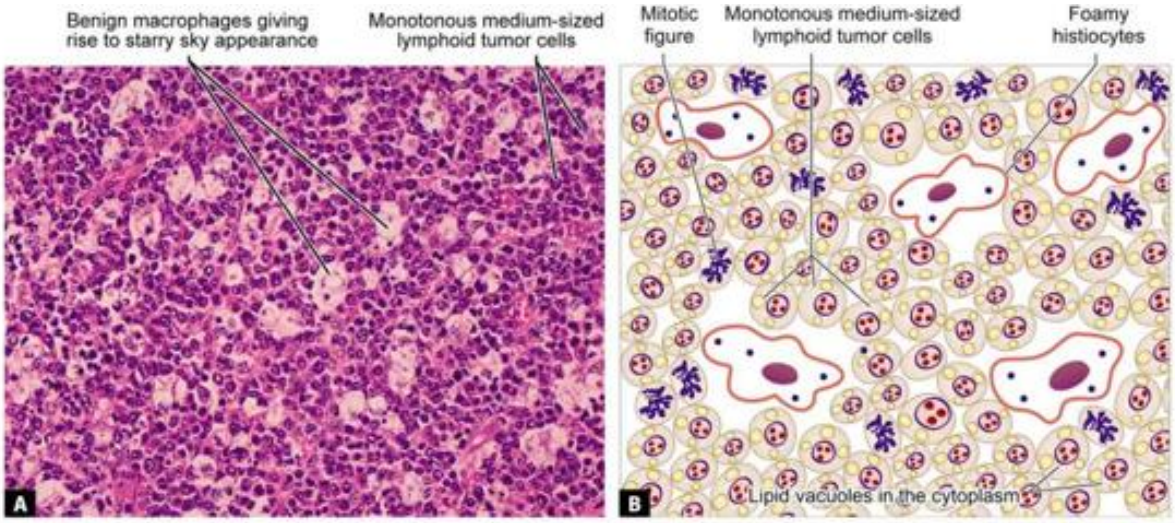
Immunophenotype

- Express surface **IgM, monotypic κ or λ light chain.**
- **Positive for common B-cell antigens** (CD19, CD20, and CD22).
- **Positive for CD10 and *BCL6*.**
- *BCL2* negative.

Cytogenetic and Molecular Genetic Features (Fig. 12.27)

Translocations of c-MYC gene

- MYC (*c-MYC*) is a **proto-oncogene** on chromosome 8.
- **Most common translocation t(8:14) (q24; q32).**
- **Translocations of *c-MYC* gene,** converts proto-oncogene into **MYC** oncogene, which leads to **overexpression of MYC protein** (oncoprotein). This causes **uncontrolled cell proliferation** and stimulation of apoptosis.
- **Mutations inactivate *p53*.**
- **Poor prognostic factors:**
  - Involvement of blood, bone marrow and central nervous system.
  - Bulk of the disease-unresected tumor of more than 10 cm in diameter.
  - High serum LDH levels.
  - Presence of residual disease after excision.



Figs. 12.26A and B: (A) H&E; (B) Diagrammatic. Burkitt lymphoma composed of medium-sized lymphoid cells admixed with benign macrophages giving a "starry sky" appearance.

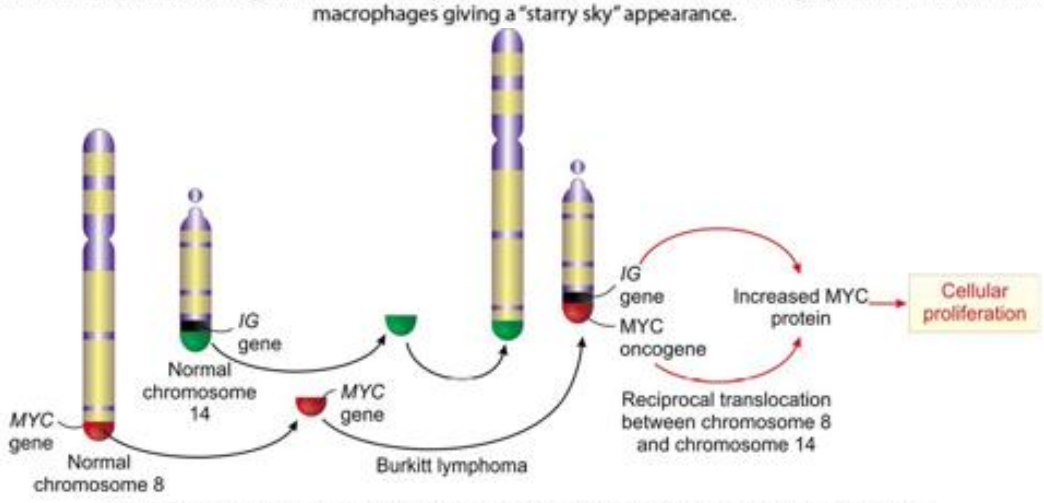


Fig. 12.27: Chromosomal translocation and activated MYC oncogene in Burkitt lymphoma.

BL: Aggressive B-cell lymphoma, three clinical variants. (1) endemic, (2) sporadic and (3) immunodeficiency-associated. Endemic: involves jaw and associated with EBV.

BL: Medium sized B-cells. Starry sky pattern.

BL: Translocation of c-MYC gene.

BL: Prognosis—very aggressive but responds well chemotherapy.

MATURE T-CELL AND NK CELL NEOPLASMS

Peripheral T-Cell Lymphoma (PTCL), NOS

Mainly involves lymph node.

Microscopy

- **Lymph node** with **effacement of the normal architecture.**
- P**aracortical or diffuse infiltration** by neoplastic T-cells.
- **Neoplastic T-cells.**
  - **Small, intermediate to large cells** with sparse or abundant; clear, eosinophilic or basophilic.
  - **Vesicular or hyperchromatic nuclei**, **prominent nucleoli.**

Immunophenotype

- Lack TdT (expressed by immature T-cells).
- Express pan T-cell-CD2, C3, CD5 and either α β or γ δ T cell receptors (TCR).

Mycosis Fungoides

- **Cutaneous T-cell lymphoma.**
- Lymphoid cells with **irregular nuclear outlines.**
- Limited to **skin.**

Age: Most are adults or elderly.

Microscopy

- **Epidermis** (**epidermotropism**) **and upper dermis** is infiltrated by neoplastic T-cells.
- Groups of neoplastic cells in the epidermis—**Pautrier's microabscess.**
- Tumor cells have **convoluted (cerebriform) nuclear contours.**

Sézary Syndrome

Rare disease is defined by the triad namely:

1. **Widespread exfoliative erythroderma**
2. **Generalized lymphadenopathy**
3. **Presence of characteristic Sézary cells** in the skin, lymph nodes and peripheral blood.

Prognosis

Aggressive disease and most die of opportunistic infections.

Peripheral T-cell tumors constitute >15% of non-Hodgkin lymphomas. NK cell tumors are very rare.

PTCL: Clinical features
- Fifth to seventh decade.
- Generalized lymphadenopathy

PTCL prognosis: Highly aggressive with a poor response to therapy.

Mycosis fungoides and Sézary syndrome: T-cell neoplasms with skin involvement.

Mycosis fungoides has three stages:
1. Patch stage
2. Plaque stage
3. Tumor stage

Immunophenotype: Express pan-T-CD2+, CD3+ and CD5.

Sézary cells are neoplastic T-cells with cerebriform nuclei.

Immunophenotype: Tumor cells express-CD2+, CD3+ and CD5+.

Hodgkin Lymphomas

CC: Describe and discuss the pathogenesis, pathology and the differentiating features of Hodgkin's and non-Hodgkin's lymphoma.

Definition: Malignant lymphoid neoplasms with following characteristics:
- **Minority** (1–3%) **of specific neoplastic cells** (Hodgkin cells and **Reed-Sternberg cells**).
- **Majority background of** reactive **non-neoplastic cells.**

- Usually involves **lymph nodes. The involved lymph nodes are enlarged, encapsulated with fish flesh appearance on cut surface (Fig. 12.28).**
- Majority occurs in **young** adults.

Classification (Box 12.18)

Q. Classify lymphoma and discuss Hodgkin lymphoma.

Hodgkin lymphoma (HL) is broadly divided into two types, which differ in clinical features, behavior, morphology and immunophenotype.

Cell of Origin and Immunophenotype

- **Classical Hodgkin lymphoma**
  - **Cell of origin: Germinal center or postgerminal center B-cell**
  - **Immunophenotype: CD15** and **CD 30 positive.**

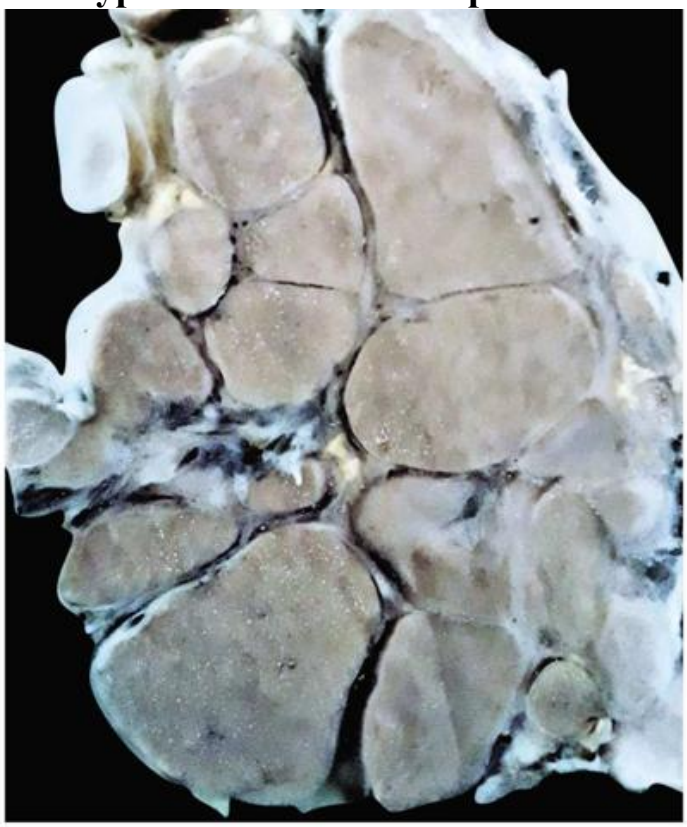


Fig. 12.28: Hodgkin lymphoma showing enlarged, encapsulated lymph nodes with fish flesh appearance on cut surface.

BOX 12.18: WHO classification (2016) of Hodgkin lymphoma.

- Classical Hodgkin lymphoma (CHL)
  - Nodular sclerosis classical Hodgkin lymphoma (NSCHL)
  - Mixed cellularity classical Hodgkin lymphoma (MCCHL)
  - Lymphocyte-rich classical Hodgkin lymphoma (LRCHL)
  - Lymphocyte depleted classical Hodgkin lymphoma (LDCHL)
- Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)

Classical HL: $CD15^+$ and $CD30^+$
NLPHL: $CD15^-$, $CD\ 30^-$, $CD20^+$, and $CD\ 45^+$.

- **Nodular lymphocyte predominant Hodgkin lymphoma**
  - **Cell of origin: Germinal center B-cell at the centroblastic stage** of differentiation.
  - **Immunophenotype: CD15** and **CD30 negative**.

Morphology of Neoplastic Cells

Q. Write short note on RS cell and its variants. Mention the types of Hodgkin lymphoma where they are seen.

Reed–Sternberg (RS) cells are neoplastic cells **(Fig. 12.29) pathognomonic** of Hodgkin lymphoma.
Appearance and description of diagnostic Reed–Sternberg cells and its variants are shown in **Figure 12.30.**
Various types of cells found in Hodgkin lymphoma are listed in **Table 12.9.**

Hodgkin lymphoma (HL) synonym: Hodgkin disease.

HL is mainly divided into:
1. Classical, and
2. Nodular lymphocytic Hodgkin lymphoma

Reed–Sternberg (RS) cells: CD 30 is the most sensitive marker and PAX 5 is the most specific marker.

CLASSICAL HODGKIN LYMPHOMA

CC (Show How): Identify and describe the features of Hodgkin's lymphoma in a gross and microscopic specimen.
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Q. Describe the gross and microscopic features of all subtypes of Hodgkin lymphoma. Compare their prognosis.

Classical Hodgkin lymphoma (CHL) account for 95% of Hodgkin lymphomas and has four subtypes.

DESCRIPTION	APPEARANCE
• Large cells (20–60 µm in diameter) • Abundant eosinophilic cytoplasm • Nucleus: - Typically two large nuclei—mirror image - Prominent eosinophilic nucleolus surrounded by a halo	 Classic RS cell
• Single, large, round nucleus with a large eosinophilic inclusion-like nucleolus • May be seen in any subtypes of CHL	 Mononuclear cell variant (Hodgkin cell)
• Abundant, lightly acidophilic or water-clear cytoplasm • Large folded or multilobed nucleus • One or more prominent eosinophilic nucleoli • Seen in nodular sclerosis of CHL	 Lacunar cell variant
• Dark eosinophilic cytoplasm with pyknotic nuclei • Strongly indicate HL but not diagnostic	 Mummified cell variant
• Highly pleomorphic cells • Large bizarre hyperchromatic nuclei • Commonly found in lymphocyte-depleted CHL	 Anaplastic/pleomorphic variant
• Abundant pale cytoplasm with popcorn nucleus • Specific to the nodular lymphocyte predominance type of HL	 Lymphocyte predominant cell (LP cell)/popcorn cell

Classical RS cell is binucleated with owl-eyed nuclei having mirror image appearance.

Lacunar cell has clear cytoplasm and seen in nodular sclerosis CHL.

LP/popcorn cell is seen in nodular lymphocyte predominant HL.

Fig. 12.30: Diagrammatic appearances and characteristic features of Reed–Sternberg cells and its variants.

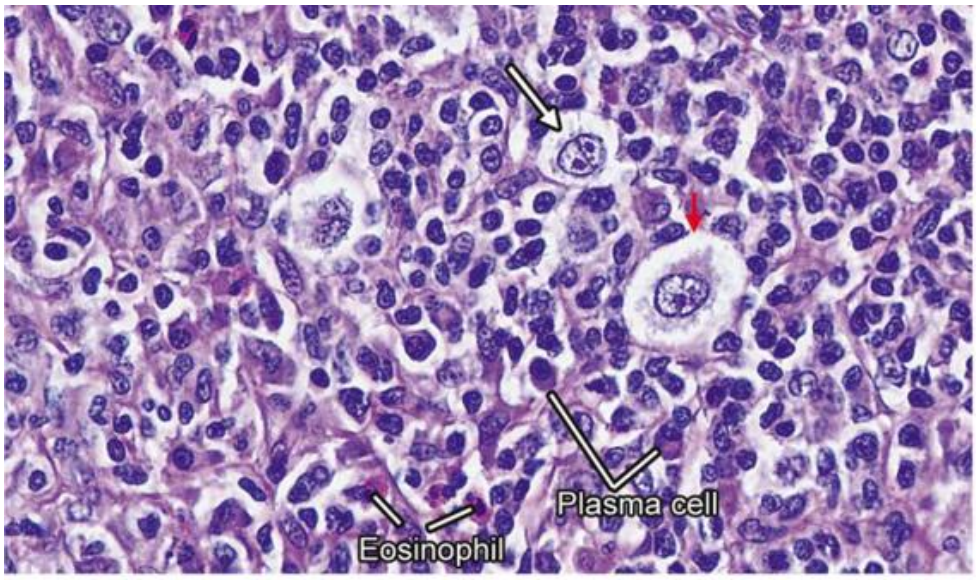


Fig. 12.29: Microscopic appearance of Hodgkin lymphoma showing RS cells (long arrow) and Hodgkin cells (short arrow) within the background of mixed population of reactive cells.

TABLE 12.9: Types of cells found in Hodgkin lymphoma.

Non-neoplastic cells	*Neoplastic cells*
• Reactive lymphocytes • Macrophages/histiocytes – Granulocytes – Eosinophils – Neutrophils • Plasma cells	• Reed–Sternberg cells (classical) • Variants – Mononuclear – Lacunar – Mummified – Anaplastic/pleomorphic – Lymphocyte predominant (LP) cell/popcorn

HL: Majority are non-neoplastic cells and minority are neoplastic cells.

Nodular Sclerosis ClassicalHodgkin Lymphoma (NSCHL)

Q. Write short note on nodular sclerosis HL.

Subtype of CHL characterized by **collagen bands** that surround **nodules** and have **lacunar cell variant of Reed–Sternberg cells.**

- Most common: **40–70%** of cases.

- Most between **20 and 30 years of age** with **equal** frequency in **males and females.**
- **Rarely associated with EBV.**
- Involves **mediastinal lymph nodes.**

Microscopy of NSCHL (Figs. 12.31A to C)

- **Loss of lymph node architecture.**
- **Sclerosis and nodules: Broad collagen** bands (sclerosis) divide the lymphoid tissue into **nodules of varying sizes and shapes**.
- **Presence of lacunar cell.**
- **Background: Small T lymphocytes, eosinophils, plasma cells, and macrophages.**

Immunophenotype

- RS cells are **$CD15^+$ and $CD30^+$; $CD45^-$ and T cell markers negative.**
- **EBV negative.**

Nodular sclerosis is the most common subtype of CHL. Lacunar cells are commonly seen.

NSCHL
- Nodules separated by broad bands of collagen
- $CD15^+$, $CD30^+$, EBV-ve and CD 45-ve

NSCHL prognosis: Better than other types of CHL, with a cure rate of 80–85%.

Mixed Cellularity Classical Hodgkin Lymphoma (MCCHL)

Q. Write short note on mixed cellularity HL.

- **Second common subtype:** 20–25% of cases.
- **More common in males**
- **Strongly associated with EBV**.
- **Older age**, with **systemic symptoms** (such as night sweats and weight loss) and **advanced tumor stage**.
- Involves **peripheral lymph nodes**.

Microscopy of MCCHL (Figs. 12.32A and B)

- Lymph node architecture obliterated.
- **Plenty of Reed–Sternberg cells** and Hodgkin cells.
- **Back ground: Small lymphocytes, eosinophils (sometimes numerous), neutrophils, plasma cells and benign macrophages (histiocytes).**

Immunophenotype

RS cells are $CD15^+$, $CD30^+$ and EBV^+ (about 70%).

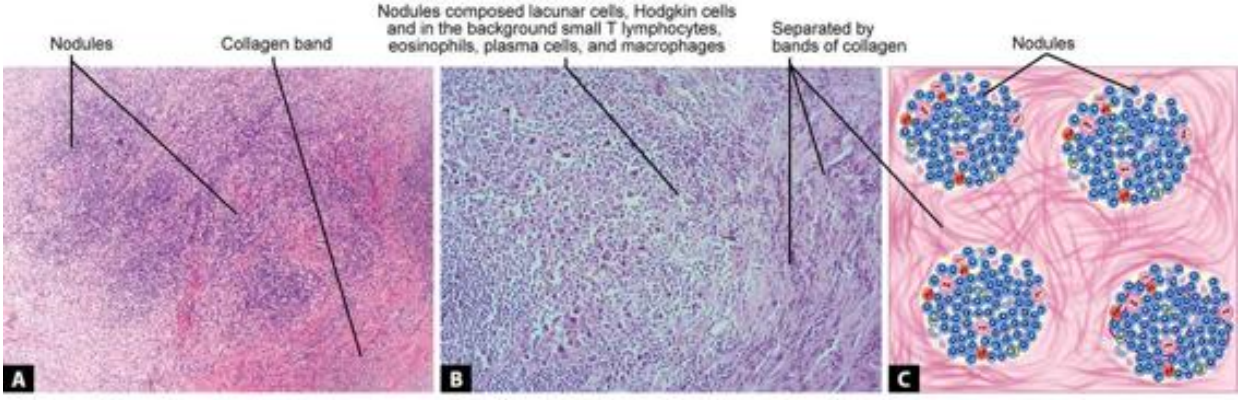
MCCHL: Scattered classical RS cells and mixed inflammatory background, CD15+, CD30+ and EBV+.

MCCHL prognosis: Very good.

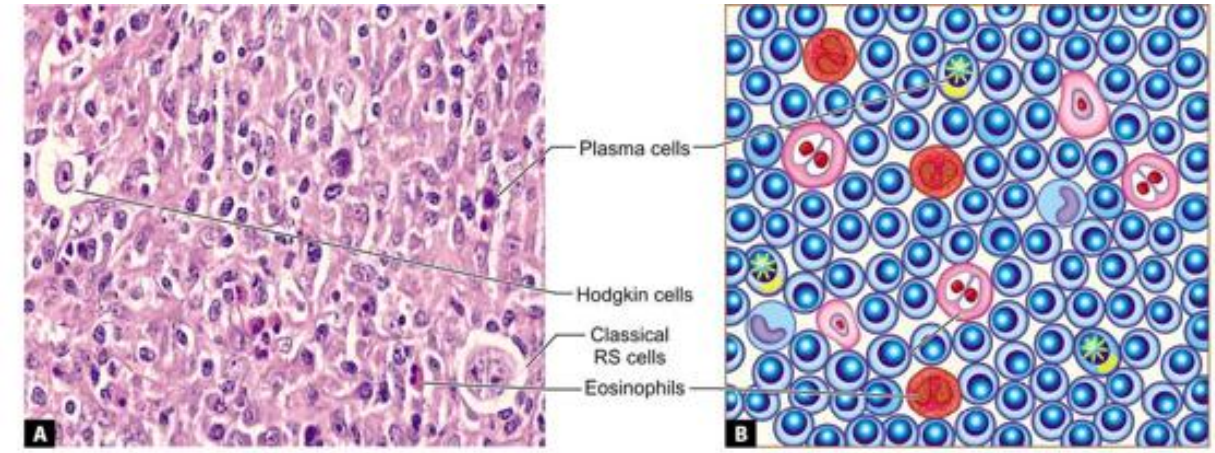
Lymphocyte-rich Classical Hodgkin Lymphoma (LRCHL)

Q. Write short essay on lymphocyte rich Hodgkin lymphoma.

- Subtype of classical Hodgkin lymphoma with **scattered Hodgkin and RS cells**.
- **Uncommon**—about 5% of classical HL.
- More in **elderly** patients, associated with **EBV in 40%** of cases.
- Involves **peripheral lymph nodes**.



Figs. 12.31A to C: (A) Low power; (B) High power; (C) Diagrammatic. Nodular sclerosis classical Hodgkin lymphoma with nodules separated by bands of collagen. Also seen are lacunar cells and RS cells in each nodule within the background of lymphocytes, eosinophils, plasma cells and macrophages.



Figs. 12.32A and B: Mixed cellularity classical Hodgkin lymphoma with classical RS cells, Hodgkin cells in the background of mixed cellular population consisting of lymphocytes, eosinophils, plasma cells and macrophages.

Microscopy of LRCHL (Figs. 12.33A and B)

- **Growth patterns:** May show two patterns.
  - **Nodular—**common
  - **Diffuse—**rare
- **Only few Reed–Sternberg cells and Hodgkin cells**.
- **Background: Abundant reactive small lymphocytes.**

Immunophenotype

CD45^{-}, CD20^{-}, CD15^{+} and CD30^{+}.

LRCHL prognosis: Good to excellent prognosis.

LRCHL:
- Uncommon.
- Few RS cells.
- Abundant lymphocytes.
- CD15^{+}, CD30^{+}, CD45^{-} and CD20^{-}.

Lymphocyte-depleted ClassicalHodgkin Lymphoma (LDCHL)

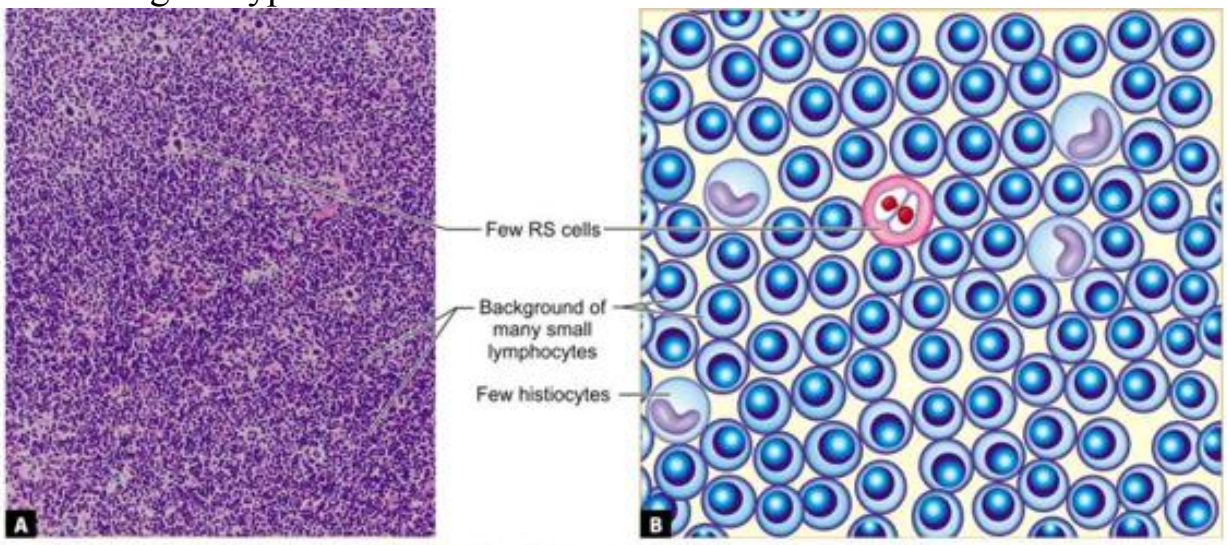
Subtype of classical Hodgkin lymphoma **rich in Hodgkin and RS cells** in a background depleted in non-neoplastic lymphocytes.

- **Rarest**—less than 5% of cases
- Predominantly in **older, HIV-positive patients**, often **EBV-associated** (over 90%)

- Predominantly **retroperitoneal lymph nodes, abdominal organs and bone marrow**.

Microscopy of LDCHL (Fig. 12.34)

- P**aucity of lymphocytes.**
- **Plenty of RS cells** or their **anaplastic/pleomorphic variants.**
- Histological types



Figs. 12.33A and B: Lymphocyte-rich classical Hodgkin lymphoma: (A) H&E shows numerous lymphocytes with few RS cells; (B) Diagrammatic. One RS cell is seen in a background of many small lymphocytes and few histiocytes.

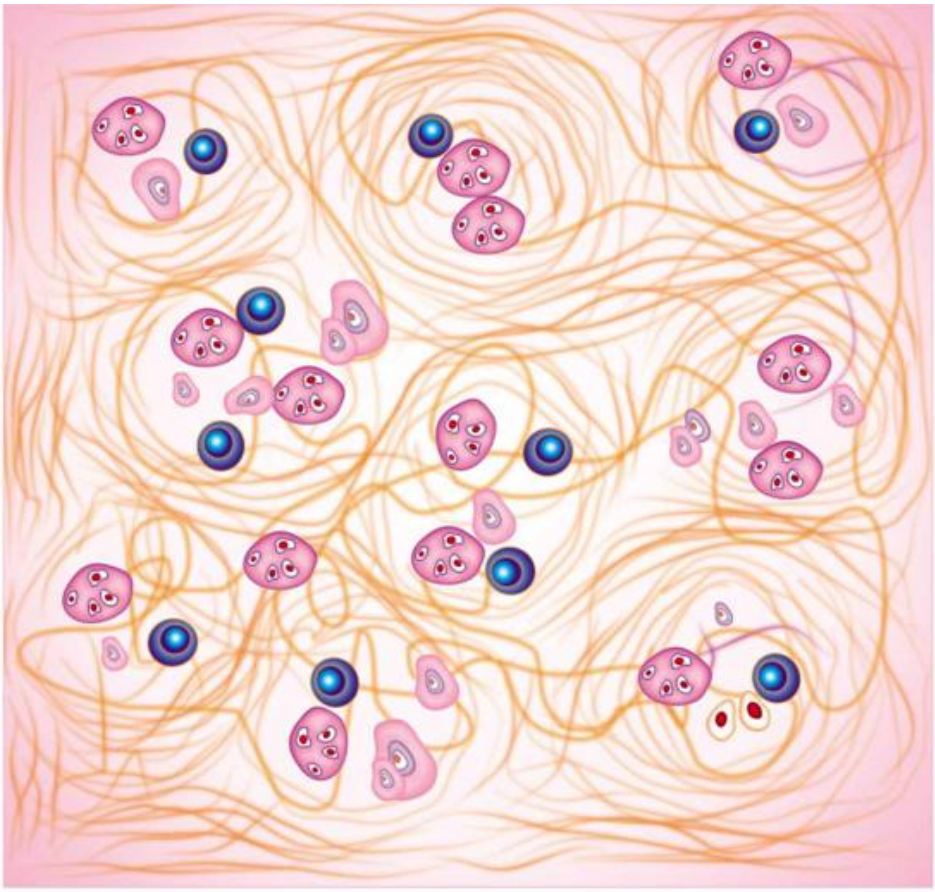


Fig. 12.34: Lymphocyte-depleted classical Hodgkin lymphoma with the pleomorphic variant of RS cells surrounded by fibrous tissue.

- **Reticular: Numerous Hodgkin and RS cells** with depletion of lymphocytes.
- **Diffuse sclerosis/fibrosis:** Hypocellular infiltrate containing bizarre RS cells with fine fibrosis.

Immunophenotype

RS cells are $CD15^+$, $CD30^+$; majority are EBV^+.

NODULAR LYMPHOCYTE PREDOMINANTHODGKIN LYMPHOMA

Q. Write short essay on nodular lymphocyte predominant Hodgkin lymphoma (NLPHL).

- **Uncommon—**5% of all Hodgkin lymphomas.
- **Not associated** with **EBV**.
- Majority males, usually **30–50 years** of age.
- Involves mainly **cervical or axillary lymph nodes**.

Microscopy of NLPHL (Figs. 12.35A and B)

- Loss of lymph node architecture.
- Nodular and/or diffuse infiltrate of **abundant small lymphocytes** with histiocytes and **scattered LP cells**.
- **Lymphocyte predominant cells** (LP cells)/"popcorn" cells **(Fig. 12.32)**:
  - Specific to NLPHL.
  - Large with relatively abundant, pale cytoplasm.
  - Single large delicate **multilobulated** nucleus **or folded nuclei** resembling bubbly outlines of popcorn kernels.
  - One or more **inconspicuous nucleoli.**
- **Hodgkin and RS cell**s are not found.

Immunophenotype

LP cell are $CD20^{+}$, $CD\ 45^{+}$ and $CD15^{-}$, $C30^{-}$ and EBV–ve. Express *BCL6*.

ETIOLOGY AND PATHOGENESIS OF HODGKIN LYMPHOMA

- **EBV:** Previous EBV infection (infectious mononucleosis) ↑ risk of HL.
- **Genetic factors:** HLA-B18 higher in HL.
- **Immune status:** HL more frequent in immunocompromised patients and autoimmune diseases (e.g., rheumatoid arthritis).

Pathogenesis (Fig. 12.36)

- **EBV and HL:** HL is associated with EBV infection.
- **Activation of nuclear factor (NF-κB) common event** in classical HL → rescue germinal center B-cells from apoptosis → **produces Reed–Sternberg cells.**
- **Accumulation of reactive cells in** response to *cytokines* (such as IL-5, IL-6 and TGF-β) and *chemokines* secreted by Reed–Sternberg cells.

LABORATORY FINDINGS

PERIPHERAL SMEAR

- *RBCs:* Normocytic normochromic anemia.
- *WBCs:* Leukocytosis occurs in 1/3rd of the patients. Eosinophilia is frequent.
- *Platelets:* Normal or increased.

Fine Needle Aspiration Cytology (FNAC)

RS cells/its variants against a background of inflammatory cells (depending on the subtype).

Spread

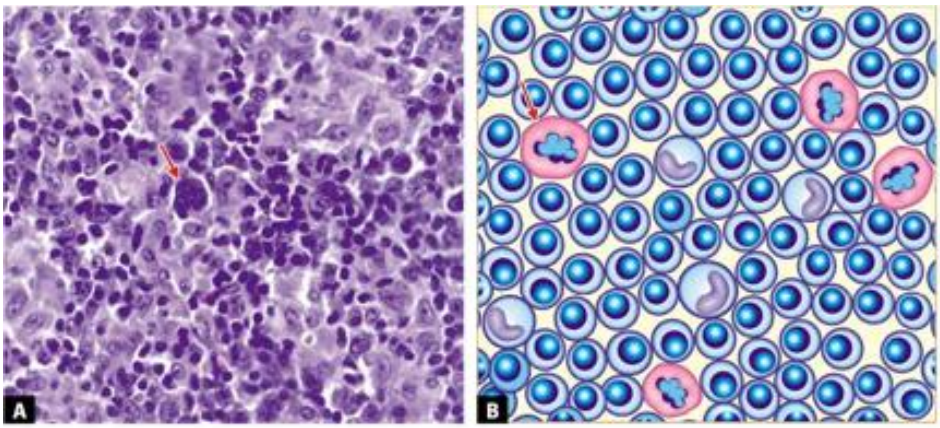
- Mainly **by contiguity**
- First nodal disease → then splenic disease, hepatic disease → and finally marrow involvement and extranodal disease.

Staging of Hodgkin Lymphoma

Clinical staging of Hodgkin lymphoma is discussed in **Table 12.10**.

Differences between Hodgkin Lymphoma and Non-Hodgkin Lymphoma

CC: Differentiating features of Hodgkin's and non-Hodgkin's lymphoma.

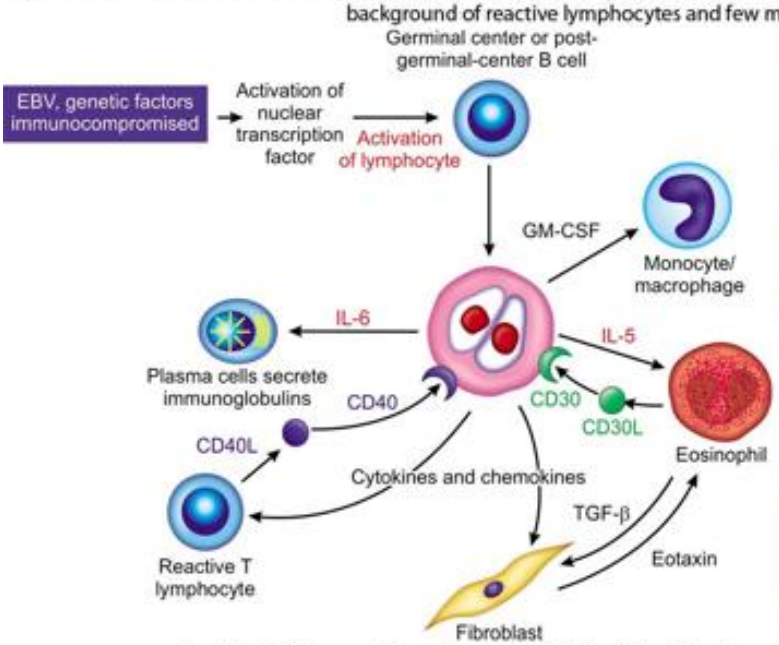


LDCHL:
- Rarest.
- Paucity of lymphocytes.
- Plenty of RS cells.
- CD15⁺, CD30⁺; majority are EBV⁺.

LDCHL prognosis: Outcome less favorable than with other subtypes.

NLPHL:
- Uncommon.
- Abundant lymphocytes.
- LP cells.
- No Hodgkin/RS cells.
- CD20⁺, CD45⁺ and CD15⁻, C30⁻ and EB -ve.

Figs. 12.35A and B: (A) H&E; (B) Diagrammatic. Nodular lymphocyte predominant Hodgkin lymphoma with 'popcorn' cells (arrow) in a background of reactive lymphocytes and few macrophages.



NLPHL prognosis: More likely to recur than the classical subtypes, but the prognosis is very good.

ESR in HL: Raised.

Bone marrow in HL: Involved in the later stages.

HL: Extranodal involvement uncommon

Clinical features:
- Painless enlargement of lymph nodes.
- Systemic/constitutional symptoms: Fever, night sweats and weight loss

HL: Pel-Ebstein fever is characterized by alternating bouts of fever followed by remissions.

Fig. 12.36: Pathogenetic mechanism and interaction of various cell types in Hodgkin lymphoma.

TABLE 12.10: Clinical staging of Hodgkin lymphomas (Cotswold revision of Ann Arbor staging classification).

Stage	*Definition*
I	Involvement of a single lymph node region or lymphoid structure (e.g., spleen, Waldeyer ring, thymus)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (the mediastinum is a single site; hilar lymph nodes are lateralized); the number of anatomic sites should be indicated by suffix (e.g., II_3)
III	Involvement of lymph node regions or structures on both sides of the diaphragm
	III_1: With or without splenic, hilar, celiac or portal nodes III_2: With para-aortic, iliac or mesenteric nodes
IV	Involvement of extranodal site(s) beyond those designated E

E, involvement of a single extranodal site, or contiguous or proximal to known nodal site of disease

TABLE 12.11: Differences between Hodgkin and non-Hodgkin lymphomas.

Sl. No.	*Characteristics*	*Hodgkin lymphoma*	*Non-Hodgkin lymphoma*

TABLE 12.11: Differences between Hodgkin and non-Hodgkin lymphomas.

Sl. No.	*Characteristics*	*Hodgkin lymphoma*	*Non-Hodgkin lymphoma*
1.	Site of involvement	Arises in a single node or chain of nodes (cervical, mediastinal, para-aortic)	Mainly involves multiple peripheral nodes
2.	Pattern of spread	Orderly spread by contiguity in a predictable fashion	Noncontiguous spread in an unpredictable fashion
3.	Mesenteric nodes and Waldeyer ring	Rarely involved	Commonly involved
4.	Extranodal involvement	Uncommon	Common
5.	Characteristic of neoplastic cells	Neoplastic cells—Hodgkin or Reed–Sternberg cells form minor tumor cell mass (1–5%)	Neoplastic cells form the major tumor cell mass

Q. List the differences between HL and NHL.

HL differs from NHL in several respects and their main differences are shown in **Table 12.11**.

SPLENOMEGALY

CC: Enumerate and differentiate the causes of splenomegaly.

The spleen is rarely the primary site of disease. Enlargement of spleen is called splenomegaly and occurs in a wide variety of systemic diseases (secondary involvement). Main causes of splenomegaly are listed in **Box 12.19**. Splenomegaly may be grouped into three degrees namely mild, moderate and massive.

Cc (Show How): Identify and describe the gross specimen of an enlarged spleen.
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Mild splenomegaly (weight <500 g)

Its causes include acute splenitis; acute splenic congestion; infectious mononucleosis, bacterial endocarditis, acute malaria and other disorders (e.g., septicemia, chronic venous congestion of spleen **(Fig. 12.37)** in

congestive heart failure, systemic lupus erythematosus, rheumatoid arthritis, intra-abdominal infections, typhoid fever and thalassemia minor).

Moderate splenomegaly (weight 500–1000 g)

Its causes include chronic congestive splenomegaly (portal hypertension or splenic vein obstruction), acute leukemias, disorders with extravascular hemolysis (hereditary spherocytosis, thalassemia major, autoimmune hemolytic anemia), amyloidosis, Niemann–Pick disease, many infections (e.g., infective endocarditis, tuberculosis, and typhoid), sarcoidosis; metastatic carcinoma or sarcoma.

Massive splenomegaly (weight >1000 g)

BOX 12.19: Main causes of splenomegaly.

Infections: Nonspecific splenitis of various blood-borne infections (particularly infectious endocarditis)

- *Acute:* Infectious mononucleosis, typhoid fever, brucellosis, bacterial septicemia
- *Subacute:* Subacute bacterial endocarditis
- *Chronic:* Tuberculosis, malaria, syphilis, kala-azar, leishmaniasis

Immunologic inflammatory disorders

- Rheumatoid arthritis
- Systemic lupus erythematosus
- Sarcoidosis
- Amyloidosis

Non-neoplastic hematological disorders

- *Hemolytic anemias:* Thalassemia major, hemoglobinopathies
- *Immune thrombocytopenia*

Congestive states related to portal hypertension

- Cirrhosis of the liver
- Splenic or portal vein thrombosis or stenosis
- Right-sided cardiac failure

Primary or metastatic neoplasm

- *Lymphohematogenous neoplasms:* Leukemia, Hodgkin lymphoma, non-Hodgkin lymphomas and lymphocytic leukemias, myeloproliferative disorders, multiple myeloma
- Primary neoplasms (e.g., hemangiosarcomas) and cysts of spleen
- *Metastatic neoplasm:* Sarcoma, carcinoma

Storage diseases

- Gaucher disease
- Niemann–Pick disease
- Mucopolysaccharidoses



Fig 12.37: Mild splenomegaly due tochronic venous congestion of spleen.

Q. Write a short note on causes of massive splenomegaly.

A spleen is considered as massively enlarged when its lower pole is within the pelvis, or which has crossed the midline into the right lower or right upper abdominal quadrants. Its causes are listed in **Box 12.20**.

Hypersplenism: It is characterized by excessive removal of red blood cells, granulocytes or platelets from the circulation. A chronically enlarged spleen usually removes excessive numbers of one or more of the formed elements of blood. This may lead to anemia, leukopenia, or thrombocytopenia. Platelets are more susceptible to sequestration in the red pulp. Hence, thrombocytopenia is more common and severe in patients with splenomegaly compared to anemia or neutropenia. Hypersplenism is characterized by splenomegaly, cytopenia(s), normal or hyperplastic bone marrow, and a response to splenectomy.

Laboratory Assessment

Complete blood count: The CBC may show cytopenia of one or more blood cell types. This suggests hypersplenism.

- **RBC count:** It may be normal, decreased (thalassemia major syndromes, SLE, cirrhosis with portal hypertension), or increased (polycythemia vera).
- **WBC count:** It may be normal, decreased (congestive splenomegaly, leukemias), or increased (infections or inflammatory disease, myeloproliferative disorders).
- **Platelet count:** It may be normal, decreased due to increased sequestration or destruction of platelets in an enlarged spleen (congestive splenomegaly, Gaucher's disease, immune thrombocytopenia), or increased in the myeloproliferative disorders such as polycythemia vera.

Other laboratory studies: It depends on the differential diagnosis of the underlying illness responsible for splenomegaly.

CLINICAL SCENARIO

Acute Lymphoblastic Leukemia

- History strongly indicative **acute lymphoblastic leukemia (ALL):** Child (**1–6 years** of age)/adult (30–40 years) presenting with **rapid onset** (few weeks) of **pallor, fatigue, bleeding, fever, and infection**. Presence of **lymphadenopathy**, hepato- or splenomegaly, CNS disease, testicular enlargement.
- Laboratory findings of **marked raised WBC count**, presence of **more than 20% lymphoblasts in the blood and bone marrow, blasts + with PAS** (block positivity) and TdT + are features of **acute lymphoblastic leukemia**.

Case 1

History: A **6-year-old** boy presents with fatigue, **bone pain, weakness** and low-grade fever of 1-week duration. Physical examination shows anemia, **sternal bone tenderness and lymphadenopathy.**

Case 2

History: A **5-year-old** boy complains of sudden onset of fever, tiredness and pallor. On examination, there are many **enlarged cervical lymph nodes**, hepatosplenomegaly, **bone tenderness, and petechial hemorrhages** on the skin.

Case 3

History: A 4-**year-old** boy is becoming increasingly lethargic for the past 1 month. On examination, he is having fever, **ecchymoses** on the skin of his lower legs and shoulder, generalized lymphadenopathy and tenderness on palpation of long bones.

Laboratory findings of cases 14 to 16: Hb 8 g/dL, hematocrit 24%, **WBC 18,200 cells/cu mm** and **platelet count of 90,000/cu mm**. Serum LDH is markedly increased. **Peripheral blood smear** shows **abnormal cells,** which are **PAS positive**. A **bone marrow** examination shows 100% cellularity with **replacement by primitive cells**. These abnormal primitive cells have scanty cytoplasm and large nuclei with indistinct nucleoli.
Following blood smear examination, the child is admitted to the medical oncology ward.

Acute Myeloblastic Leukemia

- History strongly indicative **acute myeloblastic leukemia (AML):** Adult presenting with **rapid onset** (few weeks) of **pallor, fatigue, bleeding, fever, and infection.**
- Laboratory findings of **marked raised WBC count**, presence of **more than 20% myeloblasts in the blood and bone marrow, presence of Auer rods** (not seen in all subtypes), **blasts + with MPO and Sudan Black B** are features of **acute myeloblastic leukemia**.

BOX 12.20: Causes of massively enlarged spleen (massive splenomegaly).

- Chronic myeloid leukemia
- Myelofibrosis (primary or secondary to polycythemia vera or essential thrombocytosis)
- Hyper-reactive malarial splenomegaly syndrome (tropical splenomegaly syndrome)
- Gaucher's disease
- Lymphoma (mantle cell lymphoma, marginal B-cell lymphoma), hairy cell leukemia
- Infectious diseases (e.g., chronic malaria), kala-azar (visceral leishmaniasis)
- Beta-thalassemia major
- AIDS with *Mycobacterium avium* complex
- Splenic cysts
- Leukemias: Chronic lymphocytic leukemia, hairy cell leukemia
- Others: Sarcoidosis, autoimmune hemolytic anemia

Case 4

History: A **45-year-old** man presents with **fatigue, episodes of epistaxis, bleeding from gums,** and low grade fever of 1 week duration. Physical examination reveals temperature of 37.4°C and **hepatosplenomegaly**.

Laboratory findings: Hb 7 g/dL, **WBC count 51,000/cu mm** and **platelet count 80,000/cu mm. Peripheral blood smear** shows abnormal large cells with **Auer rods**.

Chronic Myelogenous Leukemia

- History strongly indicative of **chronic myelogenous leukemia (CML):** Patient in **fifth and sixth decades of life** presenting with gradual development of **fatigue, weakness, weight loss, anorexia** and fullness of abdomen, early satiety and left upper quadrant pain or mass (due to **splenomegaly**).
- Laboratory finding of **very high WBC count** with **myeloid precursors, neutrophils and myelocyte peak, low LAP and presence of Ph chromosome is diagnostic of chronic myelogenous leukemia.**

Case 5

History: A **50-year-old** male comes to the hospital with complaints of generalized weakness, weight loss, easy fatigability, **abdominal discomfort and dragging sensation in the left hypochondrium** for the last 8 months. On examination, he is pale and has marked **splenomegaly.**

Laboratory findings: Hb 11.9 g/dL, hematocrit 36%, **WBC count 1,20,000/cu mm**, platelet count 4,12,000/cu mm. Peripheral smear examination shows characteristic blood picture **with myeloid precursors, neutrophils and myelocyte peak** confirms the clinical diagnosis. **The leukocyte alkaline phosphatase (LAP) score is low**. Chromosomal analysis shows t(9:22) positivity. He is admitted to the oncology department for treatment.

CHAPTER 13

Disorders of Hemostasis

DISORDERS OF PRIMARY HEMOSTASIS

NORMAL HEMOSTASIS

CC: Describe normal hemostasis.

- Hemostasis is the **body's response to vascular damage/injury** (for details *refer* pages 116-8 and **Fig. 5.8**).
- Includes several sequences of events at the site of vascular injury. They are as follows:
  - **Primary hemostatic plug: Platelet adhere** to subendothelial structures at the site of injury. The **platelets change their shape** and **release granule contents.** The released contents cause platelet **aggregation and form primary hemostatic plug.**
  - **Secondary hemostatic plug: Exposure of tissue factor** at the site of vascular injury **activates the extrinsic coagulation system. The fibrin formed develops into a secondary hemostatic plug.**

Terminology used in Bleeding Disorders

Petechiae (Fig. 13.1A): They are **small (1–2 mm in diameter), red to purple hemorrhagic spots** in the skin, mucous membranes (e.g., conjunctiva) or serosal surfaces. They result from blood leaking through intact endothelial lining of capillaries. They are most **commonly found with**

low platelet counts (thrombocytopenia) or defective platelet function or loss of vascular wall support (e.g., vitamin C deficiency)**.**

Q. Define and classify purpura.

Definition: Purpura (Fig. 13.1B): The term purpura means purple. They are **slightly larger (>3 mm) than petechiae**, but <1 cm in diameter. Palpable purpura suggest vasculitis (e.g., Henoch–Schönlein purpura). The causes are **thrombocytopenia, increased vascular fragility and vasculitis** (inflammation of vessels).

Purpura may be classified as nonthrombocytopenic (vascular) purpura **(Box 13.2)** and **thrombocytopenic (Box 13.3)**.

Ecchymoses/bruises (Fig. 13.1C): They are larger (1–2 cm) and result from blood escaping through endothelium into intact subcutaneous tissue. They result from trauma or hemostatic disorder and usually do not produce significant swelling as in hematoma. Extravasated RBCs in the lesions are phagocytosed and degraded by macrophages and release the hemoglobin which gives rise to red-blue color. The pigment from heme is converted into biliverdin and then to bilirubin (blue-green color) and iron from red cells forms hemosiderin (golden-brown color). These changes are responsible for the characteristic color changes in ecchymoses. A good example of an ecchymosis is a "black eye."

Hematoma (Fig. 13.1D): It is swelling formed when blood leaks from a vessel and collects within a tissue. The swelling results from a large area of hemorrhage in subcutaneous tissue or muscle. It is blue or purple and slightly raised.



Figs. 13.1A to D: (A) Petechiae which appear as small (1–2 mm in diameter), red to purple hemorrhagic spots in the skin, mucous membranes or serosal surfaces; (B) Purpura—slightly larger (>3 mm) than petechiae; (C) Ecchymoses are larger (>1–2 cm) and result from blood escaping; (D) Hematoma is swelling formed when blood leaks from a vessel and collects within subcutaneous tissue or muscle.

Petechiae are pinpoint hemorrhages seen only with thrombocytopenia.

CLASSIFICATION OF HEMOSTATIC DISORDERS(BOX 13.1)

Q. Define purpura. Describe the classification of purpura.

Q. Define and classify bleeding disorders. Describe the laboratory diagnosis of purpuras.

1. **Bleeding disorders (hemorrhagic disorders/hemorrhagic diathesis):** Bleeding disorders have an **abnormal tendency to bleed** (hemorrhage) due to failure of hemostasis.
2. **Thrombotic disorders:** They cause **thrombus formation.**

BLEEDING DISORDERS CAUSED BYVESSEL WALL ABNORMALITIES

CC: Classify and describe the etiology, pathogenesis and pathology of vascular bleeding disorders.

Vascular purpura (**nonthrombocytopenic purpura)** is group of **disorders of blood vessels** that results in **bleeding.** They should be distinguished from bleeding disorders due to abnormalities of platelets.

Classification of bleeding disorders caused by vessel wall abnormalities are presented in **Box 13.2.**

BLEEDING DISORDERS DUE TO ABNORMALITIES OF PLATELET

CC: Classify and describe the etiology, pathogenesis and pathology of platelet disorders including ITP.

Classification of Platelet Disorders

Classification of platelet disorders is discussed in **Box 13.3**.

THROMBOCYTOPENIA

Definition: Decrease in the **platelet count below** the lower limit of **150,000/cu mm (**150×10^9/L).

Clinical Features of Thrombocytopenia

- Cutaneous bleeding appears as pinpoint hemorrhages (petechiae) and ecchymoses.
- Mucosal bleeding.

BOX 13.1: Classification of disorders of hemostasis.

Bleeding disorders

- **Disorders of primary hemostasis**
  - **Vessel wall abnormalities**:
    - Congenital, e.g., Ehlers–Danlos syndrome
    - Acquired, e.g., Henoch–Schönlein purpura
  - **Platelet abnormalities:**
    - Quantitative: Thrombocytopenia (e.g., ITP, drug-induced, congenital)
    - Qualitative: Platelet function disorders:
      - Inherited, e.g., Glanzmann thrombasthenia, Wiskott–Aldrich syndrome, Bernard–Soulier syndrome
      - Acquired, e.g., uremia, drugs
- **Disorders of coagulation system (disorders of secondary hemostasis)**
  - **Congenital:** Hemophilia A, B; von Willebrand disease; other coagulation factor deficiencies (XI, VII, II, V, X)
  - **Acquired:** Vitamin K deficiency, liver disease, disseminated intravascular coagulation

Thrombotic disorders

- **Inherited:**
  - Deficiency of antithrombotic factors: Antithrombin III deficiency, protein C deficiency, protein S deficiency
  - Increased prothrombotic factors: Activated protein C (APC) resistance (Factor V mutation/factor V Leiden)
  - Prothrombin (G20210A mutation)
- **Acquired:** Fibrinolytic system defects

Hemostatic disorders are broadly classified as bleeding disorders and thrombotic disorders.

Bleeding disorders may be due to:

- Diseases of blood vessels
- Platelet disorders
- Coagulation disorders

Vascular purpuras are also known as nonthrombocytopenic purpuras.

BOX 13.2: Classification of bleeding disorders caused by vessel wall abnormalities (nonthrombocytopenic purpura).

Acquired disorders

1. Due to decreased amount of connective tissue
   - Senile purpura
   - Scurvy
   - Cushing syndrome and steroid therapy
2. Due to vasculitis
   - Henoch–Schönlein purpura
   - Infections
   - Drug reactions
3. Associated with plasma cell neoplasms
   - Amyloidosis
4. Miscellaneous
   - Simple easy bruising

Congenital/inherited disorders

- Hereditary hemorrhagic telangiectasia
- Ehlers–Danlos syndrome
- Marfan syndrome

Senile purpura is due to vessel instability.
Henoch–Schönlein purpura is characterized by hypersensitivity vasculitis and palpable purpura.

BOX 13.3: Classification of platelet disorders.

1. **Quantitative platelet disorders**
   - **Thrombocytopenia**
     - Decreased platelet survival
     - Decreased production
     - Sequestration
     - Dilutional
   - **Thrombocytosis**
2. **Qualitative platelet disorders**
   - **Hereditary**
     - Defective adhesion of platelets
     - Disorders of platelet secretion
     - Defective platelet aggregation
   - **Acquired**

BOX 13.4: Causes of thrombocytopenia.

1. Decreased platelet survival

- Immunological destruction
  - Primary autoimmune

- Acute immune thrombocytopenic purpura
- Secondary autoimmune
  - Systemic lupus erythematosus, B-cell lymphomas
  - Chronic immune thrombocytopenic purpura
  - Alloimmune: Post-transfusion or pregnancy or neonatal
  - Drug-induced: Quinidine, heparin, sulfa compounds
  - Infections: HIV infection, infectious mononucleosis, dengue fever, cytomegalovirus

- Nonimmunological destruction
  - Disseminated intravascular coagulation
  - Thrombotic thrombocytopenic purpura, hemolytic uremic syndrome
  - Mechanical destruction: Prosthetic heart valves, malignant hypertension
  - Microangiopathic hemolytic anemias
  - Giant hemangioma

2. Decreased production of platelets

- Bone marrow failure: Aplastic anemia (congenital and acquired)
- Bone marrow replacement: Leukemia, disseminated cancer granulomatous disease
- Selective impairment of platelet production
  - Drug-induced: Alcohol, thiazides, cytotoxic drugs
  - Infections: Measles, human immunodeficiency virus (HIV)
- Ineffective hematopoiesis: Myelodysplastic syndromes
  - Nutritional deficiencies: Vitamin B_{12}, folic acid deficiency (megaloblastic anemia)

3. Sequestration

- Hypersplenism

4. Dilutional transfusions

- Intracranial bleed (subarachnoid and intracerebral hemorrhage) rare but serious.

Severity of Bleeding

- **Post-traumatic** bleeding—when the platelet **count is 20,000–50,000/cu mm**.
- **Spontaneous** bleeding—when the platelet count falls **below 20,000/cu mm**.
- **Intracranial** bleeding—when platelet count is **<10,000/cu mm.**

Intracranial bleeding occurs when platelet count is <10,000/cu mm.

Causes of Thrombocytopenia (Box 13.4)

Q. Write short essay/note on causes of thrombocytopenia.

IMMUNE THROMBOCYTOPENIC PURPURA

CC: Describe the etiology, pathogenesis and pathology of ITP.

Q. Describe the etiology, pathogenesis, clinical features, laboratory findings/hematological features of immune/idiopathic thromocytopenic purpura.

- Most common form of thrombocytopenia.
- **Due to increased destruction of platelets** by **immune mechanisms**—mainly **autoimmune** mechanism.

Types of Immune Thrombocytopenic Purpura (ITP)

Acute Immune Thrombocytopenic Purpura

- **Self-limited** disease.
- **Children:** 2–4 years and seen equally in both sexes.
- Presents **1–3 weeks after viral** (measles, rubella, EBV) **infection.**
- Platelet destruction by **antiplatelet autoantibodies.**
- **Platelet count is decreased**, sometimes even below 10,000/cu mm (10×10^9/L).

Clinical Features

- **Sudden onset.**
- **Petechiae**, **gum bleeding, epistaxis** and mild fever.
- Usually **resolve** spontaneously **within 6 months**.
- **Excellent prognosis.**

Chronic Immune Thrombocytopenic Purpura

- Persistent **thrombocytopenia** for **more than 6–12 months.**
- Indolent, females are more affected than males (F:M = 3:1).
- **More common** and usually seen in **adults (20–40 years).**

Pathogenesis of ITP (Fig. 13.2)

- **Autoimmune disorder** (type II hypersensitivity reaction) characterized by formation of **antiplatelet antibodies**, directed against membrane glycoproteins (most often IIb-IIIa or Ib-IX of platelets).
- Antiplatelet antibodies in about 80% of patients and are of the **IgG type.**
- Antiplatelet antibodies act as opsonins and are recognized by IgG Fc receptors present on mononuclear phagocytes of RE system (mainly spleen) and are destroyed there resulting in thrombocytopenia.

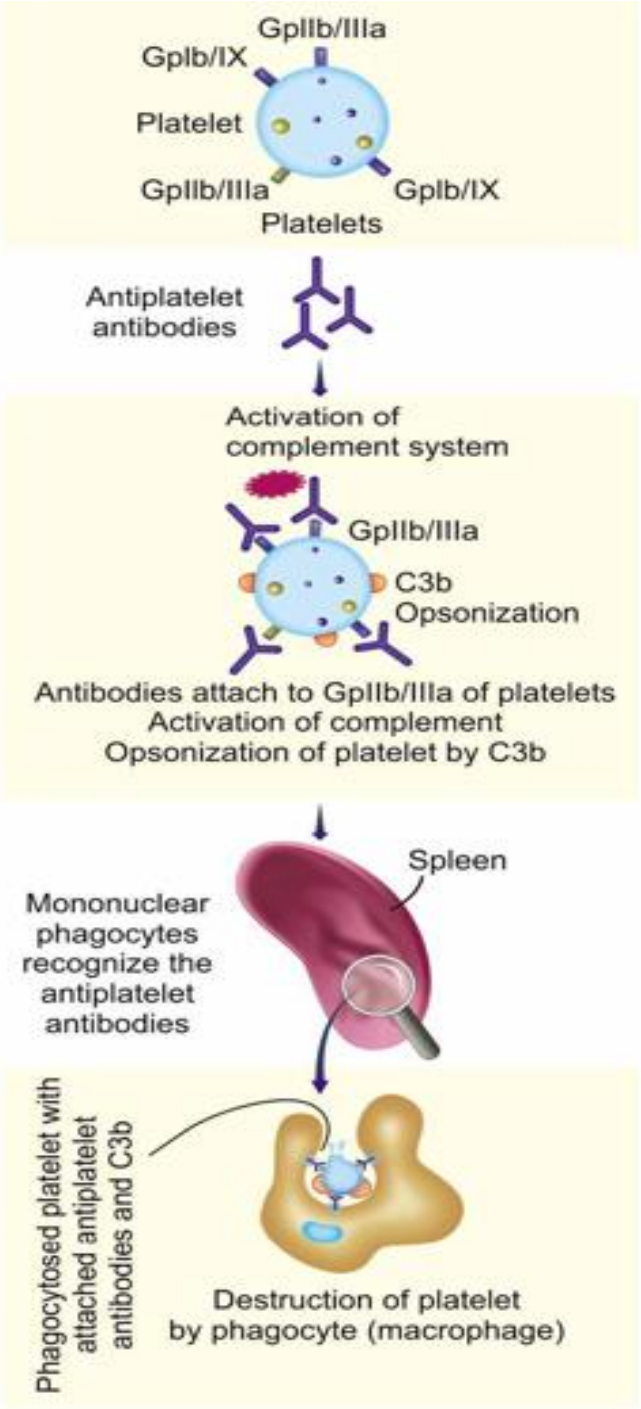


Fig. 13.2: Pathogenesis of idiopathic thrombocytopenic purpura. It is a type II hypersensitivity reaction characterized by complement dependent opsonization and phagocytosis. The antibody (uaully IgG type) binds to antigens on the platelets (most often IIb-IIIa or Ib-IX of platelets). Activation of complements produces opsonin C3b. Opsonization of platelets by antibodies and complement leads to ingestion of platelets by phagocytes of RE system (mainly spleen) via either Fc or C3b receptors. This results in destruction of platelets.

- **Splenectomy causes marked improvement** in 75–80% of patients.

Clinical Features

- More common in **females** (F:M ratio is 3:1).
- Age between **20 and 40 years**.
- Clinical features are due to thrombocytopenia: **Skin bleeding, mucosal bleeding, menorrhagia** in females, etc.

Laboratory Findings

Q. Write short note on laboratory findings in ITP.

Peripheral Blood

- **Platelet count: Markedly reduced** below 80,000/cu mm (80×10^9/L).
- **Hemoglobin:** Ranges from 7–12 g/dL.

PERIPHERAL SMEAR

- *Platelets:* **Markedly reduced** (thrombocytopenia) and **abnormally large sized platelets (megathrombocytes/giant platelets)**
- *RBCs:* **Chronic blood loss** (e.g., menorrhagia) due to ITP may **lead to microcytic hypochromic anemia**
- *WBCs:* Normal

Q. Write short note on bone marrow changes in ITP.

BONE MARROW

- ***Cellularity:*** **Hypercellular**
- ***Megakaryopoiesis:***
  - **Moderate increase in number (Fig. 13.3)** of both **immature and mature** forms of **megakaryocytes**.
  - **Immature megakaryocytes predominate large nonlobulated single nuclei and basophilic cytoplasm**.
- ***Erythropoiesis:***
  - **Prolonged bleeding** may cause anemia leading to **normoblastic erythroid hyperplasia**.

- Constant bleeding leads to **iron deficiency** and **micronormoblastic erythroid hyperplasia**.

- ***Myelopoiesis:*** Normal.
- ***Storage iron:*** Severe and chronic bleeding causes iron deficiency with reduced iron stores.

- **Bleeding time (BT): Prolonged**, but prothrombin time (PT) and partial thromboplastin time (PTT) are normal.
- **Tourniquet test: Positive.**
- **Clotting time (CT): Normal.**
- **Tests for platelet autoantibodies:** May be positive.
- **Spleen:** Normal size.

Acute ITP is seen mainly in children between 2–4 years.

Acute ITP: Autoimmune disease, sudden onset, shorter duration and usually resolves within 6 months.

Chronic ITP: Autoimmune disease and the antibodies are directed against glycoprotein IIb/IIIa of platelets.

Spleen is the major site of destruction of platelets and important site of autoantibody synthesis.

ITP: Splenomegaly and lymphadenopathy are uncommon and in their presence one should consider the diagnosis other than ITP.

ITP: Platelets markedly reduced below 80,000/cu mm.

Bone marrow in chronic ITP shows megakaryocytic hyperplasia (increased number of megakaryocytes) with immature megakaryocytes.

ITP: Bone marrow—decreased megakaryocytes—against the diagnosis of ITP.

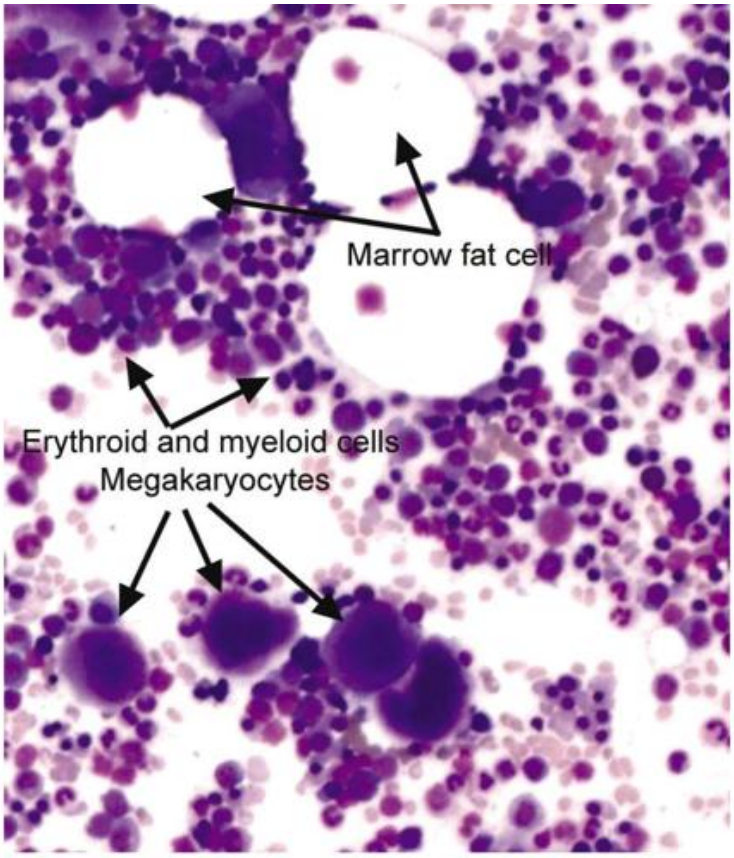


Fig. 13.3: Bone marrow in ITP showing moderate increase in number of megakaryocytes (arrows).

BOX 13.5: Causes of thrombocytosis.

Idiopathic/primary (autonomous production)

- Essential thrombocytosis
- Polycythemia vera
- Chronic myeloid leukemia

Secondary (reactive thrombocytosis)

- Iron deficiency
- Malignancy
- Following hemorrhage
- Following splenectomy

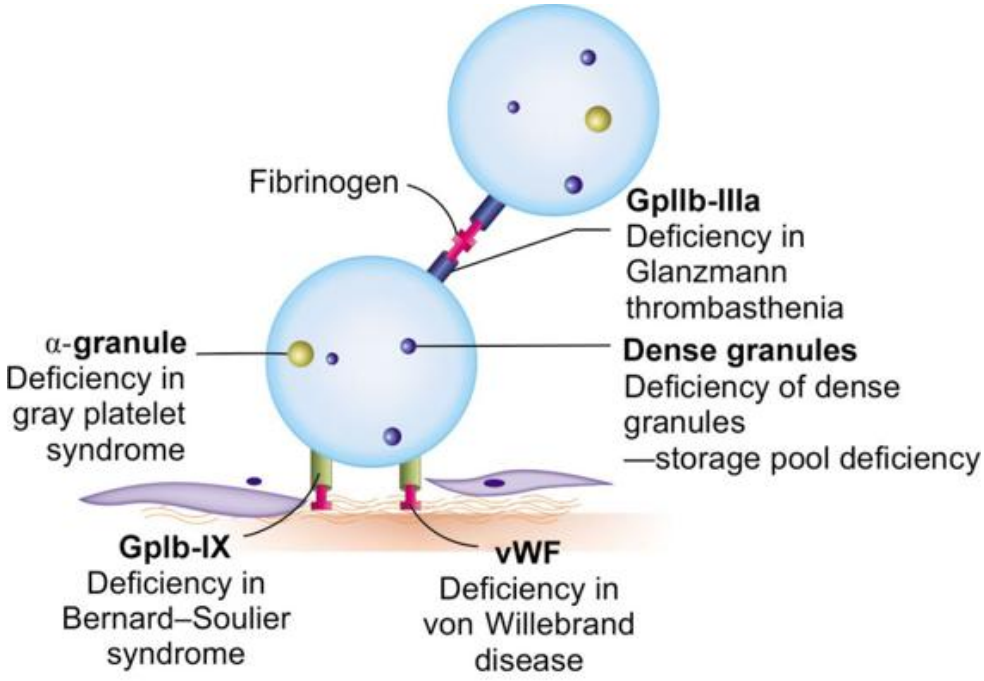


Fig. 13.4: Functional disorders of platelet.

ITP: Bleeding time prolonged, PT and APTT normal.

Aspirin blocks the cyclo-oxygenase enzyme of platelets and prevents aggregation of platelets.

Big platelets are found in Bernard–Soulier syndrome and small platelets are found in Wiskott–Aldrich syndrome.

THROMBOCYTOSIS

Q. Write short note on thrombocytosis and its cause.

Definition: Platelet count more than 4,50,000/cu mm is known as thrombocytosis.

Causes: Various causes of thrombocytosis are listed in **Box 13.5**.

QUALITATIVE PLATELET DISORDERS

Q. Write short note on qualitative/functional disorders of platelets.

Classification of platelet functional (qualitative) disorders are presented in **Figure 13.4** and **Box 13.6**.

Platelet sequence in hemostasis: Platelet adhesion → release of granule contents → platelet aggregation → primary (temporary) hemostatic plug → activation of coagulation system → fibrin → secondary (permanent) hemostatic plug.

Delta granules of platelets contain (CASE): (i) Calcium, (ii) ADP/ATP, (iii) Serotonin and (iv) Epinephrine

BLEEDING DISORDERS: DUE TO ABNORMALITIES OF COAGULATION/ CLOTTING FACTOR

CC (Show How): Differentiate platelet from clotting disorders based on the clinical and hematologic features.

Bleeding due to coagulation disorders must be distinguished from those due to platelet/vascular disorders **(Table 13.1)**. The details of tests are discussed on pages 391-3.

CLASSIFICATION OF COAGULATION DISORDERS

Classification of coagulation disorders is discussed in **Box 13.7**.

BOX 13.6: Classification of platelet functional (qualitative) disorders.

A. Hereditary

1. ***Disorders of platelet adhesion:*** Bernard–Soulier syndrome
2. ***Disorders of platelet secretion:*** Storage pool deficiency
3. ***Disorders of platelet aggregation:*** Glanzmann thrombasthenia

B. Acquired

1. ***Drugs:*** Aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), dipyridamole, sulfinpyrazone
2. ***Renal failure:*** Uremia

3. ***Hematologic malignancies:*** Myeloproliferative neoplasms and myelodysplastic syndromes

TABLE 13.1: Distinguishing clinical and hematologic features in platelet/vascular and coagulation disorders.

Characteristics	***Platelet/vascular disorders***	***Coagulation disorders***
Clinical features		
Onset	Spontaneous and develops immediately after trauma/surgery	Delayed bleeding after trauma/surgery
Type of lesion	Petechiae, ecchymoses	Hematomas
Sites of bleeding	Skin, mucous membrane	Deep tissues
• Mucous membrane	Common from nose, mouth, gastrointestinal and genitourinary tracts	Uncommon except from gastrointestinal or genitourinary tract
• Into the joint	Absent	Common in severe factor deficiencies
• Into the muscle	Following trauma	Spontaneous
Hematologic features		
Platelet count	Reduced/normal in platelet disorders and normal in vascular disorders	Normal
Clot retraction test	Absent or reduced	Reduced or absent in fibrinogen deficiency (congenital or acquired)
Capillary fragility test (Hess/tourniquet test)	Positive	Negative
Bleeding time	Prolonged	Normal
Coagulation or clotting time	Normal	Prolonged
Prothrombin time	Normal	Prolonged
Activated partial thromboplastin time (APTT)	Normal	Prolonged

Hereditary Coagulation Disorders

Usually, due to deficiency of single coagulation factor.

Factor VIII–vWF Complex

- **Factor VIII–vWF complex** has two components:
  1. Plasma **factor VIII**
  2. **von Willebrand factor (vWF).**
- **vWF protects factor VIII** and important for its stability. **Subendothelial vWF promotes platelet adhesion.**
- Whenever there is vascular endothelial injury, **plasma vWF** gets **adsorbed to exposed subendothelial matrix** and **augments adhesion of platelets.**

HEMOPHILIA

CC: Classify and describe the etiology, pathogenesis and pathology of hemophilias.

Q. Write short essay/note on hemophilia.

- Hemophilia **A and B** are **similar in both clinical and pathological features**, the **difference** being **in the deficient factor**.
- Both are **sex-linked recessive disorders** resulting in inherited deficiency of the clotting factor or synthesis of a defective clotting factor.
- **Males are affected** and **females are carriers**.

Hemophilia A (Factor VIII Deficiency)

Q. Write short essay/note on hemophilia A.

- **Common hereditary X-linked recessive** disease.
- About 30% of hemophiliacs may be due to acquired mutations.
- **Reduced amount or** activity of **factor VIII** is associated with **life-threatening bleeding**
- Bleeding is due to both inadequate coagulation and inappropriate clot removal (fibrinolysis).

Mode of Inheritance (Fig. 13.5)

- X-linked recessive disease. **Genes** for factor VIII are located **on the long arm of the X-chromosome**.

- Does not manifest when there is a normal copy of X-chromosome.
- **Males** with a defective/mutant factor VIII gene (*hemophiliac* gene) on their single X chromosome (X_H) **suffer** from hemophilia.

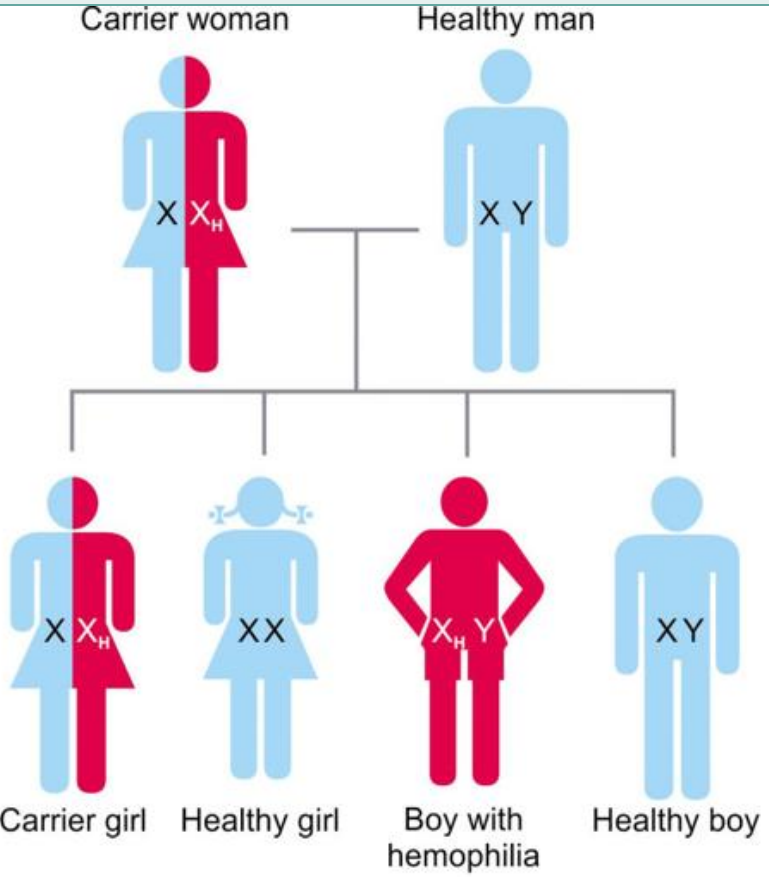
BOX 13.7: Classification of coagulation disorders.

A. Hereditary coagulation disorders

1. Hemophilia A
2. Hemophilia B
3. von Willebrand disease
4. Others

B. Acquired (secondary) coagulation disorders

1. Vitamin K deficiency
2. Liver disease
3. Others



Hemophilia A: Males are suffers and females are carriers.

Fig. 13.5: Mode of inheritance in hemophilia.

TABLE 13.2: Factor VIII level and clinical severity in hemophilia A.

Clinical severity	*Level of factor VIII activity in percentage*	*Clinical features*
Mild	>6	In the mildest form, it may be unnoticed. Bleeding develops after trauma only
Moderate	2–5	Bleeding after trauma including dental and other surgical trauma. Easy bruising
Severe	<1	Frequent and spontaneous hemorrhage into joints (hemarthrosis) and soft tissues

Normal range for factor VIII: 45–158 IU/dL.

- **Heterozygous females are carriers** and do not express the full clinical disease because of the paired normal X-chromosome.
- However, females with two copies of the defective X_H chromosome may rarely suffer from hemophilia.

Molecular Genetics

Causative mutations include deletions, inversions, point mutations and insertions.

Clinical Features

Clinical severity **depends on the level of factor VIII activity with normal range expressed as percentage (Table 13.2)**. Severe cases have less than 1% residual factor VIII activity.

Common clinical presentations include:

- Frequent and spontaneous **hemorrhage into the joints**—hemarthrosis.
- Hemorrhage into **soft tissues.**
- **Prolonged bleeding** following trauma.

Laboratory Findings

- **Bleeding time: Normal**

- **Clotting time: Prolonged,** but is not a sensitive test
- **Platelet count: Normal**
- **Prothrombin time: Normal**
- **Activated partial thromboplastin time (APTT): Increased** (normal 30–40 seconds)
- **Factor VIII assay: Essential for the diagnosis** and to assess the levels and severity of disease.
- **Fibrinogen assay:** Normal
- **FDP:** Negative
- **Detection of carriers:** By DNA markers.
  - To detect female carriers
  - Prenatal diagnosis of affected fetuses.

Complications

Due to Hemophilia

- **Deforming arthritis and contractures:** This is due to repeated bleeding into the joints. Organization and fibrosis of intramuscular hematomas → contractures of involved muscles.
- **Anemia:** Excessive, spontaneous or repeated bleeding leads to anemia.

Due to Therapy

- **Viral hepatitis: Hepatitis B, C and D** in patients who received multiple transfusions of FFP/cryoprecipitate.
- **AIDS:** In individuals who received fresh frozen plasma (FFP) or cryoprecipitate, when screening tests for HIV were not available.
- **Factor VIII inhibitors:** Makes further management difficult.

Hemophilia A: X-linked recessive disorder.

Hemophilia A: Percentage of level of factor VIII activity correlates with severity of disease.

Hemophilia A: Common presentation
- Hemarthrosis
- Hemorrhage into soft tissues

Hemophilia A:
Decreased: Factor VIII
Increased: APTT and clotting time

Causes of death in hemophilia:
- Intracranial hemorrhage
- Prolonged bleeding

Treatment of hemophilia:
- Factor VIII concentrate
- Recombinant factor VIII

Hemophilia B (Christmas Disease,Factor IX Deficiency)

Q. Write short note on Christmas disease.

- **Clinically indistinguishable from hemophilia A**
- **X-linked recessive** disorder
- **Variable clinical severity**
- Assay of factor IX should be done to diagnose Christmas disease (named after the first patient).

Laboratory Findings

Similar to hemophilia A.

- **Bleeding time:** Normal
- **Clotting time: Prolonged**
- **Platelet count:** Normal
- **Prothrombin time:** Normal
- **APTT: Increased** (normal 30–40 seconds)
- **Factor IX assay:** Factor IX is **decreased.**

Hemophilia B:
- X-linked recessive disorder
- Mutation in factor IX
- Deficiency of factor IX

Hemophilia B: Clinical features
- Usually milder than hemophilia A.
- Hemarthrosis is the common presentation.

Hemophilia B: Decreased factor IX and increased APTT and clotting time.

VON WILLEBRAND DISEASE (VWD)

Q. Write short essay/note on von Willebrand disease, its clinical features and laboratory investigations.

- Most common **inherited** bleeding disorders
- Most cases are **autosomal dominant disorders**
- Variable clinical picture with more than 20 variants.

Categories

Grouped into two major categories:

1. **Quantitative deficiency of vWF:** Decreased circulating vWF
   - Type 1—autosomal dominant, mild disorder and form about 75% of all cases
   - Type 3—autosomal recessive, severe disorder and least common type.
2. **Qualitative defects in vWF:**
   Type 2—autosomal dominant, accounts for 25% with several subtypes.

Clinical Features

- Most cases are of **mild bleeding**
- Common symptoms:
  - Spontaneous bleeding from mucous membranes (e.g., epistaxis)
  - Excessive bleeding from wounds or menorrhagia.
- In severe cases, similar to hemophilia A.

Laboratory Findings

- **Platelet count:** Normal
- **Bleeding time: Prolonged**
- **Clotting time: Prolonged**
- **Tourniquet test (Hess test): Positive** due to defect in platelet adhesion
- **APTT: Prolonged APTT**
- **PT: Normal**
- **vWF assay:** Plasma level of active vWF is **decreased**
- **Platelet function test:** Defective **ristocetin**-induced platelet **aggregation test** is **diagnostic of vWF.**

Laboratory tests in hereditary disorders are summarized in **Table 13.3**.

Three common hereditary disorders are as follows:

1. Hemophilia A (deficiency of factor VIII)
2. Hemophilia B (deficiency of factor IX)
3. von Willebrand disease (deficiency of vWF)

vWF is synthesized by endothelial cells and megakaryocytes.
vWF may be located in the plasma and subendothelial tissue.

vWF: Causes platelet adhesion and prevents degradation of factor VIII in plasma. Platelet adhesion molecule is synthesized in the Weibel–Palade bodies in endothelial cells.

vWD: Autosomal dominant disorders caused by mutations in vWF.

vWD: Increased bleeding time, clotting time and prolonged APTT. Plasma vWF is decreased. Defective ristocetin-induced platelet aggregation test is diagnostic.

TABLE 13.3: Summary of laboratory tests in hereditary coagulation disorders.

	Hemophilia A	*Hemophilia B*	*von Willebrand disease*
Bleeding time	N	N	Increased
APTT	Increased	Increased	Increased
Factor VIII	Decreased	N	Low or normal
Factor IX	N	Decreased	N
vWF	N	N	Decreased

(N: normal).

Hemophilia A, B and vWD: Prothrombin time, thrombin time and platelet count are normal. APTT increased in all the three.

ACQUIRED COAGULATION DISORDERS

Q. Write short note on acquired coagulation disorders and its causes.

Coagulation Factor Abnormalities

Usually characterized by multiple clotting abnormalities.

Vitamin K Deficiency

Vitamin K is important for the activity of four clotting factors: Prothrombin, factor VII, factor IX and factor X. In neonates, low levels of vitamin K levels may produce life-threatening hemorrhage during the first week of life known as ***hemorrhagic disease of the newborn.*** Parenteral vitamin K therapy is rapidly effective. Vitamin K is discussed in detail on pages 271-2.

Diagnosis of Vitamin K Deficiency

CC: Diagnosis of vitamin K deficiency.

Vitamin K deficiency leads to **delayed coagulation and bleeding** and is usually discovered when unexpected or excessive bleeding occurs.

Prothrombin time: It is the main laboratory test done to investigate the bleeding. Vitamin K deficiency should be suspected as the cause for bleeding when the prothrombin time is prolonged (increased) but other coagulation studies are normal. If it is suspected that bleeding is due to vitamin K deficiency, then **vitamin K should be administered parenterally**. If the bleeding stops and the PT return to normal, then it can be assumed that vitamin K deficiency is the cause. Of the vitamin K–dependent clotting factors, factor VII (extrinsic pathway) has the shortest half-life. Thus the prothrombin time is the first to be prolonged. Ultimately, the aPTT also will be prolonged as clotting factors in the intrinsic pathway become deficient.

Other coagulation tests: It may be performed in patients with excessive bleeding. These include aPTT, thrombin time, platelet count, platelet function tests, coagulation factor tests, fibrinogen, von Willebrand factor, and d-dimer.

Measurements of the level of vitamin K in the blood is not a routine test and is usually not done to determine its deficiency.

Liver Disease

Liver synthesizes all the clotting factors and severe liver disease is associated with a hemorrhagic diathesis.

Other Causes

Disseminated intravascular coagulation involves deficiency of several coagulation factors.

Vitamin K dependent coagulation factors: II, VII, IX and X.

DISSEMINATED INTRAVASCULAR COAGULATION

CC: Define and describe disseminated intravascular coagulation (DIC), its laboratory findings and its diagnosis.

Q. Write short essay/note on disseminated intravascular coagulation, etiology (list the disorders associated with DIC), pathogenesis, clinical features and laboratory investigations/diagnosis.

Definition: DIC is a widespread disorder with **combination of thrombosis and hemorrhage.**

Etiology

Develops as a secondary complication of wide variety of disorders **(Box 13.8)**.

Pathogenesis (Fig. 13.6)

Disseminated intravascular coagulation is a disorder that shows **combination of (i) thrombosis and (ii) hemorrhage.**

Thrombi/Clot Formation

Mechanism of Thrombi Formation

- **Initiation of thrombotic process:** Two major mechanisms initiate the thrombotic process of DIC namely entry of **thromboplastic (procoagulant) substances** into the circulation and widespread **endothelial injury.**

- *Entry of thromboplastic (procoagulant) substances into the circulation:* Source of thromboplastic/procoagulant substance in majority is **tissue factor**, which **activates** coagulation system.

BOX 13.8: Major disorders associated with disseminated intravascular coagulation.

Infections
- Gram-negative bacterial sepsis
- Meningococcemia and other bacteria
- Fungi, viruses, Rocky Mountain spotted fever, malaria

Obstetric complications
- Retained dead fetus
- Abruptio placentae
- Toxemia and pre-eclampsia
- Septic abortion
- Amniotic fluid embolism

Neoplasms
- Carcinomas of pancreas, prostate, lung and stomach
- Acute promyelocytic leukemia

Massive tissue injury
- Traumatic
- Fat embolism
- Burns
- Surgery

Vascular disorders
- Aortic aneurysm, giant hemangioma

Immunologic reactions
- Transfusion reactions
- Transplant rejection

Respiratory distress syndrome

Miscellaneous
- Snakebite, liver disease, acute intravascular hemolysis, shock, heatstroke, hypersensitivity, vasculitis

DIC: Sepsis, major trauma, obstetric complications and certain cancers are the common triggers.

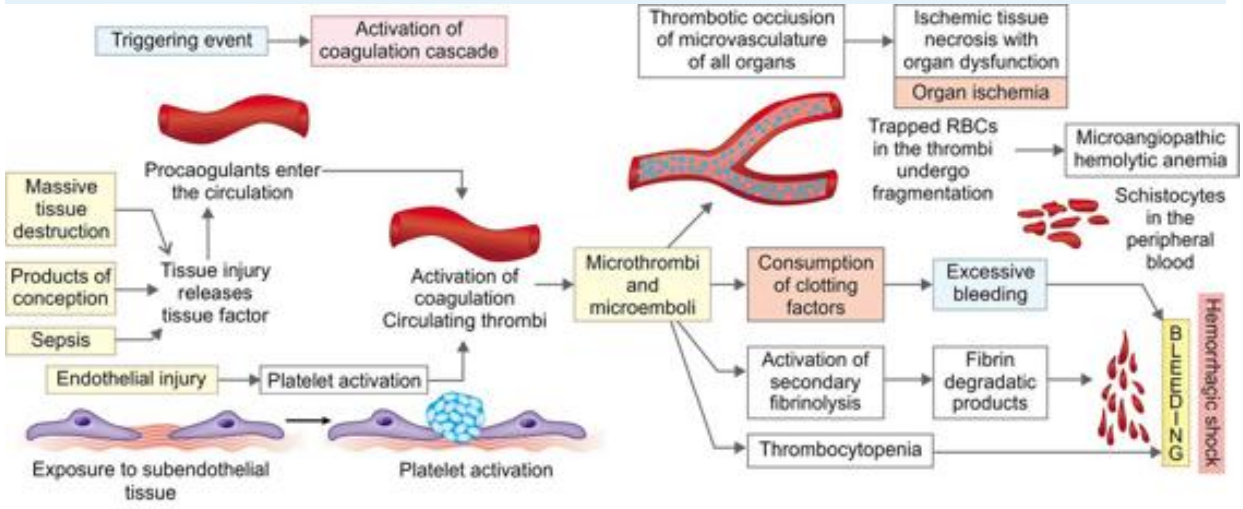


Fig. 13.6: Pathogenesis of thrombosis, ischemic tissue necrosis and bleeding in disseminated intravascular coagulation.

- *Widespread endothelial injury:* Endothelial injuries **expose the thrombogenic subendothelial matrix**.

- **Development of thrombi:**
  - Both procoagulant substances (tissue factor) and endothelial injury activate coagulation system resulting in fibrin-platelet **thrombi formation** in the microvasculature.
  - During this process, there is **consumption of clotting factors, fibrin and platelets**. Hence, it is also referred to as **consumptive coagulopathy** or **defibrination syndrome.**
- **Consequences of thrombi formation:** Widespread deposition of fibrin-thrombi within the microcirculation leads to:
  - **Ischemic necrosis:** Microvascular thrombi produces **microinfarcts or large areas of infarction** and multiorgan failure.
  - **Microangiopathic hemolytic anemia:** RBCs trapped in the intravascular fibrin-thrombi deposits undergo fragmentation. These RBCs appear as schistocytes in blood smears; but, frank hemolytic anemia is unusual in DIC.

Hemorrhagic Diathesis

- Causes of hemorrhagic/bleeding diathesis:
  - Consumption of platelets
  - Consumption of coagulation factors
  - Activation of fibrinolytic system.
- **Mechanism of hemorrhagic diathesis: Fibrin-thrombi activate secondary fibrinolytic system** and generate **plasmin.** The plasmin cleaves fibrinogen and fibrin and generates fibrin split products (FSPs) [or fibrin degradation products (FDP)]. FSPs are potent anticoagulant and have antiplatelet effect and produces hemorrhagic diathesis.

Clinical Features

- **Serious**, **often fatal**, clinical condition
- Signs and symptoms are related to:
  - **Hemorrhagic diathesis/bleeding:** Most common, manifest as ecchymoses, petechiae or bleeding from mucous membranes or at the sites of venipuncture.

- **Microvascular thrombi:** Tissue hypoxia and infarction of the organ leading to multiorgan failure.

Laboratory Findings in DIC

Screening Assays

- **Coagulation abnormalities:**
  - **APTT: Increased** as a result of consumption and inhibition of the function of clotting factors.
  - **Prothrombin time: Increased.**
  - **Thrombin time (TT): Increased** because of decreased fibrinogen.
  - **Fibrinogen:** Decreased.
- **Bleeding time: Increased** due to decreased platelet count.
- **Platelet count: Decreased** due to utilization of platelets in microthrombi.
- **Peripheral smear: Microangiopathic hemolytic anemia with schistocytes.**

Confirmatory Tests

- **Fibrinolysis abnormalities**
- Fibrin degradation/split products (**FDP**): Secondary fibrinolysis results in **generation of FDPs**, which can be measured by latex agglutination.
- **D-dimer test:** It is **specific for diagnosing DIC**.

Summary of screening tests for bleeding disorders are presented in **Table 13.4**.

DIC: Widespread thrombo-hemorrhagic disorder secondary to wide variety of disorders.

DIC:
- Consumption of coagulation factors
- Widespread thrombosis in small blood vessels

DIC laboratory findings:
- Increased: APTT, PT, BT, D-dimer
- Decreased: Platelets, fibrinogen

DIC: D-dimer test is specific diagnostic test.

Prognosis of DIC:
- Depends on the underlying disorder.

- Mortality is high in severe cases.

Treatment of DIC:

- Removal of the underlying cause
- Replacement of clotting factors and platelets.

THROMBOTIC DISORDERS: HYPERCOAGULABLE STATE

HYPERCOAGULABLE STATE (THROMBOPHILIA)

Group of inherited or acquired conditions that are associated with **increased tendency or risk to develop thrombosis** (*refer* Box 5.2 for causes of hypercoagulability).

Inherited Hypercoagulable States

Clinical Presentation

- **Thrombosis** develops at **young age** (less than 45 years)
- **Recurrent** thromboembolism
- **Family history** of thromboembolic episodes
- Thrombosis develops in the venous system and at **unusual anatomical sites** like visceral veins.

Deficiency of Antithrombotic Factors

Antithrombin (AT) III Deficiency

- Autosomal dominant disorder
- Deficiency of antithrombin—either quantitative or qualitative
- Risk of a thrombosis—20–80%.

Protein C and S Deficiency

- **Normally,** activated **proteins C** (APC) **and protein S** act as a complex which **degrades activated factors V and VIII.**
- When there is deficiency of these proteins, the activated factor V and VIII are **not neutralized**. This leads to activation of the clotting system and formation of thrombus.

Increased Prothrombotic Factors

Activated Protein C Resistance (Factor V Leiden)

- Most common genetic disorder associated with familial thrombophilia.
- **Point mutation** in the **factor V gene** causes synthesis of a **factor V variant.** This variant is known as factor V Leiden/**Leiden mutation.**
- Activated **proteins C (APC) and protein S** complex, normally inhibits activated factor V and VIII. But the variant clotting factors produced in this condition cannot be degraded.
- **Factor V variant** has normal procoagulant activity but is **resistant to inhibition by APC.**

ACQUIRED HYPERCOAGULABLE STATES

TABLE 13.4: Summary of screening tests for bleeding disorders.

Investigation	*Normal range*	*Main causes of abnormal test*
Blood count and film	Show the number and morphology of platelets and any blood disorder	Platelet disorders, leukemia or lymphoma
Platelet count	150–450 × 10^9/L	Thrombocytopenia
Bleeding time (template method)	2–9 minutes	• Thrombocytopenia • Abnormal platelet function • Deficiency of von Willebrand factor • Vascular abnormalities
Prothrombin time (PT)	11–16 seconds	Deficiency or inhibitors of factors II (prothrombin) V, VII or X or fibrinogen (I). Others include heparin, warfarin

TABLE 13.4: Summary of screening tests for bleeding disorders.		
Investigation	***Normal range***	***Main causes of abnormal test***
Activated plasma thromboplastin time (APTT)	30–40 seconds	• Deficiency or inhibitors of prekallikrein; high molecular weight kininogen; factors II, V, VIII, IX, X, XI, XII or fibrinogen (I) • Treatment with heparin, warfarin • Antibodies against clotting factors • Lupus anticoagulant
Thrombin time	15–19 seconds	Hypofibrinogenemia, DIC

Causes of the acquired hypercoagulable states **(*refer* Box 5.2).**

Antiphospholipid Antibody Syndrome (APLA/APS)

Q. Write a short note on antiphospholipid syndrome.

- Presence of antiphospholipid antibodies (APAs) in the plasma are associated with **hypercoagulable state**.
- **Antiphospholipid antibody** reacts with plasma proteins, which are bound to phospholipids **(*refer* Fig. 6.16)**.
- **Two** important antiphospholipid **antibodies: Lupus anticoagulant antibody and anti-β_2 glycoprotein antibody.**
  1. **Lupus anticoagulant antibody: Prolongs the phospholipid-dependent coagulation tests** in vitro (e.g. prolongation of APTT).
  2. **Antibodies against the phospholipid–β_2-glycoprotein complex:** It also bind to cardiolipin antigen used in the serological test for syphilis.

Types

- **Primary antiphospholipid syndrome:** No predisposing cause.
- **Secondary antiphospholipid syndrome:** Association with autoimmune diseases, such as systemic lupus erythematosus, hence known as lupus anticoagulant syndrome.

Clinical Features

- **Hypercoagulable state:** Most common acquired hematologic cause of recurrent thromboembolic events.

- **Repeated spontaneous abortions:** Normally, tissue plasminogen activator (t-PA) is necessary for the invasion of uterine blood vessels by placental trophoblastic tissue. Recurrent spontaneous abortions develop due to antibody-mediated inhibition of t-PA activity.
- **Immune thrombocytopenia.**

Laboratory Tests

Coagulation Tests

- **APTT: Prolonged**
- Factor VIII levels: Normal
- Prothrombin time: Normal
- Thrombin time: Normal
- Fibrinogen level: Normal.

Confirmatory Test

- **Test for lupus anticoagulant: Dilute Russell's viper venom test (DRVVT):** Russell's viper venom (RVV) normally activates factor X leading to fibrin clot. If **Lupus anticoagulant is present, it prolongs clotting time** by binding to RVV and preventing the action of RVV.
- **Antibodies against the phospholipid–β_2-glycoprotein complex:** Detected by enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA).

Factor V Leiden/Leiden mutation is characterized by factor V variant.
Factor V Leiden is resistant to inhibition by APC. It is associated with familial thrombophilia.

Antiphospholipid antibodies includes lupus anticoagulant antibody and anti-β_2 glycoprotein antibody.

Triad of thrombosis, recurrent spontaneous abortions and immune thrombocytopenia may be the presenting clinical features of antiphospholipid syndrome.

Immune Thrombocytopenic Purpura (ITP)

- History strongly indicative of **acute immune thrombocytopenic purpura**: Sudden development of bruising, petechiae and **mucocutaneous bleeding** in a **child following a bacterial or**

nonspecific viral (upper respiratory or gastrointestinal) **infection** without features of acute leukemia (such as anemia and bone tenderness).

- Laboratory findings of **markedly reduced platelet count** and **megakaryocytic hyperplasia with immature megakaryocytes**. In the bone marrow favors the diagnosis of **acute immune thrombocytopenic purpura**.

Case No. 1

History: A **14-year**-old male comes to the OPD with **petechial rashes of 2 days** duration. He has no history of bleeding in past. He had an attack of **viral infection 3 weeks before** this complaint.

Laboratory findings: Hb 14.5 g/dL, hematocrit 45%, RBC count 5.1 million/cu mm. WBC count 7,200/cu mm and **platelet count 75,000/cu mm**. MCV is 85 fL, MCH is 32 pg and MCHC is 31 g/dL and RDW is 14.

CHAPTER
14

Clinical Pathology

ANTICOAGULANTS

CC: Describe the role of anticoagulants in hematology.

Q. Write short essay/note on anticoagulants/various types of anticoagulants used in hematology. Mention their mode of action. List the uses of trisodium citrate.

Blood coagulates when withdrawn from the vessel and anticoagulants are used to prevent blood from clotting. Commonly used anticoagulants are listed in **Table 14.1**.

Calcium Chelating Agents

Most of the anticoagulants used in the laboratory act by binding calcium as an insoluble salt or soluble but unionized salt. Since calcium is essential for many of the steps in the clotting mechanism, chelation of calcium prevents clotting.

a. Ethylenediaminetetraacetic Acid

Commonly disodium or tripotassium salts are used in the laboratory.

Action: They bind and remove free calcium ions from blood by chelation, thereby preventing coagulation.

Advantages: Ethylenediaminetetraacetic acid (DIA) **preserves the morphology** of the blood cells even up to 3 hours and cell counts up to 12 hours after collection of the blood. Therefore, it is the **anticoagulant of**

choice for all cell counts and peripheral smear. Tripotassium salts of EDTA are more soluble and are preferred for cell counters than that of less soluble sodium salt. EDTA is used in solid form at concentration of 2 mg/mL of blood.

Disadvantages: It is more expensive than oxalate, but this is usually outweighed by the advantages. It is **not suitable for coagulation studies.**

Alterations Induced by EDTA

Peripheral smear

- **RBC:** Red cells may assume spherocytic or macrocytic form and shows crenation.
- **WBC:** Morphologic changes take place in the neutrophils within 30 minutes of blood collection. Neutrophils show separation of nuclear lobes, vacuoles and loss of granules in the cytoplasm. Monocytes and lymphocytes show irregular lobulation of nucleus and vacuoles in the cytoplasm.
- **Platelets:** They swell and disintegrate and a false decrease in their count may be observed.

Hematological tests

- **Hematocrit/PCV and mean cell volume:** Increased due to swelling of cells.
- **Osmotic fragility:** Increased
- **Total WBC count and platelet count:** Progressively decreased.

Uses

- Cell counts: RBC count, total leukocyte count, eosinophil count, platelet count.
- Hemoglobin, HbF estimation and Hb electrophoresis.

b. Oxalate

Q. Write short note on double oxalate mixture.

- **Potassium oxalate:** Most often used for chemical analysis. Shrinks RBCs and therefore not recommended for packed cell volume (PCV), erythrocyte sedimentation rate (ESR), or peripheral smear.

TABLE 14.1: Commonly used anticoagulants.

I. Calcium chelating agents	Uses
• Ethylenediaminetetraacetic acid (EDTA)	RBC count, total leukocyte count, eosinophil count, platelet count. Hemoglobin, HbF estimation and Hb electrophoresis
• Double oxalate (mixtures of ammonium oxalate and potassium oxalate)	Since, morphology of the blood cells is not well preserved, it is now replaced by EDTA
• Sodium citrate solution	Coagulation studies, ESR by Westergren method, blood bank [acid citrate dextrose (ACD) solution was used and now replaced by citrate phosphate dextrose adenine (CPDA)]
• Sodium fluoride	Estimation of blood sugar
II. Heparin	Osmotic fragility test, red cell enzyme studies (e.g., G6PD and PK deficiency), electrolyte estimation, arterial blood gas analysis

- **Ammonium oxalate:** It causes swelling of RBCs and therefore not used for PCV, ESR or peripheral smear.
- **Double oxalate:** To balance the swelling effect of ammonium oxalate and the shrinking effect of potassium oxalate, the two are combined in a mixture in the ratio of 3 parts of ammonium oxalate to 2 parts of potassium oxalate. Morphology of the blood cells is not well preserved; hence it has now been replaced by EDTA. As it is toxic, it is not used as an anticoagulant for blood transfusion. It is used in concentration of 2 mg/mL of blood.

c. Sodium Citrate

Action: Sodium citrate binds with calcium ions in the blood to form a soluble calcium citrate complex thus preventing coagulation of blood.

Disadvantages: Since it is used as a liquid which results in dilution of cellular elements, it is not suitable for cell counts, Hb, etc.

Uses

- **Coagulation studies:** Either 3.8% trisodium citrate pentahydrate or 3.2% trisodium citrate dihydrate solution is used for coagulation studies. One part of anticoagulant is mixed with 9 parts of blood (1:9, i.e., ratio of 0.5:4.5 mL) and thus, there is less dilution of the plasma.
- **ESR by Westergren method**: In the Westergren method of ESR estimation, 1 part of 3.8% sodium citrate is mixed with 4 parts of blood (e.g., 0.4 mL citrate and 1.6 mL blood/0.5 mL:mL of blood), thus maintaining a **ratio of 1:4 between citrate and blood**.
- **Blood bank:** acid citrate dextrose solution (ACD solution) was used as an anticoagulant in blood banking. This has been replaced by citrate phosphate dextrose adenine (CPDA).

d. Sodium Fluoride

Sodium fluoride acts an antiglycolytic agent which inhibits the use of glucose by blood cells.

Use: It is used for estimation of **blood sugar**.

Heparin

Heparin acts as an anticoagulant by preventing conversion of prothrombin to thrombin. It is used when almost instantaneous anticoagulation is required and to prevent any chances of hemolysis after the blood is drawn. Syringe is heparinized by taking 1 drop of heparin to wet the inner surface of the syringe and the blood is collected.

Disadvantages

- Expensive.
- Causes clumping of platelets and leukocytes, so it is not used for automated cell counters.
- Imparts bluish discoloration to the background of peripheral blood smears.
- Prevents coagulation for only a limited period of time and its effect slowly disappears as the heparin is metabolized or neutralized in the blood.

Uses

- Osmotic fragility test done for diagnosis of spherocytosis.
- Red cell enzyme studies, such as G6PD and PK deficiency.
- Electrolyte estimation.
- Arterial blood gas analysis.

BLOOD SAMPLE

Type of Blood Sample

Q. Write short note on method of obtaining plasma and serum.

1. **Whole blood:** It is used for complete hemogram (hemoglobin, ESR, platelet count, reticulocyte count, and peripheral smear evaluation), osmotic fragility test, estimation of HbF, Hb electrophoresis, Coombs test and for biochemical investigations, such as blood glucose.
2. **Serum:** It is obtained by **allowing the blood to clot** in a tube or in a vial (without adding any anticoagulant). It is used for estimation of bilirubin, creatinine, uric acid, proteins, albumin, globulin and albumin/globulin (A/G) ratio, serum enzyme levels [e.g., alkaline phosphatase, acid phosphatase, aspartate (AST/SGOT) and alanine aminotransferase (ALT/SGPT)], serum electrophoresis of proteins, lipoproteins and immunoglobulins.
3. **Plasma:** It is obtained by **centrifugation of the anticoagulated blood**. Red blood cells form the sediment and the supernatant is the plasma. It is used for coagulation studies [prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time], assay of various coagulation factors (e.g., factor VIII, IX), assay of FDP and D-dimer (in disseminated intravascular coagulation), confirmation of hemoglobinemia where plasma is colored red (e.g., PNH).

Collection of Blood

Venous blood for the tests is collected by (i) syringe or (ii) vacuum tubes.

Q. Write short note on vacutainer.

Vacutainer: These are vacuum tubes which have a colored top **(Table 14.2)**. They are used in most of the laboratories and have replaced the collection of blood by syringe. It is very essential to use appropriate vacuum tubes with proper ratio of anticoagulant and the blood for specific tests.

TABLE 14.2: Types of commonly used vacuum tubes, their color, content and uses.

Color of the top	*Anticoagulant*	*Uses*
Red	No chemicals—plain	Serum for various studies
Purple or lavender	EDTA	Routine hematological tests
Light blue	Sodium citrate	Coagulation studies
Gray	Fluoride	For glucose estimation
Green	Heparin	Osmotic fragility test, red cell enzyme study
Black	Citrate	Erythrocyte sedimentation rate

TABLE 14.3: Methods of hemoglobin estimation.

Method	*Principle*
Colorimetric method	
1. Visual colorimetric method; Sahli's method or acid hematin method, alkaline hematin method	These methods are based on measuring the color of hemoglobin or a hemoglobin derivative in the blood either visually or by photocolorimetry
2. Photoelectric method: Cyanmethemoglobin (HiCN) method, oxyhemoglobin method 3. Haldane method	
Physical (specific gravity) method	Specific gravity
Chemical method	Iron content of hemoglobin
Gasometric method	Oxygen combining capacity of hemoglobin
Cell counter/autoanalyzer	RBCs are lysed by the lysate and color is matched against inbuilt standard color. Most use cyanide-free biodegradable reagent

Capillary blood: May be collected either from the finger or heel pricks using sterile disposable lancet/needle.

Arterial blood: Is usually required for blood gas analysis and is collected from the femoral artery by disposable needle and syringe.

Complications Encountered During Blood Collection

Q. Write short note on complications of venipuncture.

- **Ecchymosis** and **hematoma** at the site of puncture.
- **Syncope or fainting.**
- **Failure to get blood** because the needle might not have entered the vessel. In obese individuals, it is often difficult to locate the vein.
- **Hemolysis of blood sample** due to narrow bore of needle, quick withdrawal of blood with excessive suction or contamination of syringe by water. Hemolyzed blood sample is not suitable for testing.

HEMOGLOBIN ESTIMATION

Q. Write short note on methods of hemoglobin estimation.

One of the important features of anemia is reduction in the red cell mass. Red cell mass can be estimated by measuring hemoglobin (Hb). There are several methods for Hb estimation utilizing different principles **(Table 14.3)**. The commonly used methods include colorimetric method namely Sahli's (outdated but still used in some places) and cyanmethemoglobin method.

Cyanmethemoglobin (Hemiglobin Cyanide; HiCN) Method

Cyanmethemoglobin is the most accurate, convenient, readily available and preferred method for estimation of hemoglobin concentration. It is the standard method used in most of the centers.

Principle: Blood is diluted in a stable standard solution of potassium ferricyanide and potassium

cyanide. **Hemoglobin is first oxidized to methemoglobin** (Hi) by the potassium ferricyanide. The cyanide ions (CN^-) of potassium cyanide convert **methemoglobin (Hi) to stable cyanmethemoglobin** (HiCN). The cyanmethemoglobin has a broad absorption, maximum being at a wavelength of 540 nm. Thus, the color of this solution is compared against a standard HiCN solution of known Hb value in a spectrophotometer or photoelectric colorimeter at 540 nm.

Apparatus: Photoelectric colorimeter with a green filter or spectrophotometer at a wavelength of 540 nm is used. Other equipments required are: Sahli's pipette (mark of 20 mm) and 5 mL pipette.

Reagents: The diluent is **detergent-modified Drabkin solution**, the composition of which is shown in **Table 14.4**.

Drabkin solution should be clear and pale yellow and when measured in the photoelectric colorimeter should give zero reading at 540 nm against water blank. The detergent enhances lysis of RBCs and decreases turbidity due to precipitation of proteins.

TABLE 14.4: Constituents of Drabkin solution (pH 7.0–7.4).

Constituents	***Quantity***
Potassium ferricyanide	400 mg
Potassium cyanide (KCN)	100 mg
Potassium dihydrogen phosphate (anhydrous)	280 mg
Non-ionic detergent (*Sterox SE*)	1 mL
Distilled water	2 liters

Advantages of the Cyanmethemoglobin Method of Hemoglobin Estimation

Q. Write short note on advantages of the cyanmethemoglobin method of hemoglobin estimation.

- Hb value obtained is **accurate,** since almost all forms of hemoglobin (hemoglobin, oxyhemoglobin, methemoglobin, carboxyhemoglobin, but not sulphemoglobin) are converted into cyanmethemoglobin.

- Being a colorimetric method, there is direct comparison with HiCN Standard. Thus, **visual error** during matching the color like in Sahli's method is **eliminated.**
- Cyanmethemoglobin is a stable compound and **color does not change with time.** So readings can be made at the operator's convenience and delay in taking reading does not alter the value.
- **Easy** to perform the test.
- The **standard is stable** and certified cyanmethemoglobin standards are available.
- Reagents are readily available.

COMPLETE BLOOD COUNTS (HEMOGRAM) (TABLE 14.5)

Q. Write short note on complete blood counts (CBC) (hemogram).

Various Parameters Obtained in Automated Cell Counters (Table 14.6)

Q. Write short note on various parameters obtained in automated cell counters.

PERIPHERAL BLOOD SMEAR EXAMINATION

Importance of Peripheral Smear Examination

CC (Show How): Perform, Identify and describe the peripheral blood picture in anemia.
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Q. Write short essay on importance of peripheral smear examination.

Peripheral smear (peripheral blood film) is the most important, valuable and frequently asked investigation in hematology laboratory.

Q. Write a short note on abnormalities of shape of red blood cells.

Peripheral smear provides the following information:

- **Red cell morphology:** Morphological features (size, shape) of RBCs are important for diagnosis of anemias and other hematologic disorders **(Figs. 14.1 to 14.3)**.
- **WBC disorders and differential leukocyte count** (DLC): Quantitative and qualitative changes in WBCs help in diagnosis of both hematologic and nonhematologic disorders.
- **Platelet number and morphology**: These features are useful in the diagnosis of bleeding disorders.
- **Cross check the CBC parameters:** It helps in cross-checking the CBC parameters derived from automated cell counters.
- Detection of **blood parasites** (hemoparasites).

Q. Write short note on poikilocytosis, anisocytosis, macrocyte and target cell. (Ans: refer Figs.14.1 and 14.2)

Stains for Blood Smear

Blood cells contain cellular structures which vary in their reaction (pH), some are acidic and others being basic. The aniline dyes used in staining blood smears are of two general classes: Basic dyes, such as methylene blue and acidic dyes, such as eosin.

Romanowsky Stains

Q. Write short essay on Romanowsky stains.

All stains which are made of combinations of acidic (eosin) and basic dyes (methylene blue) are called **Romanowsky stains.** The action of these stains depends on compounds formed by the interaction of methylene blue and eosin. Methylene blue on oxidation produces colored compounds called

azures that have the ability to combine with eosin. Oxidation is achieved during maturation/chemical treatment of the stain. The azures are responsible for different shades of staining in the smears (i.e., RBCs— pink, granules of eosinophils—red-orange, granules of basophils—bluish-black, granules of neutrophils—lilac).

TABLE 14.5: Complete blood counts.

• Hemoglobin • Total leukocyte count • RBC count • Platelet count • Differential leukocyte count • Hematocrit (Hct)/packed cell volume (PCV)	• Reticulocyte count • Peripheral blood smear – RBC morphology – Platelets morphology – Hemoparasites – Any abnormal/atypical cell

TABLE 14.6: Various parameters obtained in automated cell counters.

Hemoglobin Absolute values: • Mean corpuscular volume (MCV) • Mean corpuscular hemoglobin (MCH) • Mean corpuscular hemoglobin concentration (MCHC) • Red cell distribution width (RDW)	• Mean platelet volume (MPV) • Hematocrit (Hct)/ packed cell volume (PCV) • Total leukocyte count • RBC count • Platelet count • Reticulocyte count • Differential leukocyte count (3 part or 5 part)

Term	Description	Appearance	Condition
Normocyte	• Normal RBC is biconcave, 7.2 μm in diameter		• Normal RBC • Normocytic normochromic anemia
Microcyte	• RBCs smaller than normal and less than 6 μm in diameter		• Iron deficiency anemia • Thalassemia
Macro-ovalocyte	• Larger than normal, more than 9 μm in diameter and also oval		• Megaloblastic anemia due to vitamin B_{12} and folic acid deficiency
Anisocytosis	• Variation in size		• Severe anemia of any type

Fig. 14.1: Variation in size of red blood cells and associated conditions.

- Nuclei and structures in the blood which are stained by the basic dyes are called basophilic.

- Structures that take up only acidic dyes are called acidophilic or eosinophilic.

Romanowsky group includes the following stains:

- ***Leishman stain*** contains acetone-free methyl alcohol which acts as a **fixative**. Since, acetone destroys the cells, methyl alcohol should be free from acetone.
- ***Giemsa stain***
- ***Wright stain***
- ***Jenner stain***
- ***Jenner–Giemsa stain***

The differences between the various Romanowsky stains are mainly in the proportion of the reagents and in their preparation.

Hemoparasites

Q. Write short essay on hemoparasites.

Hemoparasites include (i) malarial parasites, (ii) microfilaria, (iii) trypanosomes, (iv) *Leishmania donovani* and (v) babesiosis.

1. **Malaria (***refer* pages 101-4 and **Fig. 4.12):** Malarial parasites can be demonstrated in the peripheral smear; most common are *Plasmodium vivax* and *plasmodium falciparum*.
   - ***Plasmodium vivax:*** In the peripheral blood the trophozoites, schizonts and rarely gametocytes of plasmodium vivax may be found.
   - ***Plasmodium falciparum:*** In falciparum infections usually only ring stage and gametocytes may be demonstrated.
2. **Filariasis:** *Microfilaria* **(***refer* **Fig. 5.6B)** can also be demonstrated in peripheral blood.
3. **Trypanosomes:** These are motile flagellate protozoa.
4. ***Leishmania donovani* (LD):** It causes **kala-azar.** Amastigote forms known as LD bodies can be found in the reticuloendothelial cells of the bone marrow, spleen and buffy coat preparations of peripheral blood.
5. **Babesiosis:** It is a malaria-like parasitic disease caused by babesia.

Procedure for preparation of blood smear, its staining and steps in examination are provided in **Appendix 3**.

RETICULOCYTE COUNT

Q. Write short essay on reticulocyte, its morphology, staining method, normal values and its importance/utility. List the conditions in which reticulocyte count is increased.

Reticulocytes are **immature, non-nucleated RBCs** released from bone marrow. They are slightly larger than the mature RBCs. They continue to synthesize hemoglobin after loss of the nucleus.

Methods of Reticulocyte Count

- **Visual method: Staining in living** state is known as **supravital staining.** Reticulocytes contain ribosomes and RNA and can be stained by supravital stains in the live and unfixed state. When blood is briefly incubated in supravital stains, such as **new methylene blue or brilliant cresyl blue** solution, the RNA is precipitated **(***refer* **Fig. 11.22)** as a dye-ribonucleoprotein complex. On microscopy, the complex appears as a dark blue network (reticulum or filamentous strand or granular material). Reticulocytes stain ***polychromatic*** with Romanowsky stains and hence, the term ***"polychromatophil"*** is used to indicate their presence in peripheral smear.

Term	Description	Appearance	Condition
Normal	• Biconcave, flattened on smear		• Normal
Poikilocytosis	• Variation in shape		• Severe anemia
Spherocytes	• Smaller and stain darker without any central pallor		• Hereditary spherocytosis • Autoimmune hemolytic anemia
Target cells or leptocytes	• Only the periphery and the central regions of the cell appear hemoglobinized		• Thalassemia • Sickle cell anemia • Hemolytic anemia • Post-splenectomy • Liver disease
Sickle cells	• Thin, elongated, slightly curved and have shape of a sickle		• Sickle cell anemia
Bite cells	• Pairs of spicules		• G6PD deficiency
Schistocytes (fragmented cells)	• Small, irregular shaped, triangular or speculated cells		• Microangiopathic hemolytic anemia (e.g. DIC, TTP)
Acanthocytes	• RBCs with few spicules of uneven (irregular) length and shape		• Abetalipoproteinemia • Liver disease
Echinocyte/ Burr cell	• Very small irregular shrunken cells with pointed projections. Resemblance to the small thorny 'burrs'		• Uremia
Elliptocyte or ovalocyte	• Oval in shape		• Hereditary ovalocytosis • Hemolytic anemia
Pencil-shaped cells	• Elongated thin cells (exaggerated ovalocytes)		• Iron deficiency anemia
Teardrop and pear-shaped cells	• These abnormal RBCs have a teardrop or pear-like shape		• Myelofibrosis • Marrow infiltration
Stomatocytes	• Red cells with a slit-like area of central pallor		• Hereditary stomatocytosis • Liver disease

Fig. 14.2: Variation in shape of red blood cells and associated conditions.

- Automated method.

Normal reticulocyte count: Reticulocyte count is expressed as percentage of total red cells. Normal is 0.5– 2.5% and in newborn (cord blood) it is 1–7.0%.

Causes of increased and reduced reticulocyte count are listed in **Table 14.7**.

Term	Description	Appearance	Condition
Normochromic	• Cell with central 1/3 pallor		• Normal RBC
Hypochromic	• Central pallor more than 1/3		• Iron deficiency anemia • Thalassemia • Anemia of chronic disorder
Polychromasia	• Cells stained purple and are larger than normal		• Hemolytic anemia • Response to therapy in deficiency anemia

Fig. 14.3: Variation in color of red blood cells.

TABLE 14.7: Causes of increased and reduced reticulocyte count.

Causes of increasedreticulocyte count	*Causes of reducedreticulocyte count*
• Hemolytic anemias • Hemolytic crisis • Hemorrhage • Following treatment in iron/folic acid/vitamin B_{12} deficiency anemias. Highest counts are found on 6th/7th day of treatment and indicate marrow response to hematinics	Due to decreased erythropoietic activity • Aplastic anemia • Aplastic crisis due to parvovirus (hereditary spherocytosis and sickle cell disease) • Pure red cell aplasia • Fanconi anemia • Myelofibrosis

HEMATOCRIT (Hct)

Q. Write short essay/note on definition, methods and significance of PCV/hematocrit.

Definition: Hematocrit **(packed cell volume**/erythrocyte volume fraction**)** is the **ratio of the volume of red cells to that of the whole blood**.

It indicates relative volume of red cells and plasma (e.g., in anemia red cells are reduced with corresponding reduction in the hematocrit).

Methods of Estimation of Hct/PCV

- Macromethod using Wintrobe tube.
- Micromethod using capillary tube.
- Automated analyzer.

Wintrobe Method

Q. Write short essay/note on Wintrobe PCV tube and its uses.

Wintrobe tube (Figs. 14.4A to D): It is a special thick-walled glass tube measuring **11 cm in length** and an internal diameter of 2.5 mm, with a capacity of 1 mL. This is calibrated at 1 mm intervals up to 105–110 mm. It has bold markings in ascending order from top as 0, 10, 20, 30,...100 for ESR determination and descending order from the top as 100, 90, 80,...10, 0 for **PCV estimation**.

Anticoagulants used: EDTA, dried heparin or double oxalate.

Principle: Anticoagulated whole blood is centrifuged at a standard speed. RBCs which are heavier than white cells, platelets and plasma, sediment at the bottom and the volume of red cell mass denotes the hematocrit.

Normal range for PCV: Hematocrit is expressed as a percentage (e.g., 45%) or as a decimal fraction (e.g., 0.45). It is useful for evaluating absolute values like MCV and MCHC.

- Adult males 38–47%
- Adult females 36–46%
- Infants 45–70% (cord blood)

Causes of increased PCV: Polycythemia vera rubra and secondary polycythemia. Decreased PCV is seen in anemia.

Uses of Wintrobe Tube and Hematocrit

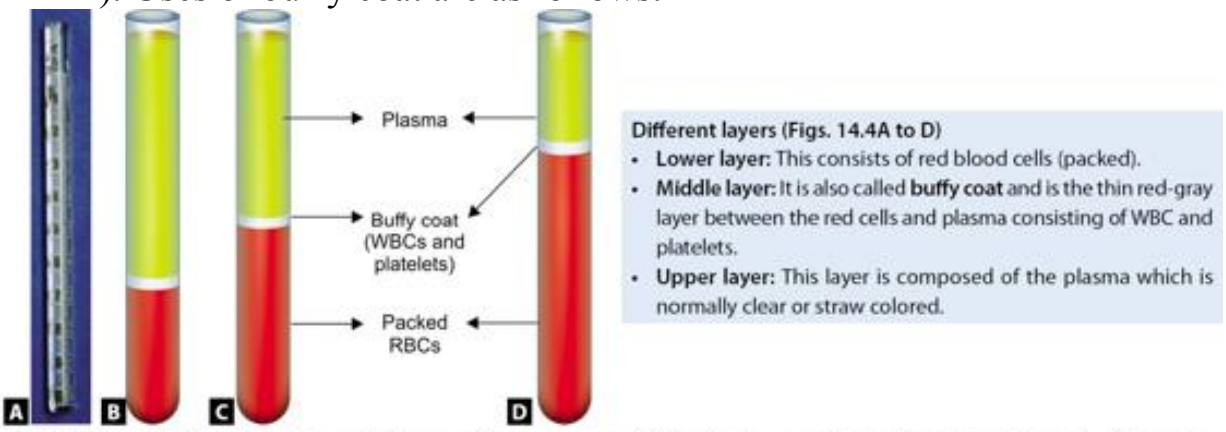
- **For estimation of hematocrit** which is useful for the following:
  - **Anemia:** To determine the presence of anemia, to assess its severity and to assess response to therapy.

- Presence or absence of **polycythemia**.
- **Checking the accuracy of hemoglobin value** (Hb in g/dL × 3 = hematocrit).

- PCV which is also required **to determine** the **red cell indices** namely mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC).

Q. What is buffy coat? Mention its importance.

- **Buffy coat obtained by hematocrit** gives an approximate indication of the number of WBCs (normally 0.1 mm of this layer = 1000 WBC/cu mm). Uses of buffy coat are as follows:



Figs. 14.4A to D: (A) Wintrobe tube and diagrammatic appearance of different columns after the blood is centrifuged in (B) anemia; (C) normal; and (D) polycythemia vera.

- Thickness of the **buffy coat increases with marked leukocytosis**, as in CML, CLL and acute leukemias. Absent or minimal buffy coat implies leukopenia.
- In subleukemic leukemia, a smear made from the buffy coat has greater concentration of WBCs and identification of abnormal cells is easier.
- LE cell test.

- **ESR estimation:** However, the Westergren method is preferred as it is more accurate.

ERYTHROCYTE SEDIMENTATION RATE

Q. Write short essay/note on normal values, indications, anticoagulants used, stages and factors affecting erythrocyte sedimentation rate.

Erythrocyte sedimentation rate (ESR) estimation is a commonly used nonspecific test in routine clinical practice. ESR is a useful but nonspecific marker of underlying inflammation. However, other high-sensitivity inflammatory markers (e.g., C-reactive protein) are presently used to detect or monitor disease (e.g., cardiovascular disease and metabolic syndrome).

Principle: When anticoagulated blood is placed in a vertical tube and is allowed to stand, RBCs settle towards the bottom of the tube. The speed of sedimentation of red cells in plasma over a period of 1 hour is measured by the length of the sedimented RBC column and is expressed in millimeters. RBCs have net negative charge on their surface and tend to repel each other. The repulsive forces are partially or totally counteracted if there is an increase in the positively charged plasma proteins.

Factors Affecting ESR (Box 14.1)

- **Plasma factors:** An accelerated ESR is favored by elevated levels of fibrinogen, globulins and cholesterol (which increase the positive charge of plasma) whereas albumin and lecithin retard ESR.
- **Red cell factors:** The sedimentation rate is directly proportional to the weight of the cell aggregates and inversely proportional to the surface area.
- **Number:** Anemia increases the ESR and polycythemia decreases.
- **Size:** Microcytes sediment slower than macrocytes.
- **Rouleaux formation:** It accelerates the ESR.
- Red cells with an **abnormal or irregular shape**, such as sickle cells or spherocytes, do not exhibit rouleaux formation and have low ESR.
- **Technical factors:** ESR tube must be kept vertical; otherwise it results in inaccurate ESR.

Stages of ESR

Sedimentation occurs in three stages:

1. **Stage of aggregation/rouleaux formation:** In the initial 10 minutes, there is little sedimentation as rouleaux form and the size of the rouleaux formed influence the speed of sedimentation.
2. **Stage of settling:** For about 40 minutes, settling occurs at a constant rate.
3. **Stage of packing:** Packing of RBCs occur in the final 10 minutes.

Methods

Two commonly employed methods are as follows: **Westergren and Wintrobe methods**.

Westergren Method

Q. Write short note on Westergren pipette/tube.

Westergren tube is a straight glass pipette (open at both ends) 30 cm in length, bore of 2.55 mm and calibrated in millimeters from 0–200. The capacity of tube is about 1 mL. The tube is vertically placed on the Westergren rack.

BOX 14.1: Factors that affect ESR.

Factors that increase ESR: Old age, pregnancy, anemia, macrocytosis, raised fibrinogen
Factors that decrease ESR: Microcytosis, polycythemia, marked leukocytosis, low fibrinogen

BOX 14.2: Indications for ESR.

- Suspicion of infectious, inflammatory or neoplastic conditions
- To monitor disease activity (e.g., rheumatic fever, tuberculosis)
- As a diagnostic criteria (e.g., temporal arteritis)

Anticoagulant used: 3.8% trisodium citrate.

Ratio of blood and anticoagulant: 2 mL of whole blood in 0.5 mL of 3.8% trisodium citrate (or 1.6 mL of blood + 0.4 mL of citrate). Blood to anticoagulant ratio should be 4:1.

Indications for ESR estimation (Box 14.2).

Normal range for erythrocyte sedimentation rate (Table 14.8).

Causes of Increased ESR

Causes of increased ESR is listed in **Table 14.9**.

Causes of Decreased ESR

- Polycythemia vera

- Sickle cell disease
- Hypofibrinogenemia.

LE CELL TEST

Q. Write short note on LE cell.

In autoimmune diseases (e.g., SLE, rheumatoid arthritis), variety of antibodies are found. One of them is an antinuclear antibody (ANA) which cannot penetrate intact cells. ANAs can react with nuclei of damaged cells and converts nuclear chromatin into homogeneous material. In vivo, ANAs act on the nuclei of damaged cells and denatures it into homogeneous material. This nuclear material is phagocytosed in the presence of complement by phagocytic leukocyte (neutrophil or macrophage). The LE cell **(***refer* **Figs. 6.17A and B)** is any **phagocytic leukocyte** (neutrophil or macrophage) **that has engulfed the denatured nucleus of an injured cell**. **In the tissue, they are known as LE bodies or hematoxylin bodies**.

Tart cell: LE cell has to be differentiated from a tart cell **(***refer* **Fig. 6.17C)**. It is a monocyte/neutrophil containing a phagocytosed nucleus of another cell. The nuclear material is not homogeneous and it retains its chromatin pattern. It may be found in healthy individuals.

Significance: LE cells may be demonstrated in **SLE, rheumatoid arthritis**, other **autoimmune disorders, hepatitis and penicillin sensitivity.**

BONE MARROW EXAMINATION

NC: Enumerate the indications and describe the findings in bone marrow aspiration and biopsy.

Bone marrow examination is essentially done to confirm or rule out a hematologic disorder. It also helps in evaluation of non-hematological disorders (e.g., metastasis). Bone marrow may be obtained by:

- **Aspiration:** Bone marrow aspiration is a simple, easy and safe procedure.
- **Trephine biopsy** is indicated in conditions where the aspiration either fails to yield marrow or to confirm some of the diseases (where biopsy findings are diagnostic).

Bone Marrow Aspiration

Q. Write short essay on bone marrow aspiration study.

Bone marrow needles: Needles commonly employed for the aspirations of the marrow are Salah needle and Klima needle.

Sites for Bone Marrow Aspirate

Usual sites for bone marrow aspiration are as follows:

- Sternum
- Posterior superior iliac spine
- Iliac crest
- Anterior superior iliac spine
- Spinous process of lumbar vertebra.

In **infants**, upper end of the tibia is the ideal site for marrow aspirate.

Indications for bone marrow aspiration is listed in **Box 14.3**.

Q. Write short note on indications for bone marrow aspiration.

Contraindications for bone marrow aspiration:

- Hemophilia.
- Congenital hemorrhagic disorders.

Dry Tap

Q. Write short answer on causes of dry tap in bone marrow aspiration.

TABLE 14.8: Revised normal range for erythrocyte sedimentation rate.

Age	*Men*	*Women*
Below 50 years	15 mm/hour	20 mm/hour
Above 50 years	20 mm/hour	30 mm/hour
Above 85 years	30 mm/hour	42 mm/hour

TABLE 14.9: Causes of increased ESR.

Markedly increased in:
- Multiple myeloma
- Macroglobulinemia
- Hyperfibrinogenemia

Moderately increased in:
- Infective diseases like tuberculosis
- Chronic inflammatory diseases
  - Rheumatic fever, osteomyelitis
  - Autoimmune diseases: Rheumatoid arthritis, SLE, etc.
- Neoplasia

BOX 14.3: Indications for bone marrow aspiration.

Diagnostic
- **Primary hematolymphoid disorders**
  - **Red cell disorders:** Nutritional anemia (e.g., megaloblastic anemia), pure red cell aplasia
  - **White cell disorders:** Subleukemic/aleukemic leukemia, diagnosis and classification of acute leukemias
  - **Megakaryocytic disorders:** ITP and other thrombocytopenias
  - **Myeloproliferative neoplasms:** Polycythemia vera, chronic myeloid leukemia, idiopathic thrombocythemia
  - **Myelodysplastic syndromes**
  - **Plasma cell neoplasms:** Multiple myeloma, Waldenström macroglobulinemia
- **Systemic diseases: Storage disorders** (e.g., Gaucher, Niemann–Pick disease)
- **Staging of lymphoid malignancies and solid tumors:** Lymphoma, metastatic deposits (e.g., carcinoma prostate, breast, lung, kidney)
- **Detection of infection and/or source of pyrexia of unknown origin (PUO)**
  - **Parasitic disorders:** Kala-azar
  - **Fungal disorders:** Histoplasma
  - **Mycobacterial infection**
- **Iron store**
- **Miscellaneous disorders:** Pancytopenia or unexplained cytopenias, PUO

Post-treatment follow up: To know the response to therapy and follow up in cases of leukemia, aplastic anemia and agranulocytosis

Therapeutic: Bone marrow transplant

BOX 14.4: Indications for trephine biopsy.

- Aplastic anemia
- Myeloproliferative neoplasms—to study reticulin fibrosis in myelofibrosis
- Myelodysplastic syndromes
- Pre- and post-bone marrow transplantation
- Pyrexia of unknown origin (granuloma of tuberculosis)
- Bone morphology in chronic renal failure, osteoporosis and osteomalacia
- Staging
  - Lymphoma
  - To detect metastasis in cancer patients
- Acquired immune deficiency syndrome (AIDS)

During aspiration, if the marrow is not obtained it is called a dry tap. Dry tap is common in:

- Hairy cell leukemia.
- Myelofibrosis (marrow has been replaced by fibrous tissue).

Complications of bone marrow aspiration and/biopsy

- Local infection.
- Hemorrhage.
- Cardiac tamponade or mediastinitis.

Bone Marrow Trephine Biopsy

Marrow trephine biopsy is performed by one of the trephine biopsy needles like Jamshidi needle or Westerman–Jensen needle or Islam needle. The biopsy obtained consists of a core of bone with marrow. These are excellent for morphological evaluation as well as for special stains. Trephine biopsy tissue is decalcified and processed like other histopathological tissue containing bone. Sections are stained with hematoxylin and eosin stain, reticulin stain, Masson's trichrome stain (for fibrous tissue). Immunocytochemical staining can be performed especially for acute leukemias.

Sites of Trephine Biopsy

- Posterior superior iliac spine (most commonly used site).
- Anterior superior iliac spine.
- Spinous process of vertebra.

Indications for Trephine Biopsy (Box 14.4)

Q. Write short note on indications for bone marrow trephine biopsy.

Findings in bone marrow aspiration and biopsy of RBC disorders, WBC disorders and hemostatic disorders are duscussed in Chapters 11, 12 and 13 respectively.

OSMOTIC FRAGILITY TEST

Q. Write short note on osmotic fragility test.

The normal red cell membrane is unstretchable and is freely permeable to water. RBCs are not lysed in normal buffered (pH 7) saline solution (9 g/L NaCl). The osmotic fragility test measures the ability of RBCs to withstand lysis when suspended in buffered solutions of various concentrations of sodium chloride (NaCl). The pattern of lysis helps to determine whether the shape of RBCs is normal or abnormal. Spherocytes and stomatocytes hemolyze with higher concentration of NaCl compared to normal red blood cells.

Principle

- In osmotic fragility test, small volumes of blood are mixed with large excess of buffered saline of varying concentration.
- When the red cells are suspended in buffered hypotonic solutions (i.e., the strength of the saline solution is decreased below normal saline), their volume progressively increases till a "critical hemolytic volume" is reached. At this critical point, the red cell membrane ruptures and liberates hemoglobin into the supernatant solution. The ability of normal RBCs to withstand hypotonic solution, lies in its biconcave shape which allows considerable water to enter the RBC before it stretches enough to break or lyse.
- If RBCs are spherocytic, there is loss of membrane which causes reduction in their surface area. The spherocytes cannot expand as much as normal biconcave RBCs and there is decrease in the critical hemolytic volume. When the strength of the saline solution is decreased (hypotonic) spherocytic red cells (which do not have normal biconcave shape) will begin to **hemolyze more than normal red cells.** The sooner hemolysis occurs, the greater the osmotic fragility of the cells. Incubation of blood overnight at 37°C makes the osmotic fragility test (OFT) more sensitive.

Interpretation

- **Normal:** Lysis of fresh RBCs begins at 0.5% of NaCl solution and is complete at 0.3%. It may be normal in patients with mild HS.
- **Increased osmotic fragility:** Spherocytes having decreased surface/volume ratio, have a limited capacity to expand in hypotonic solutions. Lysis of spherocytes begins early at a higher concentration of NaCl as compared to normal biconcave red cells. This is known as **increased osmotic fragility** and shows a **shift to right** of normal range in the graph **(***refer* **Fig. 11.23)**. Increased osmotic fragility indicates that the red cells are spherocytic. Increased osmotic fragility is observed in **hereditary spherocytosis (hemolysis begins at 0.6 to 0.8% compared**

to normal 0.5% of NaCl solution), hereditary elliptocytosis and other **conditions associated with spherocytosis, like in autoimmune hemolytic anemia, ABO hemolytic disease of newborn and burns.**

- **Decreased osmotic fragility:** Conversely, hypochromic and flat red cells have an increased capacity to expand in hypotonic solutions. They lyse at a lower concentration than do normal cells, and thus have decreased osmotic fragility. The graph shows **shift to the left** of normal range and indicate that the red cells are flattened or hypochromic. This type of RBCs have increased surface/volume ratio and are found in **iron deficiency, thalassemia (target cells have increased surface volume), sickle cell anemia, liver disease and reticulocytosis.**

Use: For the diagnosis of spherocytosis. However, it is time-consuming, tedious and cannot distinguish between the causes of spherocytosis. This test may be negative if there are only few spherocytes in the peripheral blood. Sensitivity of the test can be increased by incubation the red cells at 37°C for 24 hours before performing the test. This test is termed as osmotic fragility test after incubation.

LABORATORY EVALUATION OF HEMOSTATIC DISORDERS

Q. Discuss laboratory diagnosis in a case of bleeding disorder.

The clinical laboratory evaluation is an integral part of the diagnosis and management of patients with bleeding disorders. Laboratory tests for hemostatic disorders depend on the component of hemostasis that is involved **(Table 14.10)**.

Most bleeding disorders are caused by one of three defects: (i) a defect in platelet number or function, (ii) a defect in platelet–vessel wall interactions (i.e., an abnormality in the adhesive interactions between platelets and the vessel wall) or (iii) a defect or deficiency in coagulation factor. The diagnosis of bleeding disorders requires a battery of tests **(Table 14.11)** which include screening tests done in all patients with history of bleeding, followed by specific tests to identify the exact nature of the disorder.

Approach to bleeding disorder is shown in **Flowchart 14.1**.

Tests for Platelet Component

Platelet Count

It is obtained on anticoagulated blood either manually or by electronic particle counters.

- **Normal range of platelet count:** 1,50,000–4,50,000 platelets/cu mm.
- **Causes** of thrombocytopenia (*refer* **Box 13.4**).

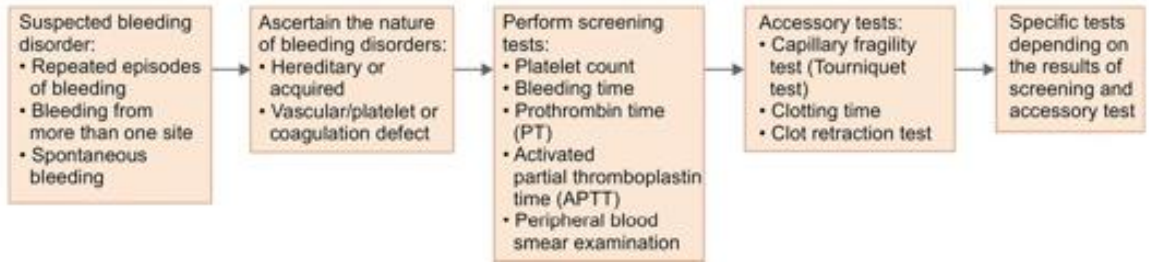
TABLE 14.10: Laboratory tests for hemostatic disorders depending on the component involved.

Platelet component	*Plate-vessel wall interaction*	*Coagulation component*
• Platelet count • Platelet aggregation test • Clot retraction test	• Capillary fragility test (Tourniquet test) • Bleeding time	• Prothrombin time (PT) • Activated partial thromboplastin time (APTT) • Prothrombin consumption time • Factor assay

TABLE 14.11: Laboratory tests in bleeding disorders.

Screening tests	*Accessory tests*	*Others*
• Platelet count • Bleeding time • Prothrombin time (PT) • Activated partial thromboplastin time (APTT) • Peripheral smear	• Capillary fragility test (Tourniquet test) • Clot retraction test	• Prothrombin consumption time • Factor assay

Flowchart 14.1: Approach to bleeding disorder.



Platelet Aggregation

These tests measure the ability of platelets to aggregate in response to agonists like thrombin and form the basis for qualitative tests of von

Willebrand factor. They are used for classification of congenital qualitative disorders of platelets.

Clot Retraction Test

Q. Write short essay/note on clot retraction test and causes of impaired clot retraction.

After the coagulation of blood, the clot under the action of thrombosthenin (a substance released from platelets) undergoes contraction and starts retracting within one hour. The clot shows 50% retraction in 2–4 hours. This process is completed in 18–24 hours with separation of serum. Subsequently, the fibrin clot dissolves due to fibrinolysis and the RBCs sink to the bottom. Clot retraction is dependent on normal platelet number, platelet function, concentration of fibrinogen and the activity of the fibrinolytic pathway.

- **Normal value:** Normal clot retraction shows more than 50% of serum separated at the end of 24 hours. A normal clot is firm, rubbery, elastic and not easily broken.
- **Interpretation:** Absent or reduced clot retraction is seen in:
  - Fibrinogen deficiency (congenital or acquired).
  - Thrombocytopenia.
  - Thrombasthenia.

Tests for Platelet and Vascular Component

Capillary Fragility Test (Hess Test/Tourniquet Test)

Q. Write short essay/note on capillary fragility test (Hess test/Tourniquet test).

It measures the ability of capillaries to withstand the increased stress.

Procedure: Sphygmomanometer cuff is tied to the upper arm above the elbow and the cuff is inflated to 80 mm for 5 minutes. Release the pressure after 5 minutes. The number of petechiae present in a circle of 5 cm diameter on the flexor aspect of forearm (below the bend of the elbow) is noted.

- **Normal:** 0–5 petechiae.

- **Interpretation:** Positive test is indicated by more than 10 petechiae and is found in:
  - Vessel wall abnormalities: Vascular purpura, scurvy.
  - Platelet disorders: Thrombocytopenia, defective platelet function.

Bleeding Time

Q. Write short essay/note on bleeding time, its definition, normal range and methods.

Bleeding time (BT) is used as screening test for disorders of platelet-vessel wall interactions. It measures the time required for bleeding to stop after a standardized superficial cut of the skin capillary bed.

Methods: Duke's method (obsolete), Ivy's method and template method (method of choice).

Template Method for Bleeding Time

Template is a disposable blade fitted on to a holder made of plastic and is used for the test. The blade projects through the bottom so that the incision made through the slit is 9 mm long and 1 mm deep.

Principle: A small skin cut of a standard size and depth is made and the oozing blood is wiped with a filter paper. Bleeding stops when the capillaries contract and platelet plug seals the vessel.

Normal range: 2–9 minutes.

Uses: Tests for bleeding time is prone to problems of reproducibility, sensitivity and specificity. Though, it is one of the screening tests, it is not recommended as routine preoperative screening test. This test evaluates the defects of primary hemostasis. Thus, bleeding time measures the platelet-vessel wall interactions (integrity of capillary and platelet function).

Note: The bleeding time usually is not prolonged in patients with coagulation factor deficiencies.

Interpretation: Prolonged bleeding time is found in:

- **Platelet disorders:**
  - **Quantitative:** Thrombocytopenia. If platelet count is below 50,000/ mm^3, BT should not be performed as bleeding may be difficult to

stop.
    - **Qualitative:** von Willebrand disease, Bernard–Soulier syndrome, Glanzmann's thrombasthenia.
- **Primary vascular disorders:** Ehlers–Danlos syndrome.
- **Platelet-vessel wall interactions:** von Willebrand disease.
- **Others:** Afibrinogenemia, severe hypofibrinogenemia, uremia, aspirin.

Tests for Coagulation Component

Complete blood count and peripheral blood smear examination is important in case of any hemostatic disorder. It can provide useful information. For example, fragmented red cells in DIC, leukemic cells, thrombocytopenia.

Coagulation or Clotting Time (Lee–White Method)

Q. Write short essay/note clotting time and its normal range.

It measures the time taken for the fresh blood to clot.

Normal: 4–11 minutes.

Disadvantages

- **Not a sensitive test:** It is one of the oldest tests and is not sensitive as it fails to detect mild/moderate coagulant defects. Hence, it is obsolete now and not recommended as a screening test. PTT is a more sensitive test for assessment of the coagulation cascade. Clotting time is prolonged only with severe deficiency of factor VIII, IX or fibrinogen (afibrinogenemia) and in heparin therapy.
- **Misleading:** Normal value may be obtained in mild-to-moderately severe hemophilia A and B and it does not exclude major factor deficiency.

Quick's One Stage Prothrombin Time

Q. Write short essay/note on prothrombin time (PT) and its normal range.

Principle: Prothrombin time (PT) is the time taken by citrated plasma to clot after the addition of tissue thromboplastin and calcium. It tests ***extrinsic and common pathway*** of coagulation system.

Normal range: 11–16 seconds.

Reporting of prothrombin time: Prothrombin time may be reported in different ways:

- Patient PT and control PT in seconds.
- Ratio of patient PT to control PT.

Q. Write short note on INR.

International normalized ratio (INR): Due to the inherent variation in the sensitivity of thromboplastin reagents, it is advisable to report the PT in INR. It provides a uniform scale inspite of using different sources of thromboplastin. WHO recommended that each thromboplastin should have ISI (International sensitivity index) value. INR is calculated using the formula: INR = (PT patient in seconds/ PT normal plasma in seconds) ISI.

Uses of Prothrombin Time

- **Screening test to evaluate coagulation disorders:** It measures coagulation factor I, II, V, VII and X. Deficiency of any one of these factors leads to prolongation of PT. It should be used along with PTT.
- **To monitor oral anticoagulant therapy.**
- **To evaluate liver function:** Liver disease can result in deficiency of the coagulation factors. Hence, PT should be performed before a *liver biopsy* and *prolonged* PT is a contraindication for liver biopsy.

Interpretation: Prolonged PT is seen in:

- Liver disease.
- Administration of oral anticoagulants like coumarin.
- Vitamin K deficiency: Obstructive jaundice, hemorrhagic disease of the newborn.
- Deficiency of factors I, II, V, VII and X.
- Disseminated intravascular coagulation (DIC).

Activated Partial Thromboplastin Time(Partial Thromboplastin Time)

Q. Write short essay/note on activated plasma thromboplastin time (APTT) and its normal range.

Principle: Activated partial thromboplastin time (APTT) is the time taken for citrated plasma to clot in the presence of a surface activator (kaolin), phospholipid and calcium. Partial thromboplastin time (PTT) is a measure of the ***intrinsic*** and ***common coagulation pathways*.**

Normal range: 30–40 seconds.

Reporting: The patient's value is always to be reported with the control values in seconds. A prolongation of the patient value >8 seconds of the control value is considered as abnormal.

Uses

- **Best single screening test for coagulation disorders.** This test is abnormal with deficiencies of II, V, VIII, IX, X, XI and XII.
- For screening hemophilia A and B.
- For detecting coagulation inhibitors.
- For monitoring anticoagulant therapy like heparin.

Interpretation: Common causes of a prolonged APTT are as follows:

- Inherited coagulation disorders—deficiency of factor II, V, VIII, IX, X, XI, XII (e.g., hemophilia A, hemophilia B).
- von Willebrand disease.
- Disseminated intravascular coagulation.
- Heparin therapy.
- Vitamin K deficiency.
- Liver disease.
- Oral anticoagulant therapy.

Summary of screening tests for bleeding disorders are presented in **Table 14.12**.

TABLE 14.12: Summary of screening tests for bleeding disorders.

Investigation	*Normal range*	*Main causes of abnormal test*
Platelet count	150–450 × 10^9/L	Thrombocytopenia
Bleeding time (template method)	2–9 minutes	Thrombocytopenia, abnormal platelet function, deficiency of von Willebrand factor, vascular abnormalities
Coagulation/clotting time (Lee–White method)–not a sensitive test	4–11 minutes	Severe deficiency of factor VIII, IX or fibrinogen (afibrinogenemia) and in heparin therapy
Prothrombin time (PT)	11–16 seconds	Deficiency of factor II, V, VII or X
Activated plasma thromboplastin time (APTT)	30–40 seconds	Deficiency of factor II, V, VIII, IX, X, XI, XII, heparin, antibodies against clotting factors, lupus anticoagulant

URINE ANALYSIS

CC (Show How): Describe abnormal urinary findings in disease states and identify and describe common urinary abnormalities in a clinical specimen.
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Urine analysis (urinalysis) reflects the state of function of the kidneys and urinary tract. It provides information about metabolic or systemic (nonrenal) disorders. Urine examination should precede all other invasive/non-invasive diagnostic investigations for renal function.

Methods of Collection

Q. Write short essay/note on urine sample collection.

- **Midstream specimen:** This is used for all types of urine examination. After voiding first half of urine into the toilet, a part of the next voided urine is collected as midstream sample.
- **Clean-catch specimen:** This is the method of collection for culture and sensitivity of urine. The external genitalia are cleaned with soap and water and specimen is collected as mentioned in the midstream sample.
- **Catheter specimen:** It is used for culture and sensitivity in bed-ridden patients or patients with urinary tract obstruction.
- **Collection from infants:** It is usually collected by a clean plastic bag attached around the genitalia. For bacteriological examination urine is aspirated from bladder by needle.

Preservation of Urine

Q. Write short essay/note on preservative for urine collection.

Urine sample should be **examined within 2 hours** of collection. If delay is likely to occur, it should be preserved by one of the following methods:

- Refrigeration without any preservative
- Use of preservatives like toluene (add few drops to form a thin layer on the urine surface), concentrated HCl (in the ratio of 1mL for 125 mL of

urine), thymol and formaldehyde (add 1 drop for 15 mL of urine—preserves cells and casts).

Examination of Urine

Urine examination consists of: (i) physical examination, (ii) chemical examination and (iii) microscopic examination.

Physical Examination

Q. Write short essay/note on physical examination of urine.

Volume

Q. Write short answer on volume of urine.

Q. Write short answer on oliguria.

A healthy adult excretes about 600–2000 mL of urine in 24 hours. In infants, the volume is 300–600 mL/day. Volume is measured by collecting 24-hour urine samples in a measuring cylinder.

- ***Polyuria:*** Increased urine output (>2 liters in 24 hours). Its causes include physiological (e.g., increased fluid intake) or pathological (e.g., diabetes mellitus, chronic renal diseases, diuretic therapy, diabetes insipidus, primary aldosteronism).
- ***Oliguria*:** Decreased urinary output (less than 500 mL in 24 hours). Its causes include restricted intake of fluid, excessive loss of fluid (e.g., in hemorrhage, burns, dehydration and shock) renal diseases (e.g., acute glomerulonephritis, nephrotic syndrome, acute tubular necrosis as in shock, burns, crush syndrome, incompatible blood transfusion and heavy metal poisoning) and Addison's disease.
- ***Anuria*:** Markedly diminished urine output, usually less than 125 mL in 24 hours. Causes include renal ischemia, tumors and renal stones.

Color (Table 14.13)

Normal urine is **straw to amber colored** due to the presence of urochrome pigment, excretion of which is generally proportional to the metabolic rate.

Q. Write short essay/note on chyluria.

Chyluria is a rare condition in which the **urine contains lymph. Obstruction to lymph flow** and rupture of lymphatic vessels into the renal pelvis, ureters, bladder or urethra may be associated with chyluria. The causes include **filariasis, abdominal lymphadenopathy and tumors**. The amount of lymph determines the color of urine which may range from clear to opaque or milky.

Lipiduria: In nephrotic syndrome, urine shows fat globules which are triglycerides (neutral fat) and cholesterol. It may also be observed in patients with bone fractures.

Specific Gravity

Q. Write short answer on specific gravity of urine including normal values and methods of estimation.

TABLE 14.13: Conditions associated with color changes in urine.

Color	***Condition***
Colorless	Dilute urine as in polyuria (e.g., diabetes mellitus, diabetes insipidus)
Dark brown	Oliguria
Smoky (red or red-brown)	Red blood cells (hematuria)
Cola colored	Intravascular hemolysis
Yellow-brown	Bilirubin
Orange-brown	Urobilin/urobilinogen
Dark colored on standing	Alkaptonuria (presence of homogentisic acid)
Milky	Pus or chyle (chyluria)

Specific gravity depends on the number, density and weight of the solute particles in the urine. It is used as a measure of the concentrating power of the kidney. Normal specific gravity of a 24-hour urine sample is 1.003–

1.035, average being 1.016. Specific gravity provides information about the renal status and hydration.

Methods: Specific gravity (SG) is measured by: (i) urinometer, (ii) refractometer and (iii) dipstick method. Urinometer and refractometer methods are more accurate as compared to the dipstick method.

Q. Write short answer on causes of increased and decreased specific gravity of urine.

Causes of increased specific gravity: Glycosuria (e.g., diabetes mellitus), proteinuria (e.g., nephrotic syndrome), dehydration (e.g., restricted fluid intake, diarrhea, vomiting, fever and excessive sweating).

Causes of decreased specific gravity: Excessive fluid intake, diabetes insipidus and end-stage kidney (e.g., chronic glomerulonephritis, chronic pyelonephritis, bilateral polycystic kidneys and hypertension).

Q. Write short answer on low fixed specific gravity of urine and its importance.

Isosthenuria: When **specific gravity of urine is low and fixed at 1.010**, this is known as isosthenuria. In this condition, the urine whose specific gravity (concentration) is neither greater (more concentrated) nor less (more dilute) than that of protein-free plasma (1.008–1.012). It is indicative of severe renal damage (chronic renal failure) with disturbance of both the concentrating and diluting abilities of the kidney. It reflects damage to the kidney's tubules or the renal medulla.

Q. Write short answer on hyposthenuria.

Hyposthenuria: Hyposthenuria is the secretion of urine of low specific gravity (<1.008) due to inability of the kidney to concentrate the urine normally. It is closely related to isosthenuria in which the urine has a relatively low specific gravity, though not necessarily equal to that of plasma. Therefore, unlike isosthenuria, this condition is not associated with renal failure as the kidney tubules have altered the glomerular filtrate. Hyposthenuria indicates that the kidney can dilute the urine but is unable to concentrate, i.e., proximal renal tubule and loop of Henle function is retained but the tubules are unresponsive to ADH, either from a primary

ADH deficiency (central diabetes insipidus) or lack of responsiveness of renal tubules to ADH due to renal tubular disease or inhibitors of ADH (nephrogenic diabetes insipidus).

Chemical Examination

Proteinuria

Q. What is proteinuria? Name the methods of its detection.

The presence of detectable protein in the urine is known as **proteinuria**. It indicates glomerular injury. If urine is turbid, filter or centrifuge it before testing.

Test for Protein in Urine (Box 14.5)

Q. Write short answer on tests for proteinuria.

BOX 14.5: Tests for protein.

- Heat and acetic acid test
- Sulfosalicylic acid test
- Heller's test
- Dipstick method

Heat and acetic acid test

Principle: Heat-induced **coagulation of proteins** and precipitation. Coagulation can be further enhanced when drops of acetic acid are added.

- ***Procedure:*** Take a 5 mL test tube and fill 2/3rd with urine. Acidify by adding a few drops of 3% acetic acid if urine is alkaline. Boil upper portion for 2 minutes (lower part acts as control). If precipitation or turbidity appears, add a few drops of 3% acetic acid.
- ***Interpretation:*** If turbidity or precipitation disappears on addition of acetic acid, it is due to phosphates; if it persists after addition of acetic

acid, then it is due to proteins. The test is semiquantitative and can be graded from traces to 4+ depending upon amount of protein.

Sulfosalicylic acid test

Q. Write short answer on sulfosalicylic acid test.

- This test **detects all types of proteins** (albumin, globulin, glycoproteins and Bence–Jones proteins).

Principle: Cold precipitation of proteins by a strong acid. More sensitive and reliable than heat and acetic acid test.

- ***Procedure:*** Take 2.5 mL of urine in a small test tube. Slowly pour 2.5 mL of 3% sulfosalicylic acid. Wait for 10 minutes.
- ***Interpretation:*** Presence of a **cloudy precipitate** indicates the presence of proteins in urine. However, it also precipitates mucus and Bence–Jones proteins.

Heller's test

Principle: Cold precipitation of proteins by a strong acid.

- ***Procedure:*** Take 5 mL of urine in a test tube and add a few drops of concentrated nitric acid.
- ***Interpretation:*** Presence of proteins is indicated by a **white ring** at the junction of urine and acid.
- ***Precaution:*** Filter urine if turbid as it interferes with the final reading.

Dipstick method

Principle: The test is based on the protein error of pH indicators. At a constant pH any color change in the indicator is due to protein. The reagent strip is impregnated with an indicator tetrabromophenol blue or tetrachlorophenol-tetrabromosulfophthalein buffered to pH 3.0. At this pH it is yellow in the absence of protein. If urine contains protein (which will elicit a pH change), it forms a complex with the indicator turning its color to green or bluish-green.

Quantitative Estimation of Proteins in Urine

Q. Write short answer on Esbach's albuminometer.

Protein excretion in a 24-hour urine sample is required in suspected cases of nephrotic syndrome (>3.5 g/24 hours) and orthostatic/postural proteinuria. This may be carried out by Esbach's albuminometer method.

Principle: Cold precipitation of proteins by a strong acid.

Procedure: Fill the albuminometer with urine up to the mark U. Add Esbach's reagent up to the mark R. Stopper the Esbach's albuminometer, mix and allow it to stand for 24 hours. Take the reading from the level of precipitation in the albuminometer and divide the value by 10 to get the percentage of total proteins.

Categories of Proteinuria

- **Proteinuria quantification:** Classified depending on the amount of protein in the urine as **heavy** (>4 g/day), **moderate** (1–4 g/day) and **mild** (<1 g/day) proteinuria.
- **Qualitative categories of proteinuria:** Classified depending on the structure involved as (i) **renal** (glomerular causes/pattern, tubular cause/pattern), (ii) **prerenal** and (iii) **postrenal**.
- **Types of glomerular proteinuria.**

Q. Write short essay/note on selective proteinuria.

- **Selective proteinuria:** In this type, only intermediate-sized (<100 kDa) proteins (such as albumin, transferrin) leaks through the glomerulus.
- **Nonselective proteinuria:** It is characterized by leakage of range of different proteins including larger proteins (e.g., immunoglobulins) through the glomerulus.

Causes of Proteinuria (Box 14.6)

Q. Write short note/answer on causes of proteinuria.

Bence–Jones Proteins

Bence–Jones proteins are light chains of immunoglobulins, secreted in **multiple myeloma.** It may also be found in macroglobulinemia and

lymphoma. Bence–Jones proteins precipitate at temperature between 40°C and 60°C, and redissolve near 100°C. It reappears on cooling to 40–60°C.

Microalbuminuria

Screening tests for urine protein are not sensitive enough to detect very small quantities of protein. **Microalbuminuria is the presence of albumin in urine above the normal level but below the detectable range of conventional methods.** It is defined as the **persistent elevation of the urinary albumin excretion of 20–200 mg/L** (or 20–200 mg/min) in an **early morning urine sample**. It indicates early and possibly reversible glomerular damage.

Causes of microalbuminuria

- **Diabetes mellitus:** In diabetic patients, presence of microalbuminuria is associated with **increased cardiovascular mortality** and is a risk factor for **renal mortality.** Early detection can predict the development of renal complications in diabetics.
- **Essential hypertension:** In hypertensive patients, microalbuminuria **predicts cardiovascular morbidity and mortality**.

Reducing Substances in Urine

Reducing substances are those compounds which **reduce cupric ions (from copper sulfate in Benedict's reagent) in an alkaline solution to cuprous ions (cuprous oxide)**. Such substances may be sugar or nonsugar.

- **Sugar:** These include: Glucose, fructose, pentose, galactose, lactose, pentose and maltose. Sucrose gives negative result with Benedict's test and glucose oxidase. Special tests are required to differentiate the sugar occurring in urine.
- **Nonsugar:** Ascorbic acid, uric acid, urates, glucuronides, chloroform, formaldehyde, salicylates, streptomycin, phenol, PAS, homogentisic acid (alkaptonuria) and creatinine.

BOX 14.6: Causes and amount of proteinuria.

1. **Renal**
   A. **Glomerular damage:** Due to increased permeability of glomerular capillary wall
      - **Heavy proteinuria:** Nephrotic syndrome
      - **Moderate to heavy:** Acute rapidly progressive glomerulonephritis, chronic glomerulonephritis, malignant hypertension, SLE
      - **Mild to moderate:** Acute glomerulonephritis, nephrosclerosis

   B. **Tubular damage:** Inability to absorb low molecular weight proteins by tubules
      - **Moderate:** Heavy metal and vitamin D intoxication
      - **Mild:** Acute and chronic pyelonephritis, acute tubular necrosis, polycystic kidney disease, hypokalemia
2. **Prerenal**
   A. **Overflow proteinuria:** Develops due to the overflow of excess levels of a protein in the circulation. In this protein in the urine may be hemoglobin, myoglobin, or immunoglobulin
      - **Immunoglobulin: Moderate proteinuria** in plasma cell dyscrasias (Bence–Jones protein-light chain of immunoglobulin)
      - **Hemoglobin:** In intravascular hemolysis
      - **Myoglobin:** In skeletal muscle trauma
      - **Lysozyme:** Acute myeloid leukemia M4 or M5

   B. **Hemodynamic proteinuria:** Due to alteration of blood flow through glomeruli
      - **Mild asymptomatic proteinuria:** After excessive exercise, postural (orthostatic) albuminuria, congestive cardiac failure, high fever, exposure to cold, dehydration
3. **Postrenal**
   Inflammatory or neoplastic lesions of renal pelvis, ureter, bladder, prostate or urethra

BOX 14.7: Tests for reducing substances in urine.

- Benedict qualitative test
- Dipstick method

BOX 14.8: Causes of glycosuria.

Glycosuria with hyperglycemia

- Endocrine disorders
  - **Diabetes mellitus**
  - Acromegaly
  - Cushing's syndrome
  - Hyperthyroidism
  - Hyperadrenocorticism
  - Functioning α- or β-cell pancreatic tumors
  - Pheochromocytoma
- Non-endocrine diseases
  - Increased intracranial tension (brain tumor or hemorrhage)
  - Liver disorders
  - Drugs: Corticosteroids, adrenocorticotrophic hormones, thiazides
  - Alimentary glycosuria

Glycosuria without hyperglycemia

- Renal glycosuria

- Pregnancy

Blood glucose level varies between 70 and 120 mg/dL. This may increase to 120–160 mg/dL after a meal. Normally, all the glucose in the blood is filtered through glomerulus and reabsorbed at proximal tubules. If the renal threshold (the lowest blood glucose level that will result in glycosuria) is exceeded (usually greater than 180–200 mg/dL), the excess glucose will not be reabsorbed into the blood and will be eliminated in the urine as in cases of diabetes mellitus. The presence of detectable amounts of **glucose in urine** is termed **glycosuria**.

Tests for Reducing Substance

Q. Write short note on test for detection of glycosuria.

Tests for reducing substance is discussed in **Box 14.7**.

Benedict qualitative test (semiquantitative)

Q. Write short note on principle of Benedict test.

This test detects the presence of reducing substances in urine and is not specific for glucose.

Principle: The copper sulfate present in the Benedict's reagent reacts with the **reducing substances** in the urine which **convert cupric sulfate to cuprous oxide** in hot alkaline media. Thus, this test is based on the reduction of cupric ions in Benedict's solution to cuprous ions. In the absence of reducing substances in urine, the color of the reagent remains blue.

Procedure: Take **5 mL of Benedict's** (qualitative) reagent in a test tube. Boil to exclude presence of reducing substance in reagent. Add **8 drops (0.5 mL) of protein-free urine.** Boil the mixture for 5 minutes and allow to cool. The **ratio** of 5 mL Benedict's reagent and 8 drops (0.5 mL) of urine **is important because it is a semiquantitative test.** Note the color of the precipitate which is cuprous oxide formed due to reduction of cupric sulfate of Benedict's reagent.

Interpretation: The change of color from blue to green, yellow and orange/red depends on the amount of sugar present.

Dipstick method

Principle: Diastix/multistix/dipstix contains: (i) Glucose oxidase (ii) peroxidase (iii) chromogen: O-toluidine (clinistix) or potassium iodide (multistix/diastix). Glucose present in the urine is oxidized by atmospheric oxygen in the presence of glucose oxidase to gluconic acid and hydrogen peroxide. The hydrogen peroxide, in the presence of peroxidase, oxidizes the reduced chromogen present in the dipstick to various shades of purple (oxidized chromogen). The color change depend on the amount of glucose (semiquantitative) present in the urine.

Cause of Glycosuria (Box 14.8)

Q. Write short note on causes of glycosuria.

The substances which may give positive reaction with Benedict's test but negative with glucose oxidase strips are nonsugar substances, such as ascorbic acid, glucuronic acid, homogentisic acid, salicylates, phenylketonuria and tyrosinemia.

Renal glycosuria: Renal glycosuria is a benign condition due to a reduced renal threshold for glucose. It is unrelated to diabetes and not accompanied by the classical symptoms of diabetes. Therefore, it should not be mistaken as diabetes mellitus.

Alimentary glycosuria: In certain individuals, blood glucose level may rapidly increase after meals resulting in its spill over into urine. This condition is known as alimentary (lag storage) glycosuria. It may be seen in some normal individuals and some patients with hepatic diseases, hyperthyroidism and peptic ulcer.

Fructosuria: Presence of fructose in urine. It is detected by Seliwanoff's test. *Causes* include high intake of fruits like grapes and oranges.

Lactosuria: Presence of lactose in urine. It is detected by Rubner's test. *Causes*—suckling infants and nursing mothers.

Pentosuria: Presence of pentose in urine. It is detected by Bial's test. *Cause*—ingestion of fruits.

Ketone Bodies

BOX 14.9: Tests for ketone bodies.

- Rothera's test
- Gerhardt's test (ferric chloride test)
- Hart's test
- Dipstick method

Q. Write short note on ketone bodies found in urine and tests for its detection.

The presence of ketone bodies in the urine is a measure of the metabolic rather than renal function. Whenever there is a defect in carbohydrate metabolism, the fat is used as a source of energy. When increased quantities of fat are metabolized, there is increased production of ketone bodies which begin to accumulate in the blood and are subsequently excreted in the urine.

Ketone bodies are three metabolically related compounds:

- **Acetoacetic (diacetic) acid.**
- **β-hydroxybutyric acid.**
- **Acetone**.

If urine is left at room temperature, acetoacetic acid slowly converts into acetone.

Tests for Ketone Bodies

Tests for ketone bodies is discussed in **Box 14.9**.

Rothera's test

Q. Write short note on Rothera's test.

Principle: Acetoacetic acid (diacetic acid) **and acetone react with sodium nitroprusside** in presence of an alkali to form a purple color compound.

Procedure: Take 4 mL of urine in a test tube. Add a few crystals of sodium nitroprusside and saturate the urine with ammonium sulfate by mixing vigorously. Overlay with few drops of liquor ammonia along the wall of the tube.

Interpretation: Development of a **purple ring** indicates the presence of **acetoacetic acid/acetone** or both. A brown or red color is of no significance.

Gerhardt's test (ferric chloride test)

This is neither a very specific nor a sensitive test. The test **detects acetoacetic acid.**

Principle: Acetoacetic acid reacts with ferric chloride to give a deep red color.

Hart's test

This test detects β-**hydroxybutyric acid**, which the above two tests fail to detect. β-hydroxybutyric acid is usually accompanied by acetoacetic acid.

Principle: β-hydroxybutyric acid is converted into acetone which **reacts with sodium nitroprusside and liquor ammonia** to give purple red color.

Dipstick method

Principle: Dipstick contains buffers and sodium nitroferricyanide, which react with acetoacetic acid in the urine to form a pink-maroon color in 15 seconds. The **strips detect acetoacetic acid and not acetone**. However, acetone can be detected if glycine is incorporated into it.

Causes of Ketonuria (Box 14.10)

Q. Write short note on causes of ketonuria. List the nondiabetic causes.

Bilirubin (Bile Pigment)

Q. Write short note on bile pigments in urine.

Tests for bilirubin in urine provide information concerning metabolic or systemic disorders, especially liver function. **Bilirubin is a breakdown product of hemoglobin and is normally not present in urine.** Even trace amounts are clinically significant and only conjugated bilirubin is found in urine. Bilirubinuria causes yellow-brown to greenish-brown urine and forms yellow foam on shaking. Bilirubin is found only in freshly voided urine which upon standing is oxidized to biliverdin.

Tests for Bilirubin

Tests for bilirubin is discussed in **Box 14.11**.

Fouchet's test

Principle: Fouchet's reagent contains **trichloroacetic acid and ferric chloride.** In an acidic medium ferric chloride oxidizes **bilirubin** to produce a dark green colored **biliverdin.**

Dipstick method

Principle: The test is **based on a diazo reaction.** Bilirubin in the urine couples with a diazotized dichloronaniline (content of strip) in a strongly acidic medium to form colored compound namely azobilirubin.

Causes of Bilirubinuria (*refer* page 409)

- **Obstructive jaundice:** Urine shows bilirubin without urobilinogen. Bilirubin is of conjugated type.
- **Hepatocellular jaundice:** In acute viral hepatitis, bilirubin appears in the urine before the jaundice is clinically apparent. In a patient with pyrexia of unknown origin bilirubinuria indicates hepatitis.

BOX 14.10: Causes of ketonuria.

Diabetic ketonuria
- Diabetic ketoacidosis

Nondiabetic ketonuria
- Starvation
- Prolonged vomiting or diarrhea
- Infant and children
  - Prolonged febrile illness
  - Toxic states accompanied by vomiting or diarrhea
  - Glycogen storage disorders (von Gierke's disease)
- Hyperemesis of pregnancy

Lactic acidosis
- Shock, diabetes mellitus, renal failure, liver disease, infection, and drugs (e.g., phenformin and salicylate poisoning)

BOX 14.11: Tests for bilirubin.

- Fouchet's test
- Dipstick method

BOX 14.12: Tests for urobilinogen.

- Ehrlich's test
- Dipstick method

BOX 14.13: Tests for blood.

- Benzidine test
- Orthotoluidine test
- Dipstick method

- **Hemolytic jaundice: Bilirubin is absent in urine.** This is because in hemolytic anemia the hyperbilirubinemia is due to unconjugated bilirubin which is not water insoluble. Hence, not excreted in the urine.

Urobilinogen

Q. Write short note on urobilinogen in urine.

Urobilinogen is normally present in urine in trace amount (1–2 mg/dL) and is insufficient to cause a significant positive reaction. Whenever the liver is unable to efficiently remove the reabsorbed urobilinogen from the portal circulation (e.g., liver diseases, hemolytic anemia) more urobilinogen than normal is routed through the kidney and hence, excreted in the urine.

Tests for Urobilinogen

Tests for urobilinogen is discussed in **Box 14.12**.

Ehrlich's test

Principle: Ehrlich's reagent reacts with urobilinogen and forms a pink-colored aldehyde complex.

- ***Interpretation:*** Pink to cherry-red color indicates presence of urobilinogen.

Dipstick method

Principle: This method is based on the Ehrlich's test. The strip is coated with p-amino benzaldehyde with a color enhancer. It reacts with urobilinogen in a strongly acidic medium to produce a pink-red colored compound. The color is matched with the color chart on the container of the dipsticks.

Urobilinogen is normally excreted in the urine in traces.

Causes of Increased Urobilinogen in Urine(*refer* page 409)

Q. Write short note on urobilinogen in urine or in jaundice.

- **Hemolytic anemias** (without bilirubin in urine): Thalassemia, sickle cell anemia, hereditary spherocytosis.
- **Liver diseases:** Preicteric phase of infective hepatitis, drugs or toxic hepatitis, cirrhosis.

Causes of Decreased/Absent Urobilinogen in Urine

In **obstructive jaundice,** bilirubin does not reach the intestine and hence, is not converted into urobilinogen.

Bile Salts

Q. Write short note on bile salts in urine and test for its detection.

Bile salts are composed of mixture of bile acids and glycine or taurine. Two important bile salts are sodium and potassium salts of glycocholate and taurocholate. Normally, bile salts are not present in urine.

Hay's Sulfur Test

Q. Write a short note on Hay's sulfur test.

Principle: Bile salts have unusual property of **lowering the surface tension of urine** markedly even when present in small concentrations. This property is made use of in the Hay's test.

Interpretation: **Sulfur powder sinks to the bottom** of test tube in the presence of bile salts in urine.

Causes: Hepatocellular and obstructive jaundice (*refer* page 597).

Tests for Blood in Urine

Q. Write short note on tests for blood in urine.

These tests detect hematuria, hemoglobinuria or myoglobinuria.

Hematuria: Presence of abnormal number of red cells in urine, e.g., renal stones, renal cell carcinoma.

Hemoglobinuria: Presence of free hemoglobin in urine, e.g., intravascular hemolysis.

Myoglobinuria: Presence of myoglobin in urine, e.g., crush injury to muscle, strenuous exercise.

Tests for Blood

Tests for blood is discussed in **Box 14.13**.

Benzidine test

Q. Write short answer on principle of benzidine test.

Principle: The test depends upon the ability of **heme compounds** derived from hemoglobin to **catalyze the oxidation of benzidine by hydrogen peroxide.**

- ***Procedure:*** Dissolve a small amount (knife-point full) of benzidine in 2 mL of glacial acetic acid and add an equal volume of 3% hydrogen peroxide. From this, take 2 mL in another test tube and add 2 mL of previously boiled and cooled urine and mix.
- ***Control for the test:*** If the test is negative, add a drop of blood to the above test tube. It should give blue color. This is to confirm the potency of reagents especially hydrogen peroxide and excludes a false negative reaction.
- ***Interpretation:*** The appearance of **blue color** indicates the presence of blood.
- ***Precaution:*** Presence of hypochlorite (bleach) and microbial peroxidase can cause false positive results. **Benzidine is carcinogenic**.

Orthotoluidine test

Principle: Peroxidase-like activity of heme present in hemoglobin **liberates oxygen from hydrogen peroxide** in the reagent. The liberated **oxygen changes the color of orthotoluidine.**

- ***Interpretation:*** The appearance of blue or green color indicates the presence of blood.

Dipstick method

Very small amount of hemoglobin/RBCs are detected by dipstick method.

Principle: The test is based on the **liberation of oxygen from peroxide in the reagent strip by peroxidase-like activity of heme** present in hemoglobin. The liberated oxygen changes the color of the chromogen. The reagent area is impregnated with organic peroxide and the chromogen is orthotoluidine/tetramethylbenzidine.

Organic peroxide $\xrightarrow{\text{(peroxidase from Hb)}}$ $H_2O + O$

O + chromogen $\xrightarrow{\text{(Orthotoludine)}}$ Oxidized (colored compound)

Causes of Hematuria

Q. Write short note on causes of hematuria.

Urine is red colored in severe hematuria and smoky in mild hematuria. RBCs are demonstrable in urinary sediment. Causes of hematuria are shown in **Box 14.14**.

Hemoglobinuria

Hemoglobin is too large to pass through the glomerular filter. If the renal threshold is exceeded the hemoglobin can pass into the urine which becomes cola colored.

Interpretation: If the supernatant is colored pink, it indicates hemoglobinuria.

Note: Hemoglobin is precipitated when the urine is 80% saturated with ammonium sulfate but myoglobin is not.

Causes of hemoglobinuria: Any cause of hemolysis may cause hemoglobinuria and the presence of hemoglobinuria indicates significant

intravascular hemolysis. These include paroxysmal nocturnal hemoglobinuria (PNH), paroxysmal cold hemoglobinuria (PCH), incompatible blood transfusion, severe burns, autoimmune hemolytic anemia (AIHA), march **hemoglobinuria,** snake bite, blackwater fever (falciparum malaria).

Multistix reagent strips for urine testing: These have single/multiple discrete cellulose squares which are impregnated with reagents for testing glucose, protein, pH, specific gravity, hemoglobin, ketone bodies, bilirubin and urobilinogen. There are single test strips, e.g., diastix for glucose and multistix for multiple tests.

Automated urinalysis: Fully automated urine analyzer provided with automatic functions are presently available, e.g., Uriplus.

Microscopic Examination

Q. Write short note on microscopic examination of urine.

Microscopic examination of urinary deposit or sediment is an essential part of urine examination. The deposits are divided into two main groups; (i) organized deposits and (ii) unorganized deposits.

Organized Deposit (Figs. 14.5 to 14.8)

Organized deposit consists of: (i) **cells** [blood cells (red cells and white cells) epithelial cells (renal, transitional, and squamous)], (ii) **casts** (with or without inclusions), (iii) **crystals** and (iv) **other** abnormal cells or formed elements.

Cells

They are expressed as number of cells per low-power or high-power field.

1. ***Red blood cells (RBCs):*** Presence of RBCs (more than 2/hpf) in the urine indicates bleeding at any point in the urinary system from the glomerulus to the urethra. In **glomerular diseases**, the urine show **red**

cells with cellular protrusions or fragmentationand are named as **dysmorphic** (distorted morphology) red blood cells.

2. ***White blood cells (WBCs):*** Increased number of WBCs (mainly neutrophils more than 5/hpf) in urine is known as **pyuria**. It is indicative of **urinary tract infection.** The causative organism of infection may be identified by bacteriological examination. When accompanied by **leukocyte casts** or mixed leukocyte–epithelial cell casts, increased urinary leukocytes are considered to be of **renal origin.**

BOX 14.14: Causes of hematuria.

Renal diseases

- **Glomerular causes**
  - Acute glomerulonephritis
  - Renal infarct
  - Bacterial endocarditis with kidney involvement
  - Malignant hypertension
- **Nonglomerular causes**
  - Polycystic kidneys
  - Renal cell carcinoma
  - Renal stones (nephrolithiasis)
  - Trauma (including renal biopsy)
  - Renal tuberculosis
  - Hydronephrosis

Lower urinary tract/prostatic diseases

- Bladder stones (lithiasis)
- Cystitis
- Urethritis
- Carcinoma bladder
- Carcinoma prostate

Blood disorders

- Bleeding disorders—coagulation disorders, severe thrombocytopenia
- Acute leukemia
- Sickle cell disease

Miscellaneous

- Instrumentation of urinary tract

Cast and its appearance	Significance
Hyaline cast 	• Nonspecific. Present in normal urine • Acute glomerulonephritis (AGN) • Chronic glomerulonephritis (CGN) • Fever • Congestive heart failure
Waxy cast 	• End stage kidney • Chronic/advanced renal failure • Amyloidosis
Cellular casts	
1. Red blood cell (RBC) cast 	• Always indicates renal disease • AGN • Lupus nephritis • Goodpasture syndrome • Subacute bacterial endocarditis • Renal infarction
2. Leukocyte (WBC) cast 	• Acute pyelonephritis • AGN (proliferative glomerulonephritis) • Interstitial nephritis
3. Epithelial cast 	• Acute tubular necrosis/injury • Viral disease (e.g., cytomegalovirus disease) • Drug • Interstitial nephritis • Transplant rejection
Granular cast (finely or coarsely granular)	
Coarsely granular  Finely granular 	• Tubular damage • CGN • Nephritis with tubular injury • Renal failure
Fatty cast 	• Nephrotic syndrome • Fabry's disease • Other nephritis
Telescoped (mixed) sediment 	• Proliferative GN (systemic lupus erythematosus, polyarteritis nodosa) • Hypersensitivity reaction • Subacute bacterial endocarditis
Bacterial cast 	• Diagnostic of acute bacterial pyelonephritis • Intrinsic renal infections
Broad cast (renal failure casts) 	Progressive renal failure with compensatory hypertrophy of nephron (poor prognosis)

Fig. 14.5: Urinary casts and their significance.

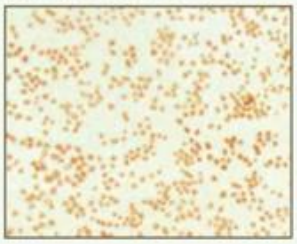
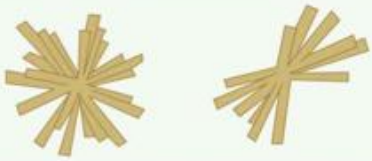
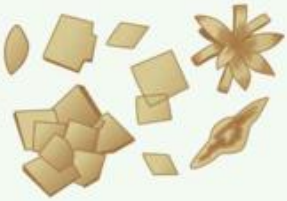
Crystal in normal acidic urine	Condition
Amorphous urates 	• Normal acidic urine • Concentrated urine as in fever and dehydration
Crystalline urates (sodium, potassium and ammonium) 	• Normal acidic urine
Crystalline uric acid 	• Normal acidic urine • Post-chemotherapy for lymphoma and leukemia
Calcium oxalate 	• Normal acidic urine • Large numbers in severe chronic renal disease or ethylene glycol or methoxyflurane toxicity/ poisoning • Nephrolithiasis

Fig. 14.6: Normal crystals found in acidic urine.

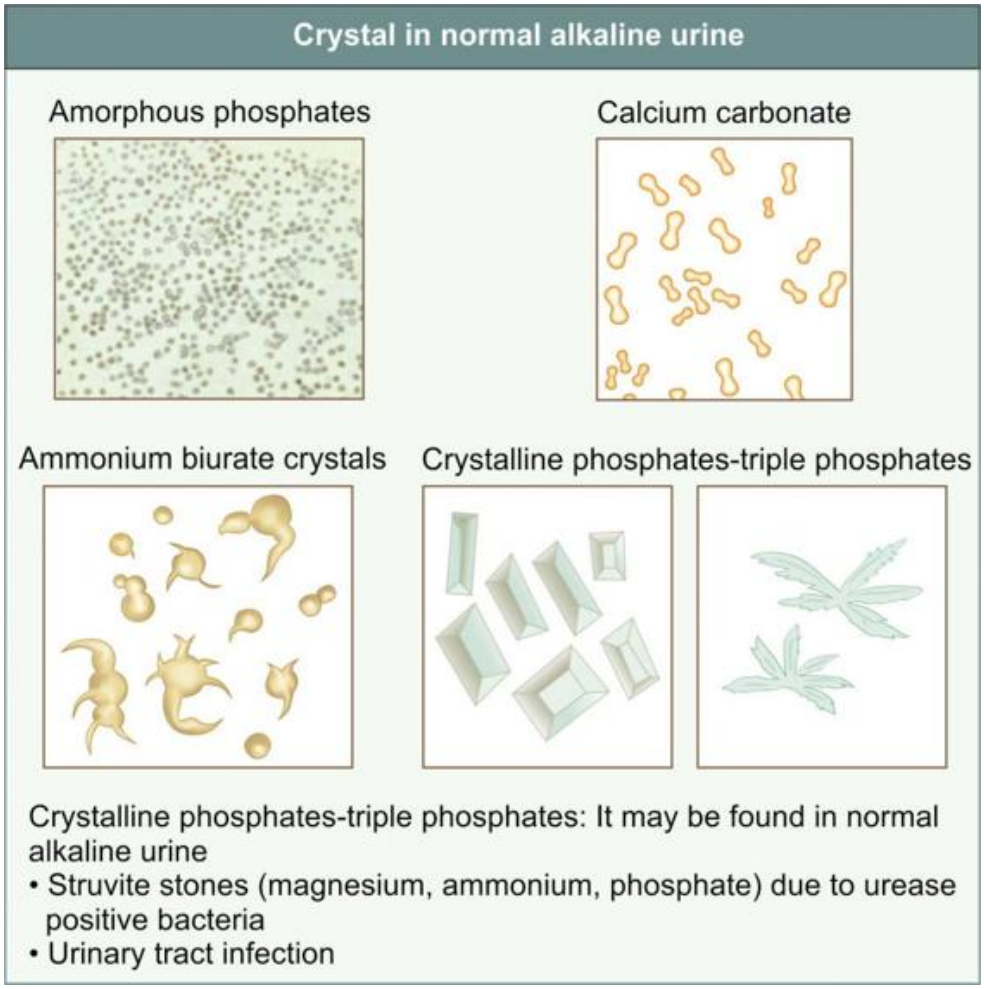


Fig. 14.7: Normal crystals found in alkaline urine.

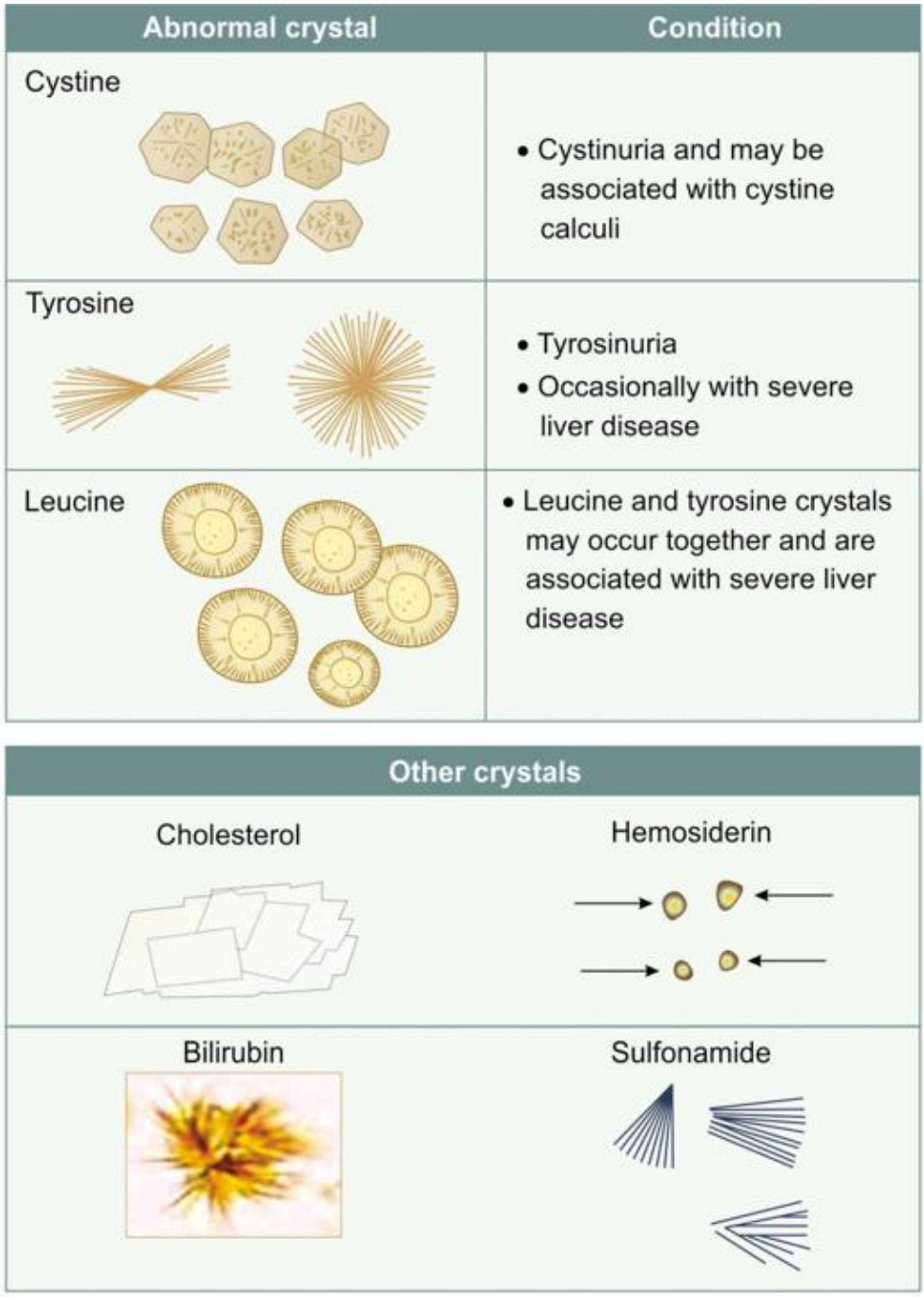


Fig. 14.8: Abnormal crystals found in urine.

Causes of pus cells in urine (pyuria): Pyelonephritis, urethritis, cystitis, urinary tract infection (UTI).

Glitter cell is a **swollen neutrophil with its cytoplasmic granules showing constant Brownian motion**. This results in a shining or glittering appearance of the cell. Causes of glitter cell include dilute or

hypotonic urine, chronic pyelonephritis and lower urinary tract infections.

3. ***Epithelial cells:*** These are derived from the urinary tract (transitional and renal) or genital tract (squamous). A few (0–2/hpf) transitional cells from the bladder may be present in the normal urine and squamous cells from the vulva and vagina usually contaminate a routine specimen from women.
   Malignant cells may be seen in tumors of urinary bladder.

Casts (Box 14.15 and Fig. 14.5)

Q. Write short note on urinary casts and crystals.

They are one of the organized elements which are **formed only in the kidney** and are **indicative of a renal disease.** They are formed by solidification of **Tamm Horsfall protein**, a glycoprotein secreted in the distal convoluted tubules and collecting tubules. These proteins form a fibrillar meshwork (basic matrix) and can trap any elements including cells, cell fragments or granular material. **Casts** are cylindrical structures with **parallel sides** and rounded ends. They vary in size, shape and appearance. **Cylindroids** probably represent abortive casts. They have one tapering end while the other end is round and the sides are not parallel. The casts may have only proteins (hyaline and waxy casts) or have trapped granular debris (granular casts), epithelial cells (epithelial casts), leukocytes (leukocyte casts), red blood cells (RBC casts) or fat droplets (fatty casts).

Crystals (Figs. 14.6 to 14.8)

These are not usually present in urine. Crystals of oxalates, urates and cystine are present in patients with history of renal stone, while urates alone are present in *gout.* Oxalates, urates and cystine are present in acidic urine while phosphates, calcium carbonates and ammonium urates are present in alkaline urine.

Unorganized Sediments

Unorganized sediments consist of crystalline or amorphous material, the nature of which varies according to pH of the urine (acid or alkaline). Majority of these have little diagnostic or prognostic significance.

BODY FLUIDS

CC: Describe abnormal findings in body fluids in various disease states.

Body fluids are lubricating fluids present within the body cavities. Normally, a small amount of fluid is present within the body cavities which keep the surfaces moist and lubricated so that the movement of the adjacent or the opposing membrane surfaces occur with minimal friction. Increase in the volume of the fluid in these cavities is known as **effusion**. The commonly examined body fluids in the laboratory include **pleural, pericardial, peritoneal and synovial fluid.**

Specimen Collection

The body fluid is collected in a clean, dry container under aseptic precautions and atraumatically to avoid mixing with fresh blood. The fluid is collected in the following three sterile test tubes:

BOX 14.15: Classification of casts.

- **Matrix:** Hyaline, waxy
- **Cells:** RBCs and its remnants, leukocytes (neutrophils, lymphocytes), renal tubular epithelial cells, mixed cells (RBCs, neutrophils, and renal tubular cells)
- **Inclusions:** Granules (proteins, cell debris), fat globules (triglycerides, cholesterol esters), hemosiderin granules
- **Pigments:** Hemoglobin, myoglobin, bilirubin

TABLE 14.14: Causes of pleural effusion.

Transudate	*Exudate*
Congestive cardiac failure	**Infections: Bacterial pneumonia, tuberculosis**
Cirrhosis of liver	Pulmonary infarction
Hypoproteinemia	Autoimmune disorders: Systemic lupus erythematosus, rheumatoid arthritis
Pulmonary embolism	**Malignancy: Secondaries from carcinoma lung,**

	breast, etc., mesothelioma and lymphoma
	Others: Acute pancreatitis, trauma to chest wall

Common causes are highlighted in bold words

- Chemical examination: Fluoride tube.
- Microscopic examination: EDTA tube.
- Bacteriological examination: Plain tube (without anticoagulant).

They should be examined as early as possible to prevent chemical changes, growth of bacteria and disintegration of cells.

PLEURAL FLUID

Causes of Pleural Effusion

Nonspecific inflammatory pleural effusion is more common than specific effusion. Various causes of pleural effusion are presented in **Table 14.14**.

Examination of Pleural Fluid

It consists of:

- Physical examination (volume, color and appearance)
- Chemical examination
- Cell counts (microscopic examination)
- Microbiological examination
- Cytological examination

Physical Examination

Note: volume, color and appearance (it applies also to pericardial and peritoneal fluid).

Color: Pleural, pericardial and ascitic (peritoneal) fluids are usually clear and straw colored.

- Uniform **blood stained fluid suggests malignancy** involving the organs/tissues surrounding the respective body cavity.
- **Turbid** fluid may be due to high cell count or high protein content.
- **Chylou**s with milky appearance usually indicates high lipid content due to lymphatic obstruction.

Appearance: The effusion may be broadly divided into **transudate** and **exudate**. It is important to differentiate whether the fluid is a transudate or

exudate (*refer* **Table 3.3**).

- **Transudate** is usually seen in all body cavities with diseases like heart failure and hypoalbuminemic conditions (e.g., nephrotic syndrome). Cirrhosis results in prominent ascites, but may also cause pleural effusion. ***Transudates*** are clear, pale yellow, or straw-colored and do not clot on standing. Transudates are mostly due to circulatory disturbances.
- **Exudate usually suggests infection or malignancy**. ***Exudates*** are opaque or turbid due to increased proteins, leukocytes, or presence of malignant cells. They may clot on standing due to high fibrinogen content. Frank pus with putrid odor appear in empyema. Exudates can be produced due to: (i) inflammatory processes, (ii) primary and metastatic tumors in serous membranes or serous cavities, or (iii) following a trauma, surgery, or other invasive diagnostic procedures.
- **Bloody or hemorrhagic fluid:** It indicates traumatic tap or malignancy.
- **Milky or chylous fluid:** It is characterized by accumulation of a milky or chylous fluid. It usually develops due to obstruction of lymphatic duct (due to injury, carcinoma, or lymphoma). It characteristic shows triglyceride level >110 mg/dL. Microscopically it shows lymphocytosis and fat droplets that stain positive with fat stains such as Sudan III.
- **Viscous fluid:** It is seen in mesothelioma.

Chemical Examination

Protein estimation: This helps to differentiate transudate from exudate. It is essential to differentiate exudates and transudates. Exudates have at least one of these criteria [Light's criteria, i.e., (i) pleural fluid protein/serum protein ratio >0.5, (ii) pleural fluid LDH/serum LDH ratio >0.6 and (iii) pleural fluid LDH above 2/3rds of upper limit of normal for serum LDH]. There is a direct relation between levels of lactate dehydrogenase in pleural fluid with degree of pleural inflammation. Transudates do not have any of these criteria.

- **Exudate:** If the fluid is an exudate, further investigations are required. These include **cell counts, cytological examination, estimation of glucose**, test for tuberculosis, Gram smear, and culture.
- **Transudates**: They are usually seen in congestive cardiac failure and cirrhosis.

Glucose estimation: Low glucose in the body fluids usually suggests bacterial infection (**empyema,** including tuberculosis), malignancy or nonspecific inflammation.

Cell Counts (Microscopic Examination)

Total leukocyte count: It should be performed on all body fluids. ***Cell count*** is done similar to total WBC count using improved Neubauer chamber. Normally, few mesothelial cells (lining cells of body cavities) and lymphocytes are seen.

Differential WBC count: Centrifuge the body fluid and from the sediment prepare the smears (at least 2). **Stains** employed includes: (1) **Leishman's stain:** Stain one smear with Leishman's stain and count 100 cells and express the differential count and (2) **Gram's stain/acid-fast stain.** These stains are useful in suspected cases of infective/tubercular infections respectively.

- **Neutrophils**: Very high leukocyte count (>50,000/µL) with predominance of neutrophils suggests effusion complication bacterial pneumonia (usually empyema).
- **Lymphocytes**: Presence of many small, mature lymphocytes with few mesothelial cells is suggests tuberculosis.
- **Hemorrhagic pleural fluids** having more than 100,000 red cells/µL is seen in pulmonary infarction, malignancy, or traumatic tap.

Transudate and Exudate

- **Transudates**: Usually leukocytes are <1,000/µL and are mostly small, mature lymphocytes.
- **Exudate**: Predominance of neutrophils (>50%) indicates an acute process (e.g., bacterial pneumonia), whereas predominance of lymphocytes indicates a chronic process (e.g., tuberculosis, malignancy).

Microbiological Examination

- **Gram's smear and culture:** It should be done on exudates (especially on purulent, blood-stained, or cloudy fluids). Culture is done to identify the organism in cases of effusion due to infections.

- **Ziehl–Neelsen stain:** Useful for tuberculous pleural effusions. It culture for tubercle bacilli is negative in a suspected case of tuberculosis, polymerase chain reaction for mycobacterial DNA or increased level of adenosine deaminase (released in pleural fluid from activated lymphocytes) will be useful for establishing the diagnosis.

Cytological Examination

Procedure: The pleuralfluid is centrifuged; smears are made from the sediment and fixed immediately in absolute alcohol. The smears are stained by Papanicolaou stain. Hematoxylin and eosin stain or Giemsa may also be used. Cytospin is a better alternative for making smears.

The common malignancies that metastasize to pleura are carcinoma of lung and breast, and lymphoma. Pleural effusion is produced due to obstruction of lymphatics by tumor cells. Usually, effusion is blood-tinged or hemorrhagic.

Pleural Fluid Findings in Malignant Pleural Effusion

Q. Pleural fluid findings in malignant pleural effusion.

- **Often hemorrhagic**, with a high erythrocyte count (>100,000/mm^3).
- Shows the characteristics of exudates such as **high protein content** (>3.0 g/dL), high LDH levels (>200 IU/L), low glucose levels (<60 mg/dL) and **a total leukocyte count exceeding 1000/mL**.
- **Predominant cells are lymphocytes (>50%)**.
- Cytological examination may show **malignant cells**. The smears are stained by Papanicolaou stain. Hematoxylin and eosin stain or Giemsa may also be used. Cytospin is a better alternative for making smears.

Pleural biopsy: Percutaneous needle biopsy of the parietal pleura is indicated if the examination of exudative pleural effusion does not yield a specific diagnosis. It is done when there is suspicion of tuberculosis or malignancy.

Pleural fluid findings in common pleural effusion are discussed in **Table 14.15**.

PERITONEAL FLUID

Excessive (pathological) accumulation of fluid in peritoneal cavity is termed ascites, and the accumulated fluid is called as ascitic fluid.

TABLE14.15: Pleural fluid findings in common pleural effusion.

Causes	*Appearance*	*Cells*

Special studies			
Exudate			
Tuberculosis	Straw-colored	5000-10000/µLmostly lymphocytes and few mesothelial cells	Ziehl–Neelsen stain; culture; polymerase chain reaction
Effusion in bacterial pneumonia	Turbid	5000-40000/µL mostly neutrophils	Gram stain; culture
Empyema	Purulent	>25000/µL;mostly neutrophils	Gram stain; culture; low pH; low glucose
Malignancy	Bloody	Lymphocytes, malignant cells	Cytology, pleural biopsy
Transudate			
Congestive heart failure	Clear	Few mesothelial cells	Nil

TABLE 14.16: Causes of ascites.

Transudate	*Exudate*
Cirrhosis of liver	Bacterial peritonitis (primary or secondary)
Congestive cardiac failure	Tuberculosis
Hypoproteinemia	Malignancy: Lymphoma, hepatocellular carcinoma, metastatic carcinoma (e.g., ovary, colon)
	Others: Abdominal injury, biliary peritonitis (rupture of gallbladder), pancreatitis, etc.

Causes of Ascites

Causes of ascites are presented in **Table 14.16**.

Examination of Ascitic Fluid

Steps and physical examination are same as that for pleural fluid as mentioned above.

Chemical Examination

- **Proteins:** In ascitic fluid, traditional division of fluid as transudate or exudate depending upon its protein content alone is not always sufficient. It is **better to use albumin gradient** (calculated as serum albumin minus ascitic fluid albumin).
- **Lactate dehydrogenase:** It is raised in **spontaneous bacterial peritonitis** (i.e., without any obvious source of infection), **secondary bacterial peritonitis** (i.e., with identifiable source of infection) and in **metastatic carcinoma** to peritoneum.
- **Amylase:** Normally, amylase levels in ascitic fluid are similar to serum amylase. If the amylase level in ascitic fluid is three times more than serum amylase, the cause of ascites is most likely to be due to pancreatic disease (e.g., acute pancreatitis).
- **Bilirubin:** If the bilirubin in ascitic fluid is more than 6.0 mg/dL and if the ascitic fluid bilirubin/serum bilirubin ratio more than 1.0, it suggests perforation of biliary tract (biliary peritonitis). The ascitic fluid will be bile-stained.

Cell Counts (Microscopic Examination)

Cell count is performed to distinguish ascites due to cirrhosis from that due to spontaneous bacterial peritonitis. If the total leukocyte count > 500/µL and absolute neutrophil count >250/µL, it favors the diagnosis of spontaneous bacterial peritonitis.

Microbiological Examination

- **Gram stain**: It is positive in about ¼ of cases of spontaneous bacterial peritonitis.
- **Culture and sensitivity.**
- **Ziehl–Neelsen stain** for tuberculous bacilli. Laparoscopic biopsy is more useful for the diagnosis of tuberculous peritonitis.

Cytological Examination

Procedure is same as that for pleural effusion. Malignant cells can be found in malignant ascites.

Pericardial effusion and hemopericardium are discussed on pages 491-2.

CEREBROSPINAL FLUID EXAMINATION

Cerebrospinal fluid (CSF) is formed within the ventricles and circulates in the subarachnoid space (between arachnoid and pia matter) and in the ventricles. The total volume of CSF in adults ranges from 90–150 mL. It is a medium for transfer of substances from brain and spinal cord into the blood.

Importance of CSF examination: Analysis of the CSF is of diagnostic importance in conditions like meningitis or primary/metastatic tumor of CNS with CSF involvement.

Collection of CSF: CSF is usually obtained by lumbar puncture (LP) using an LP needle under strict aseptic conditions.

Lumbar puncture needle: The needle measures 10–12 cm in length. In children, a shorter needle is used. It has a needle and a stilette. The stilette has a pin which fits into the slot of the head of the needle and helps to keep the needle patent.

Sites

- **Lumbar puncture:** In **adults**, CSF is normally collected in the midline of the lower back in the **3rd lumbar space** and in children in the 4th lumbar space.
- **Cisternal puncture:** It is done in spinal cord block, vertebral deformity or infections in the tissues where lumbar puncture is usually done.

BOX 14.16: Indications for lumbar puncture.

Diagnostic indications

- Infection
  - Meningeal infection:
    - Bacterial
      - Pyogenic
      - Tuberculosis
      - Syphilitic: To differentiate general paresis of insane, tabes dorsalis and meningeal syphilis
    - Viral
    - Fungal

– Encephalitis
- Subarachnoid hemorrhage
- Primary or metastatic malignancy (e.g., acute leukemia, lymphoma)
- Demyelinating diseases: Multiple sclerosis and subacute sclerosing panencephalitis (SSPE), Guillain–Barre syndrome
- Spinal canal blockage leading to elevated intracranial tension (spinal cord tumors)
- For injecting the radio-opaque dye for myelography

Therapeutic indications

- Spinal anesthesia
- Intrathecal injection of chemotherapeutic drugs for CNS prophylaxis/relapse of ALL, lymphomas

BOX 14.17: Contraindications for lumbar puncture.

- Raised intracranial pressure
- Local infective lesion
- Disseminated sclerosis
- Brain tumor
- Cardiorespiratory compromise
- Bleeding diathesis which is not been corrected

BOX 14.18: Complications of lumbar puncture.

- Herniation of cerebellum through the foramen magnum due to raised intracranial pressure
- Hematoma, either extradural or subdural
- Introduction of infection by the LP needle through the infected skin or subcutaneous tissue
- Post-puncture headache
- Failure to obtain CSF (dry tap)

v **Ventricular puncture:** It is performed in infants who have open fontanella.

Method of Collection

Normally CSF is collected in three/four sterile test tubes. The amount of CSF collected should not exceed 6–8 mL. Tube 1 for estimation of protein and glucose and serology, tube 2 is used for preparation of smears to stain with the Gram stain or other stains and for culture and sensitivity, tube 3 for cell counts and differential counts and tube 4 (if indicated) for special tests, such as the cryptococcal antigen, serologic test for syphilis, molecular tests or other serologic studies and cytology.

Indications for Lumbar Puncture

Q. Write short note on indications for lumbar puncture.

Indications for lumbar puncture is listed in **Box 14.16**.

Contraindications for Lumbar Puncture

Contraindications for lumbar puncture is listed in **Box 14.17**.

Complications of Lumbar Puncture

Complications of lumbar puncture is listed in **Box 14.18**.

Examination of CSF

Physical Examination

CSF Pressure

The normal CSF pressure in adults is 90–180 mm of water in the lateral position, while in infants and children it ranges from 10 to 100 mm of water reaching the adult level by 6–8 years.

- **Causes of raised CSF pressure:** Meningitis, cerebral edema, mass lesions in brain, etc.
- **Causes of decreased CSF pressure*:*** Dehydration, circulatory collapse, etc.

Color and Appearance

- Normal CSF is clear and watery.
- Turbidity of CSF is due to pus or RBCs.
- **Xanthochromia** (yellow CSF) results from: (i) Old hemorrhage, (ii) obstructive jaundice, (iii) excess of protein, (iv) Froin syndrome.

Differentiation of subarachnoid hemorrhage from a traumatic puncture: The presence of blood in CSF may be due to trauma during lumbar puncture or subarachnoid hemorrhage.

- In traumatic tap, first few drops are hemorrhagic and subsequent ones are clear, while in subarachnoid hemorrhage blood is uniformly mixed with CSF.
- After centrifugation, the supernatant fluid is clear with a traumatic tap, whereas the supernatant appears xanthochromic (a faint pink, orange or yellow color caused by release of hemoglobin from hemolyzed red blood cells) in subarachnoid hemorrhage.

Clot Formation

Normal CSF does not clot. When blood-brain barrier is disturbed, fibrinogen appears in CSF. Fibrinogen gets converted to fibrin and forms clot.

Causes of fibrin clot

- Meningitis: In tuberculous meningitis, the clot is fine, delicate and typically described as cob-web appearance. In purulent meningitis, large clot is formed.
- Tumors of CNS.
- Polyneuritis.

Microscopic Examination

Total cell count: Normal CSF usually contains no cells, although cell count of 0–5 lymphocytes/L is considered as normal. An **increased cell count** is known as **pleocytosis.** Total cell count should be done immediately on undiluted CSF, since after a few hours, pus cells stick to each other and to the sides of the tube or degenerate. Cell counts are performed in a manual counting chamber, either in a Fuchs–Rosenthal or improved Neubauer chamber.

Significance

Neutrophils are increased in acute pyogenic meningitis.

Lymphocytes are increased in viral, syphilitic, tuberculous and fungal meningitis.
Increased cell count should always be confirmed by bacterial or serological tests. India-ink preparation is used for diagnosis of cryptococcal meningitis. In tuberculous meningitis, acid-fast stain can detect tuberculous bacilli.

Biochemical Evaluation

Proteins are elevated in meningitis and glucose level is reduced due to utilization by the microbes. These changes are more marked in pyogenic meningitis.
Chloride reduction in tuberculous meningitis is due to general chloride deficiency because of dehydration rather than any specific effect of mycobacteria.

Refer **Table 27.1** for CSF changes in various meningitis. Interpretation of CSF findings with clinical scenario are presented on pages 875-7 of Chapter 27.

SEMEN ANALYSIS

CC (Show How): Describe and interpret the abnormalities in semen analysis.
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Q. Write short note on semen analysis.

Semen (seminal fluid) consists of spermatozoa (sperms) and the fluid part. About 40% cases of infertility are due to abnormalities in semen and therefore, semen analysis is the first test to be performed while investigating for infertility. **Defect** of sperms may be **quantitative** (absence of sperms, lack of enough sperms) or **qualitative**.
Indications for semen analysis is listed in **Box 14.19**.

BOX 14.19: Indications for semen analysis.

- Assessment of fertility/infertility
- Determine the effectiveness of vasectomy
- Determine the suitability of semen for artificial insemination

- Medicolegal purpose: In alleged rape cases, vaginal pool smears are examined to detect sperms
- For selection of assisted reproductive technology (e.g., in vitro fertilization, gamete intrafallopian transfer technique)

Collection of the Sample

Q. Write short note on collection of semen for analysis.

Patient is asked to collect the semen by masturbation after a minimum of 2 days and a maximum of 7 days of sexual abstinence. Specimen should be collected in a clean, dry, wide-mouthed plastic/glass container. Collection of condom sample is not advisable because they often contain spermicidal agents which impair the sperm motility.

Examination of Semen

Q. Write short note on various test/parameters of the semen with normal range.

Physical Examination

- ***Liquefaction*:** Immediately after ejaculation, the semen is normally a semisolid coagulated mass. At room temperature, the semen usually begins to liquefy (become thinner) within a few minutes and completely liquefies within 15 minutes. Within 30 minutes it becomes more homogeneous and watery.
- ***Semen viscosity*:** Fresh semen is fairly viscid and the viscosity can be estimated by gently aspirating semen into a wide-bore (approximately 1.5 mm diameter) plastic disposable pipette, allowing the semen to drop by gravity. Normal semen falls drop by drop and if viscosity is abnormal, the drop will form a thread more than 2 cm long. Normal viscosity is important, since increase in viscosity affects sperm motility.
- ***Appearance*:** Freshly ejaculated semen is an opaque, white gray and viscid fluid. After liquefaction it has homogeneous gray-opalescent appearance. Semen may have red-brown color when red blood cells are

present (hemospermia) or yellow in patients with jaundice or ingestion of certain vitamins or drugs.

- ***Semen volume:*** Normal volume ranges from 1.4–1.7 mL per ejaculate. Low semen volume may be due to obstruction of the ejaculatory duct, congenital bilateral absence of the vas deferens or can also be due to difficulty in collection. High semen volume may be due to active exudation in cases of inflammatory lesions of the accessory organs.
- ***Semen pH:*** Alkaline and ranges from 7.2–8 (>7.2). The pH should be measured after liquefaction, preferably after 30 minutes.

Microscopic Examination

Q. Write short note on microscopic examination of semen.

- ***Sperm aggregation or agglutination*:** The adherence either of immotile spermatozoa to each other or of motile spermatozoa to mucus strands, nonsperm cells or debris is considered to be nonspecific aggregation and should be noted. **Agglutination** refers to **motile spermatozoa sticking to each other,** head-to-head, tail-to-tail or in a mixed way. Any motile spermatozoa that stick to each other by their heads, tails or midpieces should be recorded.
- ***Cellular elements other than spermatozoa:*** During microscopic examination search should be made for the presence of cells other than spermatozoa. Some of these cells may be clinically relevant, which includes epithelial cells from the genitourinary tract and "round cells" (leukocytes and immature germ cells).
- ***Assessment of sperm motility:*** Motility of the sperms helps in penetration of cervical mucus and migration of the sperms into the fallopian tube. In normal semen, 38–42% of sperms should be motile (progressively motile and non-progressively motile). This is assessed by placing a drop of liquefied semen on a clean glass slide with a coverslip placed over it and examining under microscope. According to the WHO (2010), the **motility of each spermatozoon (plural is spermatozoa) is categorized as progressively motile (PR), nonprogressively motile (NP) and**

immotile (IM). This grading system has replaced the older grading system of a, b, c or d (I to IV) grades.

- ***Sperm vitality:*** It is important to know whether immotile spermatozoa are alive or dead. Normally, 55–63% live forms are observed. Vitality of the spermatozoa is estimated by identifying those with an intact cell membrane and is especially important for samples with less than about 40% progressively motile spermatozoa. The percentage of live spermatozoa is assessed either by dye exclusion or by hypotonic swelling.

Q. Write short note on sperm count.

- ***Total sperm count:*** Sperm count is carried out in an improved Neubauer chamber using a Thoma pipette in a dilution of 1 in 20 (as for total leukocyte count) using semen diluting fluid. **Normal range of sperm count:** 33–46 millions/ejaculate.
  - **Aspermia:** No semen.
  - **Azoospermia:** No spermatozoa in the ejaculate.
  - **Oligospermia:** Total number of spermatozoa below the lower reference limit.
- ***Sperm morphology*:** Smear prepared from semen is fixed and stained with Papanicolaou stain to identify the morphological features. Normal spermatozoa have a head, neck, middle piece (midpiece), principal piece and endpiece. Since the endpiece is difficult to see under light microscope, the spermatozoa can be considered to consists of a head (and neck) and tail (midpiece and principal piece). Normally, more than 30% of sperms have normal morphology. For a spermatozoon to be considered as normal, both its head and tail must be morphologically normal. All other forms should be considered as abnormal.

Q. Write short answer on abnormal forms of sperm.

Classification of abnormal sperm morphology: Abnormal spermatozoa usually have a lower fertilizing capacity. The abnormalities in morphology can be classified as:

1. **Head defects:**
   - **Abnormal size:** Large or small head.
   - **Abnormal shape:** Pointed/tapered, round, pyriform, amorphous.

- **Acrosomal defects:** Vacuolated acrosomal region, abnormality of acrosomal areas.
- **Abnormal number:** Double-headed.

2. **Neck and midpiece defects:**
   - Sharply bent neck.
   - Asymmetrical insertion of the midpiece into the head.
   - Thick or thin or irregular midpiece.
3. **Tail (principal) piece defects:** These abnormalities include short or bent or coiled or double tail.

Tests for Antibody Coating of Spermatozoa

The agglutination of spermatozoa may be the result of the antisperm antibodies (ASAs) or other factors present in the semen. ASAs in the semen belong to IgA and IgG types and it may also be found in sperm-free fluids (seminal plasma, blood serum and cervical mucus).

Tests for antibodies on spermatozoa ("direct tests"): Two direct tests include the mixed antiglobulin reaction (MAR) test and the immunobead (IB) test.

Tests for antisperm antibodies in sperm-free fluids ("indirect" tests).

SPUTUM EXAMINATION

Q. Write short essay/note on sputum examination and its importance.

Sputum is a highly specialized watery, colorless and odorless product of the respiratory tract. Expectorated sputum is always abnormal and it consists of mucus and a variety of cellular and noncellular materials. It is the most frequently received specimen from the respiratory tract. Both its collection and examination are advantageous as samples are easily obtained, cost effective and its cellular content is representative of the entire respiratory tract.

Indications for Sputum Examination

Q. Write short answer on indications for sputum examination.

- Smear and culture **identification of causative organisms** in suspected infection (e.g., pneumonia, tuberculosis).
- Cytological examination for **malignant cells, viral inclusions, asbestosis.**

Sputum Collection

The patient is instructed to cough up to get the sputum proper and the same is collected in a wide-mouthed sterile, glass/plastic container with screw cap.

In those patients who cannot produce sputum spontaneously by deep coughing, a specimen of sputum may be induced. This is done by inhalation of appropriate solvents which are aerosolized to stimulate sputum production.

Early morning sputum sample is preferred for routine examination and 24-hours sample for the demonstration of tubercle bacilli by concentration method.

Examination of Sputum

TABLE 14.17: Types of cells in sputum and their significance.

Type of cell	*Significance*
Pus cells (neutrophils)	Numerous pus cells indicate pyogenic infection of the respiratory tract
Eosinophils	Increased eosinophils are seen in asthma and parasitic infections of the lungs
Lymphocytes	Increased lymphocytes are seen in early pulmonary tuberculosis
Red blood cells	If increased, indicate hemorrhage (bleeding) into the lungs or the bronchi
Heart failure cells (hemosiderin-laden macrophages)	• Chronic venous congestion of the lungs • Pulmonary infarction and pulmonary hemorrhage

TABLE 14.17: Types of cells in sputum and their significance.

Type of cell	*Significance*
Anthracotic pigment-laden cells (carbon- laden)	Increased in coal workers pneumoconiosis and those who live in smoky polluted atmosphere

BOX 14.20: Liver function tests.

A. Tests for excretory functions
- Bilirubin metabolism:
  - Serum bilirubin estimation
  - Bilirubin in feces
  - Bilirubin and urobilinogen in urine
- Bromosulphthalein (BSP) excretion
- Bile acids (bile salts)

B. Tests of liver cell injury
- Enzymes that detect hepatocellular injury
  - Transaminases (aminotransferases): Serum aspartate transaminases (AST), serum alanine transaminases (ALT), AST/ALT ratio
  - Lactate dehydrogenase (LDH)
- Enzymes that detect cholestasis
  - Serum alkaline phosphatase
  - γ-glutamyl transpeptidase
  - 5'-Nucleotidase

C. Tests of metabolism and biosynthesis
- Proteins secreted into the blood
  - Serum proteins: Albumin, globulin and albumin: globulin (A:G) ratio
  - Clotting factors: Prothrombin time
- Hepatocyte metabolism: Serum ammonia
- Lipid and lipoprotein metabolism
- Carbohydrate metabolism

D. Immunological tests

E. Ancillary diagnostic test: Alpha-fetoprotein (AFP)

Sputum examination consists of (i) physical examination, (ii) microscopic examination and (iii) culture study.

Physical Examination

- **Quantity:**
  - In bronchiectasis, large amount of purulent sputum is coughed out.
  - Large amount of watery sputum with pink tinge suggests pulmonary edema.

- **Appearance/color:** Colors of sputum vary in different pathological conditions.
- **Odor or smell:** Foul smelling sputum is observed in bronchiectasis and lung abscess and is due to anaerobic bacterial infections.

Microscopic Examination

Staining of sputum: Two to three smears are made on a clean dry glass slides and are stained with:

- Leishman stain or Wright stain for differential count.
- Other stains (depends on the clinical/pathological features).
  - Gram's stain for microorganisms.
  - Ziehl–Neelsen stain for acid-fast tubercle bacilli.
  - Special stains for fungi.
- Papanicolaou stain for study of malignant cells.

Cells

Normal sputum consists of a few neutrophils, few lymphocytes, carbon-laden macrophages, occasional eosinophils and red cells. Various types of cells seen in sputum and their significance are shown in **Table 14.17**.

LIVER FUNCTION TESTS

CC (Show How): Describe and interpret the abnormalities in liver function tests.
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Q. Name liver function tests.

Liver function tests (LFTs) are groups of laboratory tests designed to give information about the state of a patient's liver **(Box 14.20)**. Because of multiple and complex functions of the liver, it is necessary to perform several tests for an accurate diagnosis, to assess the severity of the disease and its prognosis.

Tests for Excretory Functions

Bilirubin Metabolism

Serum Bilirubin Estimation

It is not a sensitive test for liver dysfunction and may not reflect the degree of liver damage.

Normal values: Total serum bilirubin in adult is <1 mg/dL of which conjugated bilirubin is less than 0.25 mg/dL.

***van den Bergh reaction*:** Bilirubin estimation is based on **van den Bergh diazo reaction** by spectrophotometric method. Diazo reagent consists of diazotised sulfanilic acid which forms a conjugated azo compound with the porphyrin rings of bilirubin.

- **Direct van den Bergh reaction**: Water-soluble conjugated bilirubin gives direct van den Bergh reaction with diazo reagent within one minute.
- **Indirect van den Bergh reaction**: Alcohol-soluble unconjugated bilirubin gives positive result with indirect van den Bergh reaction.

Causes of hyperbilirubinemia (*refer* Box 19.1).

Bilirubin in Feces

Significance: Absence of fecal excretion of the pigment is seen **in obstructive jaundice** which gives rise to clay-colored stool.

Bilirubin in Urine

Significance: Bilirubinuria is **not seen in normal subjects**. Unconjugated bilirubin is not excreted in the urine (e.g., hemolytic anemia) but conjugated bilirubinuria occurs only when there is raised serum conjugated bilirubin (e.g., obstructive jaundice). Bilirubinuria **appears in** patients of **hepatitis before** the patient becomes **jaundiced.**

It is detected in the laboratory by Fouchet's test, foam test or dipstick method (*refer* page 398).

Urobilinogen in Urine

Significance: Urobilinogen is **normally excreted** in the urine in traces (1–2 mg/dL). An **increase** in urobilinogen in the urine is found in **hepatocellular disorders** (e.g., alcoholic liver disease,

cirrhosis) and **hemolytic anemias**. In obstructive jaundice with complete biliary obstruction, urobilinogen disappears from the urine.
It is estimated by Ehrlich's test or by dipstick method (*refer* page 398-9).

Bromosulfthalein Excretion

Bromosulfthalein (BSP) is a dye that follows the same routes of binding, conjugation and excretion as that of bilirubin. BSP is intravenously injected and venous blood after 45 minutes is tested for percentage of injected dye remaining in the blood. Nowadays, this test is not done due to the availability of enzyme estimations which are better indicators of hepatic dysfunction. BSP excretion test is **valuable only** i**n** the diagnosis of **Dubin–Johnson syndrome.**

Bile Acids (Bile Salts)

Primary bile acids (cholic acid and chenodeoxycholic acid) are formed from cholesterol in the hepatocytes. These bile acids are secreted in the bile. In the gut, they undergo deconjugation by the colonic bacteria and produce secondary bile acids (deoxycholic acid and lithocholic acid). About 90% of these bile acids are reabsorbed through **enterohepatic circulation** and reach the liver. About 10% are excreted in the feces.

Significance: Raised blood **levels of bile acids produce itching** (pruritus). Serum bile acids are **raised in hepatobiliary diseases with cholestasis**. Their presence in the urine can be detected by Hay's sulfur test (*refer* page 399).

Tests of Liver Cell Injury

Estimation of serum enzymes is the most sensitive and useful test for diagnosis of various types of liver (both hepatocellular or cholestatic) injury.

Enzymes that Detect Hepatocellular Injury

Transaminases (Aminotransferases)

Hepatocellular damage/necrosis is assessed mainly by estimation of transaminases **(Table 14.18)**.

Serum aspartate transaminase (AST)

This was formerly known as glutamic oxaloacetic transaminase (SGOT). It is a **mitochondrial enzyme found in the heart, liver, skeletal muscle and kidney** and is liberated into the blood whenever there is damage to their parenchymal cells. Thus, AST may rise in acute necrosis or ischemia of other organs apart from liver cell injury. Normal serum level is 0.20–0.65 μkat/L (12–38 U/L).

Serum alanine transaminase (ALT)

This was formerly called glutamic pyruvic transaminase or SGPT. It is a **cytosolic enzyme** mainly **found in the liver**. Thus, serum ALT is fairly specific for liver tissue and is of greater value in assessing liver cell injury. Normal serum level is 0.12–0.70 μkat/L (7–41 U/L).

AST/ALT ratio

It is usually of little value except if the ratio is >3 (with ALT <300 U/L), is suggestive of **alcoholic** hepatitis.

Significance: Uses of transaminase estimations are:

- Early diagnosis of viral hepatitis
- Alcoholic liver disease
- Cirrhosis

Lactate Dehydrogenase (LDH)

It occurs in the cytoplasm of all cells, highest concentration being found in liver, heart, skeletal muscle, kidney and RBCs. Thus, it is not specific for liver diseases.

Significance

- Marked increase in metastatic carcinoma to the liver.
- Moderate increase in cirrhosis, obstructive jaundice and acute viral hepatitis.

Enzymes that Detect Cholestasis

Serum Alkaline Phosphatase

It is produced by many tissues (bone, liver, intestine and placenta) and is excreted in the bile. Normal serum alkaline phosphatase (range in adults 33–96 U/L) is mainly derived from bone.

Significance

- **Increased** levels can be found in **diseases of bone, liver and in pregnancy**
- In the absence of bone disease and pregnancy, an **elevated** serum alkaline phosphatase level (3–10 times normal) indicates **biliary tract obstruction**
- Mild-to-moderate increase is observed in parenchymal liver diseases (hepatitis, cirrhosis and with hepatic metastasis)

A combination of serum transaminases and alkaline phosphatase is very useful in the diagnosis of injury to liver cells.

TABLE 14.18: Common cause of elevated serum transaminases.

Degree	*Serum levels*	*Common causes*
Mild	<100 U/L	Chronic hepatitis C, hemochromatosis, fatty liver (steatosis)
Moderate	100–300 U/L	Apart from above, it may be found in alcoholic hepatitis, autoimmune hepatitis, Wilson disease
Severe	>300 U/L	Acute viral hepatitis, drug toxicity, toxins

γ-glutamyl transpeptidase (γ-GT)

Liver is the primary source of the serum enzyme, γ-GT. Its serum level parallels serum alkaline phosphatase. So it is used to confirm that raised serum alkaline phosphatase is due to **hepatobiliary disease**. It is also raised in patients with **alcohol abuse** even without liver disease.

5'-nucleotidase

It is also a phosphatase derived from the liver. It is useful to distinguish alkaline phosphatase of hepatic origin from that of bone.

Tests of Metabolism and Biosynthesis

Liver is the main site of synthesis of amino acids and plasma proteins, lipids and lipoproteins, carbohydrates and vitamins.

Plasma Protein Synthesis

Hepatocytes synthesize several proteins including albumin, fibrinogen, prothrombin, alpha-l-antitrypsin and acute phase reactant proteins. Levels of these plasma proteins are reduced in extensive liver damage. Estimation of the following will be useful in the diagnosis of liver disorders:

Serum Proteins

Routinely done estimations are:

- **Total** serum **proteins** (normal 6.7–8.6 g/dL)
- **Serum albumin** (normal 3.5–5.5 g/dL) is produced solely by the liver.
- **Serum globulin** (normal 2–3.5 g/dL)
- **Albumin/globulin** (A/G) **ratio** (normal 1.5–3:1).

They are evaluated by serum/immunoelectrophoresis. Previously, thymol turbidity and flocculation tests were used for their estimation.

Significance: Hypoalbuminemia develops with chronic inflammatory disorders such as **cirrhosis** and **chronic hepatitis.**

Clotting Factors

Both prothrombin time and partial thromboplastin time, reflect the activities of various clotting factors. They are prolonged in hepatocellular disease.

Prothrombin Time

It depends on hepatic synthesis of clotting factors and intestinal uptake of vitamin K (fat-soluble vitamin).

Prolonged prothrombin time: Obstruction of the bile duct and intrahepatic cholestasis, which result in vitamin K deficiency due to impaired lipid absorption, are associated with prolonged prothrombin time. With parenteral administration of vitamin K, prothrombin time will return to normal, if the prolongation is due to obstruction. If there is no

improvement in prothrombin time, it indicates severe hepatocellular disease.

Serum Ammonia

Ammonia can be metabolized only in the liver and is derived mainly from amino acid (received from the diet and tissue breakdown) and nucleic acid metabolism. Ammonia is a toxic substance ultimately converted into nontoxic urea which is readily excreted. **Increased blood level of ammonia** is found when most of the liver is destroyed as in **acute fulminant hepatitis**, **cirrhosis** and **hepatic encephalopathy**. The elevated levels are due to inability of severely damaged liver to convert ammonia to urea.

Serum Gamma Globulins (Immunoglobulins)

Increased in most forms of **chronic liver disease.**

- IgA is raised in cirrhosis.
- IgG is markedly raised in chronic active hepatitis.
- IgM is markedly increased in primary biliary cirrhosis.

Lipid and Lipoprotein Metabolism

Liver synthesizes cholesterol and cholesterol esters, phospholipids and triglycerides. These lipids are insoluble in water and are carried in circulation as high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL).

Total Serum Cholesterol (Normal <200 mg/dL)

- Raised in cholestasis, probably due to retention of cholesterol which is normally excreted in the bile.
- Decreased in acute and chronic diffuse liver diseases and in malnutrition.

Carbohydrate Metabolism

TABLE 14.19: Reference range of liver function tests.

Name of the test	*Normal range*
Serum bilirubin	Total bilirubin less than 1 mg/dL Conjugated bilirubin less than 0.25 mg/dL
Serum enzymes • Serum aspartate transaminase or AST • Serum alanine transaminase or ALT • Serum alkaline phosphatase	0.20–0.65 µkat/L (12–38 U/L) 0.12–0.70 µkat/L (7–41 U/L) 33–96 U/L
Serum proteins • Total serum proteins • Serum albumin • Serum globulin • Albumin/globulin (A/G) ratio **Total serum cholesterol**	6.7–8.6 g/dL 3.5–5.5 g/dL 2–3.5 g/dL 1.5–3:1 <200 mg/dL

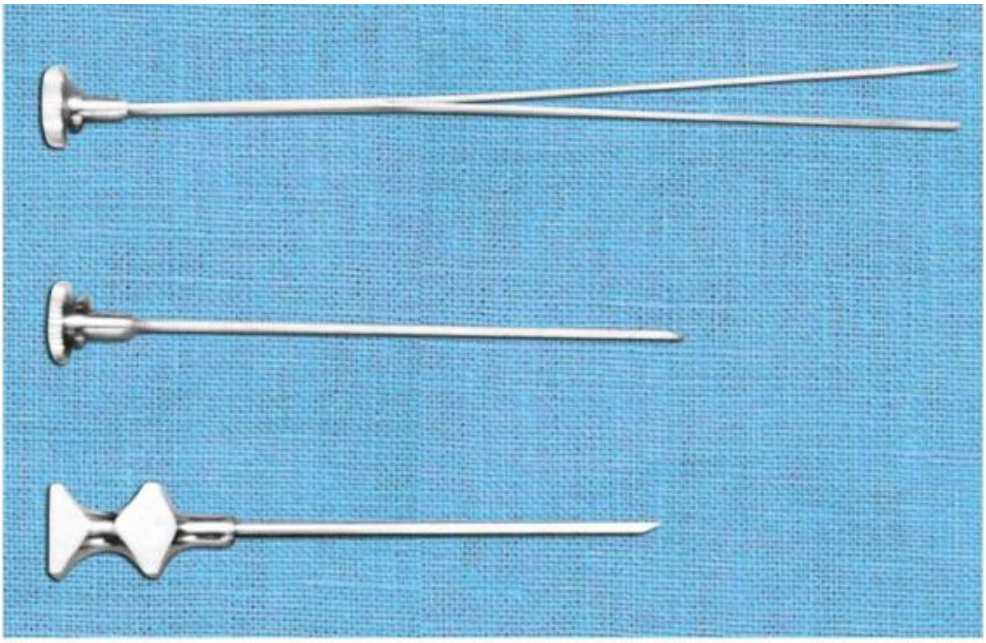


Fig. 14.9: Vim Silverman's needle showing prongs—split needle, stilette and cannula (top to bottom).

Liver also plays a central role in carbohydrate metabolism. Blood glucose level is reduced in fulminant hepatitis. Impaired glucose tolerance and relative insulin resistance is found in chronic liver disease.

Immunologic Tests

Diseases of liver may be associated with various immunological abnormalities.

Specific Antigens/Antibodies

- **Hepatitis B surface antigen (HBsAg)**, **hepatitis B core antibody (anti-HBc), hepatitis Be antigen (HBeAg)** in hepatitis B infection.
- Antibodies to *Entamoeba histolytica* can be demonstrated in amoebic liver abscess.

Ancillary Diagnostic Tests

Alpha Fetoprotein

It is an α-globulin found in fetal blood. It originates in fetal liver, GI tract and yolk sac. It is one of the tumor markers for hepatocellular carcinoma, but not a specific marker, as it may be found in non-neoplastic and other neoplastic conditions.

FNAC and/or Liver Biopsy

Reference range of liver function tests are shown in **Table 14.19**.

Liver Biopsy

Percutaneous needle biopsy of the liver through an intercostal approach is done under local anesthesia. It is a simple, safe procedure for assessment of liver histology for the diagnosis of various liver disorders.

Before the biopsy: Prothrombin time (PT) should be performed before liver biopsy. If it is high, patient should be given parenteral vitamin K and test should be repeated. If PT is prolonged by more than 3 seconds than the control, it is a contraindication for liver biopsy.

Needle: Vim Silverman's needle is used for liver biopsy (**Fig. 14.9**).

Indications for Liver Biopsy

Q. Indications for liver biopsy.

- Unexplained hepatomegaly, splenomegaly, jaundice.
- Cirrhosis of liver, to assess the type, etiology and activity of the disease process.
- Chronic hepatitis: For identifying, grading of inflammatory activity, staging and evaluation of the course.
- Pyrexia of unknown origin (PUO): A liver biopsy may be helpful in the diagnosis of miliary tuberculosis, sarcoidosis or malignancy.
- Idiopathic hemochromatosis: To determine iron stores in liver.

Contraindications

Q. Contraindications for liver biopsy.

- Congenital coagulation disorders, e.g., hemophilia A and B.
- PT prolonged by more than 3 seconds over control.
- Obstructive jaundice: Liver biopsy may cause leakage of bile from liver into the peritoneum causing severe and fatal bile peritonitis.
- Massive ascites.
- Patients with severe cough where diaphragm movements cannot be controlled.
- Uncooperative patients.
- These are relative contraindications. Hydatid cyst liver
- Hemangioma liver

Complications

- Hemorrhage from the biopsy site.
- Bile peritonitis in patients with obstructive jaundice.
- Referred pain to shoulder from right pleura.

BOX 14.21: Causes of raised blood urea nitrogen (BUN).

- **Prerenal azotemia:** Congestive cardia failure, shock, depletion of salt and water
- **Renal azotemia:** Impaired kidney function
- **Postrenal azotemia:** Obstructive lesions of urinary tract

- **Increased production of urea:** High protein diet, increased catabolism of proteins (e.g., trauma, burns)

BOX 14.22: Causes of raised serum creatinine.

- Azotemia, e.g., prerenal, renal and postrenal
- Large amount of consumption of meat
- Active acromegaly and gigantism

RENAL FUNCTION TESTS

CC (Show How): Describe and interpret the abnormalities in renal function tests.
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Q. Name renal function tests.

- **Urine analysis** (both routine and microscopic *refer* pages 393-402).
- **Blood chemistry:** Impaired renal function causes elevation of end-products of protein metabolism which is known as **azotemia.** These include:
  - Blood urea (normal range 20–40 mg/dL).
  - Blood urea nitrogen (BUN): Normal range 10–20 mg/dL.
  - Creatinine (normal range 0.6–1.2 mg/dL).
- **Renal clearance tests.**
- **Concentration and dilution tests.**

Blood Chemistry

Impaired renal function causes elevation of end-products of protein metabolism which is known as **azotemia.** These include:

- Blood urea (normal range 20–40 mg/dL).
- Blood urea nitrogen (BUN): Normal range 10–20 mg/dL. Causes of raised blood urea nitrogen (BUN) are listed in **Box 14.21**.
- Creatinine (normal range 0.6–1.2 mg/dL). Causes of raised serum creatinine are listed in **Box 14.22**.

Renal Clearance Tests

These tests measure the **glomerular function** and renal blood flow.

- Glomerular filtration rate (GFR) is the rate at which fluid passes into the nephron after filtration and it measures renal excretory function. The rate of glomerular filtration is measured by the capacity of the kidneys to clear waste products or foreign materials (inulin, mannitol, creatinine and urea) from the blood into the urine.
- Clearance of a solute from plasma into the urine equals GFR, if the solute is freely filtered through the glomerulus and neither secreted into nor absorbed from the renal tubules.
- From the determination of blood concentration of the test material and the quantity eliminated in the urine, "clearance" can be calculated and is expressed in terms of milliliters of blood cleared per unit time.

The various tests are:

Inulin Clearance Test

Inulin is ideal for the measurement of GFR because it is neither secreted nor reabsorbed by the tubule. Mannitol can also be used.

Creatinine Clearance

Creatinine is normally released into the plasma by muscle metabolism and is filtered through the glomerulus. Normally, the clearance of endogenous creatinine approximates the glomerular filtration rate. It is easy and routinely employed method for GFR estimation.

Urea Clearance Test

Urea clearance test is less sensitive test.

Concentration/Dilution Tests

These tests are used to evaluate the tubular function. The ability of the nephron to concentrate or dilute urine is dependent on the tubular cells in the renal medulla as well as antidiuretic hormone (ADH).

Urine Specific Gravity

Urine specific gravity (normal range 1.003–1.035) is a measure of capacity of the tubules to reabsorb water, thus concentrating the urine (*refer* pages 394-5). In chronic tubular damage it remains fixed at 1.010. If the patient's urine contains no sugar or protein and has a specific gravity of 1.025 or higher, a concentration test is unnecessary.

Concentration Test

In this test, an artificial deprivation of fluid is induced in a patient for more than 20 hours. With normal tubular function, water is selectively absorbed and urine will have high solute concentration with specific gravity of 1025 or more. With tubular damage, the specific gravity remains constant irrespective of water deprivation.

Dilution Test

In this test, excessive fluid is given to the patient which will result in a lower solute concentration in a normal individual. The resulting specific gravity will be 1.003 or less. In tubular damage, the specific gravity remains constant irrespective of excessive water intake.

Renal Biopsy

It is used to establish the nature and extent of renal disease. Biopsy is performed transcutaneously with ultrasound guidance. Biopsy material is examined under light microscopy (fixed in neutral buffered formaldehyde), electron microscopy (fixed in glutaraldehyde) and by immunofluorescence (tested for IgG, IgA, IgM, C3, fibrin, and κ and λ light chains by using appropriate antibodies). Indications, for renal biopsy are presented in **Box 14.23** and contraindications and complications of renal biopsy are presented in **Table 14.20**.

TABLE 14.21: Thyroid function tests.

Test	*Significance*

TABLE 14.21: Thyroid function tests.

Test	*Significance*
Serum thyroid hormones (normal values)	
• Thyroid-stimulating hormone/thyrotrophin (TSH 0.4–5.0 µU/mL)	Increased in primary hypothyroidism, Hashimoto thyroiditis. Decreased in hyperthyroidism
• Thyroid hormone levels: Free thyroxine (FT4) (5.4–11.7 µg/dL), triiodothyronine (T3) (77–135 ng/dL)	Increased in hyperthyroidism and decreased in hypothyroidism
• Thyrotrophin releasing hormone (TRH) stimulation test (0.4–5.0 µU/mL)	In primary hypothyroidism an exaggerated prolonged rise of already increased TSH level is observed. In secondary hyperthyroidism (pituitary), there will be no rise in the decreased TSH level
• Thyroglobulin (Tg)	Increased in well-differentiated thyroid carcinoma and hyperthyroidism. Decreased following total thyroidectomy or destruction of thyroid by radiation
• Calcitonin level	Used for diagnosis of primary/recurrence of medullary carcinoma of thyroid or metastases after the removal of the primary. Increased in some paraneoplastic syndromes (e.g., carcinoma of lung), hypercalcemia of any cause and C cell hyperplasia
Thyroid autoantibody tests	
• Antimicrosomal antibody • Antithyroid peroxidase antibody (TPOAb) • Antithyroglobulin antibody (TgAb) • TSH receptor antibody (TRAb)	Diagnosis and monitoring of autoimmune thyroid diseases, Hashimoto thyroiditis: Antimicrosomal or antithyroid peroxidase antibodies, Graves' disease: Anti-TSH receptor antibodies
Radioactive iodine uptake (RAIU)*(10–35% in 24 hours)*	Increased in Graves' disease, toxic multinodular goiter and adenoma and early thyroiditis. Decreased in hypothyroidism, late thyroiditis

BOX 14.23: Indications for renal biopsy.

- Nephrotic syndrome/glomerular proteinuria in adults
- Nephrotic syndrome in children with atypical presentation/ not responding to treatment (steroids)
- Chronic renal failure with normal-sized kidney
- Unexplained acute renal failure
- Differential diagnosis of acute nephritic syndrome
- Isolated hematuria/non-nephrotic proteinuria
- Unexplained renal insufficiency when kidney dimensions are near normal on ultrasonography
- Impaired functioning of renal transplant
- Asymptomatic hematuria when other diagnostic tests cannot detect the cause
- Involvement of kidney in systemic diseases (e.g., systemic lupus erythematosus, amyloidosis)

TABLE 14.20: Contraindications and complications of renal biopsy.

Contraindications	*Complications*
• Coagulation disorders • Thrombocytopenia • Uncontrolled hypertension • Solitary kidney • Rena neoplasms to avoid spread along needle track • Large and multiple renal cysts • Acute urinary tract infections • Urinary tract obstructions • Kidney <60% predicted size	• Pain • Infection • Bleeding into the urine • Bleeding around kidney • Arteriovenous fistula • Accidental biopsy of other organ or perforation of organs such as liver, spleen • Rarely death

THYROID FUNCTION TESTS

CC (Show How): Describe and interpret the abnormalities in thyroid function tests.
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Q. Write short essay on thyroid function tests.

Thyroid function tests (TFTs) is a collective term for test used to check the function of the thyroid gland. Various tests and their significance are mentioned in **Table 14.21**.

Indications

Screening for Thyroid Dysfunction

Thyroid function tests are performed to screen patients suspected of having thyroid disease.

The indications for screening include:

- Neonates for congenital hypothyroidism
- Patients with autoimmune disease
- Individuals with strong family history of thyroid diseases
- Patient with suspected hyperthyroidism/hypothyroidism
- Conditions linked to thyroid disease such as atrial fibrillation or anxiety disorder

Surveillance

- Women with a history of postpartum thyroiditis.
- Post-neck irradiation.
- Post-destructive treatment (radioiodine or surgical) for thyrotoxicosis.

Monitoring

One of the common reasons for assessing thyroid function is to monitor therapy.

- Monitoring treatment of **hyperthyroidism** with antithyroid drugs.
- Patients with primary **hypothyroidism** who are taking thyroxine hormone replacement therapy.

Serum Thyroid Hormones

Thyroid Stimulating Hormone/Thyrotrophin

Thyroid stimulating hormone (TSH) is secreted by the anterior pituitary gland. It increases production and release of thyroxine (T_3 and T_4) form the thyroid. Its secretion is inhibited by high levels of T_3 and T_4. It is stimulated by high levels of TRH and low levels of T_3 and T_4.

Use: To assess true metabolic status. It is the most important test to assess function and monitor thyroid hormone replacement therapy.

- **Increased:** Thyroid failure is characterized by decrease in the level of T_3 and T_4 with increased TSH.
  - Primary hypothyroidism

- Hashimoto thyroiditis.

- **Decreased:** In hyperthyroidism TSH level is suppressed and may be undetectable.

Thyroid Hormone Levels

- **FT_4 (Free thyroxine):** It is produced only by the thyroid gland. It travels in plasma bound to protein or in free form. It is converted to T_3 (active) and RT_3 (inactive) peripherally in blood and tissues.
- **T_3 (Triiodothyronine):** T_4 is converted to T_3 in the peripheral tissue. Most of it is transported in plasma bound to protein and only 0.3% in free form.

Use: Its level reflects secretory activity.

- **Increased in hyperthyroidism**
- **Decreased in hypothyroidism**.

Thyrotrophin Releasing Hormone Stimulation Test

Thyrotrophin releasing hormone (TRH) secreted by hypothalamus, which stimulates the release of TSH from the pituitary. TRH secretion is inhibited by high levels of T_3 and T_4 and stimulated by low levels of T_3 and T_4. Serum TSH is measured before and after the intravenous administration of TRH.

Use (Box 14.24): To differentiate two forms (whether due to tumor or not) of thyrotrophin-induced hyperthyroidism.

- In primary hypothyroidism an exaggerated prolonged rise of already increased TSH level is observed.
- In secondary hyperthyroidism (pituitary), there will be no rise in the decreased TSH level.

Thyroglobulin

Thyroglobulin (Tg) is secreted by thyroid follicular cells and is involved in iodination and synthesis of thyroid hormones.

Use: To predict the outcome of therapy for hyperthyroidism.

- **Increased:**
  - Well-differentiated thyroid carcinoma

- Hyperthyroidism

- **Decreased:** Total thyroidectomy or destruction of thyroid by radiation.

Calcitonin Level

It is secreted by the parafollicular C cells of the thyroid. It decreases the bone-resorption by osteoclasts and causes decreased serum calcium.

Use: Diagnosis of primary/recurrence of medullary carcinoma of thyroid or metastases after the removal of the primary.

Increased in:

- Some paraneoplastic syndromes (e.g., carcinoma of lung)
- Hypercalcemia of any cause
- C cell hyperplasia.

Thyroid Autoantibody Tests

The different types of thyroid autoantibodies responsible for the autoimmune thyroid disorders are:

- Antimicrosomal antibody.
- Antithyroid peroxidase (TPO) antibody (TPOAb): They are involved in the tissue destructive process associated with hypothyroidism in Hashimoto and atrophic thyroiditis.
- Antithyroglobulin (Tg) antibody.
- TSH receptor (TR) antibody (TRAb): These antibodies were previously known as thyroid stimulating immunoglobulin (TSI) or long-acting thyroid stimulators (LATS).

Laboratory assessment of antithyroid peroxidase antibodies is replacing the antimicrosomal and anti-thyroglobulin antibodies. Uses of thyroid autoantibodies measurement is listed in **Box 14.25**.

Radioactive Iodine Uptake

T_4 contains high iodine and the thyroid gland must take-up a large amount of iodine from the circulating blood for the synthesis of T_4. The iodine uptake activity of thyroid can be measured by administering orally a low dose of radioactive iodine (or technetium) and the radioactivity over the thyroid is measured at specified intervals. The amount of radioactivity that is taken up by the thyroid gland is known as radioactive iodine uptake (RAIU). Normal uptake ranges from 10 to 35% in 24 hours. A very high RAIU is seen in hyperthyroidism, while a low RAIU is seen hypothyroidism.

Use

- Evaluation of hyperthyroidism
- Differentiate Graves from toxic goiter
- Function of a thyroid nodule as hot or cold.

Increased: Graves disease, toxic multinodular goiter and adenoma and early thyroiditis.
Decreased: Hypothyroidism, late thyroiditis.

Contraindication: RAIU is contraindicated in children and during pregnancy/lactation.

Thyroid Scintiscanning

In addition to the radioactive iodine uptake, an isotype (99mTc-pertechnetate) can be administered and its distribution within the thyroid gland is assessed by gamma counter.

BOX 14.24: Uses of TSH estimation.

- Screening for thyroid function
- Screening of hypothyroidism in newborn
- Diagnosis of hypo- and hyperthyroidism
- Monitor thyroid hormone replacement therapy

BOX 14.25: Uses of thyroid autoantibodies measurement.

- **Diagnosis and monitoring of autoimmune thyroid diseases**
- **Hashimoto thyroiditis:** Antimicrosomal or antithyroid peroxidase antibodies
- **Graves' disease:** Anti-TSH receptor antibodies

TABLE 14.22: Common thyroid function tests in normal and pathological states.

TABLE 14.22: Common thyroid function tests in normal and pathological states.

Thyroid functional state	*TSH*	*Free T_4*	*T_3*	*Tg*
Euthyroid	Normal	Normal	Normal	Normal
Hyperthyroidism-Graves' disease	Decreased or undetectable	Increased	Increased	Increased
Hypothyroidism	Increased	Decreased	Decreased	Normal or decreased
Hashimoto thyroiditis hypothyroidism	High	Normal or decreased	Normal or decreased	Normal or decreased

Use

- Differential diagnosis of high RAIU thyrotoxicosis
  - Uniform or diffuse increase in uptake in Graves' disease
  - Multiple discrete areas of increased uptake in toxic multinodular goiter
  - Single area of increased uptake in adenoma.
- Evaluation of solitary nodule of thyroid
  - Hyperfunctioning: Hot nodule
  - Non-functioning: Cold nodule (~20% are malignant).

Common thyroid function tests in normal and pathological conditions is shown in **Table 14.22**.

LABORATORY VALUES OF CLINICAL IMPORTANCE

Some important common laboratory investigations are provided below which will help in interpreting the results during examinations as well as during clinical practice. The term 'reference values' has replaced older terminology 'normal values/ranges'. A variety of factors can influence reference values and it varies between laboratories depending on the laboratory methods, mode of standardization and other factors. This is especially the case with enzyme assays. The reference or "normal" ranges given here may, therefore, not be appropriate for all laboratories and they

should only be used as general guidelines. Hence, reference values provided by the laboratory performing the test should be used in the interpretation of laboratory results. Most clinical laboratories and all medical and scientific journals use SI system. Since, conventional units are still used in many laboratories in many developing countries, in this section, laboratory values are given in both conventional and international units. Many analytes are measured in either serum (the supernatant of clotted blood) or plasma (the supernatant of anticoagulated blood).

Hematology and Coagulation (Table 14.23)

TABLE 14.23: Hematology and coagulation.

Component (specimen)	*Reference value*	
	Conventional	*SI units*
RBCs AND HEMOGLOBIN		
RBC count		
• Males	4.5–5.5 × 10^{12}/L (mean 5.0 × 10^{12}/L)	
• Females	3.8–4.8 × 10^{12}/L ... (mean 4.3 × 10^{12}/L)	
RBC diameter	6.7–7.7 µm (mean 7.2 µm)	
• RBC indices (absolute values)		
• Mean corpuscular volume (MCV)	82–100 fL	
• Mean corpuscular hemoglobin (MCH)	27–32 pg	
• Mean corpuscular hemoglobin concentration (MCHC)	31–35 g/dL	
RBC lifespan	120 days	
Erythrocyte sedimentation rate (ESR) (whole blood)		
• Westergren, 1st hour		
– Males	0–15 mm 1st hour	
– Females	0–20 mm 1st hour	

– Children	0–10 mm 1st hour	
• Wintrobe, 1st hour		
– Males	0–9 mm 1st hour	
– Females	0–20 mm 1st hour	
Ferritin (serum)		
• Males	20–300 ng/mL	20–300 µg/L
• Females	15–200 ng/mL	15–200 µg/L
Folate (serum)	3–20 µg/L	3–20 ng/mL
Hematocrit (PCV)		
• Males	38–47%	
• Females	36–46%	
• Infants (cord blood)	45–70%	
	Reference value	
Component (specimen)	***Conventional***	***SI units***
Haptoglobin (serum)	40–240 mg/dL	0.4–2.4 g/L
Hemoglobin (Hb)		
• Adult hemoglobin (HbA)	95–98%	
• Males	13.0–17.0 g/dL	
• Females	12.0–15.0 g/dL	
• Hemoglobin A_2 (HbA_2)	1.5–3.5%	
• Hemoglobin, fetal (HbF) in adults	<0–2%	
• HbF, children under 6 months	<5%	
Iron, total (serum)	50–150 µg/dL	7–25 µmol/L
• Total iron binding capacity (TIBC)	310–340 µg/dL	45–73 µmol/L
• Iron saturation	20–45%	
Osmotic fragility		
• Slight hemolysis	At 0.45 to 0.39 g/dL NaCl	
• Complete hemolysis	At 0.33 to 0.36 g/dL NaCl	
• Mean corpuscular fragility	0.4–0.45 g/dL NaCl	

Reticulocytes		
• Adults	0.5–2.5%	
• Infants	2–6%	
• Newborn (cord blood)	1–7%	
Transferrin saturation		
• Male	25–56%	
• Female	14–51%	
Vitamin B_{12} (serum)		
• Body stores	10–12 mg	
• Daily requirement	2–3 µg	
• Serum level	280–1,000 pg/mL	
Autohemolysis test (whole blood)	0.4–4.50%	
Autohemolysis test with glucose (whole blood)	0.3-0.7%	
LEUKOCYTES		
Differential leukocyte count (DLC)		
• P (polymorphs or neutrophils)	40–70% (2,000–7,500/µL)	
• L (lymphocytes)	20–40% (1,500–4,000/µL)	
• M (monocytes)	2–10% (200–800/µL)	
• E (eosinophils)	1–6% (40–450/µL)	
• B (basophils)	<1% (10–100/µL)	
Total leukocyte count (TLC)		
• Adults	4,000–11,000/µL	
• Infants (full term, at birth)	10,000–25,000/µL	
• Infants (1 year)	6,000–16,000/µL	
PLATELETS AND COAGULATION		
Bleeding time (BT)		
• Ivy's method	2–7 minutes	
• Template method	2–9 minutes	
Clot retraction time (clotted blood)		

• Qualitative	Visible in 60 minutes (complete in <24 hours)	

Contd...

Component (specimen)	*Reference value*	
	Conventional	*SI units*
• Quantitative	48–64% (55%)	
Clotting time (CT)		
• Lee and White method	4–11 minutes	
D-dimer (plasma)	220–740 ng/mL	
Fibrinogen (plasma)	200–400 mg/dL	
Fibrin split (or degradation) products (FSP or FDP)	<10 µg/mL	<10 mg/L
Partial thromboplastin time with kaolin (PTTK) or activated partial thromboplastin time (APTT/aAPTT)	30–40 second	
Platelet count	150,000–450,000/µL	
Prothrombin time (PT) (Quick's one stage method)	11–16 second	
Thrombin time (TT)	15–19 second (control ± 2 sec)	

Clinical Chemistry of Blood (Table 14.24)

TABLE 14.24: Clinical chemistry of blood.

Component	*Specimen*	*Reference value*	
		Conventional	*SI units*
Alpha fetoprotein (AFP), adults	Serum	0–8.5 ng/mL	0–8.5 µg/L
Aminotransferases (transaminases)			
• Aspartate (aspartate aminotransferase, serum glutamic oxaloacetic transaminase)	Serum	12–38 U/L	0.20–0.65 µkat/L
• Alanine (alanine aminotransferase, serum	Serum	7–41 U/L	0.12–0.70 µkat/L

glutamic pyruvic transaminase)			
Amylase	Serum	20–96 U/L	0.34–1.6 µkat/L
• Total		0.3–1.3 mg/dL	5.1–22 µmol/L
• Direct (conjugated)		0.1–0.4 mg/dL	1.7–6.8 µmol/L
• Indirect (unconjugated)		0.2–0.9 mg/dL	3.4–15.2 µmol/L
CA-125	Serum	0–35 U/mL	0–35 Ku/L
Calcium—ionized	Whole blood	4.5–5.3 mg/dL	1.12–1.32 mmol/L
Calcium—total	Serum	8.7–10.2 mg/dL	2.2–2.6 mmol/L
Carcinoembryonic antigen (CEA)	Serum	<5 ng/mL in non-smokers and <10 ng/mL in smokers	
Chloride	Serum	102–109 mEq/L	102–109 mmol/L
C-reactive proteins	Serum	0.2–3.0 mg/L	0.2–3.0 mg/L
Creatine kinase (CK), total	Serum		
Males		51–294 U/L	0.87–5.0 µkat/L
Females		39–238 IU/L	0.66–4.0 µkat/L
Creatine kinase MB (CKMB)	Serum	0–5.5 ng/mL	0–5.5 µg/L
Gamma glutamyl transpeptidase (transferase) (γ-GT)	Serum	9–58 IU/L	0.15–1.00 µmol/L
Glucose (fasting)	Plasma		
• Normal		70–100 mg/dL	<5.6 mmol/L
• Impaired fasting glucose (IFG)		101–125 mg/dL	5.6–6.9 mmol/L
• Diabetes mellitus		>126 mg/dL	>7.0 mmol/L
Glucose (2-hour postprandial)	Plasma		
• Normal		<140 mg/dL	<7.8 mmol/L
• Impaired glucose tolerance (IGT)		140–200 mg/dL	7.8–11.1 mmol/L
• Diabetes mellitus		>200 mg/dL	>11.1 mmol/L
Component	***Specimen***	***Reference value***	
		Conventional	***SI units***
Glycated hemoglobin (HbA_{1c})	Whole blood	4.0–6.0%	20–42 mmol/mol Hb

Lactate dehydrogenase (LDH)	Serum	115–221 U/L	2.0–3.8 µkat/L
Muramidase	Serum	5–20 µg/mL	
5-nucleotidase	Serum	0–11 U/L	0.02–0.19 µkat/L
Phosphatases	Serum		
• Acid phosphatase		0–5.5 U/L	0.90 µkat/L
• Alkaline phosphatase		33–96 U/L	0.56–1.63 µkat/L
Prostate-specific antigen (PSA)	Serum	0–4.0 ng/mL	0–4.0 µg/L
Proteins—total	Serum	6.7–8.6 g/dL	67–86 g/L
• Albumin		3.5–5.5 g/dL	35–55 g/L
• Globulins		2.0–3.5 g/dL	20–35 g/L
• Albumin/globulin ratio		1.5–3:1	
Troponins, cardiac (cTn)			
• Troponin I (cTnI)	Serum	0–0.08 ng/mL	0–0.8 µg/L
• Troponin T (cTnT)	Serum	0–0.01 ng/mL	0–0.1 µg/L
Urea nitrogen (BUN)	Blood	7–20 mg/dL	2.5–7.1 mmol/L
Uric acid	Serum		
• Males		3.1–7.0 mg/dL	0.18–0.41 µmol/L
• Females		2.5–5.6 mg/dL	0.15–0.33 µmol/L

Contd...

Lipid Profile (Table 14.25)

TABLE 14.25: Lipid profile.

Component	***Reference value***	
	Conventional	***SI units***
Total serum cholesterol		
• Desirable for adults	<200 mg/dL	<5.17 mmol/L
• Borderline high	200–239 mg/dL	5.17–6.18 mmol/L
• High undesirable	>240 mg/dL	>6.21 mmol/L
Low-density lipoprotein (LDL) cholesterol		
• Desirable range	100–130 mg/dL	<3.34 mmol/L
• Borderline high	130–159 mg/dL	3.36–4.11 mmol/L

TABLE 14.25: Lipid profile.

Component	*Reference value*	
	Conventional	*SI units*
• High	160–189 mg/dL	4.11–4.20 mmol/L
• Very high	>190 mg/dL	>4.21 mmol/L
High-density lipoprotein (HDL) cholesterol		
• Low	<40 mg/dL	<1.03 mmol/L
• High, protective range	>60 mg/dL	>1.55 mmol/L
Triglycerides	<160 mg/dL	<2.26 mmol/L

Urea and Electrolytes (Table 14.26)

TABLE 14.26: Urea and electrolytes.

Analyte	*Reference value*	
	Conventional	*SI units*
Sodium	136–146 mEq/L	136–146 mmol/L
Potassium	3.5–5.0 mEq/L	3.5–5.0 mmol/L
Chloride	95–107 mEq/L	95–107 mmol/L
Urea	20–40 mg/dL	3.3–6.6 mmol/L
Creatinine	0.6–1.2 mg/dL	53–106 µmol/L

CHAPTER
15

Blood Banking and Transfusion

BLOOD GROUP SYSTEM

CC: Classify and describe blood group systems (ABO and Rh).

Q. Write short essay/note on blood grouping/methods of blood grouping.

Karl Landsteiner in 1900 first described the existence of major human blood groups. More than 400 red blood cell antigens have been identified, most of which are inherited in a Mendelian dominant fashion and only few are clinically important. These antigens form more than 20 genetically determined blood group systems. The two most important blood group systems are: (1) ABO and, (2) Rh systems.

ABO Blood Group System

Q. Write short essay/note on ABO blood group.

ABO Antigens

According to the ABO blood group system, there are four different kinds of blood groups: A, B, AB or O. The antigen on red cells may be A/B/AB or no antigen at all. There are two subgroups in group A, namely A1 and A2. A and B antigens are inherited as per the Mendelian laws. Each individual inherits two ABO genes, one from each parent and these genes determine the ABO antigens on their red blood cells. Absence of both antigens, A and B on the red cells are seen in blood group O.

ABO Antibodies

Natural ABO Antibodies

The serum contains naturally occurring antibodies against red cell A/B/AB antigens. These are of IgM type and do not cross the placental barrier.

Acquired ABO Antibodies

An individual lacking a particular antigen may develop an antibody after (acquired) exposure to red blood cells carrying the corresponding antigen. Exposure occurs due to parenteral introduction of red cell antigens. This may develop during:

- **Transfusion of red cells:** These acquired antibodies cause hemolytic transfusion reaction.
- **Pregnancy:** Passage of fetal red cells (having paternal antigens foreign to the mother) into the maternal circulation during pregnancy may result in mainly IgG type of antibodies. They can cross the placental barrier resulting in hemolytic disease of the newborn. The most important acquired *antibody* is anti-D which is a major cause of hemolytic disease of the newborn.

H Genes and H Antigens

The expression of A and B antigens are dependent on the presence of H gene. Most of the individuals are homozygous for the H gene. The sequence of events in the formation of A and B red cell antigens are:

- A and B red cell antigens are glycoproteins and their formation start with basic precursor substance.
- Basic precursor substance is first converted into H substance (by transferase) under the influence of H gene.
- H substance is partially converted under the influence of A and B genes (and specific transferase) into A and B antigens. Some of the H substance remains unconverted.
- Since, O group individuals do not have A and B genes, neither A nor B antigen is formed and these have only H substance (H substance remains unchanged).

Note: A, B and H antigens may be detected in the saliva and other body fluids also. Such individuals are called **secretors,** while the remaining without it are **nonsecretors.**

Bombay Blood Group

Q. Write short note on Bombay blood group.

- In 1952, Bhende, Bhatia and Deshpande discovered a new blood group known as Bombay blood group (phenotype Oh). These individuals lack the H gene and therefore, the basic precursor substance cannot be converted into H substance. This in turn results in failure to form A or B antigen.
- When their blood sample is tested for routine ABO grouping, they will be labeled as blood group O. However, their serum contains anti-A, anti-B and anti-H antibodies. These individuals therefore, should be transfused with only Bombay blood group.

ABO Grouping Technique

- ABO grouping is of two types namely (i) forward (cell) typing in which antigens on RBCs are detected and (ii) reverse (serum) typing in which antibodies in the serum/plasma is detected.
- ABO grouping is performed by making a 2% saline suspension of red cells and adding anti-A, anti-B and anti-AB sera.

Methods: The different methods available are as follows: Slide or tile technique, tube technique, microplate method, microtyping system and automated or semiautomated method.

Slide or Tile Technique

Take a slide/white tile and mark it anti-A, anti-B and anti-AB. Put one drop each of anti-A, anti-B and anti-AB sera on the marked slide. Add one drop of washed 2% red cell suspension to each antiserum. Mix each one separately with clean applicator sticks and spread the mixture over an area of 2 cm. Rock the slide gently and look for "agglutination" within 5 minutes.

Interpretation (Fig. 15.1): Blood group A, B, AB or O is interpreted depending on the agglutination in the antisera.

Controls: Each blood group test should preferably have controls, both positive and negative.

- **Positive control:** This is run by performing the above techniques by using cells of known groups A, B, AB and O. This is especially useful for testing the potency of antisera.
- **Negative control:** Run the above tests by adding saline instead of antisera. If agglutination develops, it indicates autoagglutination or pseudoagglutination.

Note:

- A group has two subgroups namely A1 and A2 which can be differentiated by using anti-A1 lectin.
- Presence of H substance can be detected by anti-H.

Rh Blood Group System

Q. Write short essay/note on Rh factor and Rh blood grouping.

Rh blood group system is the second system of clinical significance in transfusion medicine. This is a complete system of antigens which are labeled as D, d, C, c, E and e. The D antigen is highly immunogenic. Cc and Ee antigens are weak antigens and therefore, risk of sensitization to these antigens is less than the risk of sensitization to D.

Rh (D) System

Rh Antigens

- ***Rh positive (+ve):*** Individuals who have D antigen on the red cell surface are Rh positive (+). This may be present as homozygous (DD) or heterozygous (Dd) state.
- ***Rh negative (-ve):*** Individuals who lack the D antigens are called as Rh-negative.

Rh Antibodies

- In contrast to ABO system, there are no naturally occurring antibodies against Rh antigens in Rh negative individuals.

- Acquired (immune) antibodies: Rh antibodies develop after exposure to Rh antigens following transfusion (Rh +ve blood given to Rh –ve patients). They can be detected by antiglobulin test (Coombs test).

Clinical Significance

- **Rh incompatibility between donor and recipient** results in **hemolytic transfusion reactions**.
- **Rh incompatibility between mother and fetus** results in **hemolytic disease of the newborn** (HDN).

Rh (D) Typing Techniques

Methods: The different methods available are—slide or tile technique, tube technique, microplate method, microtyping system and automated or semi-automated method.

Slide or Tile Techniques

Place one drop of anti-Rh (D) reagent (should be monoclonal IgM type) on a slide/white tile. Add 1 drop of 2% red cell suspension. Mix them using a clean applicator stick. Observe for agglutination after 2 minutes.

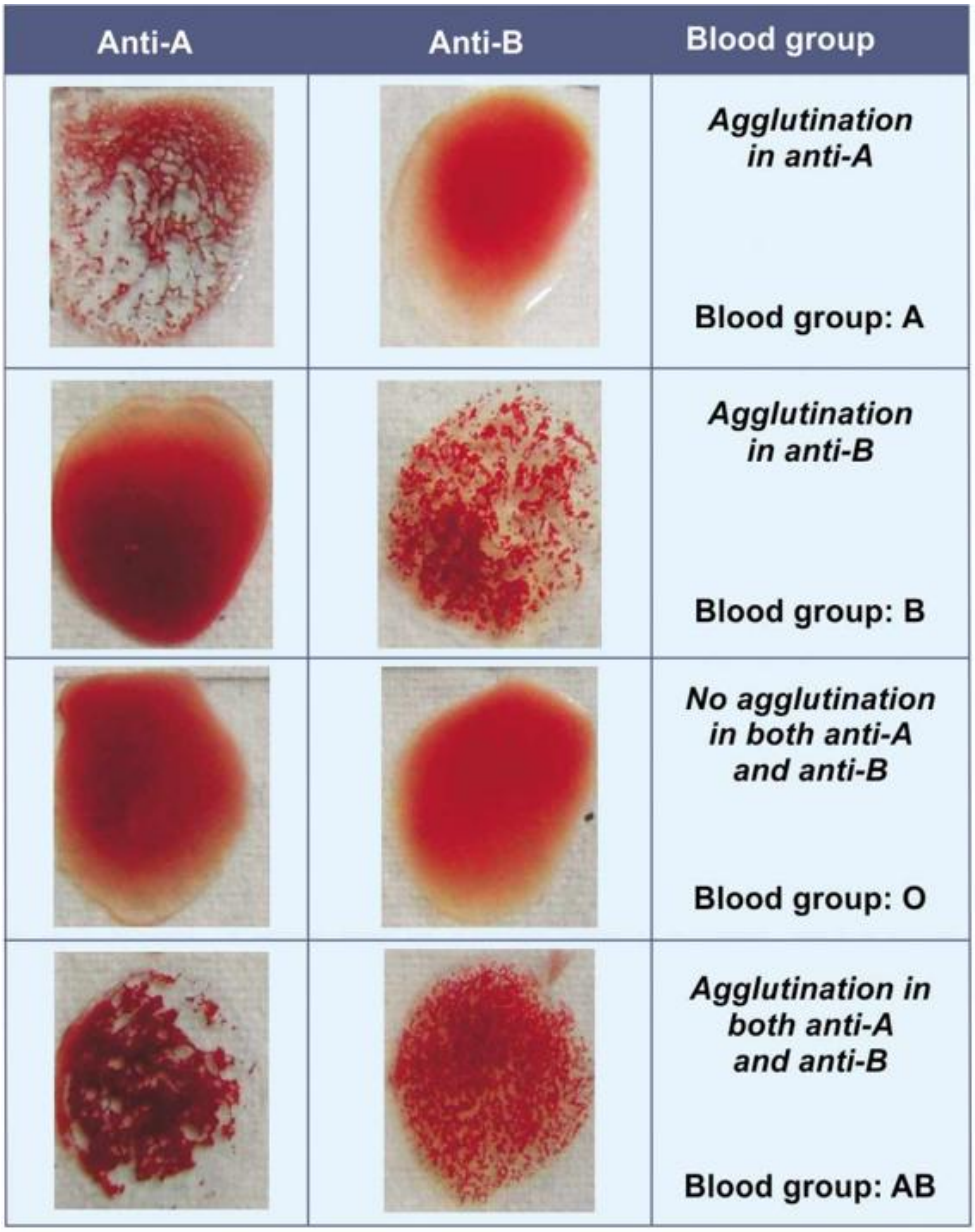


Fig. 15.1: Interpretation of blood group by slide method.

Interpretation: Presence of agglutination indicates that the blood sample is Rh +ve.

Controls: Known Rh +ve and Rh –ve samples should be run as positive and negative control.

Other Methods of ABO Grouping and Rh Typing

These include tube technique, microplate method, microtyping system and automated or semi-automated method **(Box 15.1)**. In test tube method

procedure is similar to slide method except that it is performed in a test tube.

Microplate Method

Microplate method is ideal for testing large number of blood samples and has replaced test tube method in many laboratories. Microplates are polystyrene plates having 96 small well. It saves time and cost.

Microtyping System (Gel Card)

Microtyping system (MTS) is a gel-based technology in which plastic cards contain microtubes filled with buffered dextran gel. The gel contains sodium azide (preservative) and group-specific antisera (anti-A, anti-B, anti-AB, anti-D) or antiglobulin (for Coombs test). These constituents are incorporated into gel at the time of manufacture.

Both the above methods are used for blood grouping.

Importance of Blood Group

Q. Write short answer on importance of blood group.

Blood grouping is important before blood transfusion, for pregnant women (for Rh incompatibility between Rh+ ve and Rh-ve mother) and is also of medicolegal value.

Other blood group systems: These are weak antigens and have no clinical significance. Few blood group are listed in **Box 15.2**.

TRANSFUSION MEDICINE

Transfusion medicine comprises of blood and blood component transfusion. Blood cannot be synthesized artificially. So, the source of blood is from a healthy human donor.

Blood Transfusion

Blood transfusion is the process of transferring blood/blood products from donor into the circulating system of recipient. It is important to properly collect the blood from donor, prepare its components (if required) and store

blood/components in a proper way and transfuse in such a way to avoid any risks or hazards.

Donor Selection

Q. Criteria for selection of blood donor.

Donor selection is based on medical history and few routine physical examinations (weight, blood pressure, temperature, hemoglobin) are done to know whether donor is suitable for donating blood.

- Donor should be healthy. There are three types of donors namely voluntary (should be encouraged), replacement and professional.
- Age: Donor should be between 18-65 years of age.
- Weight: Should be >45 kg.
- Blood pressure should be normal and not a diabetic on insulin or uncontrollable sugar levels.
- Time interval between each blood donation should be 3 months.
- No skin disease at the phlebotomy site.
- It is important to know whether the patient has history of diseases like hepatitis, AIDS, syphilis and if so, blood should not be obtained from them.

Collection of Blood

Blood is collected under aseptic conditions using sterile, plastic bag with anticoagulant. Now CPD-A is used as an anticoagulant. Mix the blood and anticoagulant gently and periodically during its collection.

Q. Write short answer on anticoagulants used in blood bank.

Anticoagulants used: The different anticoagulants-preservative solutions available are as follows:

- Citrate phosphate dextrose (CPD).
- Citrate phosphate dextrose adenine-1 (CPDA-1)
- Acid citrate dextrose (ACD) is not used nowadays.

BOX 15.2: Blood groups other than ABO and Rh.

- Lewis system

- P system
- KELL system
- MNS system
- Duffy system
- Kidd system
- Lutheran system
- Yt system
- Dombrok system
- Colton system
- Diego system

BOX 15.1: Various methods for ABO blood grouping and Rh typing.

Manual methods

- Traditional methods: Slide or tile technique
- Tube technique
- Microplate method
- Column agglutination technique
  - Gel technology (ID Micro Typing System)
  - Glass microbead technology
- Solid phase red cell adherence assay
- Erythrocytes magnetized technology

Automated or semiautomated method

Molecular blood grouping and genotyping

Q. Write short answer on functions of each component of CPDA-1.

Functions of various chemical used is mentioned in **Table 15.1**.

Storage of blood: Blood is stored in a refrigerator at 2°–6°C.

Predonation Check-Up

Q. Write short answer on screening of blood donor.

Donor blood: The following tests are routinely carried out on donor's blood:

- ABO and Rh grouping.
- Tests for:
  - HBsAg, anti-HCV, anti-HIV-1 and HIV-2 and serum alanine aminotransferase (ALT).
  - Malaria and syphilis.

Recipient blood: The recipient's ABO and Rh grouping are also carried out.

PRETRANSFUSION AND COMPATIBILITY TESTING

CC (Show How): Enumerate the indications, describe the principles, enumerate and demonstrate the steps of compatibility testing.

Q. Write short answer on screening of blood unit before transfusion (pretransfusion tests).

Indications: Before transfusion of any blood or its components, it is essential to know whether they are compatible with the recipient's blood. This is achieved by performing a set of procedures known **pretransfusion testing** also referred to as **compatibility testing**. These set of **procedures** (serologic and nonserologic protocols) are **required before blood is issued as being compatible**. A compatibility testing is an entire quality process **composed of many procedures (steps)**. It is designed to provide the safest blood product possible for the recipient of the transfusion. Sometimes, the term compatibility test and cross-matching are used interchangeably, but crossmatch is only a part of compatibility test. List of compatibility tests are presented in **Box 15.3**.

Crossmatching (Crossmatch Testing)

CC: Describe the correct technique to perform a crossmatch.

Q. Write short answer on crossmatching.

The term crossmatch is a crossway mixing of donor and recipient blood components. In crossmatching, the serum or plasma from the recipient is mixed with red cells from the donor. A crossmatch is only one part of pretransfusion (compatibility) testing and is very important before any blood transfusion. Crossmatching should be carried out to ensure that there are no antibodies in patient's serum that will react with the donor's cells when transfused. Crossmatching is routinely done only with donor products containing red cells, hence must be performed for red cell transfusions. **Hemolysis or agglutination at any phase or step** of the crossmatch process **indicates that the antibodies present in the recipient** interact with donor

red cell antigens and a mismatch between donor and recipient. A crossmatch is considered as compatible when there is neither agglutination nor hemolysis in testing and the donor unit is acceptable for transfusion purposes. A cross-match is considered as incompatible when there is presence of either agglutination or hemolysis.

Importance of Crossmatching

- It is the final check of ABO compatibility between donor and recipient.
- Detects the presence of any clinically significant, unexpected antibodies in the recipient's serum that may react with donor's cells, thereby preventing any transfusion reaction.
- A thorough crossmatching is performed with recipient's serum and donor's red cells.

Types

Crossmatching procedure may be divided into two major parts, namely, (i) major and (ii) minor crossmatching **(Table 15.2)**.

Crossmatch techniques: The various crossmatch techniques are listed **Box 15.4**.

BOX 15.3: Pretransfusion testing protocol (steps).

- Recipient blood sample collection: Accurate identification of patient (recipient), proper sample collection, labeling and handling
- Review of patient's past blood bank history and records (if done earlier).
- Pretransfusion testing of recipient's and donor blood specimen
  - ABO grouping and Rh typing
  - Antibody screening test of recipient's and donor's serum:
- Crossmatch: Crossmatch of recipient's sample with donor units:
  - Selection of crossmatch procedure (serologic or non-serologic)
  - Selection of blood for transfusion
  - Performance of a crossmatch: Recipient serum with donor red blood cells
- Proper labeling of blood or blood components of donor before use with the recipient's identifying information and issue

TABLE 15.1: Functions of various chemicals used in anticoagulant- preservative solution.

Chemical used	***Function***
Citrate	Prevents clotting by chelating calcium
Sodium biphosphate	Buffers the end product of glycolysis (lactic acid)

Dextrose	Needed for ATP generation for viability of red cells
Adenine	Substrate for red cell synthesis of ATP

TABLE 15.2: Types of crossmatch.

Type of crossmatch	*Donor's*	*Recipient's (patient's)*
Major crossmatch	Red cells	Serum
Minor crossmatch	Serum	Red cells

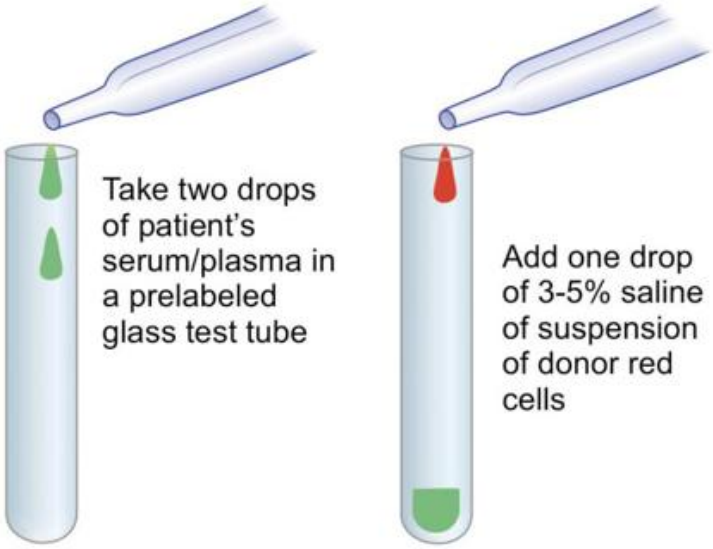


Fig 15.2: Immediate spin crossmatch. Patient/recipient serum is crossmatched with donor red cells and it is called as major crossmatch. In minor crossmatch, the donor's serum is crossmatched with patient's/recipient's red cells. Antibody screen testing on donor samples has replaced the minor crossmatch.

BOX 15.4: Crossmatch techniques.

- **Serological crossmatch**
  - Immediate spin (IS) technique
  - Saline room temperature technique
  - Albumin/low ionic strength solution (LISS) addition technique at 37°C
  - Indirect antiglobulin technique (using gel card)
- **Nonserological crossmatch**: Computer/electronic crossmatch

Serological Crossmatch Techniques

Major serological crossmatch test consists of testing the patient's (recipient's) serum with donor RBCs **(Box 15.3)**. The routinely used two

serological methods are— (i) **immediate-spin (IS) crossmatch**, and, (ii) an **indirect antiglobulin test (IAT) crossmatch**. In both these serologic crossmatch procedures, the recipient's serum or plasma is tested with the red cells from the donor unit.

Principles of Serological Testing

The pretransfusion testing is performed to detect in vitro red cell antigen and antibody reaction by observing for either agglutination or hemolysis. Only one serological method namely immediate-spin (IS) crossmatch is discussed here.

Immediate-spin Crossmatch

This is the simplest serologic test to detect most ABO incompatibility in which the **recipient's serum or plasma is mixed with donor's red cell suspensions and centrifuging immediately** (i.e., immediate spin). Then observe it for agglutination. **Absence of hemolysis or agglutination indicates ABO compatibility**. This serologic test is used to detect ABO incompatibility and is enough to rule out any ABO grouping error. In this method, there should not be any delay in the centrifugation step or reading the reaction.

Advantages: IS crossmatch procedure requires less turn-around time, workload, and reagent cost compared to full AHG crossmatch.

Disadvantages: This method **does not detect all ABO incompatibilities** and is inadequate for detection of clinically significant IgG type of antibodies.

Procedure for immediate-spin crossmatch (Fig. 15.2):

- Take two drops of patient's serum/plasma in a prelabelled glass test tube.
- Add one drop of 3–5% saline of suspension of donor red cells. Cells usually are washed once to remove any anticoagulant or plasma protein which may interfere with the testing.
- Mix the contents and incubate for 5–10 minutes for IS method or for 45–60 minutes for saline room temperature technique.
- Immediately centrifuge the tube at 1,000 rpm for 1 minute (IS method). While in case of saline room temperature technique, centrifugation is optional.

- Gently dislodge the cell button and examine the tube for the presence or absence of hemolysis or agglutination.

This procedure is known as the abbreviated or IS crossmatch.

Interpretation:

- **Positive test:** If hemolysis or agglutination is present at this stage, the test is considered as reactive/positive. It indicates that the unit selected may be **ABO incompatible** and the unit is **not considered acceptable for transfusion** (crossmatch is incompatible).
- **Negative test:** If there is **no hemolysis or agglutination**, test is considered as nonreactive/negative and the **unit is considered acceptable for transfusion** (compatible).

It is always necessary to monitor the recipient during and after transfusion so that any complications can be dealt accordingly. **Presently indirect antiglobulin technique** (by gel card technology) is used in blood banks.

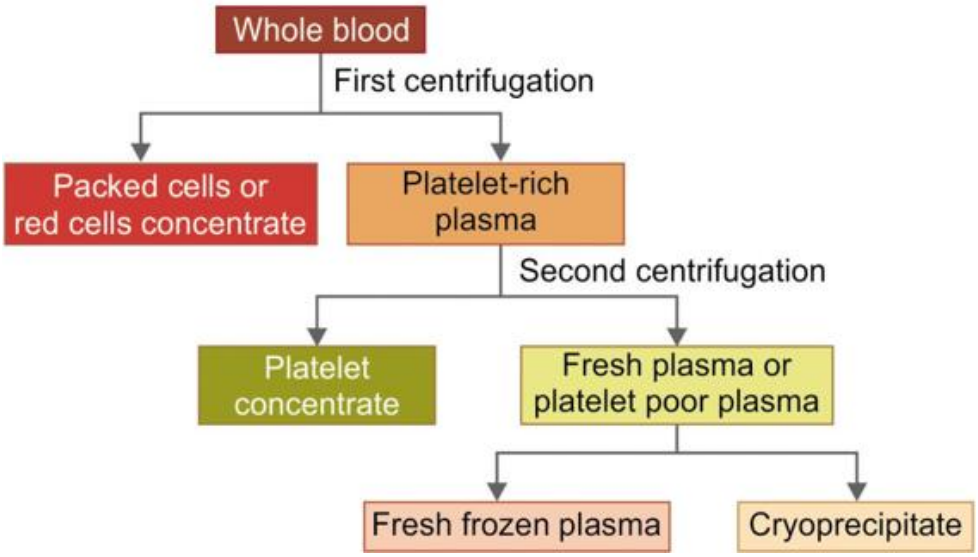
Blood Components

CC: Enumerate blood components and describe their clinical uses.

Q. Write short essay/note on blood components.

It is possible to separate different components of blood from a single unit of whole blood. These components can be used individually to help more than one patient with many purposes. Thus, red cells can be transfused to an anemic patient and plasma for a burn's patient. This also ensures that only the required components are transfused. The various blood components **(Flowchart 15.1)** are as follows:

Flowchart 15.1: Blood components.



BOX 15.5: Indications for use of packed red blood cells.

Anemia

- Hemolytic anemia especially in aplastic crisis
  - β-thalassemia major
  - Sickle cell anemia in sequestration crisis
- Hypoplastic anemia: Aplastic anemia and anemia of chronic renal disease
- Severe anemia of any cause to reduce the chances of circulatory overload
- Hypovolemia due to hemorrhage (trauma)
- Blood loss during surgery: Patients undergoing cardiac, orthopedic, and other surgeries
- Other causes: Oncology patients undergoing chemotherapy or radiation therapy, end-stage renal disease, premature infants, and neonates

TABLE 15.3: Main uses of various blood components.

Various blood components	***Main uses***
Red cell concentrates, packed red blood cells (PRBCs)	To restore tissue oxygenation
Platelets [platelet rich plasma (PRP), platelet concentrates]	To treat bleeding due to thrombocytopenia
Fresh frozen plasma (FFP)	Coagulation factor deficiencies
Cryoprecipitate	As a source of fibrinogen
Factor VIII and von Willebrand factor	Deficiency of factor VIII and von Willebrand factor
Specific factor concentrates	Specific factor deficiency

BOX 15.6: Indications for platelet concentrate.

Bleeding due to:

- Thrombocytopenia, when platelet count is less than 20,000/mm^3
  - Chemotherapy or radiation therapy or drug induced
  - Leukemia
  - Marrow infiltration, e.g., leukemia, carcinoma
  - Dilutional (e.g., massive transfusion with stored blood)
  - Hypoplastic anemia
  - Viral disease associated (e.g., dengue)
- Abnormal platelet function
- Disseminated intravascular coagulation (DIC)
- Recipients of hematopoietic progenitor cell transplants for a period after transplant
- Patients with postoperative bleeding
- Organ transplant patients (e.g., liver transplant)

- Red cells concentrate or packed red blood cells (PRBCs)
- Platelet concentrates
- Plasma
- Fresh frozen plasma (FFP)
- Cryoprecipitate

Main uses of various blood components are presented in **Table 15.3**.

Red Cells Concentrate or Packed Red Blood Cells

It is the red blood cells remaining after removal of the most of the plasma from whole blood.

Indications for Use of PRBCs (Box 15.5)

RBCs in the blood contain hemoglobin which transports oxygen to the tissues. Transfusion of PRBCs increases the mass of circulating red cells in conditions where there is impaired tissue oxygenation due to acute or chronic blood loss (e.g., hemorrhage, anemia). In the packed red cell unit, the hematocrit is between 55 and 65%. Since, the volume is only 200 mL; there is less risk of volume overload.

Platelet Concentrate

Q. Write short answer on platelet concentrate.

Platelet concentrate may be obtained from a single donor or pooled plasma. Platelets can also be obtained from a single donor by platelet apheresis. Indications for platelet concentrate transfusion are listed in **Box 15.6**.

Fresh Frozen Plasma

Q. Write short answer on fresh frozen plasma.

Fresh frozen plasma (FFP) is prepared by freezing the plasma and contains plasma proteins and all coagulation factors that include albumin, protein C and S, antithrombin and von Willebrand factor. It is indicated for replacement of coagulation factors in acquired coagulation factor deficiencies **(Box 15.7)**.

Cryoprecipitate

Q. Write short answer on cryoprecipitate.

Cryoprecipitate is a source of insoluble plasma proteins, fibrinogen, factor VIII and vWF.

BOX 15.7: Indications for fresh frozen plasma.

- Patients on anticoagulant drug therapy (Coumarin)
- Antithrombin deficiency
- Coagulopathy of liver diseases
- Vitamin K deficiency
- Microangiopathic hemolytic anemia including thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome and hemolysis, elevated liver enzymes, and a low platelet count (HELLP) syndrome
- Disseminated intravascular coagulation (DIC)

TABLE 15.4: Blood products that can and cannot transmit HIV.

Blood products which can transmit HIV	***Blood products which cannot transmit HIV***
Whole blood, packed RBCs, platelets, leukocytes and plasma	Hyperimmune gamma globulin, hepatitis B immunoglobulin, plasma-derived hepatitis B vaccine and Rho immunoglobulin

Indications for cryoprecipitate: Patients who need fibrinogen, factor VIII and vWF. Transfusion of single unit of cryoprecipitate provides about 80 IU of factor VIII.

Blood products that can and cannot transmit HIV are listed in **Table 15.4**.

TRANSFUSION-TRANSMITTED DISEASES

CC: Enumerate and describe infections transmitted by blood transfusion.

Blood is a lifesaving resource and blood components should be tested so that it makes them extremely safe and reduces the risk to minimum of the likelihood of a transfusion-transmitted disease (TTD). TTD is the infectious complications of blood transfusion. A variety of infectious agents including bacteria, viruses, parasites **(Table 15.5)**, and prion pathogens can get effectively transmitted through blood transfusions. If they are not detected in the testing process, it can cause harm and even death.

Screening Blood Donors for Transfusion-Transmitted Infections

Donor Testing

Once a donor passes the medical screen and donor questionnaire, the serologic tests should be performed for the TTD. Routine screening of donor's blood in India is presented in **Table 15.6**. Performing these tests can make blood transfusion safer than ever before.

Apart from the transfusion-transmitted diseases listed in **Table 15.5,** many other organisms may also be transmitted by transfusion. However, tests for these organisms are not routinely performed in the blood screening process. These include other viruses, such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), parvovirus B19, bacteria (presently is the leading cause of death from transfusion), parasites, such as *Babesia microti* and *Trypanosoma cruzi*, malaria, and prion diseases.

Presently performed test methods are extremely sensitive. Hence, confirmatory tests are used only to detect false-positives. These tests depend on the disease to be confirmed and include polymerase chain reaction (PCR), Western blot (WB), radioimmunoprecipitation assay (RIPA), and recombinant immunoblot assay (RIBA).

TABLE 15.5: Transfusion-transmitted infectious (TTI) agents.

Viral	*Bacterial*	*Parasitic*
Common		
• Human immunodeficiency virus (HIV) • Hepatitis B virus (HBV) • Hepatitis C virus (HCV)	*Treponema pallidum* (syphilis) is not common nowadays	*Plasmodium* species (malaria)
Other less common TTIs		
• Epstein-Barr virus (EBV) • Cytomegalovirus (CMV) • Human T lymphotrophic virus-1 (HTLV-1) • Human T lymphotrophic virus-2 (HTLV-2) • West Nile virus • Parvovirus B19 (B19V) • Dengue virus (few cases reported)	*Brucella*	• *Babesia* species • *Toxoplasma gondii* • *Trypanosome cruzi* (Chaga's disease) • *Leishmania* species
Others: Prion		

TABLE 15.6: Routine serological tests and screening methods used for testing donor blood in India.

Transfusion-transmitted diseases	*Required serological tests*	*Screening method*
Human immunodeficiency virus (HIV)	Antibodies to human immunodeficiency virus (anti-HIV 1/2 RNA) and p24 antigen	ELISA, particulate agglutination, simple rapid assays
Hepatitis B virus	Hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc)	ELISA, simple rapid assays
Hepatitis C virus	Antibody to hepatitis C virus (anti-HCV), HCV RNA	ELISA, simple rapid assays
Syphilis	Nonspecific antibody: Serological tests for syphilis (STS)	VDRL or RPR
Malaria	Trophozoite/gametocyte	Microscopy or QBC

It is important to select a safe donor to provide a safer donation. The safest donors are regular, voluntary, and nonremunerated. Blood for donation should be screened for TTDs.

Test samples: Most of the assays use either serum or plasma. The instructions with the assay must be followed strictly.

Mandatory Screening Tests

The five tests that are mandatory as per Food and Drug Administration (FDA) for the donated blood units are HBsAg, HIV Ab, HCV Ab, VDRL and malarial parasites.

Nucleic Acid Testing (NAT) or Nucleic Acid Amplification Test (NAAT)

NAT is a general term used for molecular-based methods of screening for infectious agents. A nucleic acid test (NAT) is a method useful to detect a particular genetic material (nucleic acid) of virus or bacteria which acts as a pathogen in blood, tissue, urine, etc. The NAT differs from these conventional tests in that it detects genetic materials rather than antigens or antibodies. It detects very low numbers/levels of RNA or DNA of the pathogens (e.g., HIV, HBV, HCV) in the bloodstream before the appearance of antibodies. It detects infection earlier than conventional methods thereby narrowing the window period.

ADVERSE TRANSFUSION REACTIONS

CC: Describe transfusion reactions and enumerate the steps in the investigation of a transfusion reaction.

Safety measures are usually taken at all steps of the blood collection, donor unit processing, pretransfusion testing, and patient monitoring. In spite of careful precaution and preventive measures, unfavorable adverse effects, such as noninfectious complications of transfusions occur (about 2–4% of cases) and are not always preventable. These adverse effects of blood transfusion are also known as **blood transfusion reactions or simply transfusion reactions**.

Definition: An **adverse transfusion reaction** is defined as **any adverse event** (undesirable response/effect or complications related to transfusion) **that occurs in a patient during or after the transfusion** of whole blood, blood components, or human-derived plasma products. It may range from relatively mild to severe and at times can be fatal.

Classification (Box 15.8): Transfusion reactions can be categorized into different types.

According to the time interval between transfusion and the presentation of adverse effects, transfusion reactions can be classified as:

- **Acute (immediate) transfusion reaction:** It is a transfusion reaction with **signs or symptoms occurring during or shortly after transfusion** (within 24 hours of transfusion).
- **Delayed transfusion reaction:** It is a transfusion reaction with signs or **symptoms presenting after 24 hours of transfusion**.

According to the pathophysiology/mechanism it may be further classified into:

- **Infectious and non-infectious** complications **(Box 15.8)**. Noninfectious complications of transfusions are greater risk to patients than infectious diseases.
- **Immune versus nonimmune.**

Immediate/Acute Transfusion Reactions

Definition: *Acute transfusion reaction* is defined as a reaction in which signs and symptoms occur within 24 hours of a transfusion.

Acute Hemolytic Transfusion Reaction

BOX 15.8: Transfusion reaction (complications of blood transfusion).

NONINFECTIOUS COMPLICATIONS

Immediate (acute) reactions

- ***Immunological***
  - Acute hemolytic transfusion reactions (AHTRs)
  - Acute nonhemolytic transfusion reactions
    - Febrile nonhemolytic transfusion reaction (FNHTR)
    - Allergic reaction
    - Anaphylactic
    - Transfusion-related acute lung injury (TRALI)
- ***Nonimmunological***
  - Nonimmune mediated hemolysis
  - Transfusion-associated circulatory overload (TACO)
  - Hypotension
  - Transfusion-associated sepsis (TAS)
  - Metabolic complications (hyperkalemia, hypokalemia, hypocalcemia, hypothermia)

  - Air embolism
  - Physical hemolysis

Delayed reactions

- ***Immunological***
  - Delayed hemolytic transfusion reactions (DHTRs)
  - Transfusion associated graft versus host disease
  - Post-transfusion purpura
  - HLA alloimmunization
- ***Nonimmunological***
  - Iron overload-transfusion hemosiderosis
  - Citrate toxicity
  - Thrombophlebitis

INFECTIOUS COMPLICATIONS(TRANSFUSION TRANSMITTED DISEASES)

Few of the diseases transmitted by transfusion are:

- Hepatitis (HBV, HCV and HDV)
- HIV (AIDS)
- Malaria
- Cytomegalovirus
- Syphilis

Hemolytic transfusion reaction is characterized by the destruction of transfused red cells in the recipient. It results in intravascular or extravascular hemolysis or a combination of both. Hemolytic reactions are classified as acute or delayed, and both types may stem from immune or nonimmune causes.

Definition: Acute hemolytic transfusion reaction (AHTR) is characterized by **rapid destruction of red cells** (i.e., acute hemolysis) with **symptoms developing immediately after or within 24 hours** of transfusion of red cells. The **hemolysis may be due to immune or nonimmune mechanism**.

Immediate Immune-mediated Reactions

The severity of symptoms depends on the amount and rate of incompatible blood transfused. A**s little as 10 mL of incompatible blood can cause rapid hemolysis**.

Pathophysiology: Transfusion of ABO incompatible red cells (Flowchart 15.2): The hemolysis is due to **interaction of preformed antibodies in the recipient with the donor red cell antigens**. The most severe reactions are associated with transfusion of red cells that have ABO incompatibility with the recipient.

Signs and symptoms: AHTR ranges in severity from fever to death. Patient typically develops **fever with chills**, nausea, vomiting, diarrhea, pain, dyspnea (chest tightness), tachycardia, hypotension, bleeding and hemoglobinuria. Shock and renal failure are later complications.

Causes of immune-mediated acute hemolytic transfusion reaction

- **Clerical error:** Most common cause of AHTRs is ABO incompatibility due to a clerical error.
- **Technical error: Errors in blood grouping** of recipient or donor, **improper technique a failure to detect weak antibodies.**

Acute transfusion reactions can be divided according to one of three key presenting symptoms: Fever, allergic and pulmonary **(Flowchart 15.3)**.

Acute Nonhemolytic Transfusion Reactions

Febrile Nonhemolytic Transfusion Reactions

Definition: Febrile nonhemolytic transfusion reaction (FNHTR) is defined as a rise in body temperature of 1–37°C or greater. It is an acute nonthreatening complication of transfusion and occurs during or shortly after transfusion (up to 4 hours after transfusion).

Signs and symptoms: FNHTR are a common adverse effect of transfusion. Apart from **fever,** these are usually accompanied by **chills or rigors**.

Pathophysiology of FNHTR

- **Recipient leukocyte antibodies** especially HLA antibodies (antihuman leukocyte antigen) can react with antigens on transfused donor leukocytes (lymphocytes, granulocytes) or platelets leading to FNHTR.
- **Due to release of pyrogens and cytokines**

Allergic and Anaphylactic Transfusion Reactions

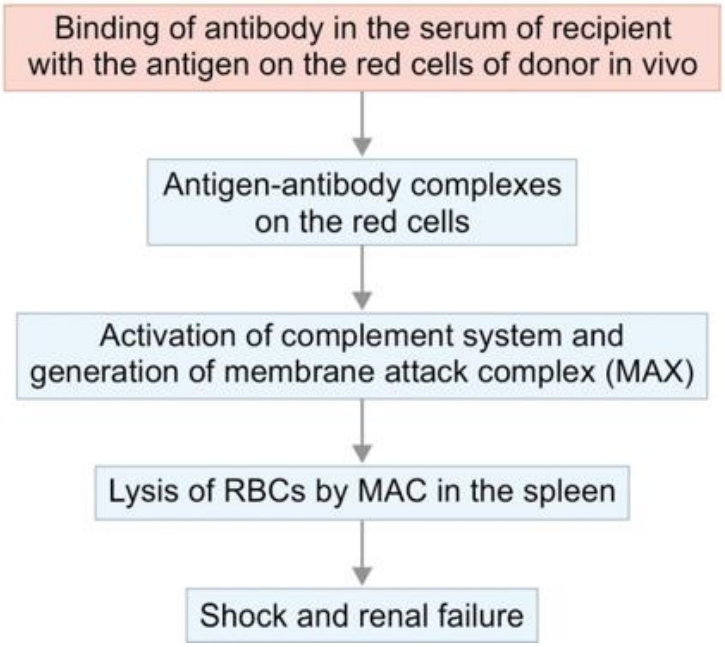
Definition: It is a type I IgE-mediated hypersensitivity reaction (HSR) against proteins (allergens) present in the donor blood.

Allergic transfusion reactions (ATRs/ALTRs) are acute, immune complications of transfusion. Allergic reactions are the **most common type**

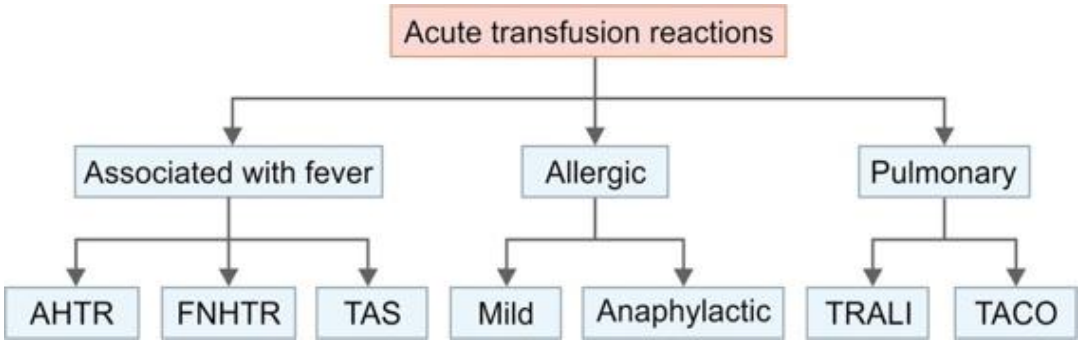
of transfusion reaction that occurs with the transfusion of blood products containing a plasma component. They **can occur with any type of blood component** including autologous RBCs.

Presenting symptoms: ATLR can present with a variety of symptoms that can range from minor urticarial effects to fulminant anaphylactic shock and death. Usually, **symptoms occur within minutes** after the transfusion is started.

Flowchart 15.2: Sequence of events in immune-mediated acute hemolytic transfusion reaction (AHTR).



Flowchart 15.3: Division of acute transfusion reactions according to one of three key presenting symptoms:Fever, allergic and pulmonary and its causes.



(AHTR: acute hemolytic transfusion reaction; FNHTR: febrile nonhemolytic transfusion reaction; TACO: transfusion-associated circulatory overload; TAS: transfusion-associated sepsis; TRALI: transfusion-related acute lung injury).

Mild allergic reactions (urticarial response) to transfusion are common. C**utaneous symptoms** include pruritus (itching), urticaria (wheals, hives

anywhere in the body), erythema, and cutaneous flushing. **Severe reactions (anaphylactoid or anaphylactic response) are rare.**

Transfusion-related Acute Lung Injury

Transfusion-related acute lung injury (TRALI) is an acute transfusion reaction presenting with acute respiratory distress and severe hypoxemia usually during or within 6 hours of transfusion in the absence of other causes of acute lung injury. TRALI can be a life-threatening or fatal transfusion reaction.

Pathogenesis of TRALI: Exact pathogenesis is not fully understood. It may be due to immune or nonimmune mechanism.

Symptoms: These include **dyspnea, hypoxemia, tachycardia**, nonproductive cough, fever, hypotension, and cyanosis. Characteristically, there are **no abnormal breath sounds**. A **chest X-ray** usually shows bilateral **pulmonary edema**. This syndrome is the **leading cause of transfusion-associated mortality.**

Nonimmunological Acute Transfusion Reactions

Nonimmune-mediated Hemolysis

Nonimmune hemolysis can also develop following transfusion. It develops **without presence/involvement of any antibodies**. Most frequently, it **presents as asymptomatic hemoglobinuria**.

Causes of nonimmune-mediated red cell hemolysis

1. **Exposure of red cells to extreme temperatures** (>50°C or <0°C)
2. **Mechanical or chemical damage with destruction of red cells**
3. **Simultaneous infusion of unapproved incompatible solutions**
4. **Bacterially contaminated blood products**: If they are transfused, they may cause hemolysis.
5. **Intrinsic red cell defect**: For example, sickle cell disease, thermal burns, glucose-6-phosphate dehydrogenase deficiency, and paroxysmal nocturnal hemoglobinuria.

Clinical signs: These include **hemoglobinemia and hemoglobinuria**. Hyperkalemia may be found in patients with renal failure. Fever may also occur.

Complications: Main complications of nonimmune hemolysis are **renal failure and cardiac arrhythmia** due to hyperkalemia.

Transfusion-associated Circulatory Overload

Transfusion-associated circulatory overload (TACO) is an acute, common, nonimmune, and preventable transfusion reaction (complication of transfusion). It presents as congestive heart failure during or shortly after transfusion.

Risk factors: Volume overload should be anticipated in at-risk patients and should be prevented. Risk of developing TACO is observed in the following:

- **Pre-existing disease:** Patients with **pre-existing heart disease, diminished cardiac reserves, end-stage renal disease, chronic anemia,** and those who are **very young or very old**, even with a small transfusion volume can produce TACO.
- **Rapid or massive transfusion** of blood.
- **Age:** Patients older than 70 years and infants.

Signs and symptoms: It present with respiratory distress and hypoxemia. Signs and symptoms include dyspnea, cough, orthopnea, cyanosis, tachycardia, chest tightness, elevated blood pressure (hypertension), pulmonary edema, distention of jugular veins, elevated central venous pressure, pedal edema and headache. TACO is also **associated with increased morbidity and mortality**.

Transfusion-associated Hypotension

Definition: Transfusion-associated hypotension (hypotensive transfusion reaction- HyTR) is defined as sudden and unexpected onset of clinically significant hypotension that occurs during transfusion of blood and blood components. It resolves quickly when the transfusion is discontinued.

Transfusion-associated Sepsis

Transfusion-associated sepsis (TAS) is an **acute nonimmune transfusion reaction**. It presents with rise in **body temperatures usually 2°C or more above normal** and rigors. A serious and potentially fatal complication of transfusion is secondary to bacterial contamination and proliferation in

donor units during storage. The transfusion of **even small quantity of blood contaminated with bacteria blood can be fatal** or cause serious morbidity.

Sources of bacterial contamination: The sources are mainly skin flora and less frequently, gut flora associated with transient bacteremia in an asymptomatic donor.

Clinical presentation: The clinical presentation of a transfusion reaction is caused by transfusion of bacteria-contaminated blood components.

Complications: Bacterial contamination of blood component may give rise to complication—shock, renal failure, DIC and death.

Delayed Reactions

Immunological

Delayed Hemolytic Transfusion Reactions

Definition: Delayed hemolytic transfusion reactions (DHTRs) also called as delayed serologic/hemolytic transfusion reactions (DSHTRs) are defined as the detection of "new" red cell antibodies at least 24 hours after transfusion of the offending unit. It is an anamnestic response after transfusion with no signs of hemolysis.

Acute hemolytic transfusion reactions are usually associated with the transfusion of ABO incompatible red cells. In contrast, in DHTRs, the symptoms appear after 24 hours and are caused due to a secondary immune response. The recipient has been immunized (primary immune response) after red cell exposure from either a prior transfusion or pregnancy.

Normally, clinically significant antibodies are detected during pretransfusion testing. However, it may not be detected because of (i) the antibody levels below the detectable levels, (ii) missed in error, (iii) less sensitivity of the test and (iv) rarely an antibody to a low-frequency antigen which are not demonstrated on the screening cells.

Time of development: Most patients develop DHTRs within the first 2 weeks after the transfusion. However, clinical DHTR may be recognized more than 6 weeks later.

Clinical presentation: DHTR are generally mild and may be overlooked. They tend to be much less severe than AHTRs. **Characteristic feature is development of fever and unexpected anemia** (unexplained or unexpected

fall/drop in hemoglobin or hematocrit) days or weeks after transfusion of an RBC component.

Transfusion-associated Graft-versus-host Disease

Definition: Transfusion-associated graft-versus-host disease (TA-GVHD) is a delayed immune transfusion reaction due to an immunologic attack by viable donor T lymphocytes present in the transfused blood component against the transfusion recipient.

TA-GVHD is a rare but highly lethal (90% mortality rate) complication of transfusion.

Clinical Manifestation

Time of manifestation: TA-GVHD usually manifests 2–30 days (usually 10–12 days) after transfusion.

Characteristic findings: The presenting symptoms features include a **maculopapular rash** (usually pruritic, typically starting centrally and extending to the extremities), watery diarrhea (accompanied by bloody stools and abdominal pain), fever, nausea, elevated liver function tests, and pancytopenia (due to marrow failure). These symptoms occur following transfusion of a nonirradiated cellular blood component.

Post-transfusion Purpura

Post-transfusion purpura (PTP) is a relatively uncommon, delayed immune complication of transfusion that occurs 5–12 days after transfusion of blood or blood products containing platelets. It presents with severe thrombocytopenia (with platelet counts below 10,000/μL) in the patient and frequently accompanied by generalized purpura, bleeding from mucous membranes, and the gastrointestinal and urinary tract.

Pathophysiology: The pathogenesis of PTP is related to the presence of platelet-specific antibodies in the patient. A patient may be previously exposed/sensitized to human platelet antigens by pregnancy or transfusion. PTP occurs when the same patient is re-exposed to the same antigen via a transfusion. PTP is a self-limiting syndrome with the platelet count generally returning to normal within 2 weeks. However, in 13–16% of cases, mortality due to intracranial hemorrhage has been reported.

Delayed Nonimmunological Iron Overload (Transfusion Hemosiderosis)

Definition: Iron overload is a delayed, nonimmune complication of transfusion. It presents with multiorgan (i.e., liver, heart, endocrine organs) damage secondary to excessive accumulation of iron.

Pathophysiology: Hemosiderosis is a condition characterized by the accumulation of excess iron in macrophages in various tissues. Iron overload, also called transfusion hemosiderosis, is a potential complication seen in patients with persistent hemolysis (e.g., thalassemia sickle cell disease) receiving long-term transfusions (who receive multiple transfusions over a period of few years). The excess iron gets deposited (accumulates) in reticuloendothelial cells of spleen, bone marrow, liver, heart, pancreas, and endocrine glands. It causes greater morbidity than the underlying anemia.

Prevention: The accumulation of toxic levels of iron stores can be prevented or reduced by use of iron chelators, therapeutic phlebotomy, or red cell exchange transfusion.

INVESTIGATION (WORK-UP) OF SUSPECTED ADVERSE TRANSFUSION REACTIONS

CC: Enumerate the steps in the investigation of a transfusion reaction.

It is very essential to *stop the transfusion* if the patient's symptoms and signs suggest a possibility of transfusion reaction. Clinical signs and symptoms of a transfusion reaction are listed in **Box 15.9**.

Initial steps (actions to be taken) in management of acute transfusion reaction are listed in **Box 15.10**.

Steps to be followed when the transfusion is discontinued are listed in **Box 15.11**.

Laboratory Investigation

The transfusion laboratory will perform relevant investigations. To rule out hemolysis from the differential diagnosis, the screening tests should be performed as listed in **Box 15.12**.

BOX 15.9: Clinical signs that suggests a transfusion reaction.

- Fever >1°C increase or >38°C, chills and rigors
- Hypotension or hypertension
- Respiratory: Wheezing, coughing, dyspnea, cyanosis
- Cutaneous: Urticaria, rash, flushing, edema
- Pain: Abdominal, chest, flank or back, infusion site
- Nausea or vomiting
- Renal: Oliguria or anuria
- Jaundice or hemoglobinuria
- Abnormal bleeding

BOX 15.10: Initial steps to follow if there is suspicion of adverse reaction.

- **Prompt recognizing of an adverse transfusion reaction**
- **Stop the transfusion**
- **Keep IV open with saline**: Maintain an intravenous line (for administration with blood) with normal saline
- **Notify immediately** to the blood center/bank and the patient's physician
- **Monitor/record vital signs**
- **Evaluate the patient** by attending physician to determine clinical intervention and potential medical management

BOX 15.13: Advantages of autologous blood transfusion.

- Prevents:
  - Transfusion transmitted diseases. Compared to allogeneic, it prevents the transmission of blood-borne pathogens
  - Red cell alloimmunization: It may be useful in patients having antibodies to very common RBC antigens and where compatible allogeneic components are not available
  - Some adverse transfusion reactions (e.g., hemolytic, febrile allergic reactions)
- Provides:
  - Compatible blood for patients with alloantibodies and rare blood groups
  - Reassurance to patients concerned about blood risks
- Supplements the blood supply

BOX 15.11: Steps to be followed when the transfusion is discontinued.

- Perform clerical check for any errors
- Visual check for hemolysis at bedside
- Collect post-transfusion sample:
  - Post-transfusion blood sample
  - Post-transfusion urine sample
- Return of the blood bag to blood center/bank

BOX 15.14: Disadvantages of autologous blood transfusion.

- Does not affect:
  - Risk of bacterial contamination

  - Risk of ABO incompatibility error
- More expensive/costly than allogeneic blood
- Results in wastage of blood, if not transfused
- Increases prevalence of adverse reactions to autologous donation. Patients are prone to develop perioperative anemia.

BOX 15.12: Initial standard laboratory investigation of a suspected transfusion reaction.

- Perform clerical check of the component bag, label, and patient sample
- Visual check for hemolysis in the blood center/bank: If hemolysis is established only in the post-transfusion sample, it indicates hemolytic transfusion reaction
- Laboratory investigation:
  - Confirmation of post-transfusion ABO-Rh
  - Post-transfusion direct antiglobulin test (DAT)

BOX 15.15: Contraindications for autologous blood transfusion.

- Evidence of infection and risk of bacteremia. Bacterial contamination is the most serious consequence of autologous transfusion
- Cardiovascular contraindications:
  - Scheduled surgery to correct aortic stenosis
  - Unstable angina
  - Severe left main coronary artery disease
  - Recent (within 6 months of donations) myocardial infarction or cerebrovascular accident
  - Patients with significant cardiac or pulmonary disease
  - Cyanotic heart disease
- Uncontrolled hypertension
- Active seizure disorder
- Hemoglobin concentration: Should not be significantly anemic (Hb level not < 11.0 g/dL)

TABLE 15.7: Sample collected following a mismatched blood transfusion and the tests performed.

Donor sample	***Post-transfusion blood sample***	***Post-transfusion urine sample***
Blood group and Rh typing	DAT	Occult blood
Crossmatching	Repeat grouping and crossmatching	Methemoglobin
Antibody screening	Serum bilirubin	Microscopy: RBC

Sample collected following a mismatched blood transfusion and the tests performed is presented in **Table 15.7**.

The hemoglobin (visual hemolysis) and antiglobulin tests are useful for screening purposes. If immune hemolytic reaction is suspected further testing/investigation are necessary.

AUTOLOGOUS BLOOD DONATION AND TRANSFUSION

CC: Enumerate the indications and describe the principles and procedure of autologous transfusion.

A voluntary donation of blood for use by the general patient population is termed as allogeneic. **Autologous transfusion is transfusion to a patient of their own blood**. Advantages, disadvantages and contraindications of autologous blood transfusion are given in **Boxes 15.13 to 15.15** respectively. The patient must not suffer from a disease that would put them at risk from this procedure. They may not be able to tolerate phlebotomy.

Preliminary Steps

1. **Age:** No minimum or maximum age limits. Each case has to be evaluated individually. The lower age limit is determined by the capacity of the child to understand and cooperate.
2. **Volume of blood to be collected:** It depends on age and weight of patient. Volume should not exceed 10.5 mL/kg of the donor's body weight.
3. **Examination:** Medical interview and examination should be structured to the special needs of donor patients. Any contraindication **(Box 15.15)** to autologous transfusion should be ruled out.
4. **Serologic testing:** ABO and Rh typing must be determined on all units. Antibody screening and crossmatch are optional if only autologous units are ordered for transfusion. Screening for blood transmissible disease is required for the first unit collected from the donor in a 30-day period.
5. **Labeling:** The unit should be clearly marked for "**autologous use only**" and carry the statement autologous donor. Units should be clearly labeled with name and age of patient-donor, identification number, name of collection center, name of hospital, date of collection and expiry, ABO and Rh group, and signature of patient-donor.

Pretransfusion Testing

The patient's blood sample should be tested for ABO and Rh grouping. It is optional to test for unexpected antibodies and crossmatching. The blood unit must be retested to confirm the ABO and Rh group using a sample obtained from segment of tubing attached to bag.

Types of Autologous Transfusion

Types of autologous transfusion are given in **Box 15.16**.

Preoperative Collection

In this procedure, the blood is drawn and stored prior to anticipated need. Patients suitable for preoperative collection are stable patients scheduled for surgical procedures in which blood transfusion is likely.

Collection schedule: Usually, a weekly schedule is used. Two or three units of blood may be collected several weeks before surgery by autologous donation. There should be sufficient interval from donation to surgery. The last collection should be done not before 72 hours of the scheduled surgery and preferably be longer. This period helps for recovery of a substantial portion of the collected red cell mass and for adequate volume repletion. During this period, iron supplementation may be needed. Erythropoietin stimulation can be used as an adjunct to autologous donation. It is to be noted that there is no indication for single unit autologous transfusion in an adult.

Indications for preoperative autologous collections are listed in **Box 15.17**.

Perioperative Blood Collection

In perioperative blood collection, the blood is collected either during or after surgery.

Acute Normovolemic Hemodilution (ANH)

In this type, one or more units of blood is collected before starting/beginning the surgery. In order to maintain the circulatory blood volume, it is replaced simultaneously with colloid or crystalloid solution. Hematocrit is allowed to come down to 25–30%. The blood collected is reinfused either during or after surgery. The blood units are reinfused in the reverse order of collection.

Thus, the first unit collected and therefore, the last unit transfused has the highest hematocrit and concentration of coagulation factors and platelets.

Indications: It is useful in cardiac surgery because:

- Due to hemodilution there is reduced loss of red cells. This is because the whole blood shed during surgery is having a low hematocrit.
- The blood collected by ANH is stored at room temperature and is returned to the patient within 8 hours of collection. Thus, there is little deterioration of platelets or coagulation factors and results in preservation of hemostasis.

Intraoperative Collection

In this type, bloodshed from the surgical (operative) field is recovered by circulatory devices. The shed blood is processed by machines called shell savers and then infused/transfused into the same patient.

Indications: This type of autologous transfusion is useful in:

- Cardiovascular surgery
- Ruptured:
  - Spleen or liver
  - Ectopic pregnancy
  - Aneurysm
- Traumatic penetrating injuries

Contraindications

- When procoagulant materials (e.g., topical collagen) are applied to surgical field, it may result in systemic activation of coagulation.
- Malignancy
- Contamination of blood with fecal matter, urine or bile, etc.

Postoperative Collection

In this type, blood is collected from drainage devices and reinfused to the patient with or without processing. Postoperative shed blood may be collected into sterile canisters and reinfused after passing through microaggregate filter. Postoperatively recovered blood is dilute, partially hemolysed and may contain high concentrations of cytokines. Hence, a

maximum of 1,400 mL of unprocessed blood can be reinfused within 6 hours of initiating collection.

BOX 15.16: Types of autologous transfusion.

- Preoperative collection
- Perioperative blood collection:
  - Acute normovolemic hemodilution (ANH)
  - Intraoperative blood collection
  - Postoperative collection

BOX 15.17: Indications for preoperative autologous collections.

Preoperative autologous collections:

- Major orthopedic procedures
- Vascular surgery, cardiac or thoracic surgery
- Radical prostatectomy

3 SECTION

Systemic Pathology

Section Outline

16. Cardiovascular Disorders
17. Respiratory Disorders
18. Disorders of Oral Cavities and Gastrointestinal Tract
19. Hepatobiliary and Pancreatic Disorders
20. Kidney and Urinary Tract Disorders
21. Male Genital Tract Disorders
22. Female Genital Tract Disorders
23. Breast Disorders
24. Endocrine Disorders
25. Bones, Joints, and Soft Tissue Tumors
26. Skin Disorders
27. Disorders of Central Nervous System and Eye

CHAPTER
16

Cardiovascular Disorders

VASCULAR DISORDERS

ARTERIOSCLEROSIS

CC: Distinguish arteriosclerosis from atherosclerosis. Describe the pathogenesis and pathology of various causes and types of arteriosclerosis.

Arteriosclerosis (hardening of the arteries) is characterized by arterial wall thickening and loss of elasticity. It may be caused by:

1. **Arteriolosclerosis:** It affects small arteries and arterioles. They may cause ischemic injury. There are two variants namely hyaline and hyperplastic arteriolosclerosis (*refer* page 447).
2. **Mönckeberg medial sclerosis (*refer* Fig. 2.23A):** It is characterized by deposition of calcium in muscular arteries seen in old age (above 50 years).
3. **Atherosclerosis:** It is the most frequent and important disease of intima.
4. **Fibromuscular intimal hyperplasia:** It is a non-atherosclerotic disease that is observed in muscular arteries larger than arterioles. It is rich in vascular smooth muscle cells (SMC) and extracellular matrix (ECM) that follows inflammation (e.g., in a healed arteritis, transplant-associated arteriopathy) or mechanical injury (e.g., associated with stents or balloon angioplasty). This can lead to significant stenosis of the vessel.

Capillaries: Maximum cross-sectional surface area.

Maximum blood volume present in the veins.

ATHEROSCLEROSIS

Q. Define atherosclerosis. Discuss the risk factors, etiopathogenesis and pathology of atherosclerosis. Briefly mention the sites and clinical significance of atherosclerosis. Describe the morphology (along with neat and labeled diagram) of atherosclerotic plaque. Mention the complications of atherosclerosis.

Q. Enumerate the risk factors of atherosclerosis and discuss the role of lipid and endothelial injury in its pathogenesis.

Definition: Atherosclerosis is primarily a progressive **disease of intima involving large and medium-sized elastic and muscular arteries**. It is characterized by **focal lipid-rich** intimal lesions called **atheromas** (atheromatous or atherosclerotic plaques).

Atherosclerotic lesion in the intima may reduce or block the lumen of the involved vessel and **can rupture to cause sudden occlusion**.

Epidemiology: Atherosclerosis is a worldwide disease seen in both developed and developing countries.

Risk Factors for Atherosclerosis

Q. Write short note on risk factors for atherosclerosis.

They were identified through several studies most important being the Framingham Heart Study and Atherosclerosis Risk in Communities Study. Risk roughly increases with the increase in number of risk factors. For example, two factors increase risk about four-fold, and three (i.e., hyperlipidemia, hypertension, and smoking), increase risk by a factor of seven.

Classification of risk factors: The risk factors may be broadly classified as modifiable, nonmodifiable and additional **(Table 16.1)**.

Modifiable Risk Factors in Ischemic Heart Disease (IHD)

These are **four** in number namely: (1) hyperlipidemia, (2) hypertension, (3) cigarette smoking, and (4) diabetes.

1. Hyperlipidemia

Increase in the serum lipids mainly cholesterol (**hypercholesterolemia**) is a major modifiable risk factor. Hypercholesterolemia can initiate atherosclerotic lesion even in the absence of other risk factors. The major components of lipids in the blood are:

TABLE 16.1: Risk factors for atherosclerosis.

A. Modifiable major risk factors	
• Hyperlipidemia • Hypertension	• Cigarette smoking • Diabetes mellitus
B. Nonmodifiable (constitutional) risk factors	
• Genetic abnormalities • Family history	• Increasing age • Male gender
C. Additional risk factors	
• Inflammation • CRP level • Hyperhomocystinemia • Metabolic syndrome • Lipoprotein (a)	• Raised procoagulant levels • Inadequate physical activity • Stressful lifestyle • Obesity • Alcohol

- **Low-density lipoprotein (LDL)** known as **"bad cholesterol"** is one of the components of serum lipid and its **higher level** is associated with **increased risk of atherosclerosis**. LDL delivers cholesterol to peripheral tissues.
- **High-density lipoprotein (HDL)** known as **"good cholesterol**" and its **higher levels are associated with decreased risk**.
  - HDL mobilizes cholesterol from the periphery (including atheroma) and excretes it through bile in liver.
  - Exercise and moderate consumption of ethanol raise HDL levels, whereas obesity and smoking lower it.

- **Serum cholesterol level:** Normal range is 140–240 mg/dL. Serum cholesterol is **strongly related to the dietary intake of saturated fat** (in the absence of genetic disorders of lipid metabolism). Risk of atherosclerosis increases with increasing serum cholesterol concentrations and lowering serum cholesterol concentrations reduces the risk. The risk of atherosclerosis can be reduced by lowering LDL or total plasma cholesterol, and/or raising serum HDL. This can be achieved either by dietary modification or by treatment with cholesterol-lowering drugs.
  - **Diet which raises blood cholesterol:**
    - **Diet high in cholesterol and saturated fats** (e.g., egg yolks, animal fats, and butter). Transunsaturated fats produced by artificial hydrogenation of polyunsaturated oils (used in baked goods and margarine).
  - **Diet which lower blood cholesterol:** Diets low in cholesterol and/or with higher ratios of **polyunsaturated fats**.
    - **Omega-3 fatty acids** (high in fish and fish oils).
  - **Cholesterol-lowering drug** [e.g., statins lowers circulating cholesterol levels by inhibiting hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase. This is the rate-limiting enzyme involved in cholesterol biosynthesis in the liver].

2. Hypertension

It is one of the major risk factors.

- Incidence of atherosclerosis increases as BP rises, and this excess risk **is related to both systolic and diastolic levels of blood pressure.**
- Control of hypertension with antihypertensive therapy reduces the risk of myocardial infarction and stroke.

3. Cigarette Smoking

It is the **most important avoidable cause** of atherosclerosis.

Atherosclerosis is **more severe and extensive among smokers than in nonsmokers.** This may be the reason for the increased incidence and severity of atherosclerosis in men compared to women. **There is a strong dose-linked relationship between cigarette smoking and ischemic heart disease. Cessation of smoking reduces the risk.**

4. Diabetes Mellitus

It is a potent risk factor for atherosclerosis. Diabetes is associated with hypercholesterolemia → increases the risk of atherosclerosis. The incidence of myocardial infarction and other atherosclerotic vascular diseases (strokes, gangrene of the lower extremities) is more in diabetics than in nondiabetics.

Nonmodifiable/Constitutional Risk Factors

- **Genetic abnormalities:** Most common inherited modifiable risk factors (hypertension, hyperlipidemia, and diabetes mellitus) are polygenic. Mendelian disorders, such as **familial hypercholesterolemia** are associated with atherosclerosis.
- **Family history: Atherosclerotic disease often runs in families.** Familial predisposition is usually multifactorial, due to genetic, environmental and lifestyle factors.
- **Increasing age*:*** Age is the most powerful independent risk factor. Clinical manifestation of atherosclerosis is usually observed after middle age and the lesions progressively rise with each decade.
- **Gender: Premenopausal women have lower incidence** of atherosclerosis-related diseases compared to males of the same age group. However, after menopause this sex difference disappears. This may be due to protective role of estrogen. However, hormone replacement therapy has no role in the prevention of coronary heart disease.

Additional Risk Factors

- **Inflammation:** It plays a role in atherogenesis and may be a risk factor.
- **C-reactive protein** (CRP) **level:** It is a marker of systemic inflammation and **predicts the risk** of atherosclerosis related diseases. CRP levels predict the risk for myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death, even in healthy individuals.
- **Hyperhomocysteinemia:** It is a rare autosomal recessive inborn error and results in elevated circulating homocysteine → premature and severe atherosclerosis.
- **Metabolic syndrome:** It is associated with central obesity, insulin resistance and is a known risk factor for atherosclerosis.

- **Increased lipoprotein (a) Lp(a) levels:** It is an altered form of LDL and is associated with increased risk. Lp(a) has structural similarity to plasminogen. It competes with plasminogen in clots and decreases the ability to form and clear clots. Increased Lp(a) levels are associated with higher risk of atherosclerosis, independent of total cholesterol or LDL levels.
- **Raised procoagulant levels:** These procoagulants include, thrombin (procoagulant and proinflammatory action), platelet activation and raised fibrinogen → increased risk.
- **Inadequate physical activity:** Lack of exercise doubles the risk.
- **Clonal hematopoiesis:** It is defined as the presence of a major clone of cells in the bone marrow that have acquired somatic driver mutations (*refer* page 204) in one or more oncogenes or tumor suppressor genes. It is found that many older individuals have clonal hematopoiesis and typically have normal blood counts despite mutations. It is observed that clonal hematopoiesis is strongly associated with an increased risk of death from cardiovascular disease. This may be due to alterations in the function of innate immune cells derived from mutated hematopoietic stem cells.
- **Stressful lifestyle:** Certain personality associated with competitive, stressful life ("type A" personality) is associated with an increased risk of coronary disease.
- **Obesity:** It is often associated with hypertension, diabetes mellitus, hypertriglyceridemia, decreased HDL and physical inactivity.
- **Alcohol consumption:** It is **associated with reduced rates of coronary artery disease**.

Pathogenesis of Atherosclerosis

Q. Write short note on pathogenesis of atherosclerosis.

There are several theories proposed for pathogenesis of atherosclerosis and few of them are:

- **Insudation hypothesis:** According to this, the focal accumulation of lipid in a vessel wall is due to insudation (transport) of plasma lipoproteins across an intact endothelium.

- **Encrustation hypothesis:** This hypothesis states that small mural thrombi are formed at the site of endothelial damage. These thrombi become organized and form plaque.
- **Monoclonal hypothesis:** The monoclonal concept postulates that single clone (monoclonal) of smooth muscle migrate from the underlying media into the intima and then proliferate. The stimulus for monoclonal proliferation may be metabolites of cholesterol.
- **Response-to-injury hypothesis:** This is the currently favored hypothesis.

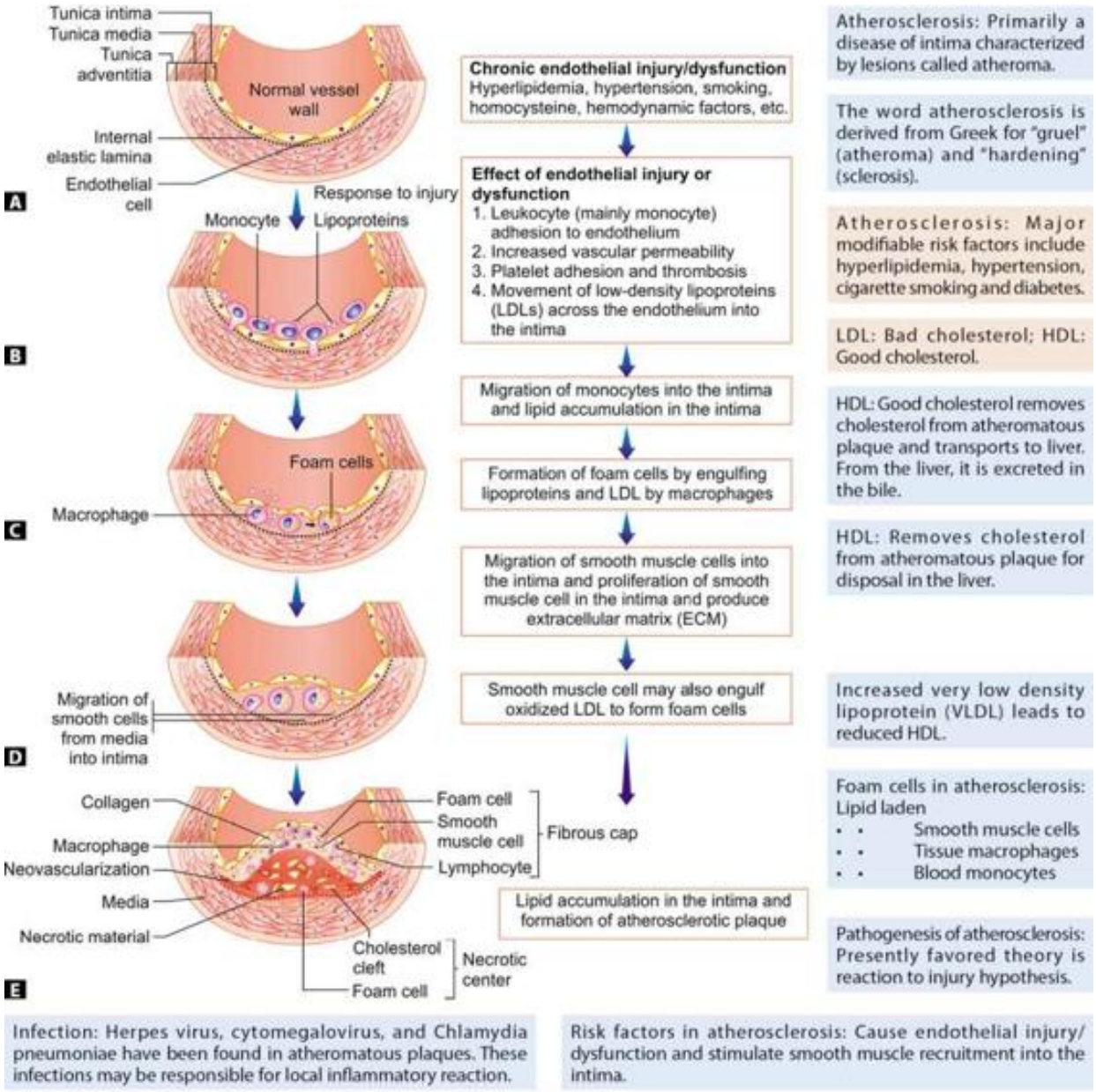
Response-to-Injury Hypothesis (Figs. 16.1A to E)

Q. Write short note on reaction-to-injury hypothesis of atherosclerogenesis.

According to this theory, **atherosclerosis develops as a chronic** (inflammatory and healing) **response of the arterial wall to the endothelial injury**. Probably accumulation of cholesterol crystals and free fatty acids in macrophages and other cells initiate inflammation. It is thought that chronic inflammation is responsible for both the initiation and progression of atherosclerotic lesions. The **sequence of pathogenic events (Figs. 16.1A to E)** is as follows:

1. **Endothelial injury and dysfunction:**
   - **Causes of endothelial injury/dysfunction:**
     - **Hemodynamic disturbances:** Plaques develop in regions having disturbed blood flow such as origin or ostia of vessels, branching points of vessel and along the posterior wall of the abdominal aorta.
     - **Risk factors: Hyperlipidemia, hypertension, toxins from cigarette smoke,** and advanced glycation end products in **diabetes** can **produce endothelial injury/dysfunction**.
     - **Others: Homocysteine,** immunocomplexes, and **infectious agents**.
   - **Effect of endothelial injury or dysfunction:**
     - **Leukocyte (mainly monocyte) adhesion** to endothelium
     - **Increased vascular permeability**
     - **Platelet adhesion and thrombosis**
     - **Movement of low-density lipoproteins (LDLs) across the endothelium into the intima**.

2. **Migration of monocytes into the intima:**
   - The **leukocytes,** which adhere at the site of endothelial injury/dysfunction, are **mainly monocytes and T lymphocytes**. Accumulation of leukocytes **initiates the atheroma formation**.
   - Locally produced **chemokines allows penetration of the endothelial layer by monocytes and lymphocytes.** The **monocytes migrate into the intima** where they are **transformed into macrophages**.
3. **Lipid accumulation in the intima:**
   - Endothelial dysfunction also allows **penetration of endothelium by lipoproteins (mainly LDL)** from blood → **LDLs accumulate within the intima** of the vessel.
   - **LDL is oxidized** by the action of oxygen free radicals produced locally by monocytes/macrophages and dysfunctional endothelial cells.
4. **Formation of foam cells and activation of macrophages:**
   - **Macrophage engulfs lipoproteins** and **oxidized LDL** from the extracellular space in the intima. Their cytoplasm becomes foamy and these cells are called as **foam cells**.
   - Some **foam cells may undergo apoptosis → release lipids** → form lipid-rich center, often called the necrotic core in atheromatous plaques.
   - **Oxidized LDL is cytotoxic to endothelial cells and smooth muscle** cells and can cause endothelial dysfunction.
   - **Oxidized LDL causes activated macrophages which produce:**
     - **Cytokine** (e.g., TNF)—**increases leukocyte adhesion**.
     - **Chemokines** (e.g., monocyte chemotactic protein 1)—accumulation of monocytes.
     - **Reactive oxygen species**—aggravate oxidation of LDL.
     - **Growth factors—**stimulate smooth muscle cell proliferation and extracellular matrix (ECM) synthesis.
5. **Migration of smooth muscle cells into the intima:**
   - Growth factor (e.g., platelet-derived growth factor) from activated platelets, macrophages, and endothelial cells causes migration of smooth muscle cells **either from the arterial media or from circulating precursors**.



Figs. 16.1A to E: Various steps in response to injury hypothesis of atherogenesis: (A) Normal; (B) Endothelial injury/dysfunction with adhesion of monocytes; (C) Migration of monocytes into the arterial intima and are transformed into macrophages. They engulf lipoproteins and oxidized LDL to form foam cells; (D) Migration of smooth muscle followed by their proliferation in the intima; (E) Fully developed atheromatous plaque.

6. **Smooth muscle cell proliferation in the intima and ECM production:**
   - Smooth muscle cells proliferate, and produce ECM (mainly collagen and proteoglycans). Many **growth factors** can **cause smooth muscle cell proliferation.** These includes: PDGF, fibroblast growth factor (FGF) and transforming growth factor-α (TGF-α).
   - Smooth muscle cell **may also engulf oxidized LDL and form foam cells**.

7. **Lipid accumulation:**
   - Occurs both **intracellularly** (within macrophages and smooth muscle cells) and **extracellularly.**
   - **Extracellular lipid** is derived **from insudation** from the vessel lumen (mainly in the presence of hypercholesterolemia) and also from **necrotic foam cells**.
   - Cholesterol in the plaque is also due to an **imbalance between influx and efflux**. High-density lipoprotein (HDL) probably transfers cholesterol from these lesions and leads to its excretion by liver.

MORPHOLOGY

Q. Write short note on fatty streaks.

Fatty streaks: They may be the earliest or precursor lesions of atherosclerosis. They are found in young children (older than 10 years) as well as in adults.

- Gross: Multiple small (~1 mm) flat yellow lesions in the intima, which may coalesce to form long streaks 1 cm or more in length. They may be mildly elevated above intima without disturbing the blood flow.
- Microscopy: Consist of lipid-filled foamy macrophages in the intima.

Atherosclerotic Plaque

Q. Differences between fatty streak and atherosclerotic plaque.

Q. Write short note on gross and microscopic features of atherosclerosis.

Q. Write short note on aortic lesions in atherosclerosis.

Gross (Figs. 16.2A and B)

- Sites: Major vessels involved in atherosclerosis in descending order are:
  - Lower abdominal aorta (abdominal aorta more than the thoracic aorta)
  - Coronary arteries
  - Popliteal arteries
  - Internal carotid arteries
  - Vessels of the circle of Willis.
- Color: White to yellow. If superimposed by a thrombus → red-brown.

- Size: Range from 0.3 to 1.5 cm in diameter. Can coalesce to form larger masses. Advanced lesions are oval and range from 8 to 12 cm in diameter.
- Shape**:** Irregular with well-defined borders.
- Distribution: Patchy (focal) and usually involve only a portion of the involved arterial wall. On cross-section → appears as an eccentric lesion.
- Composition: Soft, yellow, grumous core of lipid covered by a white fibrous cap.

Microscopy (Figs. 16.3A to C and Fig. 16.1E)

Three main components in varying proportions in different lesions:

- Cells: Smooth muscle cells, macrophages, and T-cells.
- Extracellular matrix (ECM): Collagen, elastic fibers, and proteoglycans.
- Lipid: Both intracellular and extracellular lipid.

These components occur in three regions:

- 1. Superficial fibrous cap: Composed of smooth muscle cells and collagen.
- 2. Necrotic core: It is seen deep to the fibrous cap and contains:
  - Lipid: Typical atheroma contains abundant lipid, mainly cholesterol and cholesterol esters, which is seen as empty needle shaped cleft-like spaces.
  - Debris from dead cells.
  - Foam cells (lipid-laden macrophages and smooth muscle cells).
  - Others: Fibrin, organized thrombus, and plasma proteins.
- 3. Shoulder**:** It is the peripheral region beneath and to the side of the cap. It is more cellular and contains macrophages, smooth muscle cells, and T-cells.

Neovascularization (proliferating small blood vessels): It may be seen at the periphery of the lesions near the shoulder.

Q. Write short note on complications of atherosclerosis.

Complicated plaques: Atherosclerotic plaques can undergo the following clinically important changes:

1v **Rupture, ulceration, or erosion:** Plaque protrudes into the lumen and can disturb the blood flow → resulting in turbulent flow of blood → which can damage the endothelium → cause rupture, ulceration or erosion of the intimal surface of plaques.

2v **Hemorrhage into the plaque:** It may occur due to rupture of the fibrous cap of the plaque or of the thin-walled vessels formed due to neovascularization.

3v **Thrombosis and embolism:** Ulceration/erosion/rupture of endothelial surface → exposes the blood to highly thrombogenic subendothelial collagen → favors **thrombus formation** → can partially or completely

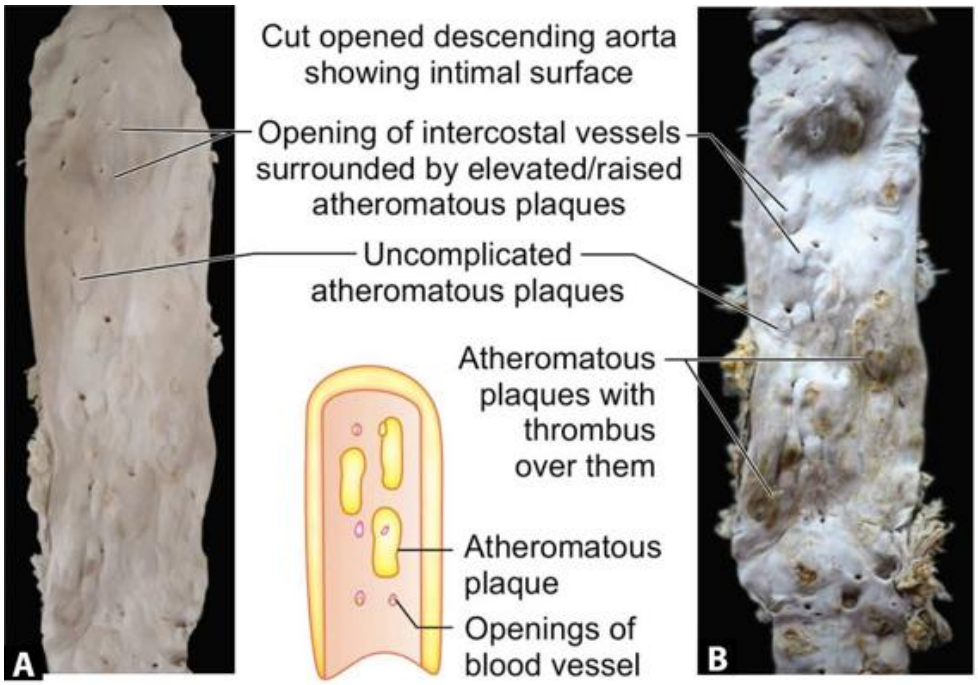
occlude the lumen (depending on the size of the lumen) → lead to ischemia. The thrombus may become organized or fragment to form thromboemboli.

4. **Atheroembolism:** Plaque rupture → discharge atherosclerotic debris into the bloodstream → results in atheroemboli.
5. **Aneurysm formation:** Atherosclerosis even though an intimal disease may cause pressure or ischemic atrophy of the underlying media. It may also damage the elastic tissue and cause weakening of the wall → result in aneurysmal dilation → which may rupture.
6. **Calcification:** It may occur in the central necrotic area of the plaque (dystrophic calcification**)**.

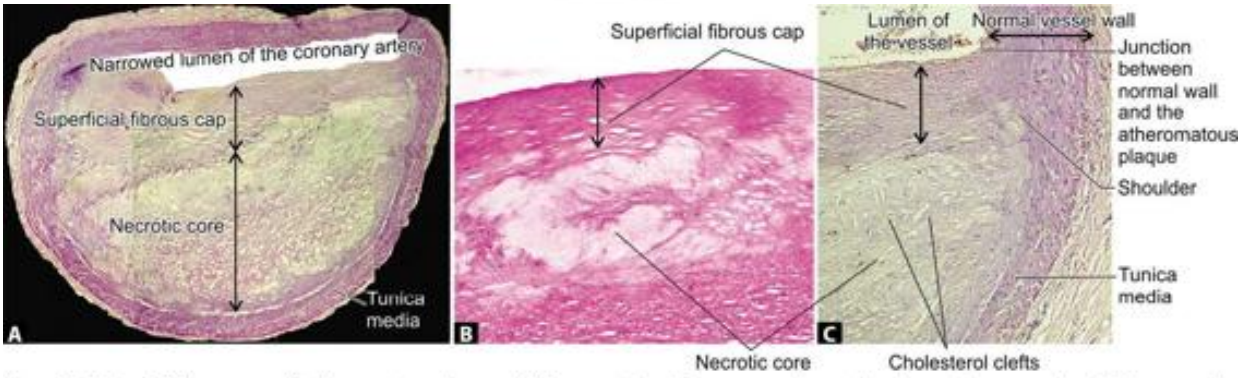
Complications and major clinical consequences of atherosclerosis are presented in **Figure 16.4**.

Clinicopathologic Manifestations of Atherosclerosis

- **Atherosclerotic stenosis**
  - Atherosclerotic plaques reduce the size of the lumen of the involved vessel. When reduction of the lumen is sufficiently severe it may lead to tissue ischemia and this lesion is termed as **critical stenosis.**



Figs. 16.2A and B: Atheromatous plaques on the intimal surface of aorta.



Figs. 16.3A to C: Microscopy of atheromatous plaque: (A) Scanner view of coronary atherosclerosis causing more than 90% narrowing of the lumen; (B) Shows central necrotic core and superficial fibrous cap; (C) Shows junction between the vessel wall and atheromatous plaque, shoulder region and cholesterol clefts in the necrotic core.

- The effects of vascular stenosis depend on arterial supply and the metabolic demand of the affected tissue. In small arteries, it may reduce blood flow leading to ischemic injury. In the coronary artery atherosclerotic lesion when produces a 70% decrease in luminal cross-sectional area, the patient may develop chest pain with exertion (*stable angina*; *refer* pages 457-8). In other site, diminished arterial blood flow can cause ischemia of the bowel (mesenteric occlusion), sudden cardiac death, chronic ischemic heart disease, ischemic encephalopathy, and intermittent claudication (diminished perfusion of the extremities).

- **Acute plaque change** (*refer* page 460 and **Fig. 16.15**)

Atheromatous plaques causing myocardial infarction and other acute coronary syndromes are often asymptomatic. Acute plaque change is the sudden change/event **occurring in an atheromatous plaque.** The composition of plaques is dynamic and it decides the risk of rupture. **Vulnerable plaques** are more likely to undergo rupture. These are plaques that have: (1) necrotic center with large areas of foam cells and extracellular lipid, (2) thin fibrous caps or contain few smooth muscle cells or (3) have clusters of inflammatory cells.

  - **Mechanical strength and stability of plaque:** Depends mainly on the collagen in the fibrous cap. The balance between synthesis of collagen against its degradation decides the integrity of fibrous cap integrity.
    - **Collagen synthesis:** It is mainly by smooth muscle cells and loss of these cells reduces the integrity and strength of fibrous cap.
    - **Collagen degradation:** Generally inflammatory cells in the plaque increase in collagen degradation and reduced collagen synthesis. This reduces the mechanical strength and integrity of the fibrous cap. Statins reduce circulating cholesterol levels and also stabilize plaques by reducing inflammation in plaque.
  - **Extrinsic factors:** Also contribute plaque changes.
    - Adrenergic stimulation raise systemic blood pressure or induce local vasoconstriction and increase the physical stresses on a given plaque.
    - Emotional stress can also contribute to plaque disruption.
- **Thrombosis:** Partial or total thrombosis over a disrupted plaque is observed in acute coronary syndromes. Mural thrombi in a coronary artery can undergo embolization.
- **Vasoconstriction:** Vasoconstriction reduces the size of arterial lumen and increases the local mechanical forces. This can cause disruption of plaque.

Disorders associated with hypercholesterolemia:
- Nephrotic syndrome
- Alcoholism
- Hypothyroidism
- Diabetes mellitus

Achilles tendon xanthoma: Pathognomonic of familial hypercholesterolemia.

C-reactive protein: Excellent marker for disrupted atheromatous plaque.

Regular exercise (brisk walking, cycling or swimming for 20 minutes two or three times a week) has a protective effect.

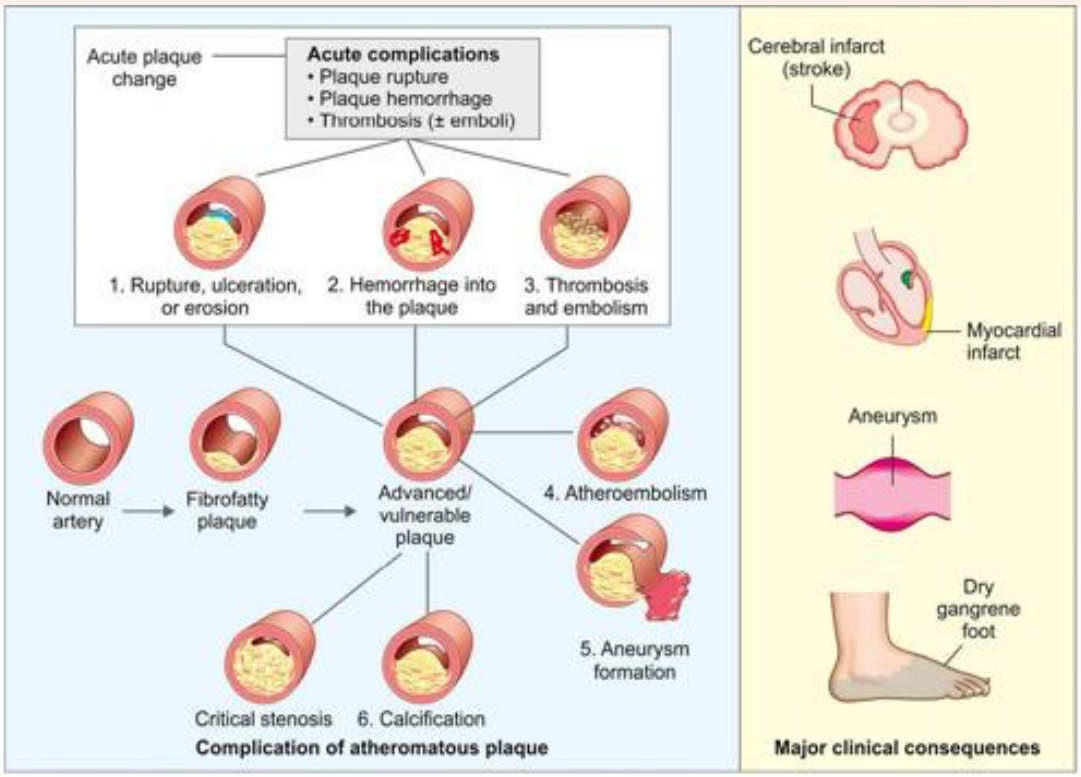
Excess alcohol consumption is associated with hypertension and cerebrovascular disease.

Fatty streaks may be the earliest or precursor lesions of atherosclerosis and may be seen in children.

Atherosclerosis: Most common site is abdominal aorta.

Atherosclerosis: Microscopy shows necrotic center covered by fibrous cap.

Complications of atherosclerosis:
- Rupture
- Hemorrhage into the plaque
- Thrombosis
- Embolism
- Aneurysm
- Calcification



Major vessels involved in atherosclerosis
- Large elastic arteries (e.g., the aorta, carotid, and iliac arteries)
- Large and medium-sized muscular arteries (e.g., coronary and popliteal arteries).

Fig. 16.4: Complications and major clinical consequences of atherosclerosis.

- Major clinical consequences of atherosclerosis:

- Myocardial infarction (heart attack)
- Cerebral infarction (stroke)
- Aortic aneurysms
- Peripheral vascular disease (gangrene of the legs)

Stable plaque:
- Have thick densely collagenized fibrous cap
- Minimal inflammation
- Negligible lipid core

Vulnerable plaque:
- Contain numerous foam cells
- Abundant extracellular lipid
- Many inflammatory cells
- Thin fibrous cap
- Few smooth muscle cells
- High-risk to undergo rupture

ANEURYSMS AND DISSECTION

CC: Describe the etiology, dynamics, pathology types and complications of aneurysms including aortic aneurysms.

Q. Define aneurysm.

Definition: An aneurysm is defined as a congenital or acquired, **localized, abnormal, permanent dilation of a blood vessel or the heart**.

Classification

Q. Write short note on aneurysm.

Aneurysms are classified by origin, location, gross appearance, composition of vessel wall, and etiology.

1. **Origin:** It can be congenital or acquired.
2. **Location:** It refers to the type of vessel involved:
   - **Artery** and the specific region involved, such as the aorta or popliteal artery
   - **Vein**
   - **Heart**.
3. **Depending on gross appearance (shape and size) (Figs. 16.5A to F):**

- **Fusiform aneurysm:** It is ovoid or fusiform dilation of vessel wall that is parallel to the long axis. Size varies in diameter (up to 20 cm) and in length. It can involve aortic arch, abdominal aorta, or iliac arteries.
- **Saccular aneurysm:** It is a localized spherical outpouchings from the portion of the vessel wall. The size varies from 5 to 20 cm in diameter and the lumen may contain thrombus.
- **Cylindrical aneurysm:** It has parallel dilatation**.**
- **Arterial dissection/dissecting hematoma:** It develops when blood enters/dissects between the layers of the arterial wall. Just like a hematoma it separates the layers of the arterial wall.
- **Arteriovenous (racemose) aneurysm:** It is a direct communication between an artery and a vein.

4. **Depending on the composition of the wall of the aneurysm:**
   - **True aneurysm** is composed of all the layers of thinned arterial wall (intima, media and adventitia) or attenuated ventricular wall of the heart.
     Examples: Atherosclerotic, syphilitic, and congenital vascular aneurysms, and ventricular aneurysms that complicates transmural myocardial infarctions.
   - **False aneurysm** (or pseudoaneurysm) is a defect in the vascular wall with a hematoma (blood-filled space forms around a blood vessel) which freely communicates with the intravascular space (**pulsating hematoma**). It usually develops after traumatic rupture or a perforating injury.
     Examples: Rupture of left ventricle, which complicates myocardial infarction or hematoma that follows trauma to artery.
   - **Arterial dissection** is characterized by entry of blood into the arterial wall through an intimal defect, which separates the underlying layers.

Q. List the causes of aneurysm.

5. **Depending on the etiology:**
   - Atherosclerotic aneurysm
   - Syphilitic aneurysm
   - Dissecting hematoma
   - Mycotic aneurysm
   - Berry aneurysm.

Pathogenesis of Aneurysms

Aneurysms develop when there is weakening of vessel walls, which may be inherited or acquired. The loss of balance between connective tissue synthesis and degradation of the vascular wall may produce aneurysm.

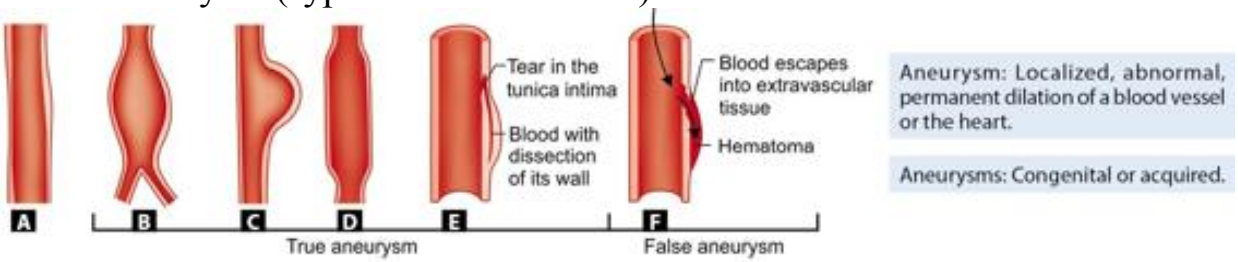
- **Inadequate or abnormal synthesis of connective tissue of the vascular wall:** TGF-β regulates smooth muscle cell proliferation and synthesis of connective tissue matrix. Mutations in TGF-β receptors or downstream signaling pathways → defective synthesis of elastin and collagen → aneurysm.
  - **Marfan syndrome** is characterized by defective synthesis of the **fibrillin** → weakening of the aortic wall → aneurysm.
  - **Ehlers-Danlos syndrome** is associated with defective type III collagen synthesis → aneurysm.
  - **Vitamin C (ascorbate) deficiency** → altered collagen cross-linking.
- **Increased degradation of connective tissue:** Increased production of matrix metalloproteinases (MMP) enzymes (e.g., by macrophages in atherosclerosis) can degrade the ECM in the arterial wall and may cause aneurysm formation. This may be seen in atherosclerotic plaque or in vasculitis.

Loss of smooth muscle cells: It may occur **due to ischemia** of the inner media as in atherosclerosis or ischemia of the outer media as in **systemic hypertension**. This results in degenerative changes in the aorta → fibrosis, increased synthesis of ECM, and accumulation of excessive amounts of amorphous proteoglycans in the medial wall. These changes in the aortic wall are called as **cystic medial degeneration.** These changes are nonspecific and can be seen in numerous conditions including Marfan disease and scurvy.

Predisposition Factors

1. **Atherosclerosis:** It predisposes to aneurysms in the abdominal aorta.
2. **Hypertension:** It predisposes to aneurysms of the ascending aorta.
3. **Others:**
   - **Trauma**
   - **Vasculitis**
   - **Congenital defects** (e.g., berry aneurysms in the circle of Willis)

- **Tertiary syphilis:** It is a rare cause of aortic aneurysms. Trepenomas has affinity for vasa vasorum of mainly thoracic aorta → obliterative endarteritis → leads to ischemic damage to the aortic media and aneurysm (syphilitic mesoaortitis)



Figs. 16.5A to F: Gross types of aneurysms: (A) Normal blood vessel; (B) Fusiform aneurysm; (C) Saccular aneurysm; (D) Cylindrical aneurysm; (E) Dissecting hematoma; (F) False aneurysm.

- **Infections (mycotic aneurysms):** The term **mycotic** (*refer* pages 124 and 132) **is a misnomer** and does not indicate that the infection is due to fungus and is usually **bacterial**. The source of infection may be:
  - Septic embolus, usually as a complication of infective endocarditis
  - Extension of an adjacent suppurative process
  - Circulating organisms directly infecting the arterial wall.

Abdominal Aortic Aneurysm

Gender and age: Abdominal aortic aneurysm **(AAA)** is more common **in men**, **in smokers**, and rare before the age of 50 years.

Causes

- **Atherosclerotic AAA:** It is a **major cause**.
  - Atherosclerotic plaque in the intima causes thinning of media and reduces the diffusion of nutrient and waste from the lumen of vessel into the arterial wall. The media undergoes necrosis and cause **weakness and thinning of arterial wall**.
  - The **excessive degradation of ECM by MMP** secreted from inflammatory cell infiltrates mainly macrophages present in the plaque.
- **Inflammatory AAA:** It is characterized by dense periaortic fibrosis accompanied by inflammatory cells (lymphocytes, plasma cells and macrophages).
- **Mycotic AAA:** It is due to **infection of the aortic** wall by **circulating microorganisms** (e.g., bacteremia from a primary *Salmonella*

gastroenteritis). The suppurative process destroys the media → aneurysm → rupture.

MORPHOLOGY OF ATHEROSCLEROTIC ANEURYSM

- Site: Aneurysm is usually observed below the origin of renal arteries and above the bifurcation of the aorta.
- Appearance:
  - Shape: Saccular or fusiform.
  - Size: May be up to 15 cm in diameter, and up to 25 cm in length.
  - Wall of the aorta: Intimal surface shows severe complicated atherosclerosis with destruction and thinning of the underlying aortic media.
  - Lumen: It frequently shows a bland, laminated, poorly organized mural thrombus.

Clinical Features and Complications

- **Rupture:** Aneurysm may rupture into the **peritoneal cavity or retroperitoneal tissues** → leading to massive, fatal hemorrhage. The risk of rupture increases as the size increases.
- **Obstruction of a branch:** It can **lead to ischemia** of tissues supplied by the obstructed vessel, e.g., iliac (leg), renal (kidney).
- **Embolism:** Atherosclerotic plaque or mural thrombus may fragment to form emboli.
- **Compression of an adjacent structure** (e.g., compression of a ureter).
- **Abdominal mass:** It may simulate a tumor.

Thoracic Aortic Aneurysms

Causes: Hypertension, Marfan syndrome, other disorders associated with mutations in TGF-β signaling pathways, and syphilis (not observed nowadays).

Clinical presentation: Symptoms depend on the encroached structure **(Table 16.2)**.

Other symptoms:

- Aortic valve dilation and valvular insufficiency.
- Narrowing of the coronary ostia → cause myocardial ischemia.
- Rupture of aneurysm → fatal.
- Syphilitic aneurysms → heart failure due to aortic valvular incompetence.

Syphilitic Aneurysm

Q. Write short note on syphilitic aneurysm aortitis.

MORPHOLOGY

- Syphilis causes aortitis and aneurysms
- Mainly affects the ascending aorta (refer page 92)
- Microscopy: It shows endarteritis and periarteritis (lymphocytes, plasma cells, and macrophages) of vasa vasorum. Obliterative endarteritis → causes focal necrosis and scarring of the media
- Medial scars → produce roughened intimal surface → tree bark appearance
- Weakened wall of the ascending aorta and aortic arch → fusiform aneurysm.

Aortic Dissection

Q. Write short note on dissecting aneurysm/hematoma/aortic dissection.

Definition: Aortic dissection develops when **blood from the aortic lumen enters into the aortic wall** and travels along the layers of the media to form a blood-filled channel within the aortic wall.

Aortic dissection may or may not be associated with aortic dilation. **Older term "dissecting aneurysm" is replaced by aortic dissection.**

It is fatal, if the dissection ruptures through the adventitia → results in hemorrhages into adjacent spaces.

Etiology

Occurs mainly in two groups: (1) men between **40 and 60 years with hypertension** or (2) **younger** patients **with connective tissue disease** of the aorta, e.g., **Marfan syndrome**.

- **Iatrogenic:** It may develop as a complication of **arterial cannulations** during diagnostic catheterization or cardiopulmonary bypass.
- **During or after pregnancy:** Cause not known.

Pathogenesis

TABLE 16.2: Clinical presentations in thoracic aortic aneurysm.

Encroached structures	*Symptoms*
Lungs and airways	Respiratory difficulties
Esophagus	Difficulty in swallowing
Irritation of or pressure on the recurrent laryngeal nerves	Persistent cough
Erosion of bone (i.e., ribs and vertebral bodies)	Pain

- **Hypertension:** It is the **major risk factor**. Aortas show medial hypertrophy of the vasa vasorum, degenerative changes in the aortic media and loss of medial smooth muscle cells.
- **Inherited or acquired connective tissue disorders:** It may be responsible **in few cases**, e.g., **Marfan syndrome, Ehlers-Danlos syndrome, vitamin C deficiency, copper metabolic defects**.
- An **aortic dissection usually starts with a tear in the intima**. The trigger for the intimal tear is not known.

MORPHOLOGY

- Site of intimal tear: It is in the ascending aorta, usually within 10 cm of the aortic valve (majority of patients).
- Characteristic of tear: (1) Transverse or oblique, (2) 1–5 cm in length, and (3) sharp with jagged edges.
- Site of dissection: It is usually between the middle and outer thirds of the tunica media.
- Extent of involvement: It can extend proximally along the aorta toward the heart and distally into the iliac and femoral arteries.

Classification (Fig. 16.6)

Two types:

1. **Type A dissections:** They have **proximal lesions** involving either **both the ascending and descending aorta or only the ascending aorta** (types I and II of the DeBakey classification). It is more common but dangerous.

2. **Type B dissections:** They have **distal lesions** which **do not involve the ascending part** and usually begin **distal to the subclavian artery** (DeBakey type III).

Complications due to Rupture

Rupture occurs through the tunica adventitia, may result in:

- **Massive hemorrhage** (e.g., into the thoracic or abdominal cavities) or
- **Cardiac tamponade** (hemorrhage into the pericardial sac).
- **Double-barreled aorta:** It may develop in few cases, when the **dissecting hematoma reenters the lumen of the aorta through a second distal intimal tear.** This creates a new false vascular channel and prevents the fatal extra-aortic hemorrhage.

Microscopy: In most cases, do not show any identifiable changes in the aortic wall. The most frequent microscopic feature is **cystic medial degeneration** without any inflammation.

Classical Symptoms

- **Sudden onset of severe pain**, beginning in the anterior chest, radiating to the back between the scapulae, and moving downward as the dissection progresses.
- Pain may be **misdiagnosed as that of myocardial infarction**.

Cause of Death

- **Rupture of the dissection** outward into the pericardial, pleural, or peritoneal cavities.
- **Retrograde dissection** into the aortic root can cause aortic valvular insufficiency, cardiac tamponade, and myocardial infarction.
- **Dissection** may also extend **into the great arteries of the neck** or into the coronary, renal, mesenteric, or iliac arteries, causing critical vascular obstruction and ischemic damage.

Morphological types of aneurysm:

- 1. Fusiform
- 2. Saccular

- 3. Cylindrical
- 4. Aortic dissection

True aneurysm: Involves all three layers of the artery or thinned wall of the heart.

Aneurysm develops due to weakening of the vessel wall either due to:
- Inadequate abnormal synthesis of connective tissue of the vessel wall or
- Increased degradation of connective tissue

Ehlers-Danlos syndrome: Defective synthesis of type III collagen.

Marfan syndrome: Defective synthesis of the fibrillin.

Fungus that invade blood vessel: Aspergillus, Candida and Mucor.

Bacteria that invade blood vessel: B. fragilis, P. aeruginosa and Salmonella.

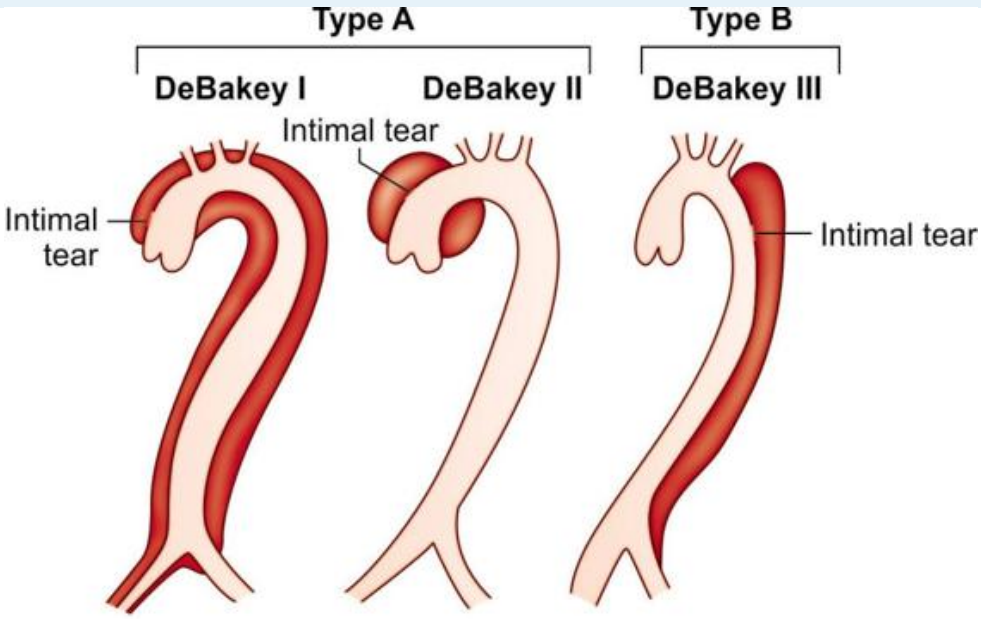


Fig. 16.6: Classification of aortic dissections. Type A (proximal) involves the ascending aorta (DeBakey I and II), Type B (distal or DeBakey III) dissections arise beyond the subclavian artery.

Atherosclerosis is the major cause of abdominal aortic aneurysm.

Complications of aneurysm:
- Rupture
- Thrombosis
- Embolization

Syphilitic aneuryms: Mesoaortitis → aortic aneurysm and aortic regurgitation.

Aortic dissection: Associated with cystic medial degeneration.

Aortic dissection: Blood enters the wall of aorta and separates its layers.

Dr Michael DeBakey is a pioneer in vascular surgery.

Aortic dissection:
- Type A dissections (DeBakey type I and II)
- Type B dissections (DeBakey type III)

Aortic dissection: Complications are due to rupture or obstruction of branches of aorta.

HYPERTENSIVE VASCULAR DISEASE

Hypertension is a common disorder and maintenance of blood pressures is important to prevent untoward consequences. Low blood pressure (hypotension) can cause inadequate perfusion of organ leading to tissue dysfunction or death. High blood pressure (hypertension) can cause end-organ damage and is one of the major risk factors for atherosclerosis.

WHO definition of hypertension: Hypertension is defined as **systolic** pressure **above 160 mm Hg and/or diastolic** pressure **above 90**.

Causes of Hypertension (Box 16.1)

- **Primary/essential/idiopathic** hypertension: It constitutes about 95% of cases.
- **Secondary** hypertension: It forms about 5% of cases and there is an identifiable cause.

The prevalence and vulnerability to complications of hypertension increase with age.

Regulation of Normal Blood Pressure

Normal blood pressure is essential for survival; it causes damage when it increases continuously above the normal range.

Factors Determining Blood Pressure

- The major factors include age, sex, body mass index and diet, particularly sodium intake, exertion, emotional state and others.
- Blood pressure depends on two hemodynamic variables, namely **cardiac output** and **peripheral vascular resistance (Flowchart 16.1)**. These two, in turn, are influenced by many genetic, environmental and demographic factors.
  - **Cardiac output:** It depends on stroke volume, and heart rate. Stroke volume in turn is influenced by the sodium homeostasis. Heart rate and contractility of myocardium (affects stroke volume) are regulated by the α- and β-adrenergic systems (also effects on vascular tone).
  - **Peripheral vascular resistance:** It is determined by functional and anatomic changes in small arteries and arterioles. Vascular tone depends on the balance between vasoconstrictors and vasodilators. Blood pressure is also influenced by tissue pH and hypoxia.

Role of Kidney

The kidneys **play an important role in the regulation of blood pressure**.

- **Renin-angiotensin system:** Kidney influences both peripheral vascular resistance and sodium homeostasis (thereby blood volume) through the renin-angiotensin system.
  - **Renin:** Whenever there is a fall in blood pressure, renin is secreted by the juxtaglomerular cells of the kidney and released into the blood circulation. It **cleaves plasma angiotensinogen to angiotensin I**, which is then converted **to angiotensin II** by angiotensin converting enzyme.
  - **Angiotensin II:** It raises blood pressure by **increasing both peripheral resistance** (direct action on vascular smooth muscle cells and causes vasoconstriction) **and blood volume** (by stimulating secretion of aldosterone by the adrenal zona glomerulosa, and increased reabsorption of sodium in distal tubules).

BOX 16.1: Major causes of hypertension (systolic and diastolic).

PRIMARY/ESSENTIAL HYPERTENSION (~ 95% of cases)

Secondary hypertension

Renal

- Acute glomerulonephritis
- Chronic renal disease
- Polycystic kidney disease
- Renal artery stenosis (renovascular hypertension)
- Renin producing tumors
- Obstructive uropathy

Endocrine

- Adrenal disorders: Cushing's syndrome, primary aldosteronism
- Pheochromocytoma
- Exogenous hormones: Glucocorticoids, estrogen
- Pregnancy-induced: Pre-eclampsia/eclampsia
- Others: Hypothyroidism (myxedema), hyperthyroidism (thyrotoxicosis), hypercalcemia, acromegaly

Cardiovascular

- Coarctation of aorta
- Polyarteritis nodosa
- Increased cardiac output
- Increased intravascular volume

Neurologic

- Psychogenic
- Increased intracranial pressure
- Sleep apnea
- Acute stress, e.g., surgery

Hypertension: Common disorder which may be idiopathic or secondary to other disorders.

- **Antihypertensive substances:** The kidney also produces substances which causes vasodilatation and have antihypertensive effect. These include prostaglandins and nitric oxide (NO). They counterbalance the vasoconstriction produced by angiotensin II.
- **Sodium homeostasis and blood volume:**
  - **When blood volume is reduced,** the glomerular filtration rate falls → leads to increased reabsorption of sodium by proximal tubules of

kidney → thereby conserves sodium and expands blood volume.

- **When blood volume is increased**, natriuretic factors (natriuretic peptides) are secreted by atrial and ventricular myocardium. They inhibit sodium reabsorption in distal tubules and cause excretion of sodium and diuresis. Natriuretic peptides also cause vasodilation and may be considered as endogenous inhibitors of the renin-angiotensin system.

Pathogenesis of Hypertension

Mechanisms of Essential Hypertension

Q. Write short note on pathogenesis of hypertension.

Essential hypertension is a **complex** and **multifactorial disorder**. Specific trigger is not known. Probable factors that play a significant role includes:

- **Decreased renal sodium excretion:** It is probably the **key feature**. Decreased excretion of sodium by kidney → leads to an **increase in fluid volume, cardiac output, and peripheral vasoconstriction** → raises the blood pressure.
- **Raised vascular resistance:** Factors that produce vasoconstriction or stimuli that cause structural changes in the vessel wall → result in an increase in peripheral vascular resistance → cause primary hypertension.
- **Genetic factors:** They play an important role in the development of hypertension. The genetic defects may be in the enzymes involved in aldosterone metabolism, sodium reabsorption and smooth muscle cell growth.
- **Environmental factors:** These include stress, obesity, smoking, lack of physical activity and heavy intake of sodium salt.

Consequences of Hypertension

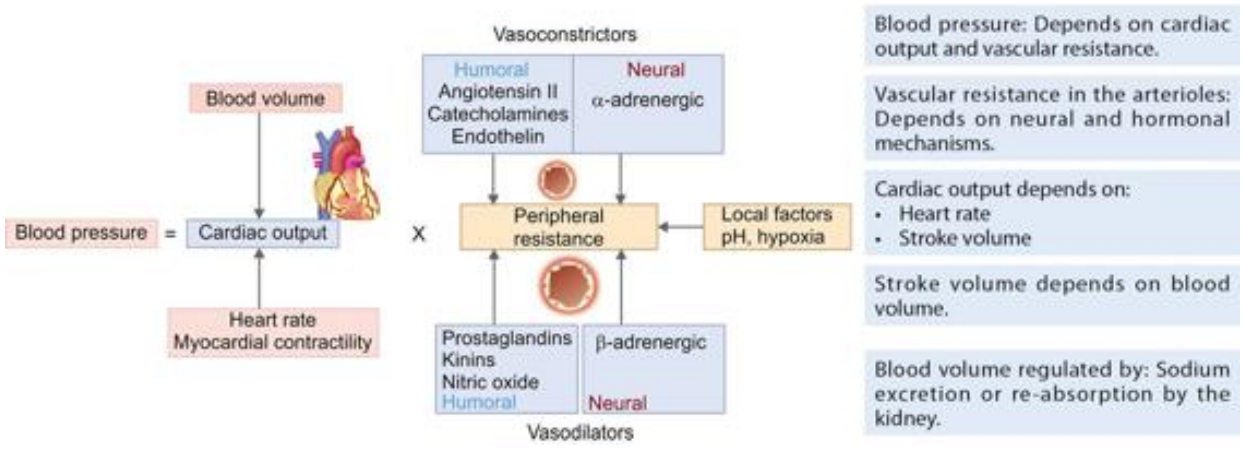
- **Risk factor:** Hypertension is one of the major modifiable risk factors for **atherosclerosis**.
- **Lesions/diseases produced:** Cardiac hypertrophy, congestive heart failure (hypertensive heart disease) and ischemic heart disease (IHD), multi-infarct dementia, aortic dissection, stroke, subarachnoid hemorrhage, hypertensive encephalopathy, retinal ischemia/infarction and renal failure.

- **Accelerated or malignant hypertension:** It is characterized by **rapid raise in blood pressure (**i.e., systolic pressure over 200 mm Hg, diastolic pressure over 120 mm Hg) → **renal failure, and retinal hemorrhages** and exudates, **with or without papilledema**.
  - It may develop in normotensive individuals but more common in patients with preexisting benign hypertension (essential or secondary).
  - It develops in ~5% of hypertensive persons. If not treated, death occurs within a year or two.

Pathogenesis of Secondary Hypertension

- **Mechanism of renovascular hypertension:**
  - Renal artery stenosis → decreased glomerular flow and pressure in the afferent arteriole of the glomerulus → stimulates renin secretion and production of angiotensin II → vasoconstriction → increased peripheral resistance.
  - Renal artery stenosis also increases sodium reabsorption → increases blood volume through the aldosterone mechanism.
- **Primary hyperaldosteronism:** It is one of the most common causes of secondary hypertension.

Flowchart 16.1: Regulation of blood pressure.



MORPHOLOGY

Q. Write short note on vascular pathology in hypertension.

Large and Medium Vessel Disease: Atherosclerosis

- Hypertension is one of the major modifiable risk factors for atherogenesis.
- Causes degenerative changes in the walls of large and medium arteries.
- Predisposes to: (1) Aortic dissection and (2) Cerebrovascular hemorrhage.

Small Vessel Diseases

Two forms can occur in hypertension: (1) Hyaline arteriolosclerosis, and (2) hyperplastic arteriolosclerosis.

- 1. Hyaline arteriolosclerosis: It is seen in the arterioles in patients with benign hypertension.
- Microscopy: It shows thickening of the wall due to homogeneous, pink hyaline material and narrowing of the lumen **(Figs. 16.7A and B)**.
- Mechanism: Chronic hemodynamic stress produced by hypertension → produces leakage of protein into the vessel wall → increased synthesis ECM by smooth muscle cell → hyalinization of the wall of arteriole.
- Consequence: The arteriolar narrowing → impaired blood supply to kidney → produces ischemia and glomerular scarring. The kidney changes in benign hypertension are called benign nephrosclerosis.
- 2. Hyperplastic arteriolosclerosis: It occurs in severe (malignant) hypertension.
- Microscopy: The blood vessels show "onion-skin," concentric, laminated thickening of the arteriolar walls and narrowing of the arteriolar lumen **(Figs. 16.7C to E)**. It may also show fibrinoid deposits and necrosis of vessel wall (necrotizing arteriolitis), particularly in the kidney.

Kidney: Plays an important role in the regulation of blood pressure.

Essential hypertension:
- Reduced renal excretion of sodium
- Increased vascular resistance
- Genetic factors
- Environmental factors

Essential hypertension: Accounts for ~95% of cases and is a multifactorial disorder.

Angiotensin II:
- Increases vascular smooth muscle tone
- Increases aldosterone secretion by adrenal → increases sodium absorption by kidney.

Hypertension: One of the modifiable risk factor for atherosclerosis. It may predispose to cardiac

hypertrophy, congestive heart failure and renal failure.

Renin: Major regulator of blood pressure is secreted by the kidneys when the blood pressure is decreased in the afferent arterioles.

Causes of hyaline arteriosclerosis:
- Benign hypertension
- Diabetic microangiography
- Old age (either normo- or hypertensive)

Blood vessels in malignant hypertension:
- Hyperplastic arteriosclerosis (onion-skin appearance)
- Fibrinoid necrosis—necrotizing arteriolitis

VASCULITIS

Definition: Vasculitis is a **heterogeneous group** of disorders **characterized by inflammation and damage of the blood vessel**. The lumen of the involved vessel is usually narrowed → may lead to ischemia of the tissues supplied.

General Characteristics

- **Primary or secondary:** Vasculitis may be primary or secondary component of another primary disease.
- **Vessel involved:** It can involve **any type, size, and location** of blood vessel. Mostly, involve small vessels (arterioles to capillaries to venules).
- **Organ involved:** Vasculitis may be **restricted to one organ** (e.g., skin), or **may involve many organ systems**.

Classification

May be classified in many ways such as: (1) According to size of the vessel involved **(Table 16.3)**, (2) role of immune complexes, and presence of specific autoantibodies, (3) granuloma formation, and (4) organ specificity.

Pathogenesis of Vasculitis

Two common pathogenic mechanisms are:
1. Immune-mediated mechanism (noninfectious vasculitis).

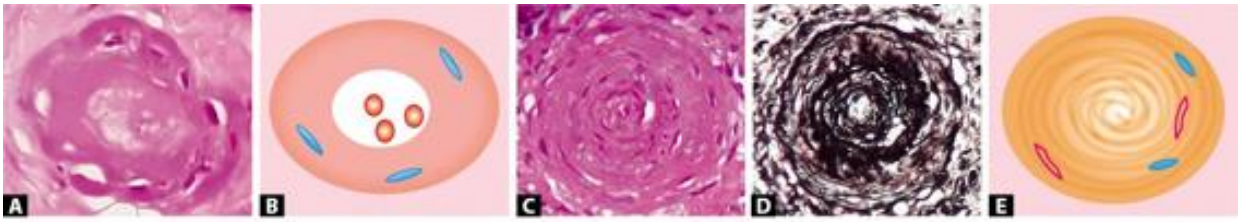
2. Direct invasion of vascular walls by infectious pathogens (infectious vasculitis).

Noninfectious Vasculitis

Mechanism: Main immunological mechanisms of noninfectious vasculitis are: (1) immune complex mediated, (2) antineutrophil cytoplasmic antibody-mediated, and (3) antiendothelial cell antibody-mediated.

Immune Complex-mediated Vascular Injury

Immune complex-mediated tissue vasculitis is characterized by deposition of immune complexes in the vessel walls. The antigen in the immune complex is not known in most of the cases.



Figs. 16.7A to E: Vascular changes (in renal blood vessels) in hypertension: [A (H&E) and B (diagrammatic)] Hyaline arteriolosclerosis in benign hypertension: The arteriolar wall is thickened, hyalinized, and the lumen is narrowed; [C (H&E), D (PAS-methanamine) and E (Diagrammatic)] Hyperplastic arteriolosclerosis in malignant hypertension: shows onion-skinning with obliteration of arteriolar lumen.

Mechanism of tissue damage:

- The **antigen-antibody** (immune) **complexes are formed** when there is little antigen excess.
- The immune complexes gets **deposited in vessel walls** → activate complement system (e.g., C5a) → chemotactic for neutrophils → phagocytose the immune complexes → release their contents → **damage the vessel wall**.
- As the process becomes chronic, mononuclear cells infiltrate the vessel wall.
- The lumen of the involved vessel is narrowed → lead to ischemic changes in the tissues supplied by the involved vessel (*refer* pages 153-6).

Causes of immune-mediated vasculitis:

- **Systemic immunological diseases** [e.g., **systemic lupus erythematosus** (SLE) and **polyarteritis nodosa**].
- **Drug hypersensitivity:** Drugs themselves may be foreign proteins (e.g., streptokinase) or they bind to serum proteins (e.g., penicillin) and behave

like antigens.

- **Secondary to viral infections:** Viral proteins may form immune complexes. Examples:
  - **Hepatitis B virus (HBV) associated polyarteritis nodosa** (PAN).
  - Cryoglobulinemic vasculitis is strongly associated with hepatitis C virus (HCV) infection.

Antineutrophil Cytoplasmic Antibodies

Antineutrophil cytoplasmic antibodies (ANCAs) are heterogeneous group of **autoantibodies directed against certain proteins** (mainly enzymes) **in the cytoplasmic granules of neutrophils and monocytes**. Two important ANCAs are:

1. **Anti-myeloperoxidase (MPO-ANCA):** MPO is a lysosomal enzyme normally involved in producing oxygen-free radicals. MPO-ANCAs can be induced by drugs (e.g., propylthiouracil). They are associated with **microscopic polyangiitis and Churg–Strauss syndrome**.
2. **Anti-proteinase-3 (PR3-ANCA):** PR3 is a constituent of neutrophil azurophilic granule. It shares homology with many microbial peptides and antibodies against microbial peptides may form PR3-ANCAs. They are associated with **Wegener's granulomatosis**.

TABLE 16.3: Classification of vasculitis according to blood vessels involved.

Examples	*Organs/vessels involved*	*Microscopic features*
Predominantly involving large vessels (aorta and large branches to extremities, head and neck)		
• Giant cell (temporal) arteritis	Temporal artery and aorta. Patients older than age 50	Granulomatous inflammation with giant cells
• Takayasu's arteritis	Aorta, aortic arch and major branches, and pulmonary arteries Patients younger than age 50	Granulomatous with some giant cells → fibrosis in chronic stages
Predominantly involving medium vessels (visceral arteries and their branches)		
• Polyarteritis nodosa (PAN)	Skin, peripheral nerve, GI tract, and renal, etc. Spares pulmonary vessels	Fibrinoid necrosis, with mixed cellular infiltrate
• Kawasaki disease	Arteritis with mucocutaneous lymph node syndrome. Usually occurs in children	Similar to PAN, fibrinoid necrosis less prominent
Predominantly involving small vessels (arterioles, venules and capillaries)		
• Granulomatosis with polyangiitis*	Upper respiratory tract, lungs, kidneys, skin, and eyes	Necrotizing or granulomatous (or both); mixed cellular infiltrate
• Churg-Strauss syndrome	Upper respiratory tract, lungs, heart, peripheral nerves	Necrotizing or granulomatous (or both); prominent eosinophils
• Microscopic polyangiitis	Necrotizing glomerulonephritis and pulmonary capillaries	Leukocytoclastic with occasional eosinophils

* Formerly called as Wegener's granulomatosis

Vasculitis with granulomatous lesions:
- Wegener's granulomatosis
- Buerger's disease
- Takayasu's arteritis
- Giant cell arteritis
- Churg–Strauss syndrome

Small vessel vasculitis:
- Wegener's granulomaosis
- Churg–Strauss syndrome
- Microscopic polyangiitis

ANCA: Autoantibodies against certain proteins in the cytoplasmic granules of neutrophil and monocytes.

Antiendothelial Cell Antibodies

They can produce **endothelial cell injury and lysis through either complement-mediated cytotoxicity or antibody-dependent cellular cytotoxicity**. For examples, Kawasaki disease and systemic lupus erythematosus.

Few examples of vasculitis are considered here.

Vasculitis: Inflammation and damage of the blood vessel.

Infections can also indirectly induce noninfectious vasculitis by generating immune complexes or triggering cross-reactivity.

Immune complex-mediated vascular injury is due to type III hypersensitivity reaction.

Hypersensitivity vasculitis most commonly involves: Post-capillary venules.

MPO-ANCA were previously called p-ANCA.

PR3-ANCA were previously called c-ANCA.

Antiendothelial antibodies:
- Kawasaki disease
- Systemic lupus erythematosus.

Giant Cell (Temporal) Arteritis

Definition: Giant cell (temporal/cranial) arteritis is a **chronic**, typically **granulomatous inflammation of medium- and large-sized arteries**.

Vessels involved: One or more branches of the carotid artery (e.g., **temporal artery)**. Can involve multiple arteries, aorta (giant cell aortitis) and its branches.

Pathogenesis: T-cell mediated immune response against an unknown antigen. Proinflammatory cytokines (TNF) and antiendothelial antibodies may also be involved.

MORPHOLOGY

Gross

Involved segment of the artery show nodular thickening of the intima → reduces the size of lumen → ischemia.

Microscopy (Figs. 16.8A and B)

- Granulomatous inflammation within the inner tunica media centered on the internal elastic lamina. It shows multinucleated giant cells (either Langhans type or foreign body type).
- Fragmentation of elastic lamina.
- Chronic inflammation consisting of T-cells and macrophages.

- Segmental distribution of inflammation with segments of relatively normal artery between the lesions.
- Intimal fibrosis in healed stage.

Clinical Features

- Manifest **after the age of 50**.
- **Fever, anemia, fatigue, headache** (most severe along the course of the superficial temporal artery) and weight loss.
- On palpation, **the involved artery is tender, thickened, or nodular**.

Diagnosis: It is confirmed by **biopsy of the temporal artery**. Responds well to corticosteroids and anti-TNF therapy.

Temporal arteritis is also known as giant cell arteritis.

Giant cell arteritis:
- Medium and large-sized arteries
- Granulomatous inflammation
- Fragmentation of internal elastic lamina
- Immunologically-mediated chronic inflammation

Giant cell arteritis: Biopsy of the involved artery is the investigation of choice.

Polyarteritis Nodosa

Definition: Polyarteritis nodosa **(PAN)** is a **systemic, necrotizing vasculitis of small and medium-sized muscular arteries** characteristically involving the **renal and visceral arteries**.

It **spares the smallest blood vessels** (arterioles, venules, and capillaries). **PAN does not involve pulmonary arteries.**

Etiology*:* Cause not known in the majority of cases. About 30% of patients with PAN have **chronic hepatitis B with HBsAg-HbsAb complexes** in affected vessels. This indicates an **immune complex–mediated** etiology in this subset.

MORPHOLOGY

Vessels involved: Vessels of the skin, kidneys, heart, liver, peripheral nerves, and gastrointestinal tract.

Gross

Nodular lesions mainly at the points of arterial branchings. Aneurysms may also be seen.

Microscopy (Fig. 16.9)

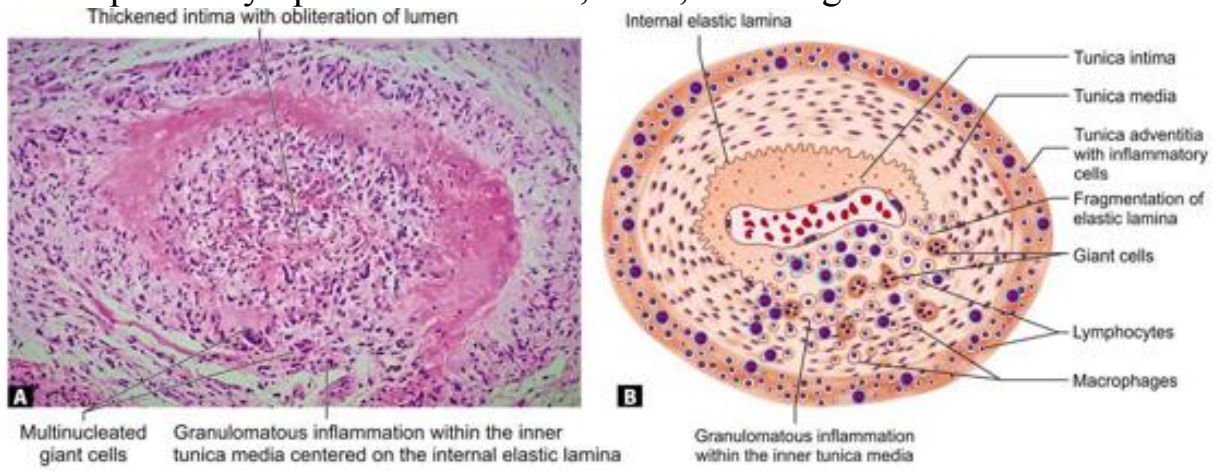
Lesions are segmental (involve only part of the vessel circumference and at branch points of arteries.

- Transmural necrotizing inflammation of small to medium-sized arteries → weakens the wall of artery → can lead to aneurysms or rupture.
- Acute phase:
  - Transmural inflammation of the arterial wall and perivascular infiltration by polymorphonuclear neutrophils and eosinophils.
  - Fibrinoid necrosis
  - Thrombosis → infarction of the tissues supplied by the involved vessel.
  - Aneurysm (up to 1 cm in size) → may rupture → produce hemorrhage.
- Late phase:
  - Subacute and chronic stages: Acute inflammatory infiltrate replaced by mononuclear cells. Fibrous thickening of the vessel wall → further occlusion of the vessel lumen.

Consequences of vasculitis: Thrombosis → impaired perfusion → lead to ulcerations, infarcts, ischemic atrophy, or hemorrhages in the distribution of affected vessels.

Clinical Features

- Usually occurs in **young adults**.
- Nonspecific symptoms are malaise, fever, and weight loss.



Figs. 16.8A and B: Microscopic features: (A) H & E staining; (B) Diagrammatic representation of giant cell arteritis.

- Specific complaints are due to **ischemia and infarction of affected tissues and organs**.

- **Renal involvement manifests as hypertension** (usually developing rapidly), renal insufficiency, or hemorrhage due to microaneurysms.
- Gastrointestinal tract involvement present with abdominal pain and melena (bloody stool).

Prognosis: It is fatal, if not treated. Immunosuppression produces remissions or cure in about 90% of patients.

Polyarteritis nodosa: Systemic, necrotizing vasculitis of small and medium-sized muscular arteries.

Polyarteritis nodosa does not involve pulmonary artery and spares small blood vessels.

Polyarteritis nodosa:
- Segmental involvement
- Transmural inflammation by neutrophils
- Fibrinoid necrosis
- Thrombus formation

Polyarteritis nodosa: Characteristically, the lesions at different stages of development (from early acute to late) coexist in different vessels or even within the same vessel.

Polyarteritis nodosa: Renal arterial involvement is a major cause of death.

Granulomatosis with Polyangiitis

Definition: Classic granulomatosis with polyangiitis (previously called Wegener granulomatosis) is a **necrotizing vasculitis**, which involves the **upper respiratory tract**, the **lungs** and the **kidneys**.

Pathological Hallmarks

- **Acute necrotizing granulomatous inflammation in the upper respiratory tract** (ear, nose, sinuses, throat) or **the lower respiratory tract** (lung) or both. It may present as chronic sinusitis and mucosal ulcerations of the nasopharynx.
- **Necrotizing or granulomatous vasculitis** affects **small to medium-sized vessels** (e.g., capillaries, venules, arterioles, and arteries). It may present with persistent pneumonitis and bilateral nodular and cavitary infiltrates.
- **Renal lesion in the form of focal and segmental necrotizing**, often **crescentic glomerulonephritis**.

Age and gender: It usually presents during **fourth to fifth decade**. **Males** are affected more often than females.

Pathogenesis: Not clear. Probably represents a form of **T-cell–mediated hypersensitivity reaction to an exogenous** (inhaled infectious or other environmental agent) **or endogenous antigen. About 95% of patients show PR3-ANCAs**, and these autoantibodies may play a role in the pathogenesis of this disease.

MORPHOLOGY

Mainly involves three organs but may be widespread involving any organ such as eyes, skin, kidney, and other organs (e.g., heart).

- 1. Upper respiratory tract lesions: They range from inflammatory sinusitis to ulcerative lesions in the nose, palate, or pharynx.
- 2. Lower respiratory tract: Lung shows multiple, bilateral, nodular cavitary infiltrates. Microscopically, it is characterized by:
  - a. Necrotizing granulomas: It consists of geographic patterns of central necrosis surrounded by a zone of fibroblastic proliferation with giant cells, reminiscent of mycobacterial or fungal infections. The granulomas may be either intravascular or extravascular.
  - b. Necrotizing vasculitis of small arteries and veins.

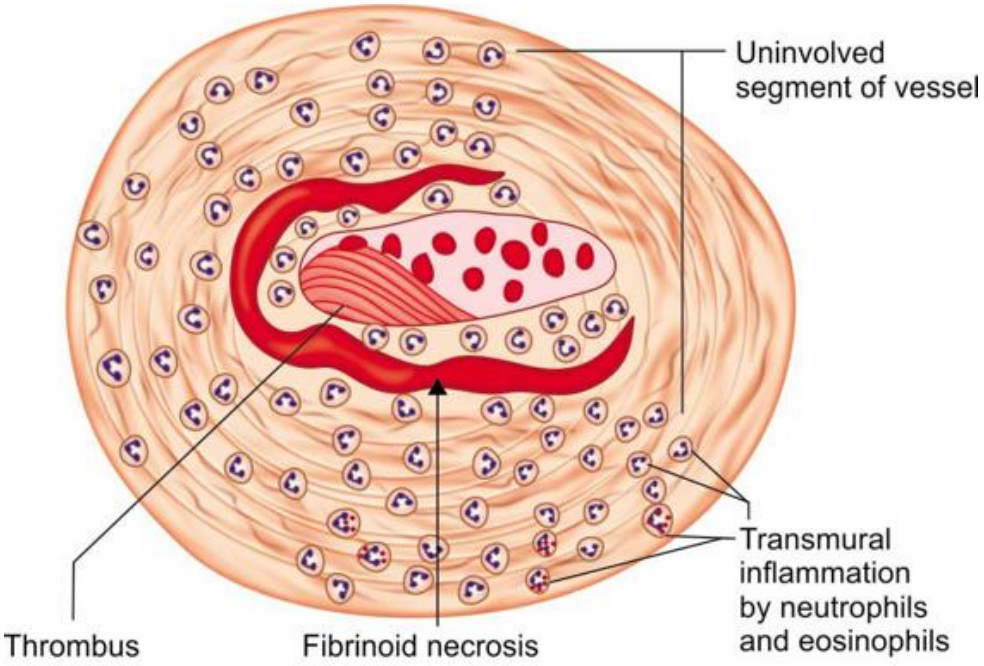


Fig. 16.9: Microscopic features (diagrammatic) of acute phase of polyarteritis nodosa.

- 3. Renal lesions:
  - a. Early lesions: Glomeruli show focal and segmental necrotizing glomerulonephritis.
  - b. Advanced lesions: They are characterized by rapidly progressive crescentic glomerulonephritis (RPGN).

Course: If not treated, it is usually rapidly fatal and majority die within 1 year.

ANCA is associated with granulomatosis with polyangiitis.

Main organs involved in granulomatosis with polyangiitis:
- Upper respiratory tract
- Lower respiratory tract
- Kidney

- Strawberry gums: Granulomatosis with polyangiitis.

VASCULAR TUMORS

Q. Write a short note on tumors of blood vessels (vascular tumors).

Tumors of blood vessels are classified as benign, intermediate grade and malignant **(Box 16.2)**.

BOX 16.2: Classification of vascular tumors.

Benign neoplasms, developmental and acquired conditions
- Hemangioma: Capillary hemangioma, cavernous hemangioma, pyogenic granuloma

Intermediate-grade neoplasms
- Kaposi sarcoma
- Hemangioendothelioma

Malignant neoplasms
- Angiosarcoma
- Hemangiopericytoma

Hemangioma

Q. Write a short note on hemangioma.

Hemangiomas are **very common benign tumors of blood vessels** and form about 7% of all benign tumors of infancy and childhood. Most of these

lesions are present from birth and increase in size as the child grows.

Types of hemangioma: Capillary and cavernous.

Capillary Hemangioma

- **Most common type of hemangioma.**
- **Sites**
  - Most commonly in the **skin, subcutaneous tissues**, and mucous membranes of the oral cavities and lips.
  - Internal organs (e.g., **liver**, spleen and kidneys).

MORPHOLOGY

Gross

- Bright red to blue in color.
- Size varies from few millimeters to several centimeters in diameter.
- May be in level with the surface of the skin or slightly raised. The skin overlying it is usually intact.

Microscopy (Figs. 16.10A and B)

- Unencapsulated lesion.
- Composed of aggregates of closely packed, thin-walled vascular channels. The size and structure of these vascular channels resemble normal capillaries.
- Capillaries are lined by flattened endothelium and their lumen is usually filled with blood. The lumens may contain partially or completely organized thrombus.
- The vessels are separated by scant connective tissue stroma.

Cavernous Hemangioma

Consists of **large, dilated vascular channels**; compared with small vascular spaces in capillary hemangiomas.

MORPHOLOGY

Gross

- Red-blue, soft, spongy masses and measure 1–2 cm in diameter.

- More frequently involve deep structures. They are found in the skin, on the mucosal surfaces and visceral organs, such as the spleen, liver and pancreas.

Microscopy (Figs. 16.11A and B)

- Consist of sharply defined unencapsulated mass.
- Composed of large, cavernous blood-filled vascular spaces, separated by a moderate amount of connective tissue stroma. Intravascular thrombosis and dystrophic calcification are common.

Endothelial cells:
- Contain Weibel–Palade bodies with von Willebrand factor
- Identified by CD31, CD34 and vWF

Hemangioma: Unencapsulated benign tumor.

Capillary hemangioma: Aggregates of closely packed thin-walled vascular channels.

Strawberry hemangioma: Type of capillary hemangioma.

Cavernous hemangioma: Large cavernous blood-filled vascular spaces.

HEART DISORDERS

HEART FAILURE

CC: Describe the etiology, types, stages, pathophysiology, pathology and complications of heart failure.

Heart failure often **termed congestive heart failure (CHF)** is a common, usually progressive condition with a poor prognosis.

Definition: Heart failure (HF) is defined as the pathophysiologic condition in which the heart is unable to eject blood (unable to generate an adequate cardiac output) delivered to it by the venous system*. This causes inadequate perfusion of tissues or increased diastolic filling pressure of the left ventricle, or both.

*Venous emptying blood into heart chambers: The inferior vena cava (IVC) empties blood into the right atrium (RA), and the pulmonary vein empties blood into the left atrium (LA).

Etiology (Box 16.3): Heart failure is a common end point for many forms of cardiac disease/disorder.

Heart failure develops when the heart is not able to pump blood at a rate sufficient to meet the metabolic demands of the peripheral tissues or can do so only at an elevated filling pressure. Inadequate cardiac output is usually accompanied by increased congestion of the venous circulation.

Types of Heart Failure

Depending on Onset

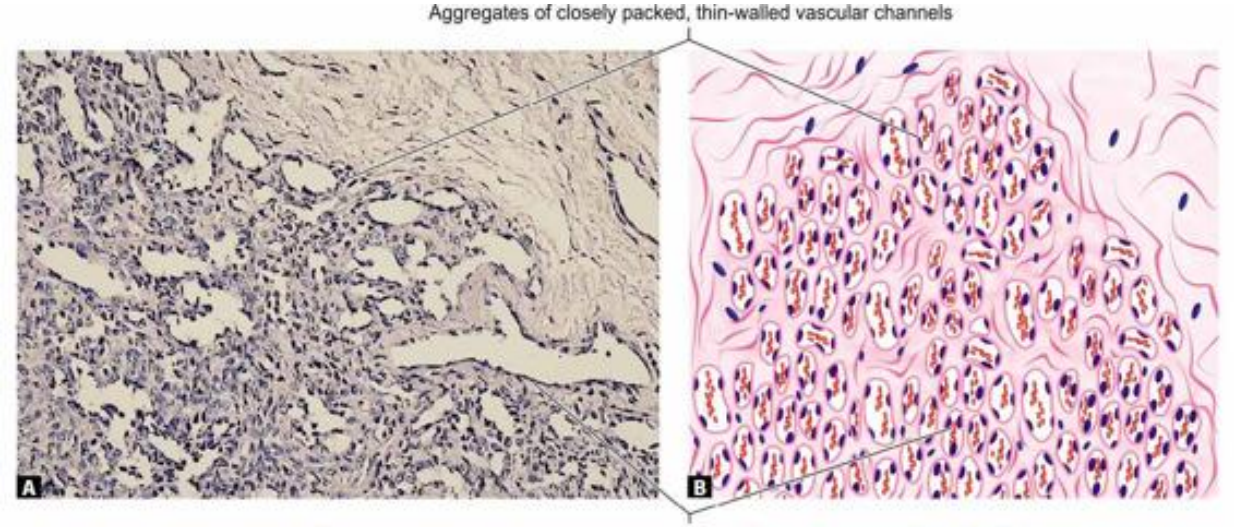
- **Chronic heart failure:** Heart failure is the common **end stage of many forms of chronic heart disease**. It is characterized by **gradual development of heart failure** and systemic arterial pressure is well-maintained, but edema develops. Examples include valvular heart disease, hypertension, ischemic heart disease and dilated cardiomyopathy.
- **Acute heart failure:** It is characterized by **sudden development of heart failure**. Examples include acute myocardial infarction and rupture of a cardiac valve.

Depending on Output

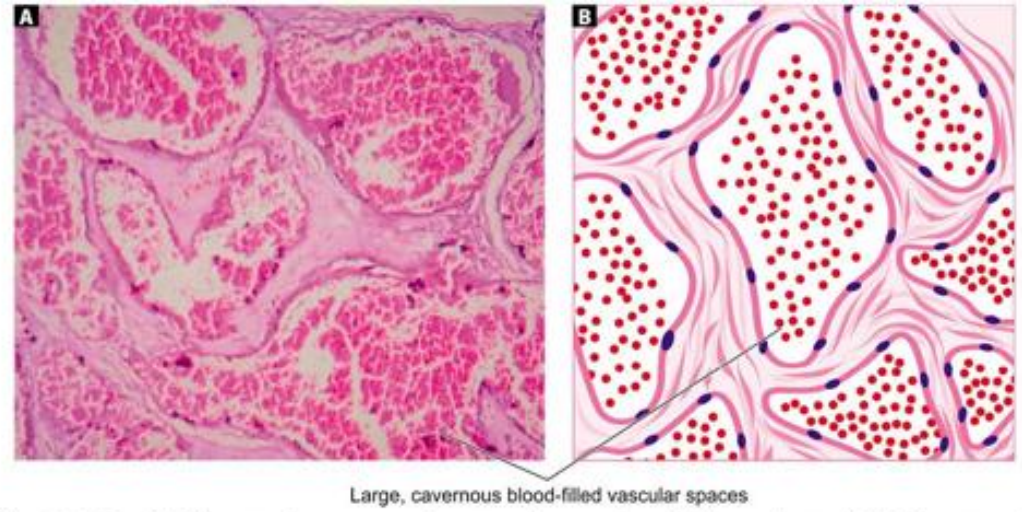
Heart failure (HF) can also be classified according to the ability of the heart to eject blood from the ventricles. **Ejection fraction (EF) is the percentage of blood volume ejected from the ventricle during systole** and is determined by dividing stroke volume by end-diastolic volume. Normally during systole, ventricles contract and eject **about 60–80%** of the blood in them at the end of diastole **(EF)**. Heart failure may result from systolic or diastolic dysfunction.

- **Systolic dysfunction with low ejection fraction:** Heart failure due to systolic dysfunction can result from **progressive deterioration of myocardial contractile function.** It is characterized by a **decrease in ejection fraction**. Patients with a low ejection fraction (EF) have significant systolic dysfunction are called HF with low EF (HFlEF). The ejection fraction in such cases is <40% (some clinicians use <50%). Heart

is not able to generate an adequate cardiac output to perfuse vital tissues. Left ventricular chamber size is increased.



Figs. 16.10A and B: Microscopic appearance of capillary hemangioma: (A) Photomicrograph; (B) Diagrammatic.



Figs. 16.11A and B: Microscopic appearance of cavernous hemangioma: (A) Photomicrograph; (B) Diagrammatic.

- Various **causes** of reduced EF due to inadequate myocardial contractile function include ischemic injury (**myocardial infarction** is the most common cause), inadequate adaptation to pressure or volume overload due to **hypertension or valvular disease**, or ventricular dilation.

- **Diastolic dysfunction with preserved ejection fraction:** It refers to an inability of the heart to adequately relax and fill. Heart failure can result from an **inability of the heart chamber to expand and fill sufficiently during diastole** (diastolic dysfunction). Patients with EF greater than 50% do not have significant systolic dysfunction and are called as HF

with preserved EF (HFpEF). It can occur singly or along with systolic heart failure. Left ventricular chamber size is decreased.

- **Causes** include **massive left ventricular hypertrophy, myocardial fibrosis, constrictive pericarditis**, or amyloidosis of heart. About 50% of CHF cases are due to diastolic dysfunction.

Left-sided, Right-sided and Biventricular Heart Failure

Heart failure can affect predominantly the left or the right side of the heart or may involve both sides (discussed on pages 455-7). In heart failure blood builds up behind the failed ventricle.

Forward and Backward Failure

When the failing heart cannot efficiently pump blood, it leads to an increase in end-diastolic ventricular volumes, increased end-diastolic pressures, and raised venous pressures. Thus, inadequate cardiac output and tissue perfusion (called forward failure) is accompanied by increased congestion or pooling of the blood in venous circulation, that is, termed as backward failure. The backward failure may cause pulmonary edema, peripheral edema, or both. The root problem in CHF is deficient cardiac function. So, every other organ is eventually affected by some combination of forward and backward failure.

Pathophysiology of Heart Failure

When cardiac workload increases or cardiac function is compromised, the cardiovascular system attempts to compensate for reduced myocardial contractility or increased hemodynamic burden through several homeostatic mechanisms. The physiologic mechanisms maintain arterial pressure and organ perfusion. In chronic heart diseases compensatory mechanisms maintain cardiac output by increasing diastolic ventricular filling pressure and end-diastolic volume. This produces characteristic signs and symptoms of heart failure. Because of the heart's capacity to compensate, congestive heart failure is usually tolerated for years. These mechanisms include:

1. **Frank–Starling mechanism** (also known as **Starling's law**)**:** It states that as a larger volume of blood flows into the ventricle (i.e., increased filling volumes), the blood will cause increased stretching of cardiac myofiber. This produces dilation of the walls of the heart, causing a greater expansion during diastole. These lengthened stretched myofibers contract more forcibly, thereby increasing cardiac output. Thus, the quantity of blood that is pumped into the aorta also increases during systole.

   Stages of heart failure

   - **Compensated heart failure:** If the dilated ventricle is capable of maintaining the cardiac output by these compensatory changes, the patient is said to be in compensated heart failure.
   - **Decompensated heart failure:** The ventricular dilatation produced is at the expense of increased wall tension. This increases the oxygen required by an already-compromised myocardium. With time, the failing myocardial muscle is not able to eject sufficient blood to meet the needs of the body, and the patient develops decompensated heart failure.

2. **Activation of neurohumoral systems:**
   - **Release of norepinephrine:** It is released by adrenergic cardiac nerves of the autonomic nervous system. Norepinephrine increases heart rate, myocardial contractility and vascular resistance.
   - **Activation of the renin-angiotensin-aldosterone system:** A reduced ejection fraction leads to reduced perfusion of kidney, causing activation of the renin-angiotensin-aldosterone system as a compensatory mechanism. This leads to retention of salt and water, with expansion of the interstitial and intravascular fluid volumes (increases circulatory volume) and increases vascular tone. This turn increases the existing pulmonary edema.
   - **Release of atrial natriuretic peptide:** It balances the renin-angiotensin-aldosterone system through diuresis and vascular smooth muscle relaxation.

Myocardial structural adaptations: Sustained increase in mechanical work due to pressure or volume overload (e.g., systemic hypertension or aortic stenosis) cause myocytes to increase in size *(hypertrophy)* with or without cardiac chamber dilation. This in turn, increases the size and weight of the heart. Myocardial hypertrophy is a compensatory response to hemodynamic

overload and it increases the myocyte contractile strength. It **occurs in association with many pathological conditions**. Cardiac myocytes adapt to increased workload by assembling new sarcomeres.

BOX 16.3: Causes of heart failure.

Common causes

- Coronary heart disease (CHD)
- Hypertension (HTN)

Less common causes

- Dilated cardiomyopathy
- Congenital heart defects
- Valvular disorder/dysfunction (e.g., due to endocarditis)
- Respiratory diseases
- Anemia (decreased oxygen carrying capacity)
- Hyperthyroidism (due to greatly increased tissue demands

Causes and patterns of hypertrophy: The pattern of hypertrophy depends on the stimulus.

- **Pressure-overload hypertrophy:** In pressure overload states such as chronic hypertension or valvular (e.g., aortic) stenosis (pressure overload), new sarcomeres are added parallel to the long axis of the myocytes, adjacent to existing sarcomeres. This **results in concentric hypertrophy** (concentric increase in wall thickness) in which the **ventricular wall thickness increases without an increase in the size of the chamber.**
- **Volume-overload hypertrophy:** In volume overload states such as, valvular regurgitation (insufficiency, shunts, or myocardial injury (volume overload), the new sarcomeres are added in series with existing sarcomeres. This increases the length of the muscle fiber. Consequently, the **ventricle dilates**. The **thickness of ventricular wall can be increased, normal, or decreased** (e.g., myocardial infarction). Thus, the best measure of hypertrophy in volume-overloaded hearts is the measurement of the heart weight rather than thickness of ventricular wall.

Functional changes: Cardiac hypertrophy is associated with increase in oxygen consumption by heart. Hence, the hypertrophied heart is susceptible to ischemia.

The above-mentioned adaptive mechanisms may be adequate to maintain normal cardiac output in the initial phase, but this capacity may be ultimately lost.

Left-sided Heart Failure (LHF)

Definition: In left-sided heart failure, the left ventricle cannot efficiently eject blood into the aorta. This causes blood to backup into the lungs ("blood builds up behind the failed heart") producing pulmonary congestion (edema).

The clinical and morphologic effects of left-sided CHF are due to passive congestion (in the pulmonary circulation), stasis of blood in the left-sided chambers, and inadequate perfusion of tissues.

Left heart failure can be categorized as heart failure with reduced ejection fraction [HFrEF (systolic heart failure)] or heart failure with preserved ejection fraction [HFpEF (diastolic heart failure)]. They can occur singly or together in one individual. It is discussed above on page 453.

Etiology: The common causes of left-sided cardiac failure are listed in **Box 16.4**.

Pathophysiology of LHF (Fig. 16.12): In left heart failure (LHF), there is ineffective pumping of the blood from the left ventricle. To compensate, left atrial and pulmonary venous pressures increases. Increasing pressure in the pulmonary veins is ultimately transmitted back to the capillaries and arteries of the lungs. It results in accumulation of blood within the pulmonary circulation. As hydrostatic pressure increases within the pulmonary veins and capillaries, fluid is forced from the capillaries into interstitial and alveolar spaces. This results in passive pulmonary congestion and edema. The capillaries in the alveolar septal get filled with blood (congestion). In chronic left ventricular failure, small ruptures of the capillaries cause escape of RBCs into the alveolar spaces. The rupture of RBCs liberates iron and is phagocytosed by alveolar macrophages. Thus, alveoli contain hemosiderin-laden macrophages (so-called heart failure cells). If capillary hydrostatic pressure of pulmonary capillaries exceeds that of plasma osmotic pressure, fluid leaks from capillaries into alveoli and can produce pulmonary edema.

The clinical and morphologic effects of left-sided CHF are a consequence of:

- **Passive congestion (blood backing up in the pulmonary circulation)** (*refer* chronic venous congestion of lung on pages 108-9).
- **Inadequate systemic perfusion leading to organ dysfunction**
- **Stasis of blood in the left-sided chambers**

MORPHOLOGY

Heart

Gross: It depends on the underlying disease process responsible for LHF [e.g., myocardial infarction or valvular deformities (valvular stenosis or regurgitation)]. Left ventricle is usually hypertrophied and can be dilated (except in failure due to mitral valve stenosis or restrictive cardiomyopathies). Left ventricular dilation can resulting mitral insufficiency and dilatation (enlargement) of left atrium.

Microscopy: These changes are nonspecific and consist of hypertrophy of myocyte and variable degree of interstitial fibrosis.

Lungs

Gross: Due to pulmonary congestion and edema, the lungs become heavy and boggy. Lungs exude a frothy pink transudate (edema) on the cut surface.

Microscopy: These include

- Perivascular and interstitial edema and is responsible for the characteristic Kerley B and C lines observed on chest X-ray in patient with CHF.
- Progressive edema of alveolar septa
- Accumulation of edema fluid in the alveolar spaces.
- In chronic heart failure, few red cells and plasma proteins escape from the leaky capillaries into alveolar spaces. These RBCs are phagocytosed and digested by macrophages. Subsequent breakdown of red cells and hemoglobin leads to the liberation of hemosiderin within macrophages. These hemosiderin-laden alveolar macrophages (also known as heart failure cells). These heart failure cells tell-tale signs (reflect) of previous episodes of pulmonary edema (refer Fig. 5.1).

Clinical Features and Complications

Symptoms during early left-sided heart failure are as a consequence of pulmonary congestion and edema and may be subtle.

Dyspnea (breathlessness or shortness of breath): It occur early and most significant (cardinal) symptom in the progression of left-sided heart failure. Difficulty breathing may be exacerbated by activity (**dyspnea on exertion**) or lying down (**orthopnea and paroxysmal nocturnal dyspnea**). Initially, there may be cough and dyspnea only on exertion. As heart failure progresses, worsening pulmonary edema may cause orthopnea [dyspnea when supine (supine position increases venous return from the lower extremities and also elevates the diaphragm), relieved by sitting or standing (hence patients usually sleep in a semi-seated position)] or paroxysmal

nocturnal dyspnea (dyspnea usually occurring at night and is a dramatic form of breathlessness, awakening patients from sleep with feelings of suffocation) and dyspnea at rest.

Respiratory system: Cough results from bronchial irritation associated with transudation of fluid into air spaces. **Fine ráles** are abnormal sounds at the base of lungs caused by the opening of edematous pulmonary alveoli and movement of air through partially fluid-filled alveoli.

Cardiovascular system: Cardiomegaly (an enlarged heart), **tachycardia, a third heart sound (S3)**. With progressive dilatation of left ventricles, the outward displacement of papillary muscles causes mitral regurgitation and a systolic murmur. Subsequent chronic dilation of the left atrium can cause atrial fibrillation. Atrial fibrillation causes stasis of the blood (especially in the atrial appendage) and favors formation of thrombi which can fragment and form emboli. These emboli can produce strokes and infarction in other organs.

Prerenal azotemia: Diminished cardiac output leads to reduced renal perfusion. This stimulates the renin-angiotensin-aldosterone axis, increasing intravascular volume and pressures. Unfortunately, these compensatory mechanisms in heart failure, exacerbate the pulmonary edema. With further progression, prerenal azotemia may develop.

Hypoxic encephalopathy: It may occur due to reduced cerebral perfusion. It is characterized by symptoms such as irritability, diminished cognition, and restlessness. This can progress to stupor and coma.

Treatment: Initially, correct the underlying cause (e.g., a valvular defect). Others includes salt restriction or drugs that decrease volume overload (e.g., diuretics), increase myocardial contractility ("positive inotropes"), or reduce afterload (adrenergic blockade or inhibitors of angiotensin-converting enzymes).

Right-sided Heart Failure

Definition: Right heart failure (RHF) is defined as the inability of the right ventricle to effectively pump venous blood into the lungs. Thus, RHF cannot provide adequate blood flow into the pulmonary circulation at a normal central venous pressure.

Causes: The right and left ventricles function in series **(Box 16.5)**. In RHF, blood builds up in the systemic venous system (vena cava and its

tributaries).

- **All the causes of LHF:** Any increase in pressure in the pulmonary circulation produced by left ventricular failure inevitably produces an increased workload (burden) on the right side of the heart (mainly right ventricle). Thus, the etiology of right-sided heart failure must include all the causes of left-sided failure and **is usually the consequence of left-sided heart failure.**
- **Corpulmonale:** Isolated right-sided heart failure is rare and usually develops **in association with** one of a variety of disorders **of the lungs**; hence it is termed as **cor pulmonale**. Corpulmonale occurs with diseases of lung parenchyma (e.g., emphysema, chronic bronchitis). Apart from parenchymal lung diseases, cor pulmonale may develop secondary to disorders that affect the pulmonary vasculature (e.g., primary pulmonary hypertension, recurrent pulmonary thromboembolism). Pulmonary disorders that increases pulmonary vascular resistance cause a high afterload on the right ventricle. This results in hypertrophy and dilation of the right ventricle and called cor pulmonale and may progress to right ventricular failure as the lung disease worsens. In cor pulmonale, myocardial hypertrophy and dilation usually restricted confined to the right ventricle and atrium. However, bulging of the ventricular septum can occur to the left side and reduce cardiac output by causing outflow tract obstruction.

Morphologic and clinical effects in isolated right-sided heart failure differ from those of left-sided heart failure in that congestion is pronounced in the systemic and portal venous systems **(Fig. 16.12)** and pulmonary congestion is minimal.

BOX 16.4: Common causes of left-sided heart failure.

- Ischemic heart disease (IHD)—most common cause
- Systemic hypertension
- Mitral or aortic valvular disease
- Primary diseases of the myocardium (e.g., amyloidosis, myocarditis).

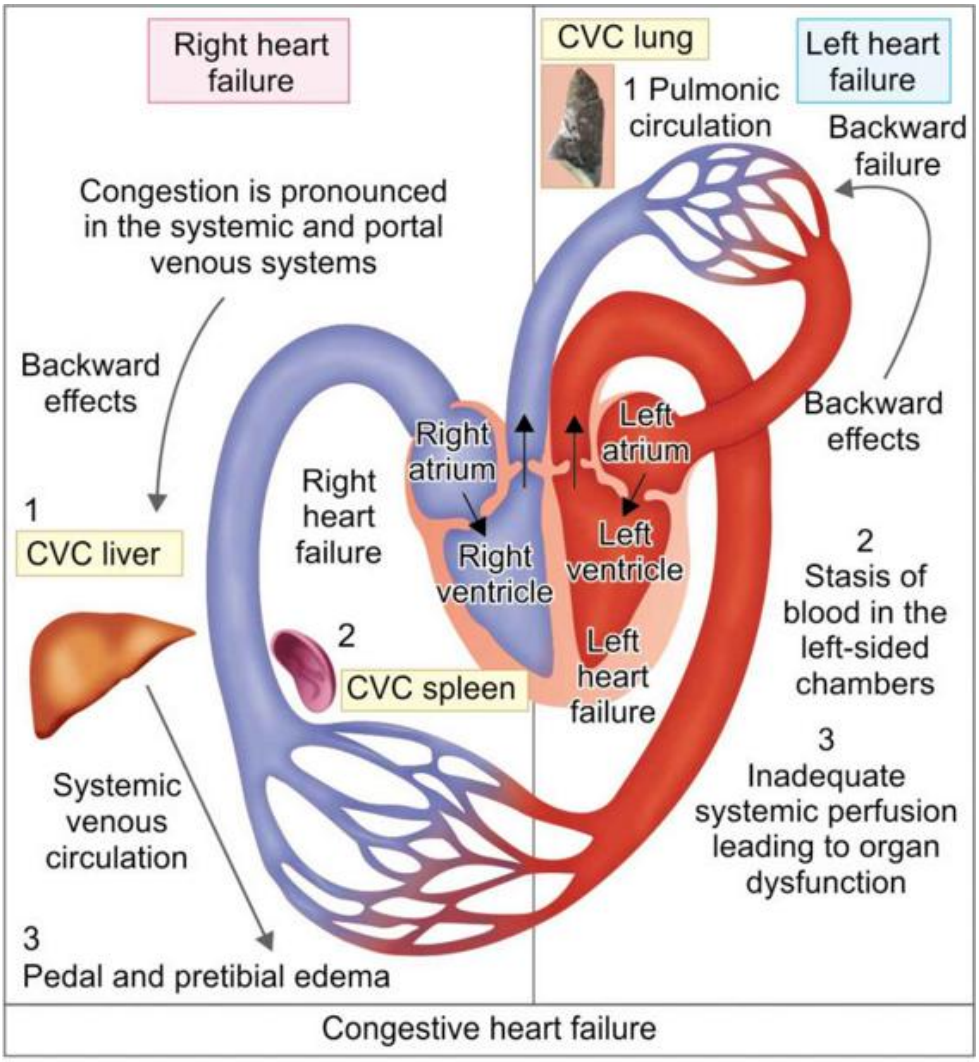


Fig. 16.12: Pathophysiology and effects on pulmonary circulations and systemic in left and right heart failure.
(CVC: chronic venous congestion)

MORPHOLOGY

Heart: Similar to left-heart failure, the morphology of heart depends on the cause. Isolated right heart failure caused by lung disease, in most cases show only hypertrophy and dilation of the right atrium and ventricle.

Liver and portal system**:** Congestion of the hepatic and portal vessels may produce morphological changes in the liver, the spleen, and the GI tract.

- Liver: Usually increased in size and weight (congestive hepatomegaly) due to prominent passive congestion. Cut shows characteristic "nutmeg liver" (refer page 109 and Fig. 5.2). It is characterized by congested red-brown centrilobular areas surrounded by peripheral paler, noncongested liver parenchyma. When there is also left-sided heart failure with hypoperfusion, severe central hypoxia produces centrilobular necrosis. In

long-standing severe right-sided heart failure, the central necrotic areas can become fibrotic, creating so-called cardiac cirrhosis.

- Spleen and GIT: Right-sided heart failure can raise the pressure in the portal vein and its tributaries (portal hypertension). This can cause tense, enlargement of spleen (congestive
- splenomegaly) (refer page 110, and Figs. 5.3 and 12.37). If severe, chronic passive congestion and edema can develop in the bowel wall and may interfere with nutrient (and/or drug) absorption.

Effusion in pleural, pericardial and peritoneal cavities**:** Systemic venous congestion in right-sided heart failure can lead to transudates (effusions) in the pleural, pericardial or peritoneal cavities (ascites).

Subcutaneous tissues: Edema of dependent portions of the body [mainly ankle (pedal) and pretibial edema] is a hallmark of right-sided heart failure. In patients who are chronically bedridden, the edema is mainly found in the presacral region. Generalized massive edema (anasarca) can also develop.

Clinical Features and Complications

Isolated/pure right-sided heart failure usually does not produce respiratory symptoms. The clinical features are due to systemic and portal venous congestion. These include enlargement of liver and spleen, peripheral edema, pleural effusion, and ascites. Venous congestion and hypoxia of the kidneys and brain due to right-sided heart failure can produce similar changes to that of hypoperfusion of left-sided heart failure. Renal congestion is more severe with right-sided than left-sided heart failure and leads to greater fluid retention and peripheral edema. As CHF progresses, patients may develop cyanosis and acidosis. In many patients biventricular CHF with symptoms due to both right sided and left-sided heart failure are observed.

Complications in advanced heart failure are shown in**Box 16.6**.

ISCHEMIC HEART DISEASE

CC: Describe the epidemiology, risk factors, etiology, pathophysiology, pathology, presentations, gross and microscopic features, diagnostic tests and complications of ischemic heart disease.

Definition: Ischemic heart disease (IHD) is a group of heart diseases in which there is an **inadequate supply of blood and oxygen to a portion of the myocardium**.

Typically, it occurs when there is an imbalance between the demand and supply (perfusion) of oxygenated blood. Cardiac myocytes produce energy

almost exclusively through mitochondrial oxidative phosphorylation. Hence, cardiac function is dependent on the continuous flow of oxygenated blood through the coronary arteries.

List of diseases included under ischemic heart disease is presented in **Box 16.7**.

Epidemiology: IHD is the **leading cause of death** in both males and females.

Ischemic heart disease: Results due to imbalance between the coronary blood supply and myocardial demand.

Coronary arteries include:
- Left anterior descending (LAD)
- Left circumflex (LCX)
- Right coronary artery (RCA)

Etiology and pathogenesis of IHD is discussed under etiology of myocardial infarction on pages 458-60.

ANGINA PECTORIS

Definition: Angina pectoris clinically present as **paroxysmal** and **recurrent attacks of substernal or precordial chest discomfort** due to **transient myocardial ischemia**, which falls short of inducing necrosis of myocardial cell.

Angina pectoris: Most common manifestation of coronary artery disease (CAD).

Variants of Angina Pectoris

Stable Angina

It is the **most common** and is also known as **typical angina pectoris**.

- **Cause: Coronary atherosclerosis** is the cause and it develops when myocardial oxygen demand increases with **increased physical activity or emotional excitement**.

Pain

- **Site: Chest pain in the substernal region**, which may radiate to the left arm, jaw, and epigastrium.
- **Duration:** It ranges from **1–15 minutes**.
- **Characteristics: Pain is relieved by rest** (which decreases demand) or **by sublingual nitroglycerin**, a strong vasodilator (which increases perfusion).

Prinzmetal Variant Angina

It is an **uncommon atypical form** of angina.

- **Cause:** Due to **spasm of coronary artery having atherosclerosis**. It is **not related to physical activity, heart rate, or blood pressure**.
- **Characteristics: Occurs at rest** and responds promptly to vasodilators.

Unstable or Crescendo Angina

BOX 16.5: Causes of right-sided heart failure.

- Cor pulmonale
- Right-sided valvular heart disease
- Right-sided disease of myocardium
- Pulmonary hypertension

- **Cause:** It is caused by the **disruption/rupture of an atherosclerotic plaque** complicated by **thrombosis/embolization/vasospasm/vasoconstriction**.
- **Characteristics: It is of prolonged duration and occurs with minimal physical activity or even at rest.**
- **Consequence: May progress to myocardial infarction** and is also referred to as **preinfarction angina**.

Angina pectoris: Three types namely:

- 1. Stable (typical)
- 2. Prinzmetal
- 3. Unstable (crescendo)

Stable angina: Most common type of angina pectoris, substernal chest pain induced by exercise.

Prinzmetal angina: Due to vasospasm of coronary arteries.

Unstable angina:

- Substernal chest pain at rest
- Multivessel disease

- Due to disrupted atheromatous plaque

MYOCARDIAL INFARCTION

Q. Classify myocardial infarction. Discuss etiopathogenesis of myocardial infarction. Add a note on enzyme changes in myocardial infarction.

Q. Write about the etiopathogenesis and pathology of myocardial infarction with diagnostic tests.

Q. Describe the morphology/sequential changes seen in myocardial infarction in time. Mention complications of myocardial infarction.

Definition: Myocardial infarction (MI) is a **coagulative type of necrosis of cardiac muscle** and is **due to prolonged severe ischemia**.

Risk Factors

- **Race:** Any race can be affected; whites are affected more than blacks. Indians are also having high-risk of IHD.
- **Age:** Its frequency **rises progressively with age** and peak is between 40 and 65 years of age. It can develop at younger age in patients with major risk factors of atherosclerosis (hyperlipidemia, hypertension, diabetes and cigarette smoking).
- **Sex:** Males have significantly higher risk than females mainly during the reproductive period. However, after menopause the risk is similar to that of males. The protective effect may be due to estrogen.
- **Other risk factors:** Refer under risk factors for atherosclerosis (*refer* pages 435-7).

Etiology

Q. Discuss etiopathogenesis of myocardial infarction.

Ischemic heart disease is caused by an imbalance between the demand and supply of oxygenated blood to myocardium **(Fig. 16.13)**.

1. Decreased/Impaired Coronary Blood Flow

Coronary arterial occlusion is the main cause of myocardial ischemia.

(a) Coronary Atherosclerosis

It narrows one or more of the epicardial coronary arteries → decreases the coronary blood flow in about 90% of cases. In these 90% of cases myocardial infarction is due to atherosclerotic narrowing of one or more coronary arteries. Hence, **IHD is often known as coronary artery disease** (CAD) or **coronary heart disease**. **Risk of IHD depends on:** (a) **extent and severity of** pre-existing (fixed) **atherosclerotic occlusion** and (b) **sudden/dynamic morphological changes** in the atheromatous plaque.

Extent and severity of pre-existing (Fixed) atherosclerotic occlusion

- **Number of coronaries affected/involved:** Atherosclerosis may affect **one**, **two or all three coronaries**.
- **Structure of plaque (Figs. 16.14A and B):** Plaques can be divided into stable and vulnerable (unstable) plaques.
  - **Stable plaques:** They have **dense collagenous and thick fibrous caps** with **minimal inflammation** and negligible underlying atheromatous **necrotic core**. These are **less likely to undergo rupture**.
  - **Vulnerable plaque (Figs. 16.14B):** They have **core with many foam cells** and **abundant extracellular lipid**. The **fibrous cap is thin** with few smooth muscle cells or groups of inflammatory cells and **increased inflammation**. These are **likely to undergo rupture**.

BOX 16.7: List of ischemic heart diseases.

1. Myocardial infarction (ischemia causes the necrosis of heart muscle)
2. Angina pectoris (ischemia is less severe to cause frank necrosis)
   - Stable angina
   - Prinzmetal variant angina
   - Unstable angina
3. Chronic IHD with heart failure
4. Sudden cardiac death due to fatal ventricular arrhythmia

Acute coronary syndrome is applied to any of the three catastrophic manifestations of IHD: (i) unstable angina, (ii) MI, and (iii) SCD.

BOX 16.6: Complications in advanced heart failure.

- Renal failure due to poor renal perfusion
- Electrolyte imbalances: Hypokalemia (due to treatment with potassium loosing diuretics) or hyperkalemia (due to drugs that promote renal absorption of potassium), hyponatremia (due to diuretics and inappropriate retention of water)
- Hepatic dysfunction (due to hepatic venous congestion and poor arterial perfusion)
- Thromboembolism (due to low cardiac output and immobility)
- Atrial or ventricular arrhythmias

- **Degree/extent of stenosis (obstruction) by atheromatous plaques:**
  - **Fixed obstruction less than 70%:** Asymptomatic.
  - **Fixed obstruction of 75% or more:** It results in **critical stenosis → precipitates ischemia by exercise** → produces symptom as chest pain—**stable angina**.
  - **Fixed obstruction of 90% and above:** It leads to **inadequate coronary blood flow even at rest-unstable angina**.
- **Rate of development of occlusion:**
  - **Slow progressive occlusion** allows time to **develop collateral circulation** to **prevent infarction**.
  - **Acute blockage—no time for collateral flow → infarction**.
- Factors which influences consequences of coronary atherosclerosis.
  - **Extent of inflammation in atherosclerosis:** Inflammation converts it to vulnerable atheromatous plaque.
  - **Thrombus** development **on the disrupted plaque**.

- **Coronary vasoconstriction**.

Sudden morphological changes in atheromatous plaque

It is known as **acute plaque change** (*refer* page 460) and is followed by **thrombosis**, produces **unstable angina**, **myocardial infarction and sudden cardiac death** in most of the patients. Hence, termed **acute coronary syndrome**.

(b) Other Causes of Coronary Artery Occlusion (Nonatheromatous Causes)

In about 10% of cases, MI occurs without atherosclerosis of the coronary vessels. It may be due to:

- **Coronary vasospasm without coronary atherosclerosis**
- **Coronary emboli:** The source of emboli is thrombi in left side of the heart. These include:
  - Left atrium in association with atrial fibrillation
  - Left-sided mural thrombus
  - Vegetations of infective endocarditis
  - Intracardiac prosthetic material
  - Paradoxical emboli: The emboli from the right side of the heart or the peripheral veins, which travel through a patent foramen ovale to the coronary arteries.
- **Diminished availability of blood or oxygen:**
  - Diminished blood volume or lowered systemic blood pressure (e.g., with hypotension or shock)
  - Hypoxemia.
  - Diminished oxygenation (e.g., due to pneumonia or CHF)
  - Diminished oxygen-carrying capacity (e.g., due to anemia or carbon monoxide poisoning).
- **Ischemia due to other causes:**
  - Vasculitis
  - Hematologic disorders like sickle cell disease
  - Amyloid deposition in vascular walls

- Vascular dissection

2. Increased Myocardial Demand

It occurs:

- With **myocardial hypertrophy**
- Increased heart rate (**tachycardia**), increased physical activity, emotional stress, etc.

Pathogenesis of MI

In **coronary arteries with pre-existing (fixed) atherosclerotic occlusion, inadequate coronary perfusion** may occur due to a **new superimposed thrombosis and/or coronary vasospasm**. In **majority of patients, MIs are caused by acute thrombosis within coronary arteries. Thrombus formation is due to acute plaque change** ***(*****Fig. 16.4)**.

Sequence of events in a typical case of MI **(Figs. 16.15Ato E)** is as follows:

1. **Acute plaque change: It is the sudden change/event occurring in an atheromatous plaque.** The initial event in pathogenesis of myocardial infarction is sudden change in the atheromatous plaques. These **acute plaque changes convert partially occlusive** atherosclerotic **plaque to produce sudden ischemia**. These changes are divided into three categories:
   a. **Rupture**, **fissuring** of plaque → exposes highly thrombogenic plaque constituents → sudden **thrombus formation → sudden occlusion of lumen**.

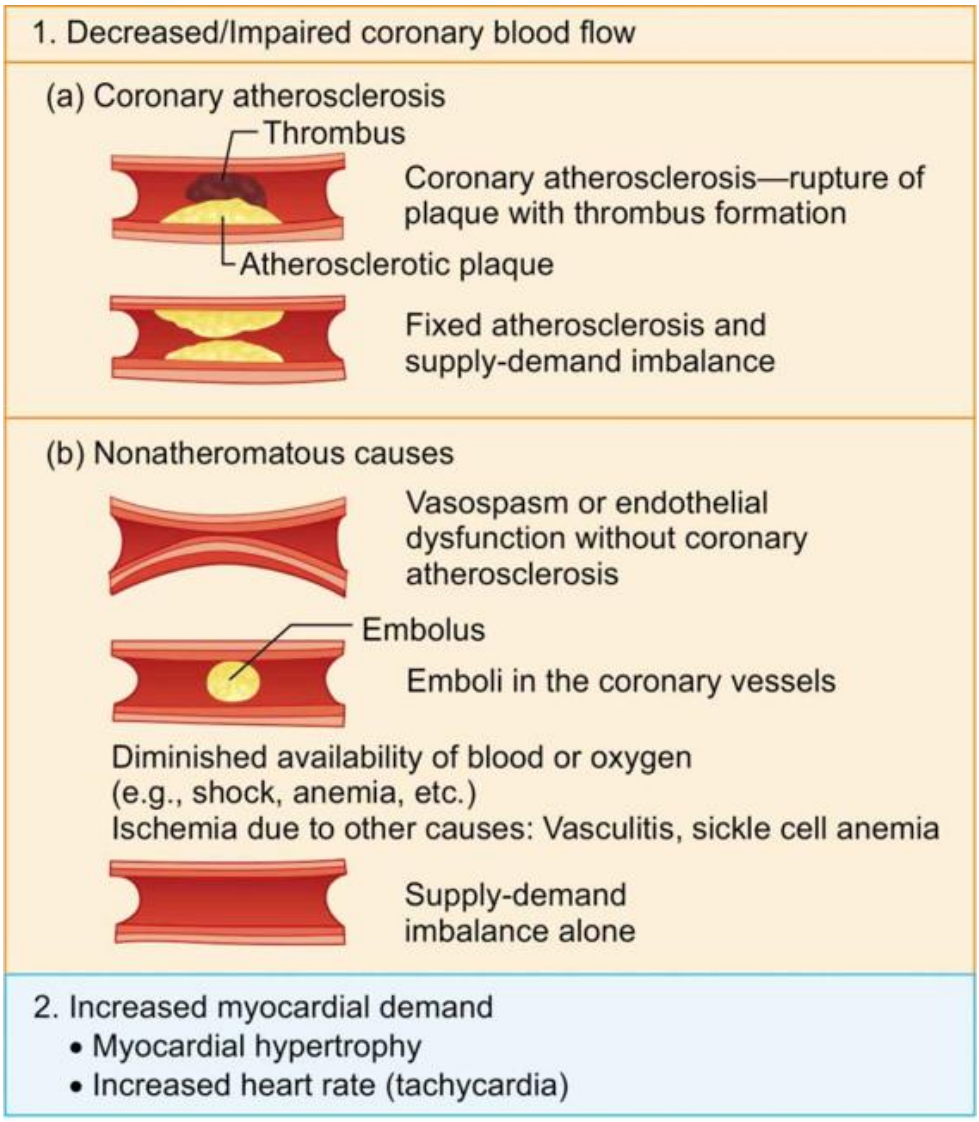
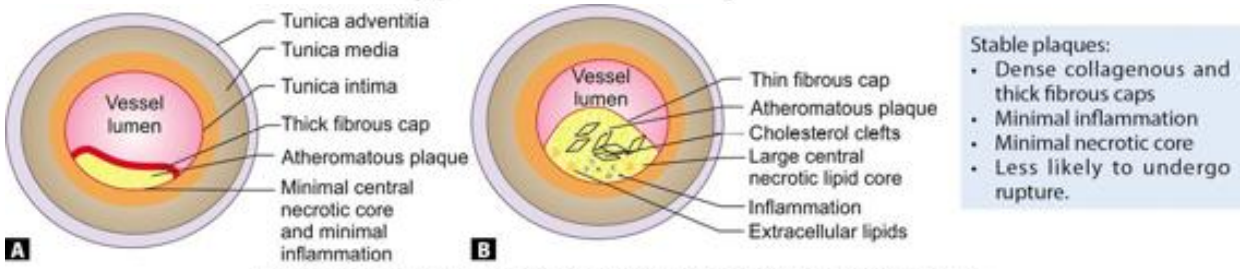


Fig. 16.13: Causes of imbalance between the demand and supply of oxygenated blood to myocardium.



Figs. 16.14A and B: Diagrammatic appearance of stable and vulnerable plaque. Cross-section of a vessel with (A) Stable atheromatous plaque; (B) Unstable atheromatous plaque.

b. **Erosion/ulceration of plaque**
   - Exposes highly thrombogenic subendothelial basement membrane
   - Sudden **thrombus formation**
   - **Sudden occlusion of lumen**.

c. **Hemorrhage into the central core of plaque** → increases the plaque size → sudden **occlusion of lumen**.

Factors that trigger acute plaque change:

- **Intrinsic:** Plaque composition and structure (namely **vulnerable plaque**).
- **Extrinsic:** Blood pressure and platelet reactivity which induces total thrombotic occlusion of already narrowed coronary artery by atheromatous plaque.

2. **Formation of microthrombi:** Acute plaque changes exposes thrombogenic subendothelial collagen → platelets adhere to the site → platelet activation and aggregation → formation of microthrombi on the atheromatous plaque → **partial or complete occlusion of the affected coronary artery**.
3. **Vasospasm: Activated platelets, endothelial cell and inflammatory cells release mediators** → cause **vasospasm** at the sites of atheroma → further narrowing of the lumen.
4. **Activation of the coagulation pathway:** Tissue factor released at the site of acute plaque change → activates coagulation system → **increase the size of the thrombus**.
5. **Complete occlusion of vessel:** Within minutes, the thrombus may completely occlude the lumen of the vessel.
6. **Myocardial necrosis:** Complete occlusion → results in **ischemic coagulative necrosis of the area supplied** by the particular coronary artery. The anatomic area supplied by that artery is called as the area at risk.

In about 10% of transmural infarction, no occlusive atherosclerotic vascular disease is found. Such infarcts are mostly due to coronary artery vasospasm or embolization from mural thrombi (e.g., in the setting of atrial fibrillation) or from valve vegetations.

Consequence of Myocardial Ischemia

These include functional, biochemical and morphological changes. Morphological changes can be divided into reversible and irreversible damage/injury.

A. Reversible Injury

These changes are potentially reversible and include:

- **Biochemical changes: Cessation of aerobic glycolysis** occurs within seconds of myocardial ischemia → **decreased production of ATP** (adenosine triphosphate) → **accumulation of** potentially **toxic metabolites** (such as lactic acid).
- **Functional disturbances: Loss of contractility** within 60 seconds → can precipitate acute heart failure.
- **Morphological changes:** They are seen at ultrastructural level such as **mitochondrial swelling, glycogen depletion and myofibrillar relaxation**. They also develop within a few minutes.

B. Irreversible Injury

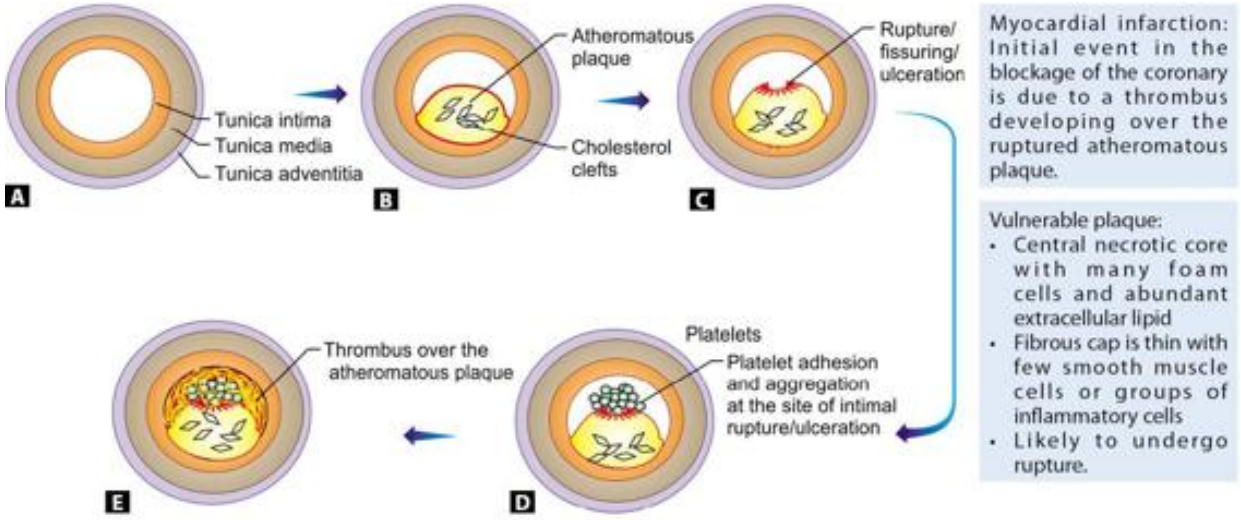
It develops only after prolonged, severe myocardial ischemia of more than 20–40 minutes.

- **Biochemical changes:** They cause **leakage of cytoplasmic proteins into the blood.** In the early phases of myocardial cell necrosis, there is breakdown of the sarcolemmal membrane → leakage of intracellular proteins (such as **myoglobin**, **LDH**, **CK**, **and troponins I and T**) into the blood. The levels of these leaked myocardial proteins in the blood is **used for the diagnosis as well as management of MI**.
- **Functional disturbances:** Arrhythmias.
- **Morphological changes: Coagulative necrosis** of cardiac muscle fibers usually complete within 6 hours of the onset of myocardial ischemia.

If blood flow is restored before irreversible injury develops, myocardium can be preserved. This is the purpose of early diagnosis and prompt intervention by thrombolysis or angioplasty.

Dominant Vessel

The **posterior third of the interventricular septum** and the **inferior/posterior left ventricle are perfused by the posterior descending artery**. The **origin of posterior descending artery may be either the RCA** (in 90%) **or the left circumflex (LCX).** The coronary artery [either RCA (right dominant) or LCX (left dominant)] which gives rise to the posterior descending artery is considered the dominant vessel.



Figs. 16.15A to E: Sequential changes in coronary artery atherosclerosis causing occlusion of lumen in ischemic heart disease: (A) Normal coronary artery; (B) Atherosclerosis of coronary artery; (C) Acute plaque change; (D) Platelet adhesion and aggregation at the site of plaque disruption; (E) Formation of occlusive thrombus.

- **Right dominant heart:** In this, occlusion of the RCA produces ischemia of posterior septal and posterior wall ischemic injury.
- **Left dominant heart:** In this, occlusion of the LCX generally produces ischemia of the left lateral wall as the posterior third of the septum, and the inferior and posterior wall of the left ventricle.

Collaterals in Heart

Three major coronary arteries are considered as end arteries. However, these epicardial vessels are interconnected by many intercoronary anastomoses (collateral circulation). These channels normally remains closed. When there is gradual narrowing of one artery, it permits blood to flow from high- to low-pressure areas through the collateral channels. This gradual dilatation of collateral can provide adequate perfusion to areas of the myocardium despite occlusion of an epicardial vessel.

Classification of Myocardial Infarct

Q. Classify myocardial infarction.

A. Depending on the thickness of myocardium involved:

- **Transmural infarct:**

- Ischemic necrosis of the **entire thickness of the ventricular wall**. However, a narrow rim (approximately 0.1 mm) of subendocardial myocardium is preserved due to diffusion of oxygen and nutrients from the ventricular lumen.
- Most myocardial infarcts are transmural.
- Usually **associated with chronic coronary atherosclerosis**, **acute plaque change**, **and superimposed thrombosis**.

- **Subendocardial (nontransmural) infarct (4 to 6 in Fig. 16.16):**
  - Ischemic necrosis of **inner one-third to one-half of the ventricular wall**.
  - Occurs due to **plaque disruption** followed by a **coronary thrombus, which undergoes lysis** or prolonged, severe reduction in systemic blood pressure. For example, shock superimposed on chronic, coronary stenosis.
- **Multifocal microinfarcts:** Develop with occlusion of small vessel (e.g., vasculitis, embolization) and may not show any changes in ECG.

Zones damaged: First necrosis in the subendocardial zone → later transmural infarct.

B. **Depending on the age of the infarct: Recent (fresh) or old (healed).**

C. **Depending on the anatomic region involved:** Anterior, posterior, lateral, septal and their combination like posterolateral.

D. **Depending on the electrocardiographic changes:**
- ST elevated myocardial infarct **(STEMI)** found in transmural infarct. ECG may have negative Q waves with loss of R wave amplitude.
- Non-ST elevated myocardial infarct **(NSTEMI)** found in subendocardial infarct and
- Electrocardiographically silent with nonspecific changes in **microinfarctions** (depending on the extent and location of the vascular involvement).

Patterns of Infarction (Fig. 16.16)

The location, size, and morphologic features of an acute myocardial infarct depend on factors such as: (i) size and distribution of the involved coronary

artery, (ii) rate of development and duration of the coronary occlusion, (iii) metabolic demands of the affected myocardium (e.g., blood pressure and heart rate) and (iv) extent of collateral blood supply.
Differences between transmural and subendocardial infarct are listed in **Table 16.4**.

MORPHOLOGY

Q. Describe the sequential changes seen in myocardial infarction.

- General features:
- Most of transmural infarcts (>50%) involve at least a portion of the left ventricle and/or interventricular septum.
- ~ 15–30% of MI, which involve posterior or posteroseptal wall also extend to involve right ventricle.
- Isolated infarction of the right ventricle and infarction of the atria are rare. In transmural infarcts, a narrow rim (about 0.1 mm) of viable subendocardial myocardium is preserved due to the diffusion of oxygen and nutrients from the lumen of the ventricles.
- **Pattern of left ventricular infarcts (Fig. 16.16):**
- Left anterior descending (LAD) coronary artery occlusion (40–50%): Infarcts involve (1 in Fig. 16.16):
  - Anterior wall of left ventricle near the apex
  - Anterior portion of ventricular septum
  - Apex circumferentially.
- Left circumflex coronary artery occlusion (15–20%): Infarcts involve (2 in Fig. 16.16) the lateral wall of left ventricle except at the apex.
- Right coronary artery occlusion (30–40%) (or its posterior descending branch): Infarcts involves (3 in Fig. 16.16):
  - Region of the inferior/posterior wall of left ventricle
  - Posterior portion of interventricular septum (inferior infarct)
  - Inferior/posterior right ventricular free wall (in some).

The sequences of the morphologic changes in acute MI and subsequent healing are summarized in **Table 16.5**.

Gross (Table 16.5)

- Within first 12 hours: No identifiable/apparent gross changes are seen. Triphenyl tetrazolium chloride (a histochemical stain) can grossly identify infarct within 2–3 hours after onset.
  - Noninfarcted myocardium appears brick-red (lactate dehydrogenase activity is preserved).
  - Infarcted area remains unstained pale (loss of dehydrogenases).
  - Old infarcts appear white and glistening.
- **By 12–24 hours (Fig. 16.17):** Grossly identifiable. Appears pale reddish-blue area (due to stagnated, trapped blood) → progressively becomes sharply defined, yellow-tan, and soft.
- After 3–5 days: Mottled with a central pale, yellowish, necrotic region with well-demarcated border of hyperemic zone (due to granulation tissue).
- By 10 days to 2 weeks: Appears soft and rimmed by a hyperemic zone of highly vascularized granulation tissue.
- After 2 weeks: Older, healed infarcts appear firm, pale gray and contracted → develops into a fibrous scar.

Microscopy (Figs. 16.18A to F and Table 16.5)

Q. Write a short note on microscopic assessment of age of myocardial infarction.

- First 6 hours: Earliest changes can be detected only by electron microscopy (ultrastructural changes)
  - Reversible injury (0–1½ hours): Relaxation of myofibrils, loss of glycogen, and mitochondrial swelling.
  - Irreversible injury (½–6 hours): Develops after 30–60 minutes of ischemia. The changes include mitochondrial amorphous matrix densities, focal disruption of sarcolemma and clumping and margination of nuclear chromatin.
- 6–12 hours: Coagulative necrosis begins and shows edema and hemorrhage. Other changes include:
  - Wavy fibers: They represent noncontractile, stretched, buckled dead myofibrils at the periphery of the infarct.
  - Vacuolization of myocardial cell (myocytolysis)**:** It is characterized by large vacuolar spaces within myocardial cell (probably water).
- **12–24 hours (Figs. 16.18B and F):** Characteristic changes of coagulation necrosis appear. These include: (1) preserved cell outlines of myocardial cell, (2) deeply eosinophilic cytoplasm and (3) pyknotic nuclei.
- 1–2 days:
  - Acute inflammatory reaction characterized by accumulation of polymorphonuclear leukocytes **(Fig. 16.18C)** at the periphery/borders of infarct.
  - Continuation of coagulation necrosis: Nuclei disappear, and striations become less prominent. Margins show contraction band necrosis.
- 3–7 days:

- Appearance of macrophages: The polymorphonuclear leukocytes are replaced by macrophages. Macrophages
- phagocytose and remove the necrotic myocardial cells and neutrophil fragments at the border of infarct.
- Granulation tissue: Appears at the periphery of infarct, and gradually extends toward the center.

- 1–2 weeks: Process of healing starts from its margins toward its center which is responsible for most advanced healing at the periphery.
  - Well-established highly vascularized granulation tissue, which consists of proliferating capillaries and fibroblasts.
  - Fibroblasts proliferate and collagen deposition proceeds.
- **2–8 weeks (Fig. 16.18D):** Increased collagen deposition and less cellularity.
- **>2 months (Fig. 16.18E):** Dense collagenous scar. In a completely healed infarct, it is not possible to determine its age.

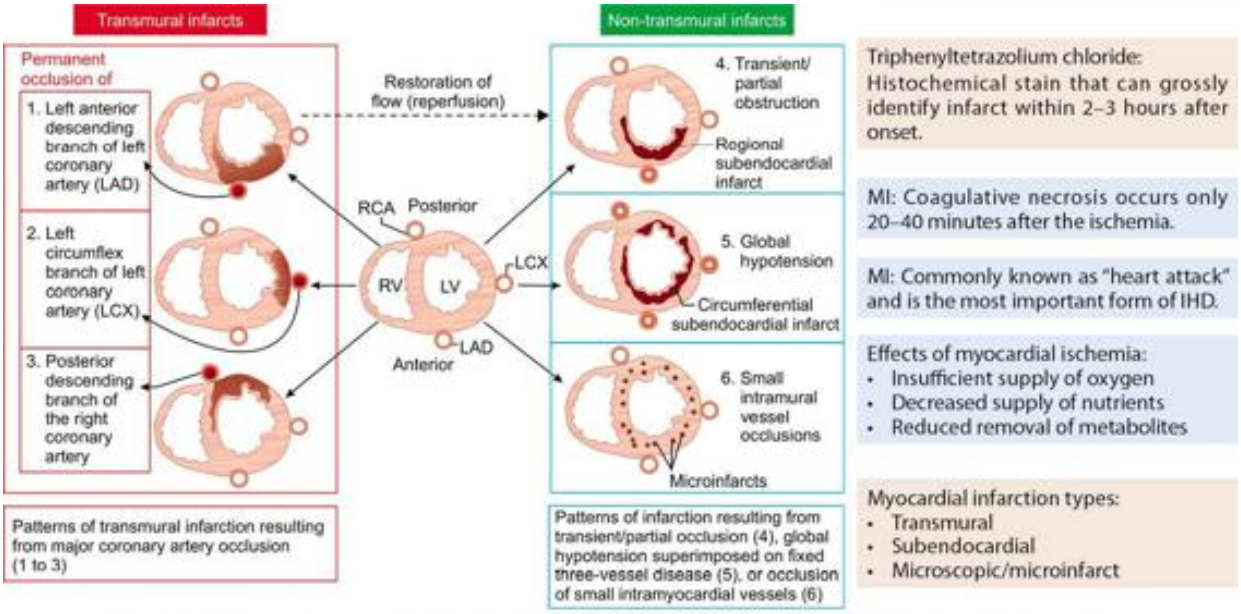


Fig. 16.16: Pattern of myocardial infarction depending on the location and nature of the diminished perfusion.

TABLE 16.4: Differences between transmural and subendocardial infarct.

Characteristics	***Transmural infarcts***	***Subendocardial infarcts***
Nature of lesion	Unifocal and solid	Multifocal and patchy
Distribution	Specific coronary artery	Circumferential
Thrombus in the coronary artery	Common	Rare
Shock	Often causes shock	Often result from hypotension or shock

TABLE 16.4: Differences between transmural and subendocardial infarct.

Characteristics	*Transmural infarcts*	*Subendocardial infarcts*
Pericarditis	Common	Absent
Cardiac aneurysm	May develop	Does not develop
Eelectrocardiography changes	Elevation of ST segment → ST elevation myocardial infarcts (STEMIs)	Non-ST elevation myocardial infarcts

Reperfusion and its Effects on MI

The ischemic myocardium can be rescued by restoring the myocardial blood flow as quickly as possible → by reperfusion (For details of reperfusion injury *refer* pages 20-1).

- **Methods of reperfusion:** It may be achieved by dissolving the thrombus using thrombolytic drugs (plasminogen activator) or angioplasty/stent placement, or coronary artery bypass graft (CABG) surgery.
- **Benefits and outcome of reperfusion:** It depends on rapidity of restoration. Reperfusion within 20 minutes of the onset of ischemia may completely prevent necrosis.

MORPHOLOGY OF REPERFUSED INFARCT

Gross

Reperfused infarcted area is hemorrhagic because the microvasculature is injured during ischemia and leaks with restoration of blood flow.

Microscopy

- Accelerates acute inflammatory responses: Neutrophils accumulate more rapidly but also disappear more rapidly.
- Contraction band necrosis: It is one of the characteristic feature. They appear as thick, irregular, transverse, intensely eosinophilic intracellular bands in necrotic myocardial cells.

Complication of Reperfusion

Q. Describe the morphological changes occurring in myocardial infarction.

TABLE 16.5: Sequence of morphologic changes in myocardial infarction.

Duration	*Gross changes*	*Light microscopic changes*
Reversible injury		
0–½ hour	None	None (electron microscope—relaxation of myofibrils; loss of glycogen and swelling of mitochondria)
Irreversible injury		
½–6 hours	None	Usually no changes but may show waviness of fibers at border (electron microscopy: mitochondrial amorphous densities)
6–12 hours	Dark mottling	Early coagulative necrosis
12–24 hours	Dark mottling	Coagulative necrosis; pyknotic nuclei; increased eosinophilia of cytoplasm; contraction band necrosis at margins; beginning of neutrophilic infiltrate
1–3 days	Mottling with yellow-tan center	Coagulation necrosis, loss of nuclei and striations; increased interstitial infiltration of neutrophils
3–7 days	Hyperemic border; central yellow-tan	Disintegration of dead myocardial cells, with disintegrating neutrophils; phagocytosis of dead cells by macrophages at the border of infarct
7–10 days	Maximally yellow-tan and soft	Phagocytosis of dead cells; formation of granulation tissue at margins
10–14 days	Red-gray depressed infarct borders	Well-established granulation tissue and collagen deposition
2–8 weeks	Gray-white scar, progressive from border toward the center of the infarct	Increased collagen deposition and decreased cellularity
>2 months	Scarring complete	Dense collagenous scar tissue

Reperfusion injury is mediated by **reactive oxygen species**, **calcium overload**, and **severe inflammatory reaction during reperfusion** (*refer* page 21).

Diagnosis of Myocardial Infraction

It is based on (1) clinical symptoms, (2) electrocardiographic changes and (3) laboratory findings.

Clinical Symptoms

- **Severe, retrosternal chest pain** that can radiate to neck, jaw, epigastrium or left arm.
- Rapid, weak pulse.
- **Profuse sweating (diaphoresis)**.
- Dyspnea due to impaired contraction of the ischemic myocardium and the resultant pulmonary edema.
- About 10–15% of cases it may be entirely asymptomatic ("silent" MIs) common in elderly and in patients with diabetes mellitus.

CC: Interpret abnormalities in cardiac function testing in acute coronary syndromes.

Electrocardiographic Changes

ECG changes in myocardial infarction **(Figs. 16.19A to C):** Inverted T waves, elevated ST segments, and Q waves.

Laboratory Findings

Q. Laboratory diagnosis of myocardial infarction.

Q. Write a short note on enzyme/biochemical markers for myocardial infarction.

These findings are mainly, due to **leakage of proteins from the necrotic myocardial cells into the blood circulation:**

1. **Cardiac troponins:** These are proteins involved in heart muscle contraction. **Increased plasma levels establish the diagnosis of myocardial infarction.**
   - **Cardiac-specific proteins are of two types: Troponins I (TnI) and T (TnT)**. They are **most sensitive and specific markers** of myocardial infarction. Levels begin to **rise at 2–4 hours** and **peaks at 48 hours**. The elevated troponin levels may **remain for 7–10 days** after acute MI.

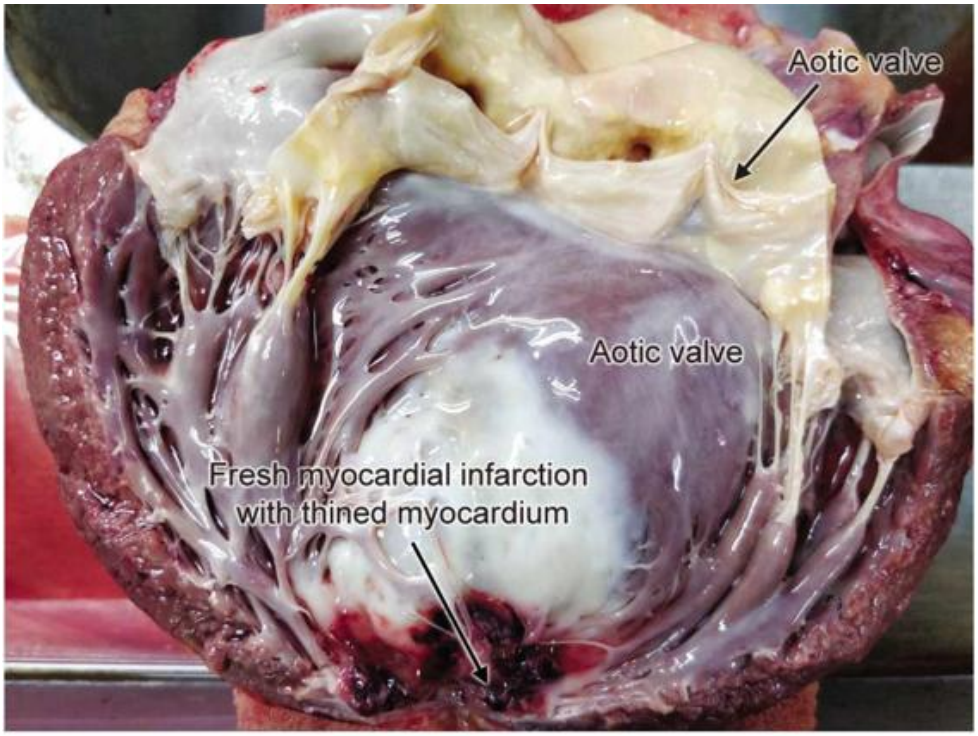
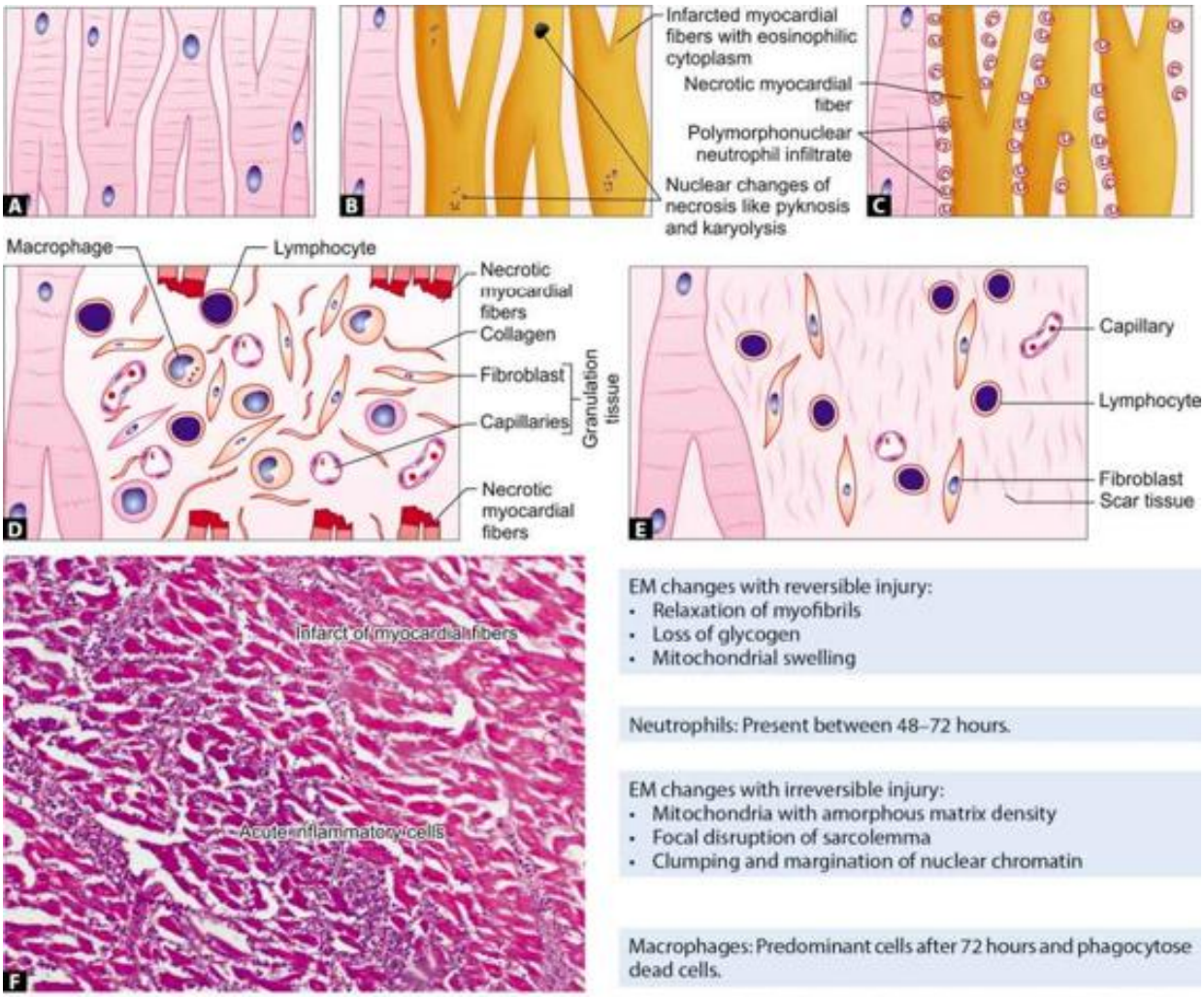


Fig. 16.17: Myocardial infarct at the apex of heart. The lumen of ventricle contains a blood clot.



Figs. 16.18A to F: Microscopic changes observed during development of a myocardial infarct: (A) Normal myocardium; (B) After about 12–18 hours, the infarcted myocardium shows myocardial fibers with eosinophilia; (C) About 24 hours after the onset of infarction, polymorphonuclear neutrophils infiltrate at the periphery of infarcted area; (D) After about 3 weeks, the infarcted area consists of granulation tissue infiltrated by lymphocytes and macrophages; (E) After 3 months or more, the infarcted region is replaced by collagenous tissue; (F) Photomicrograph of acute myocardial infarction showing coagulative necrosis with acute inflammatory infiltrate.

2. **Cardiac creatine phosphokinase (CK):** It is a **nonspecific enzyme marker** and it is present in brain, myocardium, and skeletal muscle. It has two isoforms designated "M" and "B". **MB heterodimers chiefly in cardiac** muscle (lesser amounts in skeletal muscle).
   - **MB form of creatine kinase (CK-MB)** is **sensitive but not specific**, because it is also raised with skeletal muscle injury.
   - CK-MB levels **rise within 2–4 hours** of the onset of MI, **peaks at 24 hours**, and returns to **normal within 72 hours**.
3. **Lactate dehydrogenase (LDH): It is not specific marker**. It **starts rising** after **24–48 hours**. It remains **for many days** and **returns to normal in 7–14 days**.

4. **Myoglobin:** It is an oxygen-carrying respiratory protein found only in skeletal and cardiac muscle. It is an **earliest marker of MI**, the level rises within 1–3 hours, **peaks in about 8–12 hours** and **returns to normal in about 24–36 hours**.

Various laboratory markers of MI are presented in **Table 16.6** and **Figure 16.20**.

In addition to clinical symptoms, ECG, and laboratory findings (mentioned above) other diagnostic tests may be useful evaluate cardiac function in acute coronary syndromes.

Stress Testing

Exercise ECG: An exercise tolerance test (ETT) is usually done by using a standard treadmill or bicycle ergometer protocol (recording of ECG before, during and after exercise). During this process, patient's ECG, BP and general condition are monitored. Exercise testing is useful means of assessing the severity of coronary disease and identifying high-risk individuals (i.e., post-infarct angina, poor effort tolerance, ischemia at low workload, left main or three-vessel disease, poor LV function).

Other Forms of Stress Testing

Myocardial perfusion scanning: It is performed using radioactive isotopes. It may be helpful in the (1) evaluation of patients with an equivocal or uninterpretable exercise test and (2) patients who cannot perform exercise. The scintiscans of the myocardium are obtained at rest and during stress (either exercise testing or pharmacological stress, such as a controlled infusion of dipyridamole, adenosine or dobutamine), after the administration of an intravenous radioactive isotope, such as thallium (201 thallium) or technetium (99 technetium sestamibi). The radioactive isotopes are taken up by viable perfused myocardium. If there is a perfusion defect during stress but not at rest, it indicates evidence of reversible myocardial ischemia, whereas a persistent perfusion defect seen during both phases is usually indicative of previous MI.

Stress echocardiography: It is an alternative to myocardial perfusion scanning and has similar predictive accuracy. It uses transthoracic echocardiography to identify ischemic segments of myocardium and areas of

infarction. The ischemic segments show reversible defects in contractility during exercise or pharmacological stress, whereas infarcted regions do not contract at rest or during stress.

Coronary Arteriography

It gives detailed anatomical information about the extent and nature of coronary artery disease. It usually done with a view to coronary artery bypass graft (CABG) surgery or percutaneous coronary intervention (PCI). It is done under local anesthesia and requires specialized radiological equipment, cardiac monitoring and an experienced team.

Newer Modalities

These include multiple-slice spiral computed tomographic coronary angiography (CTCA), intravascular ultrasound**,** magnetic resonance coronary angiography (MRCA) and positron emission tomography (myocardial viability is assessed using glucose metabolism).

Complications of Myocardial Infarction

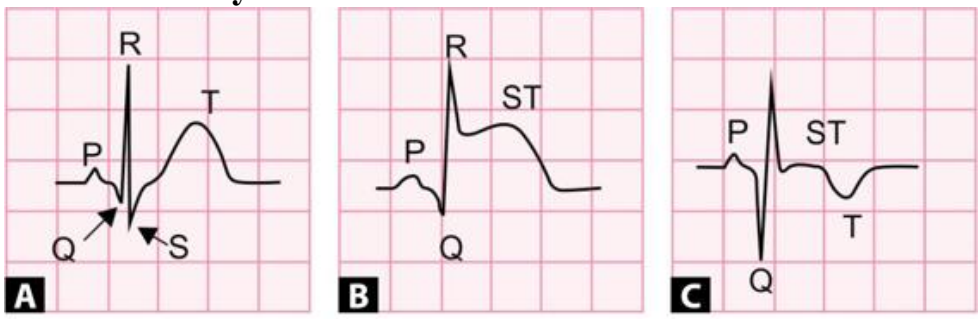
Q. Mention the complications of myocardial infarction.

The risk of complications depends on: (1) infarct size, (2) location, and (3) thickness of myocardium involved (subendocardial or transmural).

1. **Left ventricular failure and cardiogenic shock:** MI produces contractile functional abnormalities of left ventricle and is roughly proportional to the size of infarct.
   - **Mild dysfunction:** → left ventricular failure → **pulmonary edema**.
   - **Cardiogenic shock:** Severe "pump failure" occurs with a **large infarct** (>40% of the left ventricle) → cardiogenic shock → **fatal**.
2. **Arrhythmias:** Almost most MI patients develop **abnormal cardiac rhythm** → myocardial irritability and/or conduction disturbances → arrhythmias. These include **sinus bradycardia**, **tachycardia**,

ventricular tachycardia, **and ventricular fibrillation**. In inferoseptal infarcts → **partial or complete heart block**.

3. **Myocardial rupture:** Necrosis and neutrophilic infiltration → causes softening and weakening of myocardium → lead to cardiac rupture. **Most frequent during 3–7 days after transmural infarcts**.
   - **Rupture of the ventricular free wall (Fig. 16.21A):** It is most common → result in **hemopericardium and cardiac tamponade.**
   - **Rupture of the ventricular septum (Fig. 16.21B):** It is less common → lead to an **acute VSD and left-to-right shunt**.
   - **Rupture of papillary muscle (Fig. 16.21C):** It is least common → leads to acute severe mitral regurgitation.
4. **Dilatation of ventricular chamber:** Area of infarct being weak region may be disproportionately stretched → dilation of the infarct region (especially with anteroseptal infarcts) not severe enough to cause aneurysm described below.
5. **Ventricular aneurysm (Figs. 16.21D and 16.22A):** After acute transmural infarction, the affected ventricular wall may bulge outward during systole resulting in ventricular aneurysm. It develops as a late complication of large transmural infarcts.
6. **Mural thrombus (Figs. 16.20E and 16.21B):** Infarct → causes local abnormality in myocardial contractility (causing stasis) and endocardial damage (creating a thrombogenic surface) → favor **mural thrombosis** → left-sided **thromboembolism**. They may also develop **within ventricular aneurysms**.



Figs. 16.19A to C: ECG changes in myocardial infarction: (A) Normal ECG; (B) ST elevation; (C) T wave inversion and deep T wave.

TABLE 16.6: Laboratory markers of myocardial infarction.

Marker	*Onset*	*Peak*	*Normalization*	*Significance*

TABLE 16.6: Laboratory markers of myocardial infarction.

Marker	*Onset*	*Peak*	*Normalization*	*Significance*
Troponins T & I	2–4 hours	48 hours	7–10 days	Most sensitive and specific marker
CK-MB	2–4 hours	24 hours	72 hours	Sensitive but not specific
LDH	24–48 hours	Many days	7–14 days	Not specific marker
Myoglobin	1–3 hours	8–12 hours	24–36 hours	Earliest marker

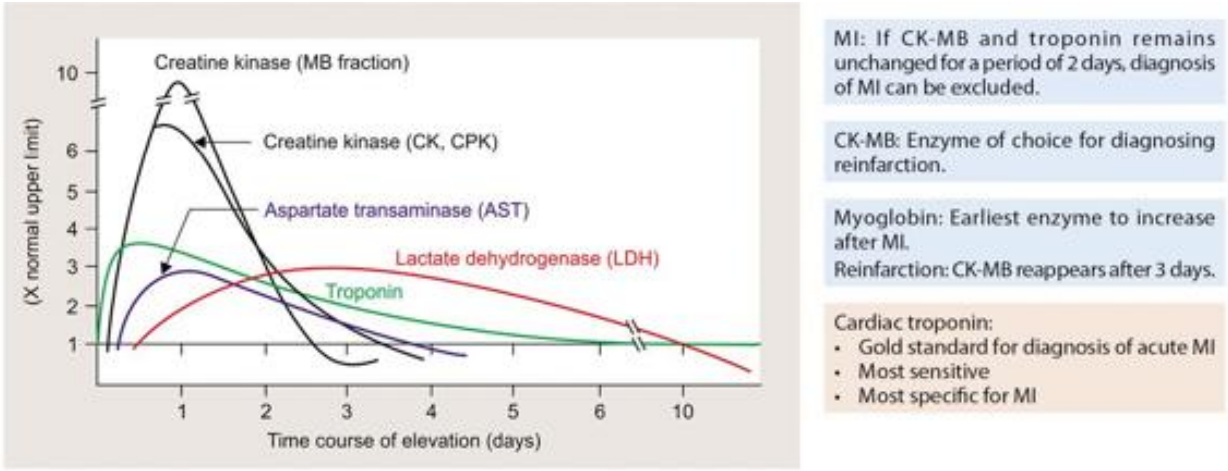


Fig. 16.20: Various enzyme levels in myocardial infarction.

7. **Pericarditis:**
   - **Early pericarditis:** A transmural myocardial infarct → can involve the pericardium → cause **fibrinous or fibrinohemorrhagic** pericarditis **(Fig. 16.21F)**. Usually develops on **second or third day**.
   - **Delayed form of pericarditis (postmyocardial infarction syndrome**/Dressler syndrome): Develops 2 to 10 weeks after infarction—**probably immunologically mediated**.
8. **Extension of infarct (Fig. 16.21G):**
   - New areas of repeated necrosis can occur adjacent to an existing myocardial infarct causing extension of infarct.
   - In extended infarct, healing is more advanced in the central zone than the periphery of the infarct. This is in contrast with that simple infarct, in which the healing is more advanced at the periphery.
   - **Cause of extension:** It may be due to retrograde propagation of a thrombus or proximal vasospasm, microemboli, or an arrhythmia.
9. **Infarction of right ventricle:** It is **unusual**. However, part of right ventricular myocardium may be involved with infarction of adjacent

posterior left ventricle and ventricular septum → venous circulation pooling → systemic hypotension.

10. **Progressive late heart failure** (chronic IHD): Usually develops due to the functional decompensation of hypertrophied noninfarcted myocardium.

Complications of myocardial infarction depending on the duration are listed in **Table 16.7**.

Q. A 56-year-old diabetic, hypertensive male was rushed to the hospital following the sudden onset of an episode of crushing substernal/central chest pain of 4 hours duration that radiated to the left arm. It was accompanied by profuse sweating. Serial measurements showed elevated CK-MB levels. He receives advanced life support measures. His course was marked by intractable cardiogenic shock and he died 4 days later.

(a) What is your probable diagnosis? (Answer: Myocardial infarction)

(b) What are the laboratory investigations that will help in arriving at the diagnosis? (refer page 465).

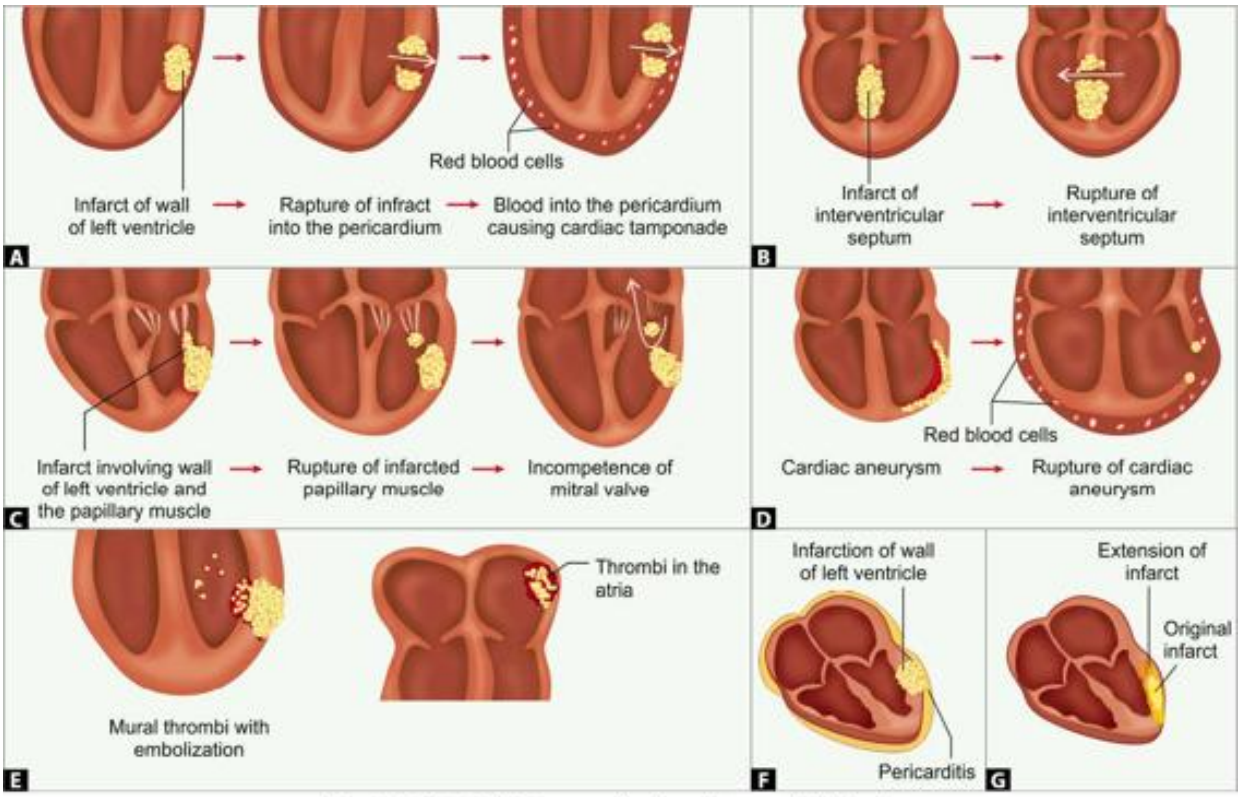
(c) What are the microscopic findings expected in the heart? (refer pages 463-4).

(d) What are the complications of this condition? (refer pages 466-7).

Chronic Ischemic Heart Disease

Progressive heart failure developing as a consequence of ischemic damage myocardium is termed as chronic IHD.

Sudden Cardiac Death



Figs. 16.21A to G: Various complications of myocardial infraction.

Definition: Sudden cardiac death (SCD) is an unexpected death from cardiac causes in individuals without symptomatic heart disease or within 1 hour of the onset of symptoms.

Cause: Usually due to fatal arrhythmia (e.g., asystole, ventricular fibrillation) occurring in IHD. **Acute myocardial ischemia is the most common cause for fatal arrhythmias.** Usually associated with coronary atherosclerosis.

IHD: Obstructive atheromatous plaques are predominantly seen within the first several centimeters of the LAD and LCX and along the entire length of the RCA.

Acute plaque change: Sudden morphological changes occurring in an atheromatous plaque. These include:

- Rupture and fissuring
- Erosion and ulceration
- Hemorrhage into central core of plaque

MI: Gross and microscopic changes develop only hours to days after the onset of ischemia.

MI: Characterized by coagulative necrosis within 24 hours.

Reperfusion injury is due to:
- Mitochondrial dysfunction—promotes apoptosis
- Myocyte hypercontracture—cytoskeletal damage and cell death
- Free radicals—damage to the myocytes
- Leukocyte aggregation—occlude the microvasculature
- Platelet and complement activation—microvascular injury

Diagnosis of MI:
- Clinical symptoms
- Electrocardiographic changes
- Measurement of serum CK-MB and cardiac troponins

MI: Ventricular fibrillation is the most common cause of death.

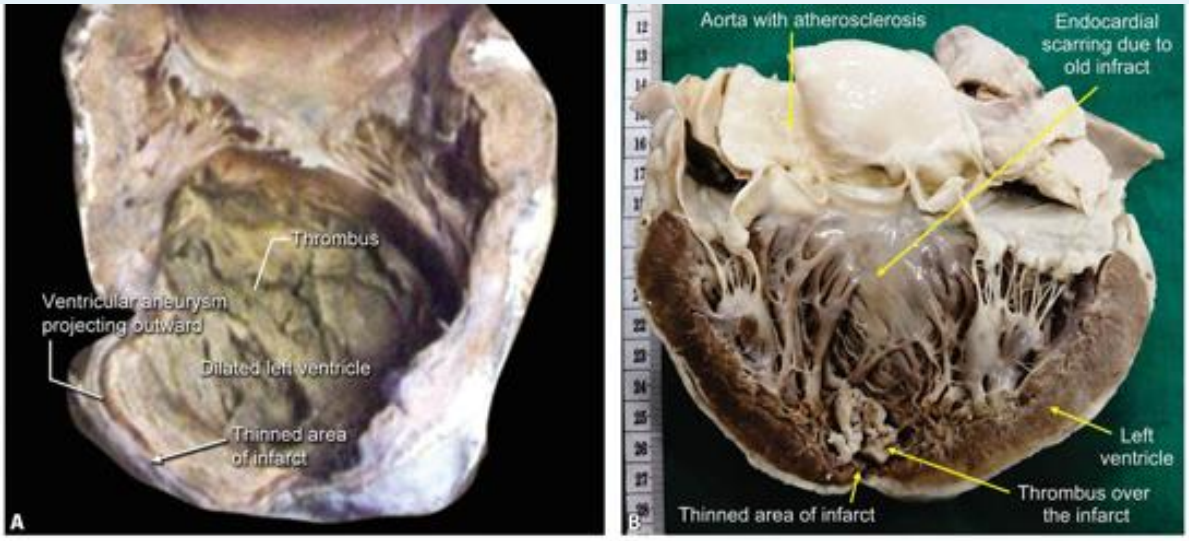
MI: Heart is maximally soft during 3–7 days; risk of myocardial rupture more during this period.

Posteromedial papillary muscle rupture:
- Due to thrombosis of RCA
- Causes mitral regurgitation

MI: Mural thrombus on the infarcted site is likely source of emboli.

Pericarditis in MI:
- Early—due to acute inflammation
- Late—autoimmune mechanism

MI is the leading cause of death and is common in diabetes mellitus.



Figs. 16.22A and B: (A) MI with ventricular aneurysm containing thrombus; (B) Myocardial infarct with a thrombus over the infarcted area.

TABLE 16.7: Complications of myocardial infarction depending on the duration.

Duration	***Complications***
First 72 hours	• Cardiogenic shock • Conductive disturbances—arrhythmias • Acute pulmonary edema (left ventricular failure) • Rupture external—hemopericardium (cardiac tamponade)
First 3 weeks	• Any of the above • Pericardial effusion • Rupture: (1) myocardial, (2) papillary muscle, (3) chordae tendineae and (4) interventricular septum • Thromboembolism
Late complications	• Repeat infarction • Cardiac aneurysm • Chronic ischemic heart disease (ischemic cardiomyopathy) • Congestive heart failure • Pulmonary hypertension • Autoimmunity—delayed form of pericarditis

Complications of MI:
- Left ventricular failure and cardiogenic shock
- Arrhythmias
- Myocardial rupture
- Dilatation of ventricular chamber
- Ventricular aneurysm
- Mural thrombus
- Pericarditis
- Extension of infarct
- Infarction of right ventricle
- Progressive late heart failure

Sudden cardiac death: Unexpected death occurring within one hour after the symptoms. Most commonly due to ventricular fibrillation.

INFECTIVE ENDOCARDITIS

CC: Describe the etiology, pathophysiology, pathology, gross and microscopic features, diagnosis and complications of infective endocarditis.

Q. Describe the etiology, pathogenesis, morphology and complications of infective endocarditis.

Definition: Infective endocarditis (IE) is defined as an **infection**, usually bacterial, **of the endocardium of heart valves or the mural endocardium**. It results in focal area of colonization or invasion by microbial agents.

Salient Features

Sites of Colonization by Microbial Agents

- **Heart valves**
- Mural endocardium
- Congenital defect (e.g., patent ductus arteriosus or coarctation of the aorta)
- Other less common sites: (i) aorta, (ii) aneurysmal sacs, (iii) other blood vessels, and (iv) prosthetic devices.

Lesions Produced

- **Vegetations:** These are lesions seen at the site of colonization or invasion. Composed of thrombotic debris and organisms.
- **Destruction** of the underlying cardiac tissues/site of colonization is often observed.

Microbes Responsible

- **Bacterial infections** (bacterial endocarditis) in most of the cases.
- **Other microbes:** Fungi, *Chlamydia*, *Rickettsiae* and other microorganisms.

Classification

A. According to the Clinical Course (Table 16.8)

It is divided into **acute or subacute** endocarditis. The clinical course depends on the virulence of the infecting microorganism and whether any underlying cardiac disease is present. Differences between acute and subacute endocarditis are listed in **Table 16.8**.

Acute Infective Endocarditis

The characteristic features are:

- **Acute** in onset
- Infection of **normal heart/cardiac valve**
- **Caused by highly virulent (suppurative) organism**. Mainly by ***Staphylococcus aureus*** and ***S. pyogenes.***
- **Affected valve is rapidly destroyed** by necrotizing, ulcerative valvular lesions.
- **Death within 6 weeks in many patients**, due to complications like acute heart failure or overwhelming sepsis.

Subacute Infective Endocarditis

The characteristic features are:

- **Insidious** in onset.
- It **involves structurally abnormal/deformed valves** (e.g., rheumatic heart disease or congenital heart disease).
- **Caused by organisms of lower virulence** (e.g., ***Streptococcus viridans*** **or** ***Staphylococcus epidermidis*****).**
- The lesions are **less destructive.**
- Pursue a **protracted course of weeks to months** and typically survive for 6 months or more. Mostly cured with antibiotics.
- Infectious **complications are uncommon**.

B. According to Anatomic Location and Causative Microbes

The clinical presentations described above are unusual today because of advance in antimicrobial therapy. So, IE is now classified according to the anatomical location and the offending organism, e.g., infective endocarditis of mitral valve due to *Staphylococcus aureus*.

Etiology

Q. Describe the etiology and pathogenesis of infective endocarditis.

Causative Organism

Many organisms can cause endocarditis, most originate from the normal flora of the body surfaces, and enter into the bloodstream in a variety of different ways. More than 90% of IE is caused by streptococci and staphylococci.

- ***Staphylococcus aureus:*** It is **virulent organisms** commonly found on the skin. It can infect either **healthy or deformed** valves. It is the major organism responsible for IE in **intravenous drug abusers**.
- ***Streptococcus viridans:*** It is present in the **normal flora of the oral cavity**. It most commonly causes endocarditis of previously **damaged** or **abnormal valves**.
- **Coagulase-negative staphylococci** (e.g., *S. epidermidis*): It is the most common cause for **prosthetic valve** endocarditis.
- **Other bacteria** commensals in the oral cavity:
  - **HACEK** group (*Haemophilus, Actinobacillus, Cardiobacterium, Eikenella*, and *Kingella*)
  - Enterococci.
- **Other agents:** It includes gram-negative bacilli and fungi.
- **Culture-negative endocarditis:** They constitutes 10–15% of cases in which no organism can be isolated from the blood.

Predisposing Factors

Three main factors which may predispose to IE are: (1) conditions that lead to bacteremia, (2) underlying heart disease, and (3) impaired host defense mechanism.

1. **Conditions with bacteremia:** Most important predisposing factors leading to the development of endocarditis are conditions that lead to bacteremia.

TABLE 16.8: Differences between acute and subacute endocarditis.

Characteristic	***Acute infective endocarditis***	***Subacute infective endocarditis***
Onset	Acute	Insidious
Condition of valve	Infection of normal heart/cardiac valve	Infection of structurally abnormal/deformed valves

TABLE 16.8: Differences between acute and subacute endocarditis.

Characteristic	*Acute infective endocarditis*	*Subacute infective endocarditis*
Virulence of organisms	Highly virulent (suppurative)	Low virulent
Lesions	Affected valve is rapidly destroyed	Less destructive
Clinical features	Clinical features of acute infection	Clinical features of complications
Course	Death within6 weeks	Protracted course of weeks
Complications	Acute heart failure or overwhelming sepsis	Infectious complications are uncommon

Sources of microbes: Transient bacteremia from any cause may lead to infective endocarditis.

2. **Underlying heart disease:** IE may involve either normal or damaged heart/valve. IE on previously normal valves develop usually with high virulent organisms. Underlying heart disease is one of the predisposing factors for IE. These diseases include:
   - **Congenital heart disease:** Unrepaired and repaired congenital defects —most common predisposing condition (e.g., valvular or septal defect).
   - **Mitral valve prolapse** (MVP) in adults.
   - **Chronic rheumatic heart disease** (mitral valve in over 85% of cases, and the aortic valve is involved in 50%).
   - **Degenerative calcific valvular diseases** (mainly in elderly).
   - Bicuspid aortic valve.
   - **Iatrogenic:**
     - Artificial (prosthetic) valves
     - Indwelling vascular catheters.
3. **Impaired host defense mechanism:** It may occur in:
   - Diabetes mellitus
   - Malignancies: Leukemia and lymphomas
   - Cytotoxic therapy
   - Neutropenia.

Prevention of IE in patients with predisposing risk factors is by prophylaxis with antibiotics.

Pathogenesis

Mechanism by which virulent organisms infect apparently normal valves is poorly understood.

The probable sequence of events **(Fig. 16.23)**, which occur with the infection of a damaged valve by less-virulent organisms is as follows:

1. **Endocardial damage and denudation:** Development of the infective endocarditis follows a predictable sequence. Endothelial damage is a common predisposing factor for the development of endocarditis. This may result from turbulent blood flow (e.g., as in valvular regurgitation), or from micro-abrasion (e.g., as in injection of a drug abuse). Damaged valve or a congenital defect creates abnormal high-velocity blood flow at the center and turbulent flow at the periphery → cause endocardial damage and denudation of endothelial surfaces.
2. **Formation of sterile thrombus:** Damaged endothelial surface exposes the extracellular matrix → attracts focal deposition of platelets and fibrin → aggregation of platelets and fibrin → formation of small sterile vegetations (sterile thrombi).
3. **Adherence of the microorganisms:** Transient bacteremia → microorganisms gain access to the circulation → adherence and deposition of microorganisms onto the sterile thrombi/vegetations (infection of thrombi).
4. **Proliferation of microorganisms within vegetations:** On the deposited microorganisms, further platelets and fibrin are laid down, which separates them from the bloodstream. Microorganisms proliferate within the vegetations and form colonies. Once infected, these vegetations enlarge and the microorganism are protected from host defense mechanisms.
5. **Formation of emboli:** The vegetation may get detached and form infective emboli → cause spread of infection to visceral organs and brain.

MORPHOLOGY

Q. Describe the morphology of infective endocarditis.

Q. Describe heart in infective endocarditis.

Gross (Figs. 16.24 and 16.25)

Valves Affected

Aortic and mitral valves (most common) and valves of the right heart (especially tricuspid) in intravenous drug abusers.

Q. Write a short note on morphology of vegetations in infective endocarditis.

Vegetations

These are hallmark/pathognomonic of IE which have following features:

- Appearance: Friable, bulky, potentially destructive vegetations (less destructive in subacute than in acute endocarditis).
- Number: They may be single or multiple and may involve one or more than one valve.
- Size: Lesions vary in size from a small, superficial deposit to bulky, exuberant vegetations.
- Sites: Vegetations form on:
  - Atrial side of the atrioventricular valves.
  - Ventricular side of the semilunar valves.
  - Points of closure of the leaflets or cusps.
- Changes in the involved valve: Edema and inflammation and in severe cases, leaflet may perforate causing regurgitation.

Microscopy

- Vegetations are composed of platelets, fibrin, cell debris, inflammatory cells, and masses of bacteria or other organisms.
- Vegetations of subacute IE may show granulation tissue at their bases indicative of healing. Later it may show fibrosis, calcification, and chronic inflammatory infiltrate.

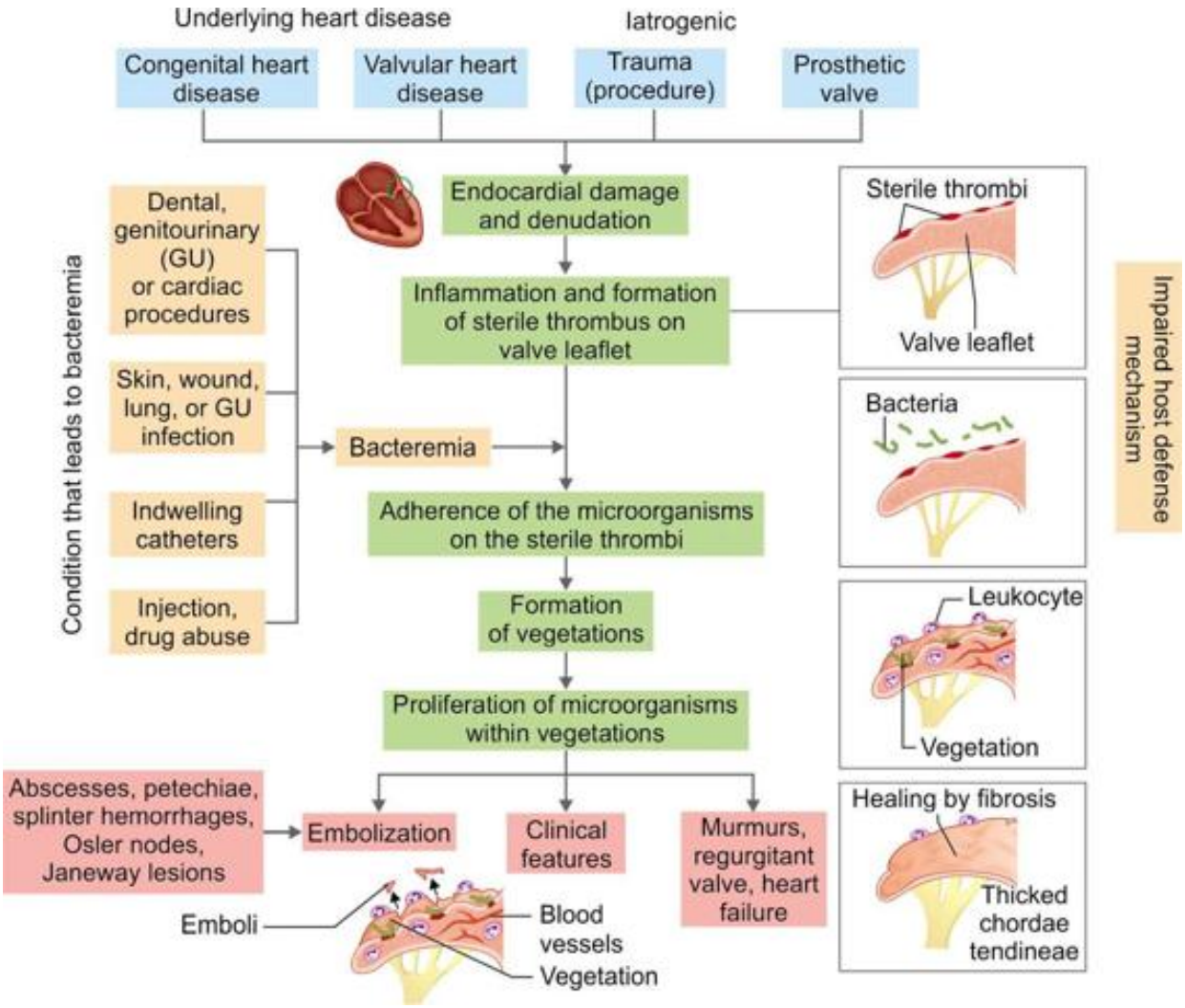
Characteristic features of four major types of vegetative endocarditis are presented in **Table 16.9**.

Complications of Infective Endocarditis

Q. Describe the complications of infective endocarditis.

These may be divided into cardiac and extracardiac.

Cardiac Complications



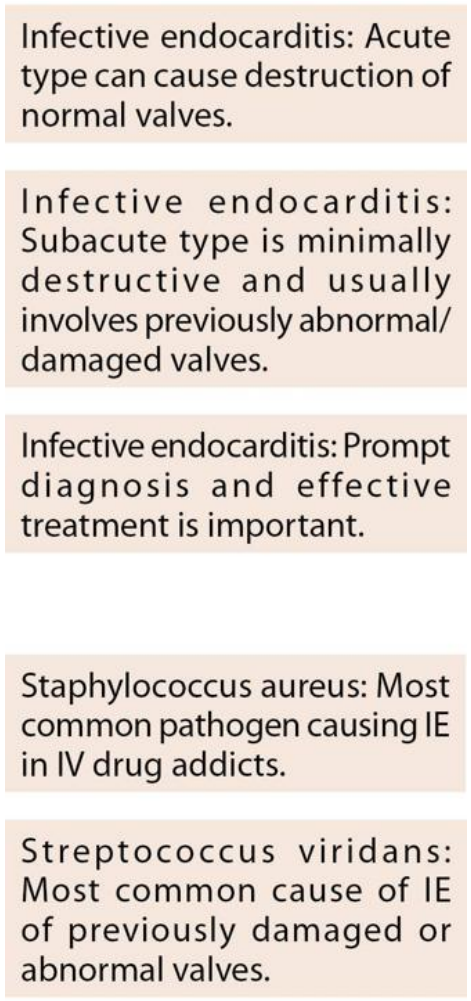


Fig. 16.23: Pathogenesis of vegetation on a valve leaflet in infective endocarditis (mainly subacute infective endocarditis).

These are **due to direct valvular damage** and **consequences of local invasion**. The infection may spread locally from valve into the valve ring, adjacent mural endocardium or chordae tendineae.

- **Ring abscess:** Vegetations may erode the **underlying myocardium** and produce an **abscess** which is known as ring abscess.
- **Perforation and rupture:** May involve valve leaflets, aorta or interventricular septa (depending on the site of infection).
- **Myocardial abscess.**
- **Suppurative pericarditis.**
- **Valvular dysfunction:** Stenosis or insufficiency.

Extracardiac Complications

Q. Extracardiac complications of subacute bacterial endocarditis.

Vegetations are usually friable and likely to be break, detach and cause embolism.

1. **Embolic complications (due to septic emboli):** Emboli contain large numbers of virulent organisms → **abscesses develop at the sites of arrest** of the emboli.
   - **Septic emboli from left side of the heart:** They enter systemic circulation and its consequences are:
     - **Septic infarcts (e.g., spleen, kidney or brain)**
     - **Mycotic aneurysms** (*refer* pages 124, 132 and 443)
     - **Small emboli (microthromboemboli) may produce tiny hemorrhagic lesions (Fig. 16.26A) namely: (1) splinter or subungual hemorrhages** and **(2) Janeway lesions** which are small erythematous or hemorrhagic nontender, macular, lesions **on the palms or soles**.
   - **Septic emboli from right side of the heart:** They enter pulmonary circulation → lead to **pulmonary abscess**.
2. **Immunological phenomena:** A hallmark of infective endocarditis is sustained high-grade bacteremia. This stimulates both the humoral and cellular immune systems.
   - **Focal segmental glomerulonephritis: Develop due to deposition of antigen-antibody complexes** in glomeruli. Grossly, the **outer surface of kidney** develops a **flea-bitten** appearance due to patchy hemorrhagic foci involving the glomeruli.
   - **Osler nodes (Fig. 16.26B):** They are small, **tender subcutaneous nodules in the pulp of the digits** and persist for hours to several days.
   - **Roth spots (Fig. 16.26C)** are **retinal hemorrhages** with white or pale centers composed of fibrin.

Clinical Features

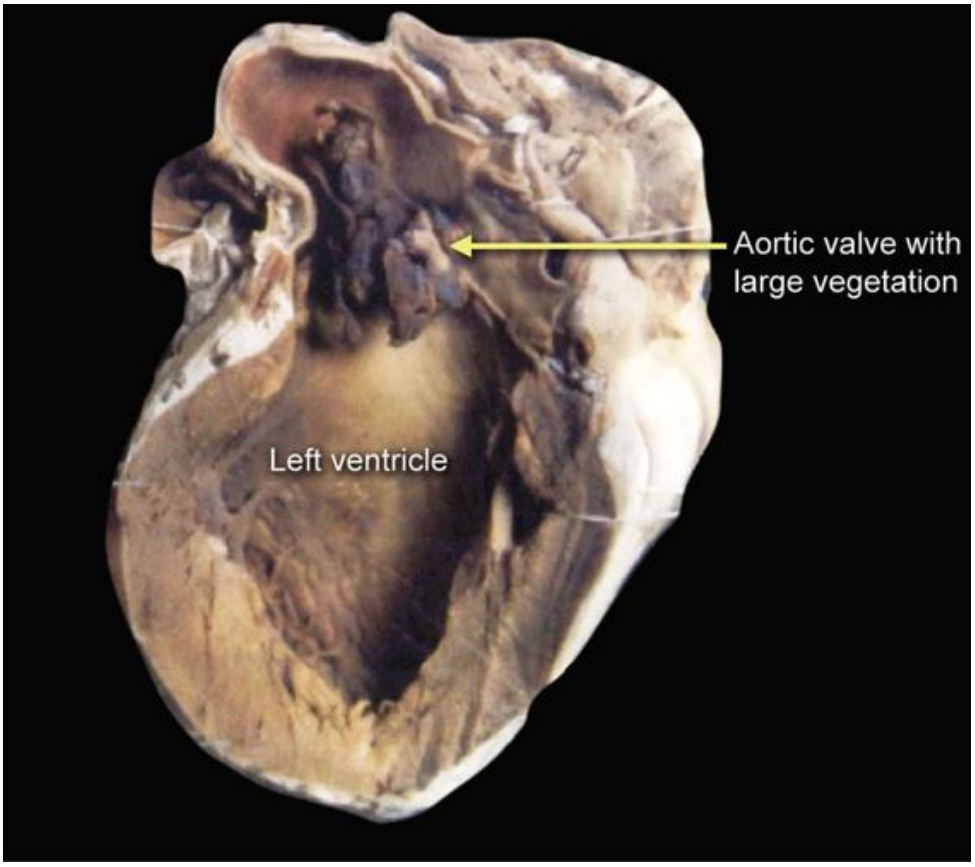


TABLE 16.9: Characteristic features of four major types of vegetative endocarditis.

Type of endocarditis	*Morphological feature*
1. Rheumatic endocarditis	Small, warty vegetations along the line of closure of valve leaflets
2. Infective endocarditis (IE)	Large, irregular on the valve cusps that can extend on to the chordae tendineae
3. Nonbacterial thrombotic endocarditis (NBTE)	Small, bland vegetations usually attached to the line of closure of valve leaflets
4. Libman-Sacks endocarditis in SLE	Small or medium-sized vegetations on either side of the valve leaflets

- Symptoms:
  - **Fever** is the most common feature of IE.
  - Acute IE develops rapidly presenting with fever, chills, weakness, and lassitude.
- Clinical examination:
  - Murmurs may be heard either due to new valvular defect or a pre-existing cardiac disease.

Diagnostic Criteria for InfectiveEndocarditis (Box 16.8)

Modified Duke criteria used for diagnosis of suspected infective endocarditis (IE). It requires either pathologic or clinical criteria. If clinical criteria are used, the criteria are as follows:

- Definite endocarditis, two major, or one major + three minor, or five minor criteria are required for diagnosis.
- Possible endocarditis = One major and one minor, or three minor criteria.

Earlier diagnosis and effective treatment prevents complications.

Prognosis: It depends on the infecting organism and whether or not complications develop.

Q. A 17-year-old male complaints of palpitation and joint pains for one year duration. Joint pain was migrating/fleeting type involving multiple major joints and was associated with redness and swelling. About 15 days back he developed low-grade fever, sometimes accompanied with chills, and dyspnea. He is not IV drug addict. On examination, there was mild pedal edema. On auscultation of heart, mid diastolic murmur was heard in the mitral region.

(a) What is the probable diagnosis? (Ans: Rheumatic heart disease and mitral stenosis with superimposed subacute infective endocarditis).

(b) Discuss the etiopathogenesis and the cardiac lesions of the condition (refer pages 470-1).

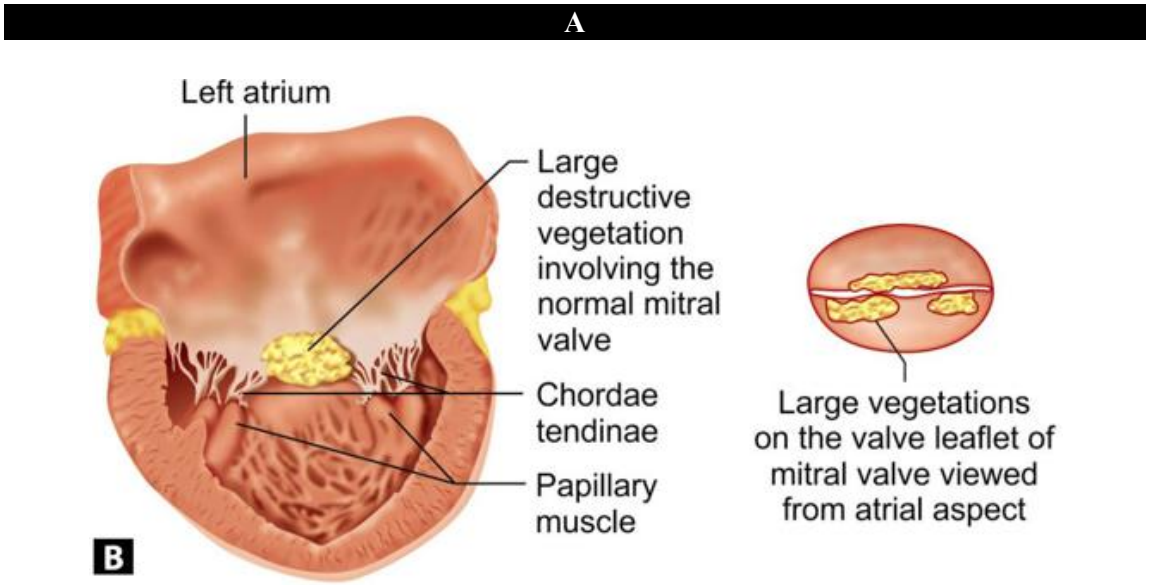
(c) List the complications (refer pages 472-3).

Infective endocarditis: Conditions which can produce transient bacteremia include:

- Obvious infection elsewhere

- Dental or surgical procedure
- Urinary catheterization
- Obstetric procedures
- Contaminated needle shared by intravenous drug users
- Trivial breaks in the epithelial barriers of the gut, oral cavity, or skin

Underlying heart disease predisposing to IE:
- Congenital heart disease
- Chronic rheumatic heart disease
- Mitral valve prolapse



Figs. 16.24A and B: Acute bacterial endocarditis. (A) Aortic valve shows large friable vegetations; (B) Mitral valve shows large friable vegetations (diagrammatic).

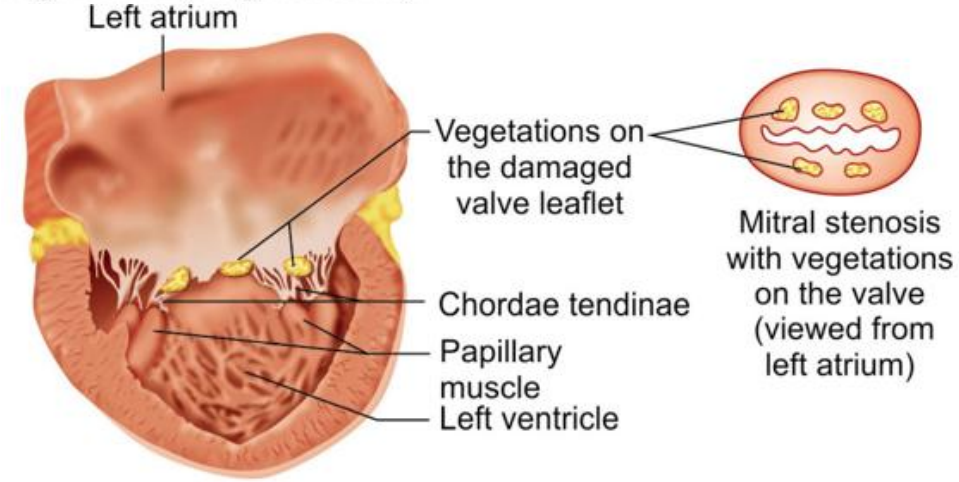
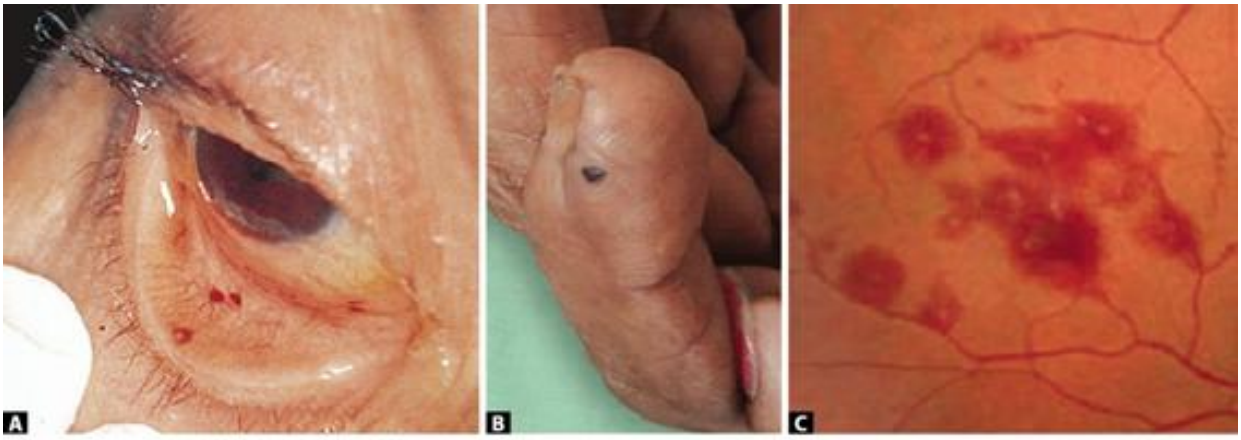


Fig. 16.25: Subacute bacterial endocarditis. Mitral valve shows vegetations.



Figs. 16.26A to C: Complications of infective endocarditis. (A) Subconjunctival hemorrhage; (B) Osler nodes; (C) Roth spots.

Infective endocarditis in intravenous drug abusers: Pathogenic organisms gain entry through skin along with the drugs, with S. aureus causing more than 50% of the infections involve tricuspid valves.

Prosthetic valve endocarditis:
- Most commonly affects aortic valve
- Most commonly caused by S. epidermidis

Infective endocarditis: Vegetations are hallmark/pathognomonic. Fever is the most consistent symptom. Blood culture is positive for the organism in majority of the cases.

Complications of IE:
- Cardiac
- Extracardiac
- – Embolic
- – Immune complex mechanism

Infective endocarditis: The "gold standard" for the diagnosis of infective endocarditis is culture of organism from a valve or other endocardial surface.

NONINFECTED VEGETATIONS

Nonbacterial Thrombotic Endocarditis

Nonbacterial thrombotic endocarditis (NBTE) is characterized by the deposition of sterile thrombi on heart valves, occurring in patients with an underlying hypercoagulable state (e.g., chronic disseminated intravascular coagulation, hyperestrogenic states, and in association with underlying malignancy such as mucinous adenocarcinomas). Though NBTE can develop in healthy individuals, it is usually associated with general debility or wasting. Hence, NBTE is **also called marantic endocarditis**.

MORPHOLOGY (FIG. 16.27A)

- The vegetations are small (1–5 mm in diameter), sterile/bland and usually occurs on previously normal valves. They are usually attached to the line of closure of leaflets. In contrast to infective endocarditis, the sterile valvular lesions of NBTE do not destruct the underlying valve.

Complications: NBTE may give rise to emboli and can cause infarcts in the brain, heart, and other organs. It can also serve as a potential nidus for bacterial colonization and lead to infective endocarditis.

Libman–Sacks Endocarditis

Q. Write a short note on Libman–Sacks endocarditis.

Endocarditis in systemic lupus erythematosus (*refer* page 164) is called Libman–Sacks endocarditis. It is characterized by the presence of sterile vegetations on the valves of patients with systemic lupus erythematosus. It develops in about 10% of SLE patients. The vegetations probably develop as a consequence of immune complex deposition. The valve adjacent to the vegetation show inflammation, often with fibrinoid necrosis. Subsequent fibrosis can produce severe deformity of valves and may resemble chronic rheumatic heart disease.

MORPHOLOGY (FIG. 16.27B)

The vegetations are small or medium-sized. The vegetations can occur anywhere on the valve surface, on the cords, or on the atrial or ventricular endocardium.

RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE

CC: Describe the etiology, pathophysiology, pathology, gross and microscopic features, criteria and complications of rheumatic fever.

BOX 16.8: Criteria for infective endocarditis.

Pathologic Criteria
Demonstration of microorganisms by culture or histologic examination in:

- Vegetation
- Embolus from a vegetation
- Intracardiac abscess

Histological confirmation of active endocarditis in vegetation or intracardiac abscess.

Clinical Criteria

Major

Positive blood cultures for IE (one of the following):

Typical microorganisms consistent with IE from two separate blood cultures:

- *Staphylococcus aureus*
- Viridans streptococci
- *Streptococcus gallolyticus* (formerly *S. bovis*), including nutritional variant strains (*Granulicatella* spp. and *Abiotrophia defectiva*)
- HACEK group: *Haemophilus* spp., *Aggregatibacter* (formerly *Actinobacillus actinomycete comitants*), *Cardiobacterium hominis*, *Eikenella* spp., and Kingella kingae
- Community-acquired enterococci, in the absence of a primary focus; OR

Persistently positive blood culture:

- For organisms that are typical causes of IE: At least two positive blood cultures from blood samples drawn >12 hours apart
- For organisms that are more commonly skin contaminants: Three or a majority of ≥4 separate blood cultures (with first and last drawn at least one hour apart)

Single positive blood culture for *Coxiella burnetii* or phase I IgG antibody titer >1:800

Evidence of endocardial involvement (one of the following):

Echocardiogram positive for IE:

- Vegetation (oscillating intracardiac mass on a valve or on supporting structures, in the path of regurgitant jets, or on implanted material, in the absence of an alternative anatomic explanation) OR
- Abscess OR
- New partial dehiscence of prosthetic valve

New valvular regurgitation

Increase in or change in pre-existing murmur not sufficient

Minor

- Fever ≥38°C (100.4°F)
- Predisposition: Predisposing heart lesion or intravenous drug use
- Vascular phenomena: Arterial petechiae, subungual/splinter hemorrhages, emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhages, Janeway lesions
- Immunological phenomena: Focal segmental glomerulonephritis, Osler nodes, Roth spots
- Microbiologic evidence, including a single culture positive but not meeting a major criterion, or for an unusual organism that can cause infective endocarditis

Q. Define rheumatic fever. Describe etiopathogenesis, pathology and complications of rheumatic heart disease.

Definition: Rheumatic fever (RF) is an **acute, post-streptococcal, immune-mediated,**

multisystem inflammatory disease. It occurs a few weeks after an episode of group A streptococcal pharyngitis.

Incidence

- **Age group:** Most common in **children between 5 and 15 years**.
- **Sex**: Both sexes are equally effected.
- **Socioeconomic conditions:** Incidence and mortality rate of RF and RHD have markedly decreased over the past century, due to improved socioeconomic conditions and rapid diagnosis and treatment of streptococcal pharyngitis.
- **Poor economy and overcrowding**: It is a predisposing factor in developing countries.
- Rheumatic fever rarely follows infections by streptococci at other sites, like skin pharynx, tonsil.

Major Phases

Two major phases:

1. **Acute rheumatic fever:** It frequently manifest as **acute rheumatic carditis.**
2. **Chronic rheumatic heart disease:** Acute rheumatic carditis **may progress to chronic rheumatic heart disease** (RHD). The deforming fibrotic valvular lesions (mainly mitral stenosis) are the principal/key features of chronic RHD.

MORPHOLOGY OF ACUTE RHEUMATIC FEVER

The lesions of rheumatic fever can be broadly divided into cardiac and extracardiac lesions.

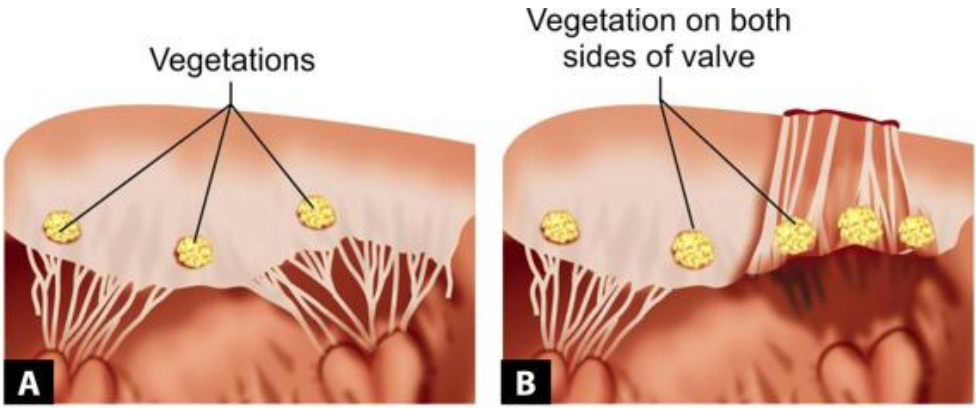
Cardiac Lesions

Q. Write a short note on rheumatic carditis.

Q. Write a short note on morphology of heart in acute rheumatic heart disease.

Cardiac lesions can be studied under: (1) Acute rheumatic heart disease, and (2) Chronic rheumatic heart disease.

Q. Write short note on Aschoff bodies (Aschoff nodule).



Figs. 16.27A and B: Vegetations in: (A) Nonbacterial thrombotic endocarditis (NBTE); (B) Libman–Sacks endocarditis (LSE).

Aschoff Body (Figs. 16.28A and B)

It is spheroidal or fusiform characteristic lesion seen in rheumatic disease.

- Size: About 1–2 mm.
- Site: During acute RF, may be found in any of the three layers, in the perivascular interstitial region of the heart.

Formation of Aschoff body

It consists of three phases

- 1. Early/exudative phase: It consists of a perivascular focus of swollen eosinophilic collagen surrounded by lymphocytes, plasma cells, and macrophages.
- 2. Intermediate/granulomatous phase: It is pathognomonic of rheumatic myocarditis. It is a granulomatous lesion, with a central fibrinoid focus associated with lymphocytes (primarily T-cells), macrophages, Anitschkow cells (pathognomonic for RF) and occasional plasma cells. Anitschkow cells are plump activated macrophages, which have abundant cytoplasm. They have round-to-ovoid nuclei in which the chromatin is disposed in a central, slender, wavy ribbon. These nuclei resemble a caterpillar when cut longitudinally (hence known as "caterpillar cells"). On cross section, these nuclei have an owl-eye appearance. Few Anitschkow cells may become multinucleated (with 2–4 nuclei) in which case, they are termed Aschoff giant cells (the cytoplasm fuses and nuclei remain intact within fused cytoplasm).
- 3. Late/healed /final phase: Aschoff body is replaced by a nodule of scar tissue.

Acute Rheumatic Heart Disease

It is characterized by pancarditis, involving all three layers of the heart (endocardium, myocardium, and pericardium).

1. Endocarditis (Fig. 16.29)

Inflammation of the endocardium (vavular/mural) is known as endocarditis. It may involve valvular (valvular endocarditis) or mural endocardium (mural endocarditis).

Valvular endocarditis

- During acute rheumatic carditis, valve leaflets become inflamed and edematous.
- All four valves may be affected, but left-sided valves (mitral and aortic) are more injured than do right-sided valves (tricuspid and pulmonary).
- Inflammatory damage causes focal loss of endothelium and fibrin deposits along the lines of closure of the valve leaflets or along the tendinous cords.
- Overlying these deposits develop small (1–2 mm) nodular or warty vegetations, called verrucae. These verrucae are firmly attached and do not get dislodged. This is in contrast to friable detachable vegetations of infective endocarditis.

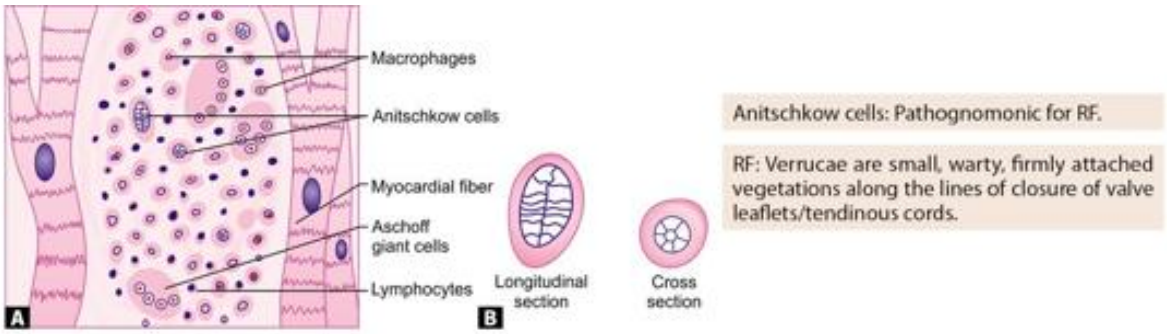
Mural endocarditis

Inflammation of the mural endocardium is less prominent than valvular lesions and most common is MacCallum plaque.

Q. Write briefly on MacCallum plaque.

MacCallum plaques (Fig. 16.29)

These are subendocardial lesions, which appear as irregular, wrinkled, map-like thickenings in the mural endocardium.



Figs. 16.28A and B: (A) Aschoff body; (B) Magnified view of Anitschkow cells; (C) Gross appearance (diagrammatic) of mitral valve and left atrium in RHD.

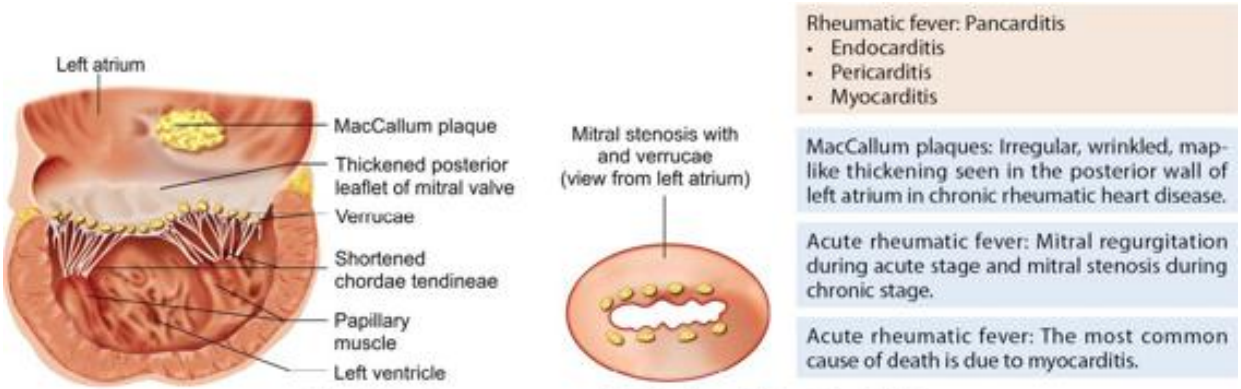


Fig. 16.29: Gross appearance of mitral valve and left atrium in RHD.

Usually seen in the posterior wall of the left atrium (in chronic RHD and not in acute rheumatic fever), just above the posterior leaflet of mitral valve. These may be formed due to damage induced by chronic regurgitation of blood. Microscopically, they may show multiple Aschoff bodies.

2. *Pericarditis*

- Inflammation of the pericardium is known as pericarditis.
- During acute RF, irregular deposits of fibrin are found on both visceral and parietal surfaces of the pericardium causing fibrinous pericarditis.
- This fibrinous pericarditis, grossly resemble the shaggy surfaces of two slices of buttered bread that have been gently pulled apart. Hence, they are commonly called as bread-and-butter pericarditis **(Fig. 16.30)**.
- Clinically, it gives a friction rub, and it never causes constrictive pericarditis.

3. *Myocarditis*

- Inflammation of the myocardium is known as myocarditis.
- Nonspecific myocarditis during acute RF may cause dilation of heart. Microscopically, myocardium may show lymphocytes and macrophages or Aschoff bodies.
- Few patients may die due to myocarditis.

Extracardiac lesions (refer page 477).

Chronic Rheumatic Heart Disease

Q. Morphology of heart in chronic rheumatic heart disease (sequale of rheumatic fever).

Myocarditis and pericarditis of acute rheumatic fever usually resolve without any sequelae. But the acute endocarditis, mainly the **valvulitis induced damage is cumulative** and with each attack of rheumatic fever, it progresses → results in structural and functional alterations of the valves.

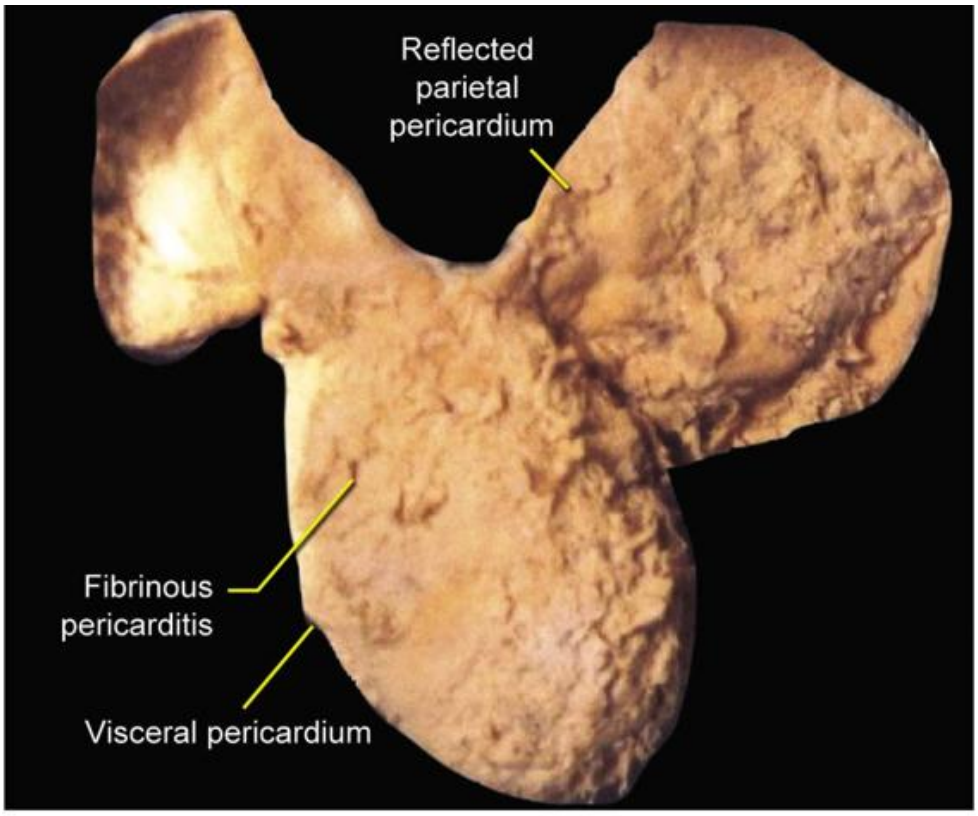
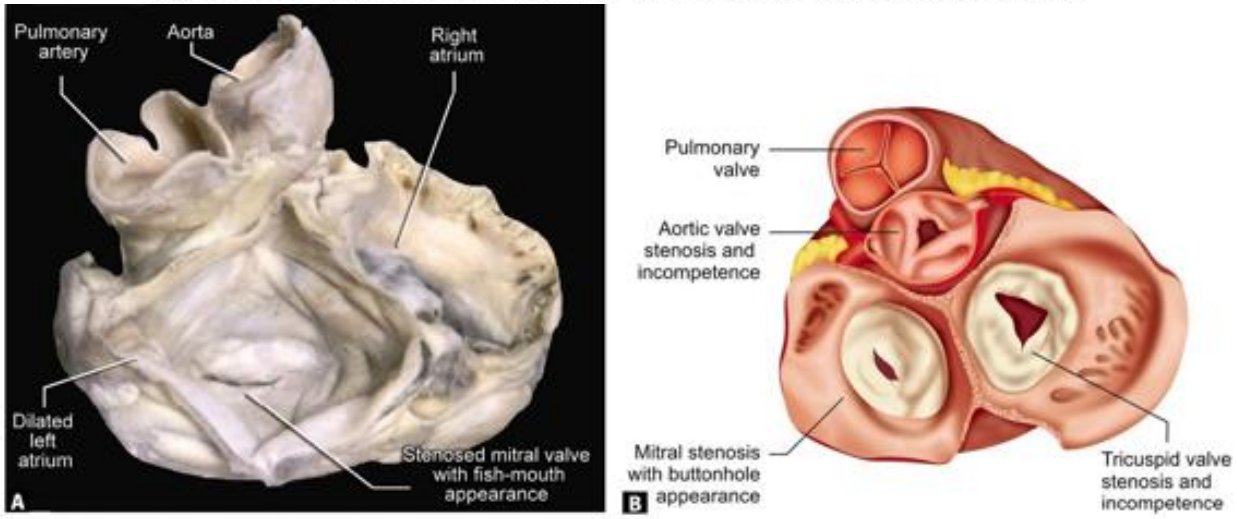


Fig. 16.30: Fibrinous pericarditis developing in rheumatic fever.



Figs. 16.31A and B: Rheumatic valvular disease viewed from above: (A) Mitral stenosis with buttonhole deformity and dilated left atrium developed as a complication of chronic RHD; (B) Multivalvular rheumatic disease affecting mitral, tricuspid and aortic valve (diagrammatic).

MORPHOLOGY OF CHRONIC RHEUMATIC HEART DISEASE

Valves Affected

- **Mitral valve (Figs. 16.31A and B) alone:** It is the most commonly (65–70% of cases) and severely affected valve in chronic rheumatic disease.
- Mitral valve along with the aortic valve in another 25% of cases.
- Tricuspid and pulmonary valve are rarely involved.

Changes in the Affected Valves

- 1. Changes in mitral valve **(Figs. 16.31A and B):**
  - Thickening of valve leaflet: It is due to fibrosis following inflammation of the valve leaflets.
  - Fusion of commissures: Healing of rheumatic lesions along the lines of closure leads to formation of fibrous adhesions/bridging of valvular commissures → result in a rigid and partially fused stenotic valve. In severe chronic rheumatic mitral valve disease, the valve orifice becomes reduced to a fixed narrow opening that has the characteristic appearance of a fish-mouth (or "buttonhole") when viewed from the ventricular aspect **(Figs. 16.31A and B)**.
  - Shortening, thickening and fusion of the chordae tendineae.
  - Consequences of mitral stenosis:
    - **Left atrium progressively dilates** → favor **mural thrombi** in the appendage or along the wall.
    - **Thrombi can dislodge → embolize**.
    - **Chronic venous congestion in the lungs**.
    - **Pulmonary hypertension → right ventricular hypertrophy and right-sided heart failure**. Left ventricle is normal in isolated pure mitral stenosis.

- 2. Changes in aortic valve **(Fig. 16.31B):** This is the second most commonly involved valve in rheumatic heart disease. Diffuse fibrous thickening of the cusps and fusion of the commissures cause aortic stenosis. The cusps may become calcified as the patient ages, resulting in stenosis and insufficiency.
- 3. **Tricuspid valve (Fig. 16.31B):** In cases of recurrent RF, the tricuspid valve may be deformed, always in association with mitral and aortic lesions.
- 4. Pulmonary valve: It is rarely affected.

Extracardiac Lesions (Figs. 16.32A and B)

Q. Write a short note on extracardiac lesion of rheumatic fever.

- Migratory polyarthritis**:** It is the usual presentation in acute rheumatic fever and is accompanied by fever. In this, one large joint becomes painful and swollen, which then

subsides and another joint gets involved for a period of days. They resolve spontaneously without any residual disability.

- Subcutaneous nodules: These are usually seen in children than in adults. The nodules are oval to spheroidal, painless and measure about 0.5–2 cm in diameter. They are attached to tendons or ligaments and are usually located at the extensor aspect of wrist, elbows, ankles or knees. Microscopically, they appear like a giant Aschoff body.
- Erythema marginatum**:** These skin lesions are non-itchy reddish rashes showing characteristic bathing suit pattern of distribution.
- Sydenham's chorea or Saint Vitus' dance: It is a neurologic disorder with involuntary rapid, jerky, purposeless movements of trunks and extremities.
- Rheumatic fever also may involve other organs like lung, pleura and arteries.

Pathophysiology of mitral stenosis:

- Narrowing of mitral valve → dilatation and hypertrophy of left atrium due to increased work in filling the ventricle during diastole.
- Chronic backup of atrial blood into the pulmonary vein → pulmonary venous hypertension → right-sided heart failure and right ventricular hypertrophy.
- Clinical features: Dyspnea and hemoptysis with rust-colored sputum (heart failure cells).

Etiology and Pathogenesis (Figs. 16.32A and B)

Q. Describe the etiopathogenesis and pathology of rheumatic heart disease.

Acute **rheumatic fever is a post-streptococcal disease**. It develops after a **latent period of 2–6 weeks after an episode of pharyngitis or tonsillitis** by **group A β-hemolytic streptococci**. It occurs most often in children.

Immunologically mediated disease: Streptococcal infection introduces the streptococcal antigens into the body which activate both antibody and T-cell–mediated reactions against streptococci.

Antibody-mediated Reactions(Type II Hypersensitivity Reaction)

Antibodies may be produced by B-lymphocytes **against various antigenic components of the *Streptococcus***. One of them produced against the **M**

proteins of streptococci seems to **cross-react with certain similar self-antigens in the myocardial cells and glycoproteins of the valves** in the heart (molecular mimicry). This may be the mechanism for pancarditis in acute rheumatic fever.

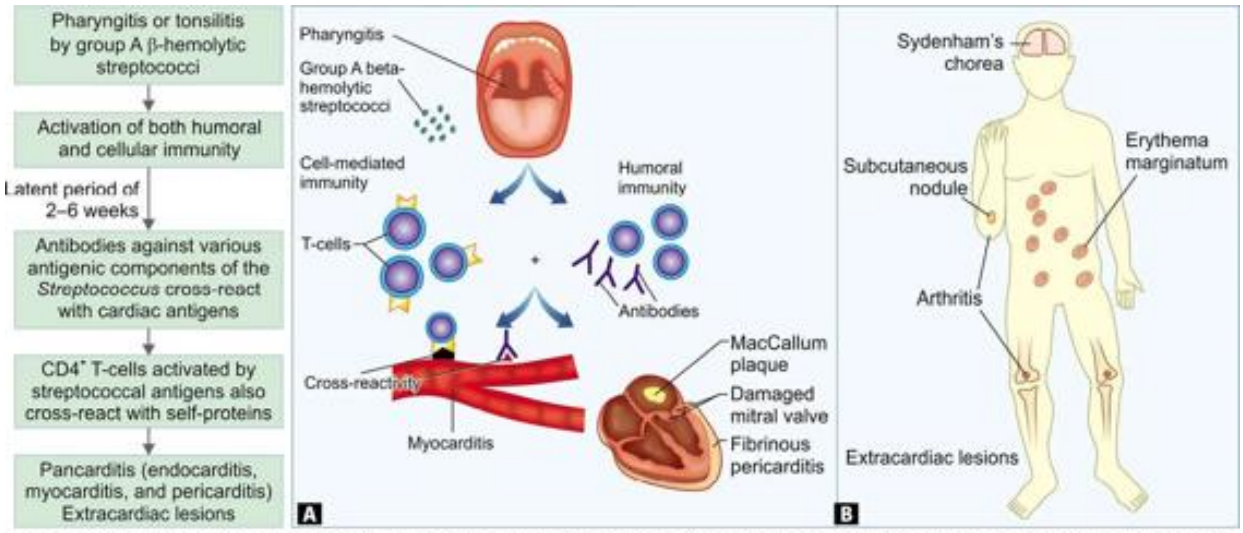
T-cell-mediated Reactions(Type IV Hypersensitivity Reaction)

$CD4^+$ T-cells are also activated by streptococcal antigens. These along with antibodies **cross react with self-proteins in the heart**. These reactions produce cytokines leading to activation of macrophages, which are seen in lesions of rheumatic fever.

Complications of Chronic Rheumatic Heart Disease

Q. Describe the complications of rheumatic heart disease.

- **Infective endocarditis:** The **damaged valves** of rheumatic heart **disease provide a site for deposition of bacteria**.
- **Mural thrombi:** They may be formed in **atrial or ventricular chambers**. Rarely, a large thrombus in the left atrial appendage may act as a ball valve and may obstruct the orifice of mitral valve.
- **Thromboemboli:** Mural thrombi may dislodge → form thromboemboli → **infarcts in various organs**.
- **Congestive heart failure:** It is associated with involvement of both mitral and aortic valves.
- **Adhesive pericarditis:** It follows the fibrinous pericarditis developed during acute attack.
- **Arrhythmias** (particularly atrial fibrillation): It is observed in mitral stenosis.



Figs. 16.32A and B: (A) Cardiac and (B) extracardiac lesions of rheumatic fever. Pharyngitis by β-hemolytic streptococci activates both humoral and cellular immunity. The antibodies produced against various antigenic components of the *Streptococcus*, can cross-react with cardiac antigens. The result is pancarditis (endocarditis, myocarditis, and pericarditis). Extracardiac lesions include polyarthritis, erythema marginatum, subcutaneous nodules and Sydenham's chorea.

Q. Write a short note on modified Jones criteria for diagnosis of rheumatic fever.

TABLE 16.10: World Health Organization criteria (2002–2003) for the diagnosis of rheumatic fever and rheumatic heart disease (based on the 1992 revised Jones criteria).

Diagnostic categories	***Criteria***
Primary episode of rheumatic fever	**Two major or one major and two minor manifestations plus evidence of preceding group A streptococcal infection**
Recurrent attack of rheumatic fever in a patient without established rheumatic heart disease	
Recurrent attack of rheumatic fever in a patient with established rheumatic heart disease	Two minor manifestations plus evidence of preceding group A streptococcal infection
Rheumatic chorea	Other major manifestations or evidence of group A streptococcal infection not required
Insidious onset rheumatic carditis	
Chronic valve lesions of rheumatic heart disease (patients presenting for the first time with pure mitral stenosis or mixed mitral valve disease and/or aortic valve disease)	Do not require any other criteria to be diagnosed as having rheumatic heart disease
Jones criteria for the diagnosis of rheumatic fever	
Major manifestations	***Minor manifestations***

TABLE 16.10: World Health Organization criteria (2002–2003) for the diagnosis of rheumatic fever and rheumatic heart disease (based on the 1992 revised Jones criteria).

Diagnostic categories	*Criteria*
1. Carditis 2. Migrating polyarthritis of the large joints 3. Sydenham's chorea 4. Erythema marginatum 5. Subcutaneous nodules	1. Clinical: Fever, polyarthralgias 2. Laboratory: Elevated erythrocyte sedimentation rate or leukocyte count or acute-phase reactants Electrocardiogram: Prolonged P-R interval
Supporting evidence of a preceding streptococcal infection within the last 45 days • Elevated or rising antistreptolysin O or other streptococcal antibody or • A positive throat culture or • Rapid antigen test for group A *Streptococcus* or • Recent scarlet fever	

Clinical Features

Rheumatic fever usually manifests as a **migrating polyarthritis and/or carditis**.

Clinical findings of **acute carditis** include pericardial friction rubs, weak heart sounds, tachycardia, arrhythmias and various cardiac murmurs (type depending on the valve involved).

Jones criteria: Presently diagnosis is based on modified Jones criteria **(Table 16.10)**. The criteria are: **evidence of a preceding group A streptococcal infection, with the presence of two of the major criteria or one major and two minor criteria**.

Laboratory Findings

There is **no specific serological test** for rheumatic fever.

- **Serological tests**: **Pharyngeal cultures** for streptococci are **negative** by the time the illness begins. The antibodies to one or more streptococcal enzymes can be detected in the sera of most patients with RF. These include:
  - **Antistreptolysin O (ASO)**
  - **DNase B**
  - **Antihyaluronidase.**
- **Acute phase reactants**

- **Raised ESR**
- **C-reactive protein**.

Course

- **Recovery: Most acute attacks subside** with 4–6 weeks.
- **Recurrence:** Clinical manifestations reappear years or even decades after the initial episode of RF.

Prognosis: Long-term prognosis has improved the outlook due to surgical repair or prosthetic replacement of diseased valves.

Q. A 10-year-old child was brought to the hospital by his mother with the complaints of migrating/shifting joint pain and swelling of the involved joint. On enquiry, the child had an upper respiratory tract infection (sore throat) and fever 3 weeks back. On examination a subcutaneous nodule was found on the extensor aspect of right elbow. On auscultation, the heart sounds were weak with tachycardia.

(a) What is your probable diagnosis? (Answer: Rheumatic fever with rheumatic heart disease)

(b) Discuss the etiopathogenesis of the condition (refer page 478).

(c) Describe the morphological features of the main organ involved in this disease (refer pages 475-6).

(d) List the complications of this condition? (refer page 478).

Old dictum is that "rheumatic fever licks the joint but bites the heart".

Acute rheumatic fever: Mitral valve is more commonly involved and responsible for 99% cases of acquired mitral stenosis. Recurrent rheumatic fever is the most common cause of mitral stenosis.

RF: Most common affected age group is between 5 and 15 years.

Rheumatic heart disease: Post-streptococcal disease in which antistreptococcal antibodies cross-react with cardiac tissues.

Changes in the mitral valve:
- Fusion of commissures
- Fixed narrow opening (fish-mouth or buttonhole)
- Shortening, thickening and fusion of the chordae tendineae.

Mitral stenosis → pulmonary hypertension → right-sided heart failure.

Atrial fibrillation: Common in mitral stenosis.

Acute rheumatic fever: Most commonly presents with migrating polyarthritis.

Acute rheumatic fever: Jones criteria used for the diagnosis.

Acute rheumatic fever: Raised ASO, DNase B titers, ESR and C-reactive protein.

CONGENITAL HEART DISEASE

Definition: Congenital heart disease (CHD) is the **abnormalities of the heart or great vessels** that are **present from birth**.

Incidence

Congenital heart diseases are the **most important and common type of heart disease in children**.

Etiology and Pathogenesis

Most CHD arise from defective embryogenesis during 3–8 weeks of gestation, when major cardiovascular structures form and begin to function. Factors that are involved in CHD are:

1. **Genetic factors:** The main causes of congenital heart disease are sporadic genetic abnormalities. These may be:
   - **Single gene mutations**
   - **Small chromosomal deletions**

- **Additions or deletions of whole chromosomes (trisomies and monosomies):** The most common genetic cause of congenital heart disease is **trisomy 21 (Down syndrome)**.

2. **Environmental factors:** These factors may be the primary cause of congenital heart disease. **Examples:**
   - **Congenital rubella infection**
   - **Gestational diabetes**
   - **Exposure to teratogens** (including drugs)
   - **Nutritional factors: During pregnancy**, consumption of multivitamin containing **folate may reduce the risk**, whereas heavy consumption of **alcohol increases the risk** of congenital heart defects.
3. **Multifactorial:** Majority of CHD may be due to combination of environmental and genetic factors.

Clinical Features

- **Severe** congenital heart diseases result in **intrauterine death**.
- Some congenital heart diseases produce clinical manifestations soon after birth, but some may not be evident until adulthood (e.g., ASD).

Congenital means "born with".

Most common genetic cause of congenital heart disease: Trisomy 21 (Down syndrome).

Classification (Box 16.9)

The structural anomalies in congenital heart disease may be divided into four major categories: (1) right-to-left shunt, (2) left-to-right shunt, (3) an obstruction and (4) malposition of heart.

Left-to-right Shunt

BOX 16.9: Classification of congenital heart diseases.

Type of congenital heart disease

1. **Left-to-right shunt**

- Ventricular septal defect
- Atrial septal defect
- Patent ductus arteriosus

2. **Right-to-left shunt**
   - Tetralogy of Fallot
   - Transposition of the great arteries
   - Persistent truncus arteriosus
   - Tricuspid atresia
   - Total anomalous pulmonary venous connection
3. **Obstructive congenital heart disease**
   - Coarctation of the aorta
   - Aortic stenosis
   - Pulmonary stenosis
4. **Malposition of heart—**very rare
   - Dextrocardia

Left -to-right shunt: Danger of shunt reversal if not corrected.

These are characterized by the flow of blood from the left (systemic) side to the right (pulmonary) side of the circulation.

Consequences

- Left-to-right shunt causes **increased pulmonary blood flow** and initially does not produce cyanosis.
- But increased pulmonary blood flow **increases the volumes and pressures** in pulmonary circulation → results in **pulmonary hypertension** → causes **right ventricular hypertrophy**.
- Later, when the **pressure in right ventricle reaches systemic levels**, the shunt changes to a new **right-to-left,** which diverts the unoxygenated blood into the systemic circulation → results in cyanosis, and hence these CHD are known as **late cyanotic congenital heart disease**, **or Eisenmenger syndrome**.
- When the pulmonary hypertension becomes irreversible, the structural defects of congenital heart disease become irreparable.

Right-to-left Shunt

If the pressure in the right side rises more than that in left, the blood flows from the right to left side (right-to-left shunt) → results in hypoxemia and

cyanosis (blue discoloration of the skin and mucous membranes) due to the mixing of poorly oxygenated venous blood with systemic arterial blood (called **cyanotic congenital heart disease**).

Consequences

- **Paradoxical embolism:** It is a condition in which emboli from the peripheral veins can bypass the lungs and enter directly into the systemic circulation (*refer* page 127). It may result in infarction and abscess in the brain.
- **Clubbing and polycythemia:** Severe, long-standing cyanosis causes clubbing of the tips of the fingers and toes (called **hypertrophic osteoarthropathy**) and **polycythemia**.

Obstructive Congenital Heart Disease

These are characterized by abnormal narrowing of chambers, valves, or blood vessels. **If the obstruction is complete**, it is called an **atresia**.
In some CHD, there may be coexistence of (e.g., tetralogy of Fallot) both obstruction (pulmonary stenosis) and a shunt (right-to-left through a VSD).
Malposition of heart are very rare type of congenital heart diseases.

Shunts: Abnormal communication between heart chambers or blood vessels. Shunts allow the flow of blood from high pressure to the low-pressure side.

Cyanosis: Blue discoloration of the mucous membranes resulting from an increased amount of reduced hemoglobin.

LEFT-TO-RIGHT SHUNTS

The common left-to-right shunt include ventricular septal defects (VSDs), patent ductus arteriosus (PDA), and atrioventricular septal defects (AVSDs).

Atrial Septal Defect

Q. Write a short note on atrial septal defect.

An atrial septal defect (ASD) is an abnormal, fixed opening in the atrial septum. It is due to incomplete formation of tissue in the atrial septum,

which allows flow of blood between the left and right atria. ASDs are **usually asymptomatic till adulthood**.

MORPHOLOGY

Classification: It is classified into three major types according to their location as: Secundum, primum, and sinus venosus.

- Secundum ASDs (90%): These result from a deficient or fenestrated oval fossa near the center of the atrial septum. The size varies, and may be single or multiple, or be fenestrated.
- Primum anomalies (5%) occur near the AV valves.
- Sinus venosus defects (5%) develop near the entrance of the superior vena cava.

Clinical Features

- ASDs cause a left-to-right shunt and results in increased pulmonary blood flow by about two to four times normal.
- A murmur may be heard due to increased blood flow through the pulmonary valve.
- ASDs usually do not produce symptoms before 30 years of age.

Complications: (1) heart failure, (2) paradoxical embolization, and (3) irreversible pulmonary vascular disease.

Prognosis: Mortality is low.

Ventricular Septal Defect

Q. Write a short note on ventricular septal defect.

- Incomplete closure of the ventricular septum is known as ventricular septal defect (VSD), is the **most common form of congenital cardiac anomaly**.
- Most (70–80%) VSDs are associated with other congenital cardiac anomalies (e.g., tetralogy of Fallot).
- Cause free blood flow from the left-to-right ventricles.

Classification

Ventricular septal defect are classified according to their size and location.

- **Membranous VSD (90%):** They involve the membranous region of interventricular septum.
- **Infundibular VSD (10%):** They lie below the pulmonary valve or within the muscular part of the interventricular septum.

Clinical Features

The clinical features depend on the size of the defect.

- **Large ventricular septal defect**
  - Usually **membranous or infundibular type** and manifest from birth.
  - Cause **left-to-right shunt** → leading to right ventricular hypertrophy and pulmonary hypertension.
  - Irreversible pulmonary vascular disease develops later, in all patients resulting **reversal of shunt**, **cyanosis**, **and death**.
- **Small ventricular septal defect**
  - May not be recognized until adult life.
  - About 50% of small muscular VSDs may close spontaneously.

Patent Ductus Arteriosus

Q. Write a short note on patent ductus arteriosus.

Normal Function of Ductus Arteriosus

In the fetus, it is an essential structure that carries the blood from the pulmonary artery to the aorta, thus bypasses the flow of blood to lungs (like the patent foramen ovale). After birth, it normally spontaneously closes and the blood from pulmonary artery flows to the lung.

Patent (Persistent) Ductus Arteriosus

It is a congenital anomaly in which the **ductus arteriosus remains open after birth (Fig. 16.33)**. PDAs may occur as an **isolated anomaly** (about 90%), **or associated with VSD, coarctation of the aorta, or pulmonary or aortic valve stenosis**. PDA produces a **characteristic continuous harsh murmur known as "machinery-like"**.

Clinical Features

Depends on diameter of PDA and the cardiovascular status of the patient. PDA is usually asymptomatic at birth, and a narrow PDA may not affect the child's growth and development. First, the shunt is from left-to-right and there is no cyanosis. But additional volume and pressure overload produces obstructive changes in small pulmonary arteries, leading to reversal of flow and its associated consequences.

Preservation of ductal patency (by administering prostaglandin E) is essential for survival of infants with congenital malformations that obstruct the pulmonary or systemic blood flow tracts. Example: In aortic valve atresia, preserved PDA supplies the major blood into the systemic circulation. Consequences of patent ductus arteriosus are presented in **Figure 16.33**.

PDA may be either life-threatening or lifesaving.

Patent Foramen Ovale

Normal Function of Foramen Ovale

- In **the fetus, it is an essential small hole in the atrial septum at the oval fossa** → produces a right-to-left shunt, though which the oxygen-rich blood from the placenta directly travels from the right-to-left atrium without passing through the non-inflated lungs.
- The **foramen ovale closes at birth** due to increased blood pressure on the left side of the heart.

Consequences of a Patent Foramen Ovale

Sustained pulmonary hypertension or transient increase in right-sided pressures (as occurs during a bowel movement, coughing, or sneezing), can produce transient right-to-left shunting, and paradoxical embolism.

RIGHT-TO-LEFT SHUNTS

These congenital diseases cause **cyanosis early in postnatal life** (cyanotic congenital heart disease). Tetralogy of Fallot is the most common and others

include transposition of the great arteries, persistent truncus arteriosus, tricuspid atresia, and total anomalous pulmonary venous connection.

Tetralogy of Fallot

Q. Write short note on tetralogy of Fallot's features and complications.

Components (Fig. 16.34): The **four main features** of the tetralogy of Fallot (TOF) are:

1. **Ventricular septal defect:** Usually large.
2. **Subpulmonic stenosis/pulmonary valvular stenosis:** It causes obstruction of the right ventricular outflow tract.
3. **Aorta overriding the VSD** and both ventricular chambers.
4. **Right ventricular hypertrophy:** It is due to the obstruction to right ventricular outflow.

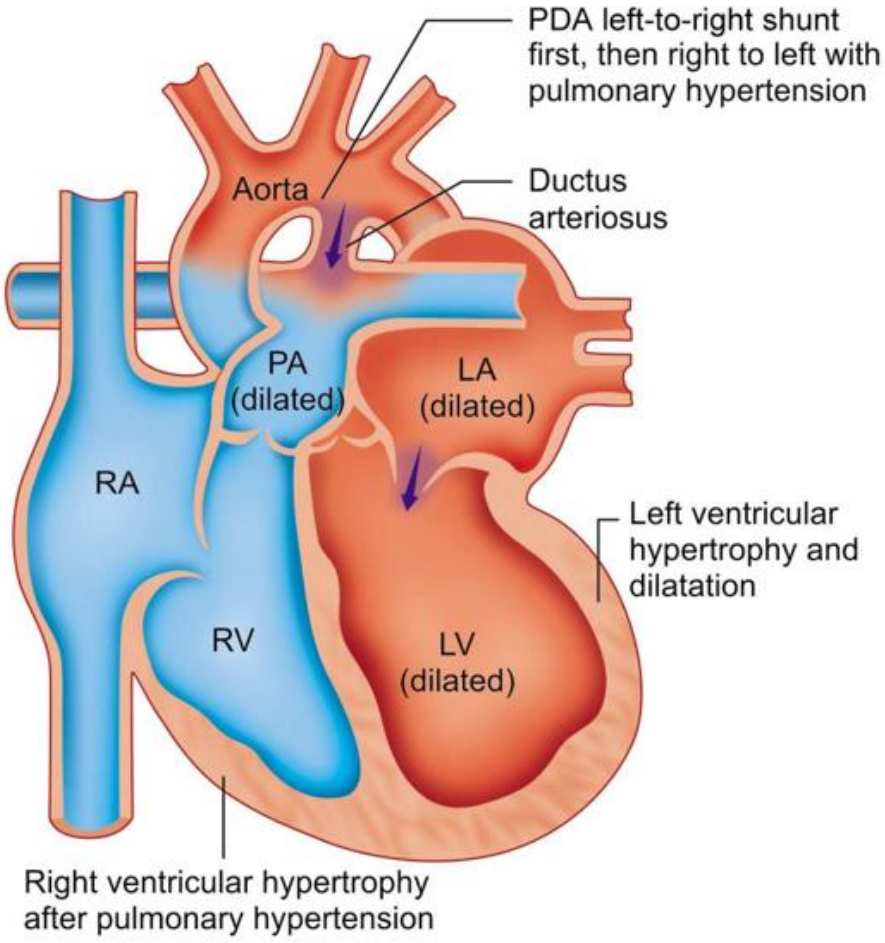


Fig. 16.33: Patent ductus arteriosus (PDA).

(LA: left atrium; RA: right atrium; LV: left ventricle; RV: right ventricle; PA: pulmonary artery).

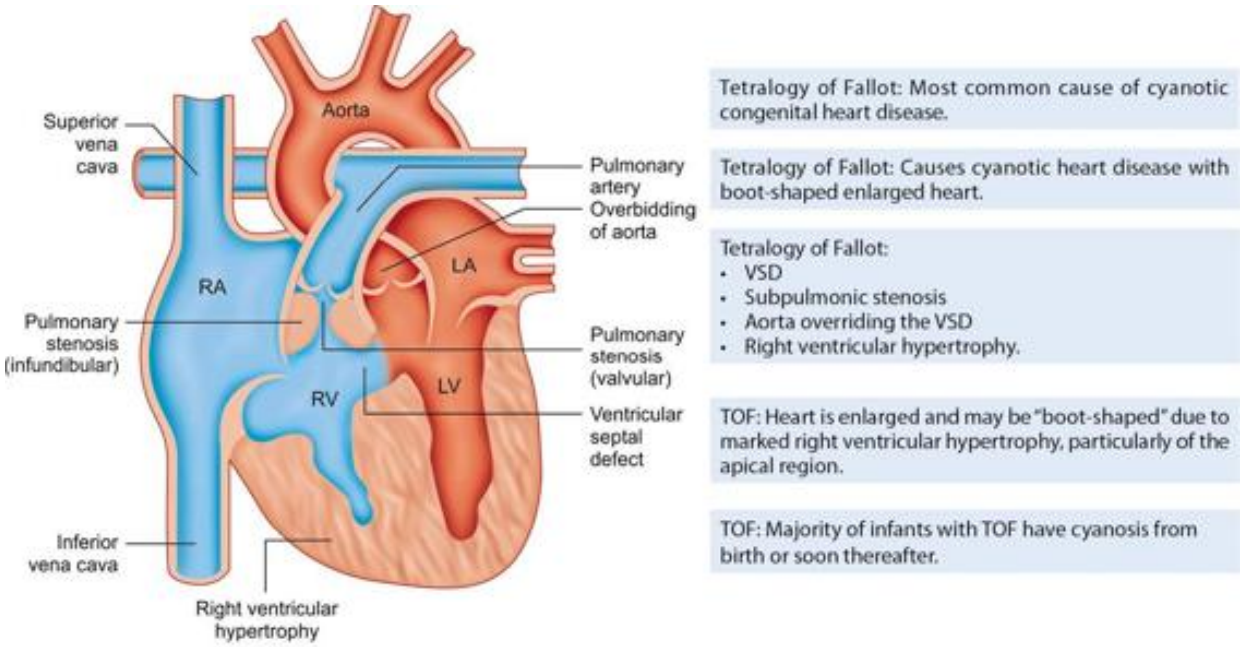


Fig. 16.34: Diagrammatic representation of tetralogy of Fallot. Four components are: (1) Ventricular septal defect; (2) Subpulmonic stenosis/pulmonary valvular stenosis; (3) Aorta overriding the VSD; (4) Right ventricular hypertrophy.
(RA: right atrium; LA: left atrium; RV: right ventricle; LV: left ventricle).

MORPHOLOGY

Heart is enlarged and may be "boot-shaped" due to marked right ventricular hypertrophy, particularly of the apical region.

Clinical Features

Clinical features depend mainly on the severity of the subpulmonary stenosis, because this decides the direction of blood flow.

- **Mild subpulmonary stenosis:** It clinically resembles an isolated VSD, and the shunt may be from left-to-right, **without cyanosis** (so-called pink tetralogy).
- **Moderate subpulmonary stenosis:** It produces increased resistance to right ventricular outflow. When the right-sided pressures approach or exceed that of left-sided pressures → right-to-left shunt develops → **producing cyanosis** (classic TOF).
- **Severe subpulmonary stenosis:**
  - Pulmonary arteries become progressively hypoplastic with small and thin walls.
  - Aorta becomes progressively larger in diameter.

- As the child grows, the size of heart increases, but pulmonary orifice does not expand proportionally. This results in progressive worsening of the obstruction.
- **Majority of infants with TOF have cyanosis from birth or soon thereafter**.

Transposition of the Great Arteries

Features: Aorta arises from the right ventricle and lies anterior and to the right of the **pulmonary artery**, which **originate from the left ventricle**.
The heart chambers and its relations are normal (concordant), i.e., the right atrium joins the right ventricle and the left atrium empties into the left ventricle.

MORPHOLOGY

- Right ventricle shows hypertrophy, because it functions as the systemic ventricle.
- Left ventricle shows atrophy (thinning of its wall) because it supports only the low-resistance pulmonary circulation.

Outlook: It depends on three factors.
1. Degree of mixing of the blood
2. Severity of the tissue hypoxia
3. Ability of the right ventricle to maintain the systemic circulation.

Without surgery, most patients die during infancy.

Transposition of great vessels:
- Aorta arises from right ventricle
- Pulmonary artery arises from left ventricle
- Atria normal.

Transposition of great arteries: Survival is incompatible unless accompanied by another minor congenital defect.

OBSTRUCTIVE CONGENITAL ANOMALIES

Congenital obstruction to blood flow may occur at:
- **Heart**

- Heart valves (e.g., stenosis or atresia of the aortic or pulmonary valves).
- Chamber of heart (e.g., subpulmonary stenosis in TOF).

- **Great vessel** (e.g., coarctation of the aorta).

Coarctation of the Aorta (Aortic Coarctation)

Coarctation **(narrowing, constriction)** of the aorta is the common structural anomaly.

Sex: It is common in males than in females (2:1). But females with Turner syndrome may have a coarctation.

Types

Two classic forms.

1. **Infantile (preductal) form (Fig. 16.35A):** It is characterized by tubular hypoplasia of the aortic arch proximal to a patent ductus arteriosus. It produces symptoms in early childhood.
2. **Adult (postductal) form (Fig. 16.35B):** It shows narrowing of the aorta, opposite the closed ductus arteriosus (ligamentum arteriosum) distal to the arch vessels.

Extent of narrowing of the aortic lumen varies from minimal narrowing to maximal narrowing with only small channel. Coarctation of the aorta may occur as a solitary defect, or accompanied by other defects such as bicuspid aortic valve, congenital aortic stenosis, ASD, VSD, mitral regurgitation, or berry aneurysms of the circle of Willis in the brain.

Clinical Features

- Depend on the severity of the narrowing and the patency of the ductus arteriosus.
- **Coarctation of the aorta with a patent ductus arteriosus (Fig. 16.35A):**
  - It usually manifests immediately after birth.
  - Through patent ductus arteriosus, unsaturated (deoxygenated) blood from pulmonary artery is delivered to the aorta distal to coarctation. This produces **cyanosis only in the lower half of the body**. The

upper half of the body is supplied by great vessels, which originate proximal to coarctation (hence no cyanosis).

- **Coarctation of the aorta without a patent ductus arteriosus (Fig. 16.35B):**
  - **Most are asymptomatic**, and may not be recognized till adult life.
  - It presents with **hypertension in the upper extremities**; and **weak pulses and hypotension in the lower extremities**.
  - The lower extremities may show features of arterial insufficiency (i.e., claudication and coldness).
  - In adults, **collateral circulation may develop between the precoarctation arterial branches and the postcoarctation arteries**.
  - These appear as enlarged intercostal and internal mammary arteries, which may produce **radiographically visible erosions ("notching") of the undersurfaces of the ribs.**
- Coarctations may be associated with **murmurs throughout systole**; sometimes with a thrill. The heart is enlarged (cardiomegaly) due to left ventricular pressure-overload hypertrophy.

Adult coarctation: Blood pressure difference between upper and lower extremity is >10 mm Hg.

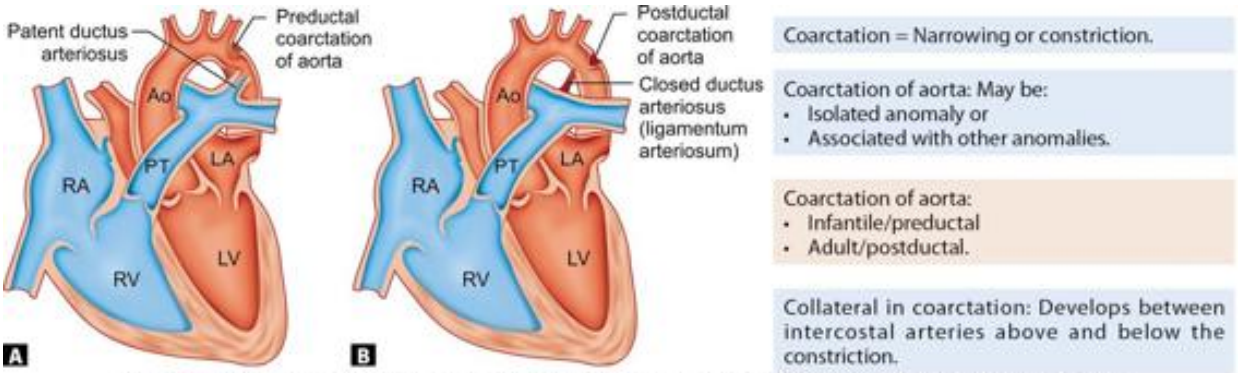
Infantile coarctation: Associated with Turner syndrome.

CARDIOMYOPATHY

NC: Classify and describe the etiology, types, pathophysiology, pathology, gross and microscopic features, diagnosis and complications of cardiomyopathies.

The term cardiomyopathy literally means heart muscle disease.

Q. Define and classify cardiomyopathy.



Figs. 16.35A and B: Coarctation of aorta: (A) With patent ductus arteriosus; (B) Without patent ductus arteriosus. (Ao: aorta; RA: right atrium; PT: pulmonary trunk; LA: left atrium; RV: right ventricle; LV: left ventricle).

Definition: Cardiomyopathies are a heterogeneous group of diseases of the myocardium that affect the mechanical and/or electrical function (intrinsic myocardial dysfunction) of the heart in the absence of any other disease. They usually (but not always) show inappropriate ventricular hypertrophy or dilatation and are due to a variety of causes that frequently are genetic.

Cardiomyopathy term should be restricted to the conditions which primarily affect the myocardium. It does not include dysfunction of heart due to other structural heart disease such as coronary artery disease, chronic myocardial dysfunction secondary to congenital or acquired valve disease, severe hypertension or pericardial abnormalities.

They manifest as failure of myocardial performance in one of the two ways: (i) mechanical (e.g., diastolic or systolic dysfunction) leading to congestive heart failure (CHF) or (ii) can cause cardiovascular death due to life-threatening arrhythmias.

Etiology: They can be genetic/inherited or have infective, toxic causes or idiopathic.

Classification: Cardiomyopathies can be classified according to etiology/underlying genetic basis of dysfunction or by functional impairments. Two fundamental forms of cardiomyopathy are:

1. **Primary cardiomyopathy:** Consists of heart muscle disease mainly involving the myocardium which may be genetic or acquired. It was observed that many cardiomyopathies were due to genetic disease. The genetic causes include mutations in myocardial proteins involved in contraction, cell-cell contacts, and the cytoskeleton. These genetic causes can lead to abnormal contraction or relaxation or to dysregulated ion transport across cell membranes. In many cardiomyopathies, the etiology may be unknown and are termed as idiopathic. Here discussion is mainly confined to cardiomyopathy that produce anatomic abnormalities in the

heart. Primary cardiomyopathy belongs to three pathologic patterns **(Figs. 16.36A to D)**:

a. **Dilated cardiomyopathy** (DCM) (including arrhythmogenic right ventricular cardiomyopathy): Most common (~90%)
b. **Hypertrophic cardiomyopathy** (HCM)
c. **Restrictive cardiomyopathy** is the least common

2. **Secondary cardiomyopathy:** Consists of myocardial disease as a part/component of generalized systemic or multiorgan disorder. These disorders form a diverse group that includes infectious or inflammatory disorders (e.g., myocarditis), immunologic diseases (e.g., sarcoidosis, amyloidosis, systemic connective tissue disease), systemic metabolic disorders (e.g., hemochromatosis), muscular dystrophies, nutritional deficiencies, chronic alcohol use.

Dilated Cardiomyopathy

Dilated cardiomyopathy (DCM) is a spectrum of heterogeneous myocardial disorders **characterized morphologicallyby progressive cardiac dilation and functionally by** depressed myocardial contractility (contractile/systolic) dysfunction). The clinicopathologic patterns are similar irrespective of the cause.

Etiopathogenesis

Many of patients with DCM are familial (genetic), but also can result from various acquires causes (both primary and secondary). These are as follows:

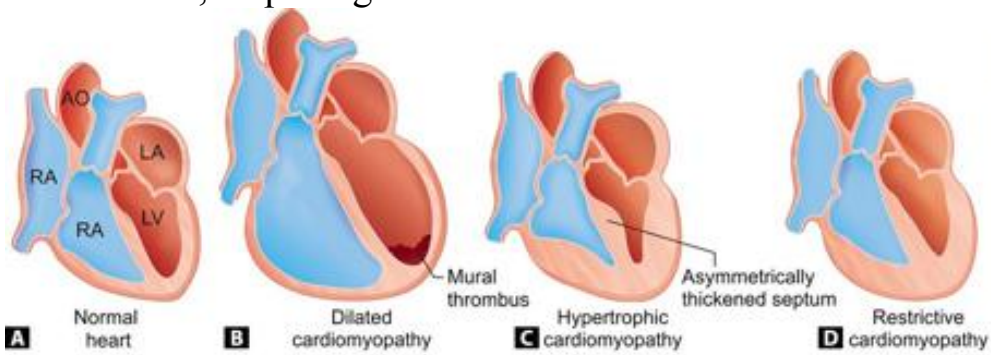
Genetic Causes

It is observed in about 20–50% of DCM. More than 50 genes have been identified that are mutated in DCM. Main mode of inheritance is autosomal dominant pattern.

- **Most common mutations affect cytoskeletal proteins or proteins that link the sarcomere to the cytoskeleton** (e.g., α-cardiac actin).
- X-linked DCM is most frequently associated with mutations in dystrophin. This is a cell membrane protein that physically binds the intracellular cytoskeleton to the extracellular matrix (ECM).

Non-genetic Causes

- **Myocarditis:** It has been documented that myocarditis (e.g., coxsackie B and other viral infections) may progress to DCM at least in some cases. However, its pathogenesis is not clear.



Spider cell: Cardiac rhabdomyoma.

Carney complex is a autosomal dominant syndrome associated with spotty skin pigmentation, cardiac myxoma and tumors of the endocrine glands.

Figs. 16.36A to D: Three major morphologic patterns of cardiomyopathy: (A) Normal; (B) Dilated cardiomyopathy; (C) Hypertrophic cardiomyopathy; (D) Restrictive cardiomyopathy.
(AO: aorta; LA: left atrium; LV: left ventricle; RA: right atrium; RV: right ventricle).

- **Toxins:** Alcohol abuse (alcohol or its metabolites especially acetaldehyde) or its associated secondary nutritional disturbance (e.g., thiamine deficiency leading to beriberi heart disease), chemotherapeutic agents doxorubicin [adriamycin, targeted cancer therapeutics (e.g., tyrosine kinase inhibitors)], cobalt (heavy metal) during inadvertent tainting (e.g., in beer production) can produce DCM.
- **Pregnancy** (peripartum cardiomyopathy).
- **Stress-provoked or tachycardia-induced:** This may occur with persistent tachycardia, hyperthyroidism, or in the fetuses of insulin-dependent diabetic mothers. Other causes include excess catecholamines (e.g., pheochromocytomas), use of cocaine or vasopressor agents (e.g., dopamine).
- **Iron overload**: In the heart, it may occur either due to hereditary hemochromatosis or from multiple transfusions.
- **Idiopathic**

MORPHOLOGY

Gross

Heart is characteristically enlarged, heavy (2–3 times the normal weight) and flabby, with dilation of all chambers.

- Conspicuous hypertrophy of both left and right ventricles is found. But the accompanying dilation, the thickness of ventricular wall may appear less than, equal to, or greater than normal wall thickness (Fig. 16.36B).
- Mural thrombi are usually seen at the apex of left ventricle and may be a source of thromboemboli.
- By definition, the secondary causes that lead to cardiac dilation such as valvular (mitral or tricuspid regurgitation) and vascular lesions (e.g., atherosclerotic coronary artery disease) are absent.

Microscopy

In DCM, microscopic features are nonspecific and usually do not point to a specific etiology.

- Myocytes: Most myocytes show hypertrophy with enlarged nuclei. Many myocytes are attenuated, stretched, and irregular.
- Fibrosis: Variable degree of interstitial and endocardial fibrosis observed. Scattered areas of replacement fibrosis; which indicates previous myocyte ischemic necrosis due to hypoperfusion.
- DCM secondary to iron overload: They show marked accumulation of intramyocardial hemosiderin. This pigment can be demonstrated by staining with Prussian blue (Perls stain) that imparts blue color to hemosiderin.

Clinical Features

Age: DCM can occur at any age, but most common between the ages of **20 and 50 years.**

Initial presentation: It usually present with slowly progressive **signs and symptoms** of CHF. These include dyspnea, easy fatigability, and poor exertional capacity.

At the end stage: Main **defect in DCM is ineffective contraction.** Thus, in end-stage, the cardiac ejection fraction usually is less than 25% (normal is 50–65%). It is common to find secondary mitral regurgitation and abnormal cardiac rhythms. Embolism from intracardiac (mural) thrombi can also occur.

Diagnosis:

- **ECHO:** Shows an ejection fraction (EF) <45% (normal >55%).
- **Chest X-ray:** Global enlargement of the heart.

Prognosis: About 50% of patients die within 2 years and about 25% survive longer than 5 years. Death is usually due to progressive cardiac failure or arrhythmia.

Treatment: Only definitive treatment is cardiac transplantation.

Arrhythmogenic Right Ventricular Cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) **is an autosomal dominant disorder.** It **manifests with right-sided heart failure (**right ventricular failure) **and rhythm disturbances** (such as ventricular tachycardia or fibrillation). It **can cause sudden cardiac death.** It has been observed that sudden deaths in about 10% athletes is due to this disorder.

Etiology: It is mainly due to mutations involving genes encoding desmosomal junctional proteins at the intercalated disk (e.g., plakoglobin), and proteins that interact with the desmosome (e.g., the intermediate filament desmin). It is thought that myocyte death is caused due to detachment of desmosomes, particularly during strenuous exercise.

MORPHOLOGY

The wall of the right ventricle is severely thinned due to the replacement of myocyte by fat and fibrous tissue.

Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is **genetic disorder characterized by** inappropriate **myocardial hypertrophy and defective diastolic filling.** In about one-third of patients there is intermittent ventricular outflow obstruction**.** It is the leading cause of left ventricular hypertrophy that cannot be explained by other clinical or pathologic causes.

Etiopathogenesis

The heart in HCM is thick-walled, heavy, and hypercontractile. This is in contrast to the flabby, poorly contractile heart in DCM. In HCM, myocardium does not relax and therefore shows primary diastolic dysfunction and usually there is preservation of systolic function. HCM has

to be clinically distinguished from disorders causing ventricular stiffness (e.g., amyloid deposition Fabry disease) and ventricular hypertrophy (e.g., aortic stenosis and hypertension).

Genetic causes: In **most cases of HCM,** there is **missense mutations in one of several genes encoding** sarcomeric **proteins that form the contractile apparatus.** In most cases of HCM, the pattern of transmission is autosomal dominant, with variable expression/penetrance. More than 400 causative mutations (most common is missense mutations) in nine different genes have been detected. All these have one common feature namely they all affect sarcomeric proteins and increase myofilament function. This results in hypercontractility of myocytes, increased use of energy, and a net negative energy balance. Of the different sarcomeric proteins mutated, three are most frequently involved and is responsible for 70–80% of HCM. These mutated sarcomere proteins include gene encoding for: (i) **β-myosin heavy chain** (β-MHC), (ii) **myosin-binding protein** C (MYBP-C) and (iii) **troponin T** (α-tropomyosin). Few genes are mutated in both HCM and in DCM (e.g., b-myosin), but in DCM the mutations depress motor function whereas in HCM there is gain of function mutations. The prognosis of HCM varies and strongly correlated with specific mutations. The sequence of events leading from these mutations to clinical and pathologic features of HCM is poorly understood.

MORPHOLOGY

Gross: Heart is always enlarged and shows massive myocardial hypertrophy (extent of hypertrophy varies in different genetic mutations) without dilatation of ventricles dilation **(Fig. 16.36C)**.

- Asymmetric septal hypertrophy: Classical features is disproportionate thickening of the ventricular septum relative to the left ventricle free wall (with a ratio of septum to free wall greater than 3:1). In about 10% of HCM, there is concentric and symmetrical hypertrophy. Though entire septum may show marked hypertrophy, it is usually most marked in the subaortic region.
- Changes in the ventricular wall**:** The left ventricular wall is thick, and its cavity is small. On longitudinal section, the normally round-to-ovoid left ventricular cavity may be compressed to assume a "banana-like" (sometimes only slit) configuration. This is due to bulging of the interventricular septum into the lumen.
- Endocardial mural plaque**:** The thickened, hypertrophied interventricular septum bulges into the left ventricular outflow tract early in ventricular systole. This obstructs the aortic

outflow tract and may produce an endocardial mural plaque in the outflow tract. This plaque is observed at the point where the anterior mitral leaflet contacts (impinges) the septal wall on the of the outflow tract during ventricular systole. There may also be associated thickening of the anterior mitral leaflet.

- Atria: Both atria are commonly dilated.

Microscopy: The characteristic microscopic features of myocardium in HCM are:

- Marked hypertrophy of myocytes, with transverse myocyte diameters frequently greater than 40 μm (normal, approximately 15 μm).
- Myofiber disarray: Haphazard myocyte and myofiber (contractile elements in sarcomeres) disarray, which is most extensive in the interventricular septum. Normally the myocytes are arranged parallelly into muscle bundles. The myofiber disarray is characterized by oblique and often perpendicular orientations of adjacent hypertrophic myocytes.
- Interstitial and replacement fibrosis.

Clinical Features

Age: It can present at any age and usually manifests during the post-pubertal period.

Clinical symptoms: In HCM, there massive left ventricular hypertrophy associated paradoxically with markedly reduced stroke volume. The **reduced stroke volume is due to impaired diastolic filling** and **overall smaller chamber size**. In about 25% of patients, dynamic obstruction to the left ventricular outflow by the anterior leaflet of the mitral valve is observed. Patient may present with exertional dyspnea due to reduced cardiac output and a secondary increase in pulmonary venous pressure. It is associated with a *harsh systolic ejection murmur* on auscultation. Even in the absence of coronary artery disease, patient may develop focal myocardial ischemia (with angina). This ischemia is due to massive hypertrophy, high left ventricular chamber pressures, and thick-walled intramural arteries.

Complications: These include atrial fibrillation (with formation of mural thrombus leading to embolization-stroke), ventricular fibrillation (leading to sudden cardiac death), infectious endocarditis of the mitral valve, and intractable congestive heart failure (CHF). HCM is an important cause of sudden cardiac death (especially in athletes younger than 35 years).

Treatment: In most patients pharmacologic intervention (e.g., β-adrenergic blockade) to decrease heart rate and contractility can be helpful. Reduction

of the septal myocardial mass by surgical excision may relieve the outflow tract obstruction and may be beneficial.

Restrictive Cardiomyopathy

It is the least common type of cardiomyopathy. Restrictive cardiomyopathy **is a group of diseases characterized by impaired ventricular filling during diastole** (the ventricular wall is ***stiffer***), ***while contractile*** (systolic) ***function is normal.*** Because the unaffected contractile function of the left ventricle, the functional abnormality can be confused with that of constrictive pericarditis or HCM.

Etiopathogenesis: This form of cardiomyopathy may be idiopathic or may be associated with systemic diseases that affect the myocardium. The various causes are as follows:

- **Amyloidosis:** It is characterized by the deposition of extracellular proteins with β-pleated sheet configuration in the interstitial tissues (*refer* pages 181-6). Cardiac amyloidosis can occur either as a part of systemic amyloidosis (e.g., multiple myeloma) or predominantly restricted (localized) to the heart (e.g., senile cardiac amyloidosis). In the localized amyloidosis, deposition of normal (or mutant) forms of transthyretin (a circulating protein synthesized by liver that transports thyroxine and retinol) in the hearts of older adult patients. This can result in a restrictive cardiomyopathy. Immunoglobulin light-chains in AL-type amyloid can also be cardiotoxic and induce myocardial dysfunction.
- **Endomyocardial fibrosis:** It is mainly seen in children and young adults in Africa and other tropical areas. It is characterized by dense diffuse fibrosis of the endocardium and subendocardium of ventricles. There may be fibrosis of the tricuspid and mitral valves. The fibrous tissue severely reduces the volume of affected chambers. Endomyocardial fibrosis may be due to nutritional deficiencies and/or inflammation related to helminthic infections (e.g., hypereosinophilia). It is the most common form of restrictive cardiomyopathy.
- **Loeffler endomyocarditis:** It is also associated with endocardial fibrosis with formation of large mural thrombi similar to those found in tropical disease mentioned above. However, it has no geographic or population predilection. In addition, it shows peripheral hypereosinophilia and tissue

infiltration by eosinophils. Probably the eosinophil granule content (especially major basic protein) released from eosinophils produces necrosis of endocardium and myocardium. This is followed by scarring, formation of thrombus over-damaged endocardium and finally organization of thrombus. In few patients, there may be hypereosinophilic myeloproliferative neoplasm caused by gene rearrangements [either the platelet-derived growth factor receptor (PDGFR)-α or -β genes]. This rearrangement produces fusion genes which constitutively express active tyrosine kinases. In these patients treatment with tyrosine kinase inhibitors can result in remission of both hematological changes and reversal of endomyocardial lesions which is otherwise appears rapidly fatal.

- **Endocardial fibroelastosis:** It is an uncommon heart disease characterized by fibroelastic thickening of the left ventricular endocardium. It is most common during first 2 years of life. In one-third of patients there may be aortic valve obstruction or other congenital cardiac anomalies. Endocardial fibroelastosis may be a common morphologic endpoint of several different disorders such as viral infections (e.g., intrauterine exposure to mumps) or mutations in the gene for tafazzin (affects mitochondrial inner membrane integrity). Diffuse involvement can lead to cardiac decompensation and death.
- **Other causes** include radiation fibrosis, sarcoidosis, metastatic carcinoma, or products/metabolites of inborn errors of metabolism (e.g., hemochromatosis).

MORPHOLOGY

Gross (16.36D)

The morphologic features are not distinctive. The ventricles are of almost of normal size or only slightly enlarged. The myocardium is firm and noncompliant and the ventricular cavities are not dilated. Dilatation of both atria is commonly found as a consequence of restricted ventricular filling and pressure overloads.

Microscopy

It shows variable degrees of patchy or diffuse interstitial fibrosis. Endomyocardial biopsy can reveal a specific etiology.

NC: Describe the etiology, pathophysiology, pathology features and complications of syphilis on the

cardiovascular system (*refer* pages 92 and 462).

PERICARDIAL DISEASE

Pericardial diseases are usually associated with a pathologic process elsewhere in the heart or surrounding structures, or are secondary to a systemic disorder. Isolated pericardial disease is unusual. The most important pericardial diseases cause accumulation of fluid (pericardial effusions), inflammation (pericarditis), and inflammatory conditions, fibrous constriction or combination of these processes.

PERICARDITIS AND PERICARDIAL EFFUSION

CC: Describe the etiology, pathophysiology, pathology, gross and microscopic features, diagnosis and complications of pericarditis and pericardial effusion.

Pericarditis

Definition: Pericarditis is the inflammation (or infiltration by cancer) of the pericardium encasing the heart (epicardial surface, parietal surface).

Etiology: It can be primary or secondary. The major causes of pericarditis are listed in **Box 16.10**.

- **Primary pericarditis** is very rare. It is typically due to viral infection and produce acute pericarditis. Tuberculosis and fungi produce chronic pericarditis.

BOX 16.10: Causes of pericarditis.

- Infectious agents**:** Viruses, pyogenic bacteria, tuberculosis, fungi, parasites
- Presumably immunologically mediated**:** Rheumatic fever, systemic lupus erythematosus, postmyocardial infarction (Dressler) syndrome
- Miscellaneous: Acute myocardial infarction, uremia, metastatic malignancies, radiation to the mediastinum, infectious processes involving other thoracic structures (e.g., pneumonia or pleuritis).

BOX 16.11: Classification of pericarditis.

Acute pericarditis

- Serous pericarditis
- Fibrinous and serofibrinous pericarditis
- Purulent or suppurative pericarditis
- Hemorrhagic pericarditis

Chronic pericarditis

- Tuberculous (caseous) pericarditis
- Chronic constrictive pericarditis
- Chronic or healed pericarditis
- Adhesive mediastinopericarditis

- **Secondary pericarditis** is most common type. It can occur secondary to a variety of disorders and uremia is the most common systemic disorder associated with pericarditis.

Classification of pericarditis: It may be classified as acute or chronic pericarditis **(Box 16.11)**:

Acute Pericarditis

It is the most frequent type of pericarditis. Depending on the morphological features (gross and microscopic characteristics) on of the pericardial surfaces and fluid, it can be divided into several types **(Box 16.11)**. The majority of cases of acute pericarditis are of uncertain etiology.

Clinical features of acute pericarditis: It presents with atypical chest pain (not related to exertion and worse in recumbency), and a prominent friction rub. When acute pericarditis is accompanied by significant fluid in the pericardial cavity, it can cause **cardiac tamponade**, which leads to declining cardiac output and consequent shock.

Serous Pericarditis

It may be accompanied by serous effusion.

Etiology: It is caused by:

- Non-infectious inflammatory diseases, e.g., rheumatic fever, and SLE
- Uremia
- Infectious processes involving other contiguous thoracic structures near the pericardium (e.g., pneumonia or bacteria pleuritis).
- Rarely viral infection elsewhere (e.g., upper respiratory tract infection, pneumonia, parotitis) may antedates the pericarditis and may be the

primary focus of infection.

- Very rarely viral pericarditis can develop as a primary infection along with *myopericarditis* in young adults.
- Tumors can produce a serous pericarditis by lymphatic invasion or direct contiguous extension into the pericardium.

Microscopy

Serous pericarditis may show mild inflammatory infiltrate of neutrophils and lymphocytes in the epipericardial fat. Pericarditis associated with metastatic malignancies may also show neoplastic cells. Usually it resolves and very rarely it may undergo organization causing fibrous adhesions of pericardium.

Fibrinous and Serofibrinous Pericarditis

It is the most common type of pericarditis and the pericardial cavity shows serous fluid mixed with variable amount of fibrinous exudate.

Etiology: Common causes of fibrinous and serofibrinous pericarditis are listed in **Box 16.12**.

MORPHOLOGY

Fibrinous pericarditis: Normal smooth, glistening pericardial surfaces are replaced by dull, fine granular roughening with fibrin-rich exudate. It produces an irregular, shaggy appearance (Figs. 16.37 and 16.30) to the pericardial surface ("bread and butter" pericarditis). Effusion fluid is fibrinous and usually rich in protein, and the pericardium contains mainly mononuclear inflammatory cells.

Serofibrinous pericarditis: It shows accumulation of larger amounts of yellow to brown turbid fluid, containing leukocytes, RBCs and fibrin.

Outcome: Fibrin may be lysed and inflammation may resolve, or can become organized.

Clinical features: Fibrinous pericarditis presents with pain (sharp, pleuritic, and position dependent) and fever. The rough inflamed pericardial surfaces produce a characteristic loud pericardial friction rub on auscultation.

Purulent or Suppurative Pericarditis

It is usually accompanied by purulent exudate that resembles pus (full of neutrophils) in the pericardial cavity.

Etiology: It is due to infection caused by microbial (pyogenic bacteria) invasion of the pericardial space. The various routes of infection are as follows:

- Direct extension of infections from neighboring structures: Examples include empyema of the pleural cavity, lobar pneumonia, mediastinal infections, etc.
- Blood spread

BOX 16.12: Common causes of fibrinous and serofibrinous pericarditis.

- Acute myocardial infarction (MI)
- Postinfarction (Dressler) syndrome (autoimmune type that develops days-weeks after an MI)
- Uremia
- Chest radiation
- Rheumatic fever, SLE
- Trauma
- Following routine cardiac surgery

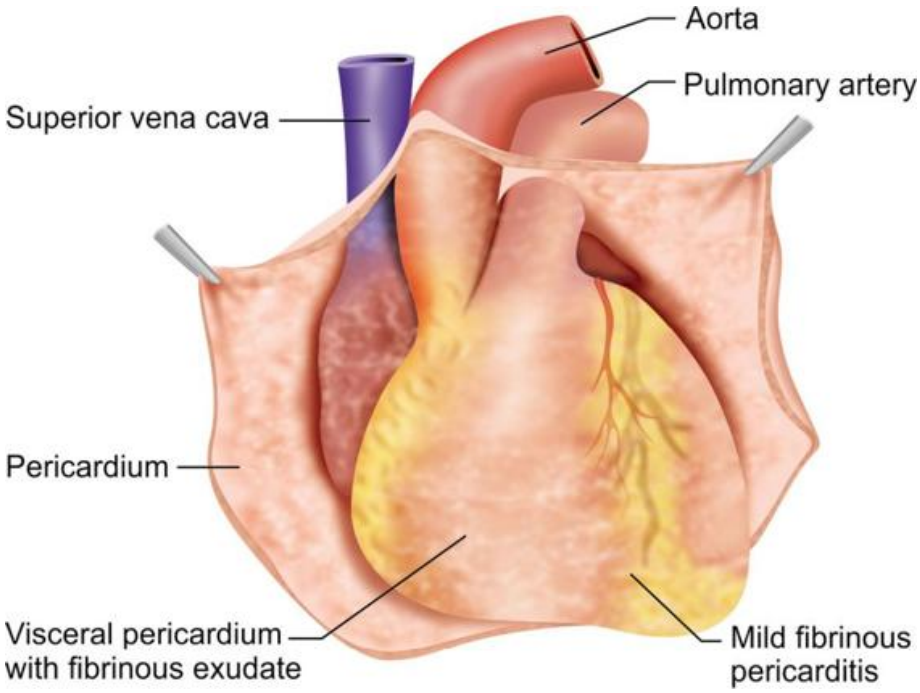


Fig. 16.37: Fibrinous pericarditis (diagrammatic).

- Lymphatic extension
- Direct infection during cardiotomy

MORPHOLOGY

Gross

The serosal surfaces of pericardium appear red, granular, and covered with the exudate. The exudate may range from a thin cloudy fluid to frank pus. The amount of exudate may be up to 400–500 mL.

Microscopy

The pericardium shows acute inflammatory infiltrate and may extend into surrounding structures to produce mediastinopericarditis.

Outcome: Complete resolution is uncommon and organization by scarring is the usual outcome. The intense inflammatory response and the scarring may lead to constrictive pericarditis.

Clinical features: During active phase, they are similar to fibrinous pericarditis. However, the frank infection can produce more marked systemic symptoms such as fever and rigors.

Hemorrhagic Pericarditis

This type of pericarditis shows an exudate composed of blood mixed with a fibrinous or suppurative effusion.

Etiology: Its causes include:

- **Spread of a malignant neoplasm** to the pericardial space is the most common cause. Cytologic examination of pericardial fluid removed through a pericardial tap often shows neoplastic cells. Most common tumors that involve the pericardium and cause malignant pericardial effusions are breast and lung carcinomas.
- **Other causes:** Bacterial infections in patients suffering from bleeding diathesis, tuberculosis, and following cardiac surgery.

MORPHOLOGY

Pericarditis due to malignancy may show an exuberant, shaggy fibrinous exudate and a bloody effusion. Metastases may be grossly found as irregular excrescences or may be grossly inapparent, especially in the case of leukemia.

Clinical features: Similar to fibrinous or suppurative pericarditis.

Outcome and complications of acute pericarditis:

- **Resolution:** It may resolve without significant sequelae

- **Cardiac tamponade:** Pericarditis can cause immediate hemodynamic complications if it there is accumulation of large amount of effusion resulting in cardiac tamponade.
- **Progression to a chronic fibrosing pericarditis**.

Chronic Pericarditis

Various types of chronic pericarditis are listed in **Box 16.11.**

Tuberculous (Caseous Pericarditis)

Etiology: Caseous pericarditis unless otherwise proved is due to tuberculosis. Very rarely, fungal infections may produce similar reaction. Pericardial involvement in tuberculosis may occur by direct spread from tuberculous foci within the tracheobronchial nodes.

MORPHOLOGY

Gross

The exudate is caseous or blood stained and tubercles may be observed on the pericardial surface.

Microscopy

It shows typical tuberculous granuloma. Granuloma consists of central caseous necrosis surrounded by epithelioid cells, Langhan's giant cells, lymphocytes (refer Figs. 2.17, 3.9 and 17.17). They are surrounded by fibrous tissue.

Complications: Caseous pericarditis may progress to a disabling, fibrocalcific, chronic constrictive pericarditis.

Chronic Constrictive Pericarditis

In chronic pericarditis, if delicate adhesions or dense, fibrotic scars occurs, it can cause obliteration of the pericardial space. When this process is severe, the heart may be completely encased by dense, fibrous or fibrocalcific scar. In such case, the heart cannot expand normally during diastole and there will be reduction in the cardiac output. This condition is called as constrictive pericarditis. These features resemble those of a restrictive cardiomyopathy.

Definition: Chronic constrictive pericarditis is a chronic fibrosing disease of the pericardium (scarring of the pericardial sac) that compresses the heart and limits the ability of the chambers in the heart to fill with blood (i.e., restricts inflow of blood to the heart).

Etiology: Causes include prior radiation therapy to the mediastinum, cardiac surgery, sequalae of a purulent or tuberculous infection of pericardium.

Pathogenesis: Constrictive pericarditis is not an active inflammatory condition. It occurs as an exuberant healing after acute pericardial injury. The visceral and parietal layers of pericardium become fused by a dense, rigid mass of fibrous tissue. This leads to obliteration of the pericardial space. The scarred pericardium may be thickened (up to 3 cm) in such a way that it narrows the orifices of the venae cavae. The fibrous tissue may show calcified areas. Because of the dense fibrous scar neither cardiac hypertrophy nor dilation can occur.

MORPHOLOGY

Heart is small and pericardium is thickened due to dense fibrosis. The fibrous scar can be up to a centimetre in thickness and obliterates the pericardial cavity/space. Sometimes calcification may be observed and in extreme cases it can resemble a plaster mold (concretio cordis).

Clinical features: Constrictive pericarditis may mimic right heart failure. Patients have small, quiet hearts and venous inflow is restricted. The rigid pericardium limits the diastolic volume of the heart. Thus, its clinical features are due to a combination of right-sided venous distention and low cardiac output (similar to the clinical features in restrictive cardiomyopathy). Cardiac output may be reduced at rest and the heart is not capable of increase its output in response to increased systemic demands. Signs of constrictive pericarditis include distant or muffled heart sounds, raised jugular venous pressure (JVP), small pulse pressure, low cardiac output, and fluid retention with ascites and peripheral edema. There may or may not be a previous history of pericarditis.

Treatment consists of surgical resection of the shell of constricting fibrous tissue (total pericardiectomy).

Chronic or Healed Pericarditis

In some cases of chronic pericarditis, organization produces either a plaque-like fibrous thickenings of the serosal membranes ("soldier's plaque") or thin, delicate adhesions. They do not produce any impairment of cardiac function.

Adhesive pericarditis: It is a milder form of healing of an inflamed pericardium. It is usually found as an incidental finding at autopsy. It is the outcome of many different types of pericarditis. The pericarditis after healing produces fibrosis in the form of mesh-like stringy adhesions between the visceral and parietal surfaces. This may completely obliterate the pericardial sac; but has no effect on cardiac function.

Adhesive Mediastinopericarditis

Etiology: It may follow infectious pericarditis, previous cardiac surgery, or mediastinal irradiation.

Pathophysiology: In this type of pericarditis, the pericardial sac is obliterated, and the external aspect of the parietal layer of pericardium is adherent to surrounding structures. This produces strains on the cardiac function. During each systolic contraction, the heart pulls both the parietal pericardium and the attached surrounding structures.

Clinical features: Theses include, systolic retraction of the rib cage and diaphragm, pulsus paradoxus, etc. The increased workload may occasionally lead to severe cardiac hypertrophy and dilation.

Pericardial Effusion and Hemopericardium

Normally, the pericardial sac contains less than 50 mL of pericardial fluid. It is thin, clear, straw-colored.

Definition: Pericardial effusions are defined as accumulations of excess fluid within the pericardial cavity.

The fluid accumulated may be either a transudate or an exudate**.** If the accumulated fluid is serous fluid it is termed pericardial effusion, if pus termed purulent pericarditis and if blood is accumulated it is termed as hemopericardium. Pericardial effusion can occur in all forms of pericarditis.

Pericardial Effusions

Etiology: Various types and the causes of pericardial effusions include are presented in **Table 16.11**.

Rate of accumulation of fluid: If the pericardium is slowly distended (long-standing) with fluid, the pericardium has time to dilate. This allows chronic pericardial effusions to accommodate even up to 2 L of fluid without any significant hemodynamic consequences or interference with cardiac function. Clinically, chronic effusions of less than 500 mL may produce only a characteristic globular enlargement of the heart shadow on chest radiograph. However, if the fluid accumulation is rapid, even 150–200 mL of fluid or blood [e.g., due to hemopericardium caused by a ruptured myocardial infarction (MI) or aortic dissection] may significantly increase intrapericardial pressure. This restricts diastolic filling, especially of the right atrium and ventricle. This can lead to fatal cardiac tamponade.

Cardiac Tamponade

TABLE 16.11: Various types of pericardial effusion and their causes.

Type of pericardial effusion	*Causes*
Serous effusion (low protein content and few cells)	Congestive heart failure, hypoalbuminemia of any cause (e.g., nephrotic syndrome)
Serosanguineous (mixture of serous fluid and blood)	Blunt chest trauma (either accidentally or after cardiopulmonary resuscitation), malignancy, ruptured MI, or aortic dissection
Chylous effusion (fluid containing chylomicrons)	Communication of the thoracic duct with the pericardial space due to mediastinal lymphatic obstruction by tumor or infection
Hemopericardium (bleeding directly into the pericardial cavity)	Ventricular free wall rupture in myocardial infarct, penetrating cardiac trauma, rupture of a dissecting hematoma of the aorta, infiltration of a vessel by tumor, a bleeding diathesis

Definition: It is a syndrome caused due to rapid accumulation of pericardial fluid, that restricts the filling of the heart. Tamponade refers to external compression of the heart chambers such that

filling is impaired.

Pathophysiology: Rapid accumulation of fluid in the pericardial cavity raise the pericardial pressure. When the pressure increase exceeds central venous pressure, the return of blood to the heart is limited. This in turn reduces cardiac output and blood pressure, and leads to **pulsus paradoxus** (an abnormal decrease in systolic pressure with inspiration).

Consequences: Hemodynamic consequences can range from a minimal symptom to sudden cardiovascular collapse and death.

Beck's triad: It consists of three classic findings in cardiac tamponade—namely (i) hypotension, (ii) distended neck veins, and (iii) muffled heart sounds.

Acute cardiac tamponade is almost always fatal unless the pericardial pressure is relieved by aspirating/removing offending pericardial fluid, via needle pericardiocentesis or surgery.

Diagnosis of Pericardial Effusion

- Chest radiograph shows a "water bottle" configuration
- ECHO is useful for detection of pericardial effusion and to determining the amount of fluid
- ECG may be useful
- Pericardial fluid analysis is similar to other body fluids (*refer* pages 402-5).

CARDIAC MYXOMA

Q. Write short note on cardiac myxoma.

- Myxomas are the **most common primary cardiac tumor** of the adult heart.
- They are **benign tumors** arising from primitive multipotent mesenchymal cells.
- **Site:** About 90% of myxomas arise in the atria, **more common in the left atrium** in the region of the fossa ovalis in the atrial septum.
- **Number: Usually single**, but can rarely be multiple.
- **Size:** Range from **small (<1 cm) to large (≥10 cm).**

MORPHOLOGY

Gross

Sessile or pedunculated. Vary from globular hard masses with hemorrhage to soft, translucent, papillary, or villous lesions having a gelatinous appearance. The pedunculated type may be mobile during systole into the atrioventricular valve producing intermittent obstruction.

Microscopy (Fig. 16.38)

Composed of stellate or globular myxoma cells embedded within an abundant acid mucopolysaccharides ground substance.

- **Clinical features:** Mainly due to **valvular "ball-valve" obstruction, embolization**, or constitutional symptoms, such as fever and malaise. Treatment is by surgical removal.

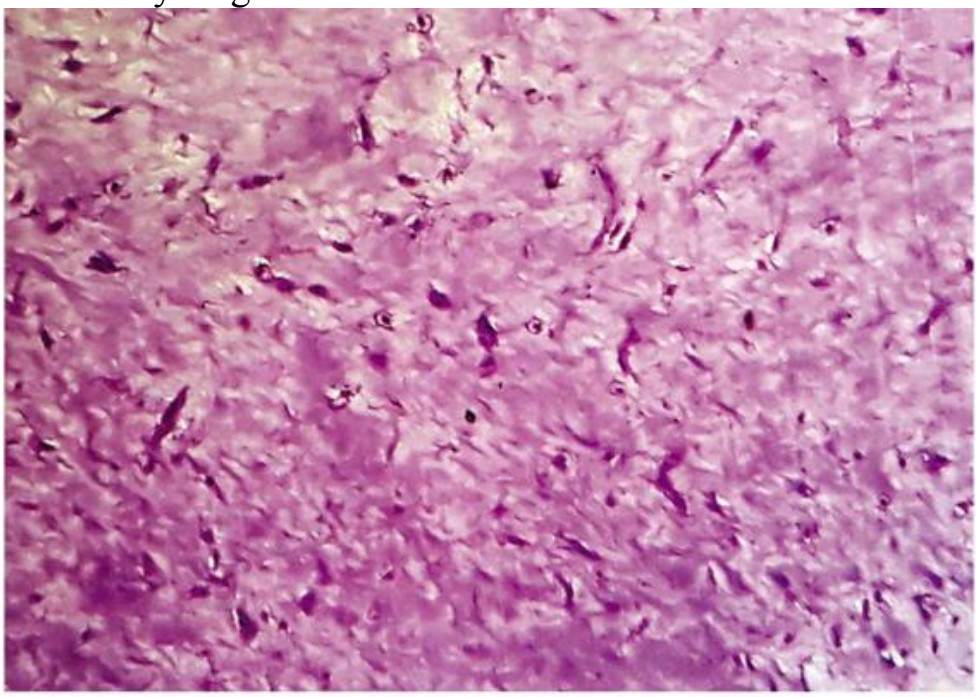


Fig. 16.38: Cardiac myxoma composed of stellate myxoma cells abundant myxoid substance.

CHAPTER
17

Respiratory Disorders

OBSTRUCTIVE LUNG DISEASES

CC: Define and describe the etiology, types, pathogenesis, stages, morphology and complications and evaluation of obstructive airway disease (OAD).

Q. Define and enumerate chronic obstructive pulmonary diseases. Describe the pathogenesis and morphology of emphysema lung.

Definition: Chronic obstructive pulmonary/lung disease (COPD) is defined as a disease state characterized by airflow limitation that is not fully reversible.

Obstructive lung diseases consist of four diseases namely (i) emphysema, (ii) chronic bronchitis, (iii) asthma and (iv) bronchiectasis. Two diseases namely (i) emphysema and (ii) chronic bronchitis are grouped together under **chronic obstructive pulmonary disease (COPD).**

EMPHYSEMA

Q. Define emphysema. Write the types of emphysema and describe its pathogenesis.

Emphysema is defined on the basis of morphological and radiological features. Different parts of airway is presented in **Figure 17.1**.

Definition: Emphysema is a **chronic lung disease** characterized by abnormal **irreversible** (permanent) **dilatation of the airspaces distal to the terminal bronchiole**. This is associated with destruction of their walls but without obvious fibrosis. Recent evidences show that small airway fibrosis is present which contributes to airflow obstruction.

Types of Emphysema/Classification(Fig. 17.1)

Q. Classify/mention the types of emphysema.

Emphysema is **classified according to its anatomic distribution** (location of the lesions) within the lobule into **four major types**: (i) centriacinar, (ii) panacinar, (iii) paraseptal and (iv) irregular.

1. **Centriacinar (centrilobular) emphysema**
   - Dilatation **involve the central or proximal parts of the acini** (formed by respiratory bronchioles), whereas distal alveoli are spared. Thus, both emphysematous and normal airspaces are present within the same acinus and lobule.
   - **Site:** Common and severe in the **upper lobes**, especially in the **apical segments**.
   - **Association:**
     - Occurs in **heavy smokers** and in association with **chronic bronchitis** and **coal-worker's pneumoconiosis**.
     - Walls of the emphysema often show **black pigment**.
     - **Inflammation around bronchi and bronchioles** is common.
2. **Panacinar (panlobular) emphysema**
   - **All the airspaces beyond terminal bronchiole** are more or less uniformly/equally dilated.
   - **Site:** In contrast to centriacinar emphysema, more common in the **lower lobes**, and is usually **most severe at the bases**.
   - **Association:** With **α_1-antitrypsin (α_1-AT) deficiency**.
3. **Distal acinar (paraseptal) emphysema**
   - Dilatation affects the **distal airspace** at the periphery of the lobule and the proximal portion is normal.
   - **Dimension**: It forms multiple, enlarged airspaces ranging **from <0.5 to >2.0 cm in diameter**. **Dilated spaces of >1 cm in size** are known as **bullae** (*refer* **Fig.17.3B**) which may rupture and cause spontaneous pneumothorax.
   - **Site:** More common **adjacent to septa**, **pleura** and at the **margins of the lobules**. **More severe in the upper lobes**.
   - **Association:** It results from forces pulling on the septa and occurs **adjacent to areas of fibrosis, scarring, or atelectasis**.

4. **Irregular (scar or cicatricial) emphysema**
   - Acinus is **irregularly involved** and may be asymptomatic.
   - **Most common form** of emphysema.
   - **Association: Occurs near the scar** and is commonly found **around old healed inflammatory process like tuberculous scars.**

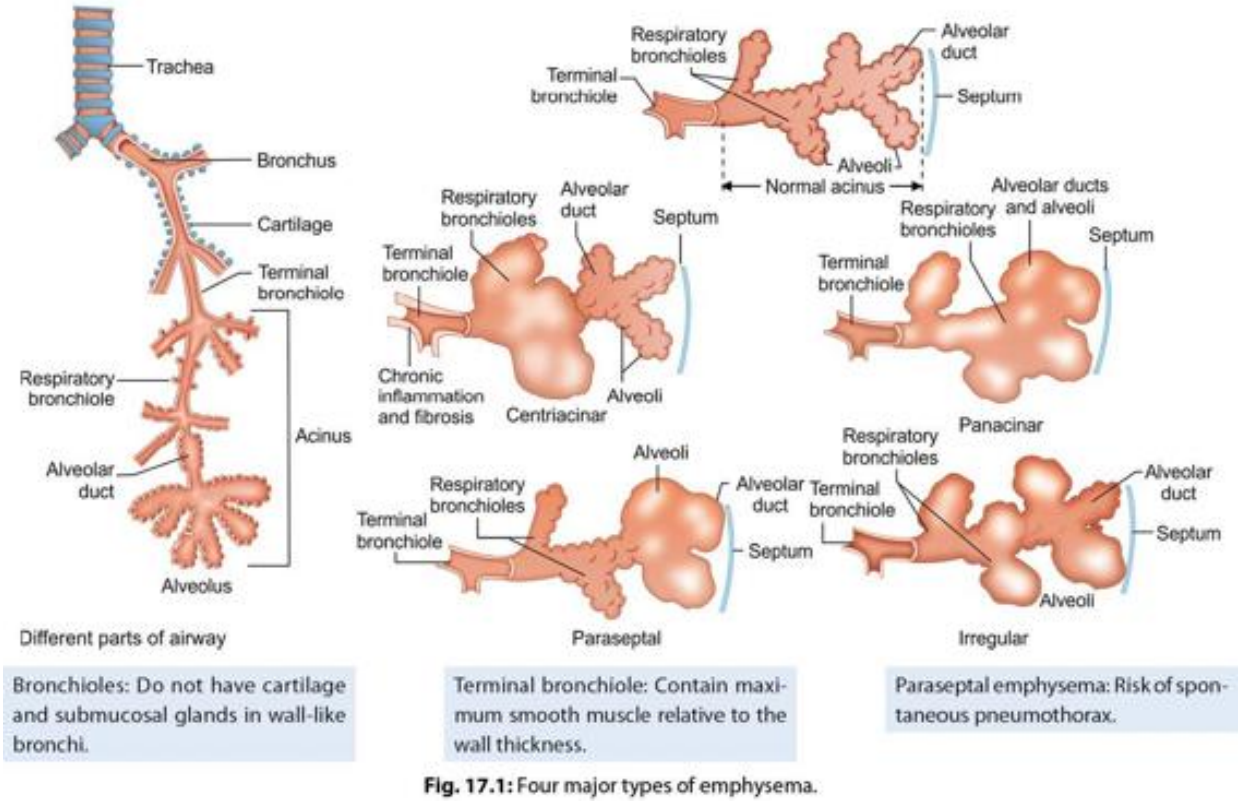


Fig. 17.1: Four major types of emphysema.

Etiology and Pathogenesis (Fig. 17.2)

Q. Etiopathogenesis of emphysema.

Clear-cut association between heavy cigarette smoking and development of emphysema is observed. Inhaled cigarette smoke and other toxic substances produce damage to the lung and produces inflammation. This leads to destruction of the lung parenchyma (emphysema) and disease of the airway (bronchiolitis and chronic bronchitis). The major event in emphysema is destruction of alveolar wall. Factors involved in the pathogenesis of emphysema are as follows:

1. **Inflammatory mediators and leukocytes**
   - **Inflammatory mediators:** Many inflammatory mediators are released in the lung (e.g., leukotriene B4, IL-8, TNF). The sources of these mediators are resident epithelial cells and macrophages.

- **Attraction of leukocytes:** Chemotactic factors (e.g., leukotriene) attract inflammatory cells from the circulation and exaggerate the inflammatory process (proinflammatory cytokines) and produce structural changes (due to growth factors).

2. **Protease-antiprotease imbalance:** Injury (e.g., by smoking or other toxic substances) activates and favors accumulation of inflammatory cells (e.g., neutrophils) and damages the epithelial cells. This leads to local release of proteases by inflammatory cells and damaged epithelial cells. The proteases can damage the connective tissue. Normally, the damage by proteases is prevented by antiprotease (e.g., α_1-antitrypsin is a major inhibitor of proteases). About 1% of patients with emphysema have deficiency of protective antiproteases.

   - **Genetic deficiency of α_1-antitrypsin:** α_1-antitrypsin is normally present in serum, tissue fluids and macrophages. A balance is maintained between protease and antiproteases. α_1-antitrypsin is encoded by a gene in the proteinase inhibitor (*Pi*) locus on chromosome 14. The *Pi* locus is polymorphic, and individuals homozygous for the Z allele, have markedly decreased serum levels of α_1-antitrypsin. This deficiency is inherited as autosomal recessive disorder. In these patients with absence of α_1-antitrypsin activity, there is excessive digestion of elastic tissues which produce emphysema. The damage is aggravated by smoking.

3. **Oxidative stress:** Contents of tobacco smoke, damage to the alveoli (apoptosis) and inflammatory cells produce oxidants. They cause further damage to the tissues and aggravate inflammation.

4. **Airway infection:** Though not involved in initiation of emphysema bacterial and/or viral infections, may exacerbate the existing inflammatory process and chronic bronchitis.

Small airways obstruction in emphysema: It is due to:

- Loss of elastic tissue in the walls of alveoli that surround respiratory bronchioles.

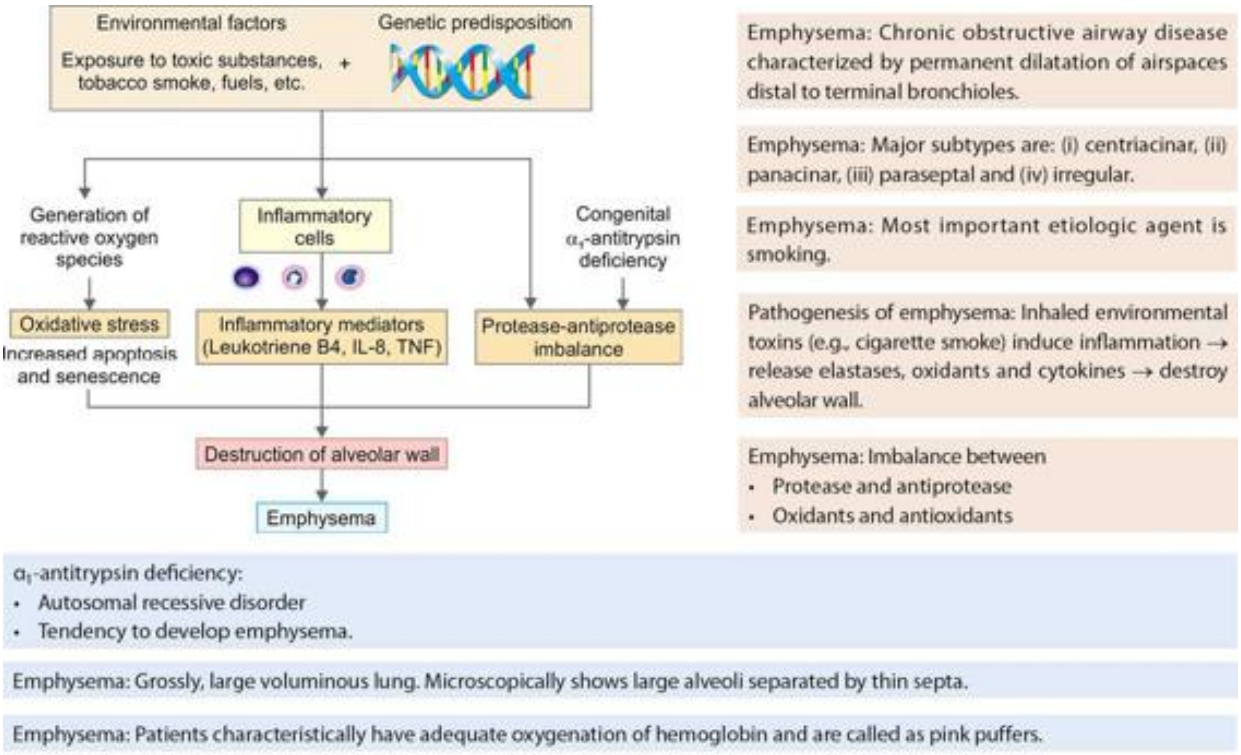


Fig. 17.2: Pathogenesis of emphysema. Exposure to environmental toxins (e.g., cigarette smoke) causes inflammatory reaction, cell death and proteolysis of extracellular matrix (ECM). α_1-antitrypsin (α_1-AT) deficiency also results in increased degradation of ECM. (IL-8: interleukin 8; TNF: tumor necrosis factor).

- Small airway inflammation may narrow the bronchiolar lumen.

MORPHOLOGY

Gross (Figs. 17.3A to C)

- Usually, **the upper two-third of the lungs** are more severely involved.
- **Bullae** are found in irregular and distal acinar emphysema.
- Advanced emphysema produces **voluminous lungs.**
- Emphysema can easily be seen on the cut surface of formalin-inflated fixed lung.

Microscopy (Figs. 17.4A and B)

- Lung shows **abnormally large alveoli** separated by thin septa. There is destruction of alveolar walls and loss of attachments of the alveoli to the outer wall of small airways. The pores of Kohn are enlarged.
- In advanced disease, **bullae may be seen**.
- There may be inflammatory changes in small airways.

Clinical Course

Manifestations appear late until at least one-third of the functioning pulmonary parenchyma is damaged.

- **Dyspnea** begins insidiously and steadily progresses.
- **Cough and expectoration**.
- **Weight loss** is common.

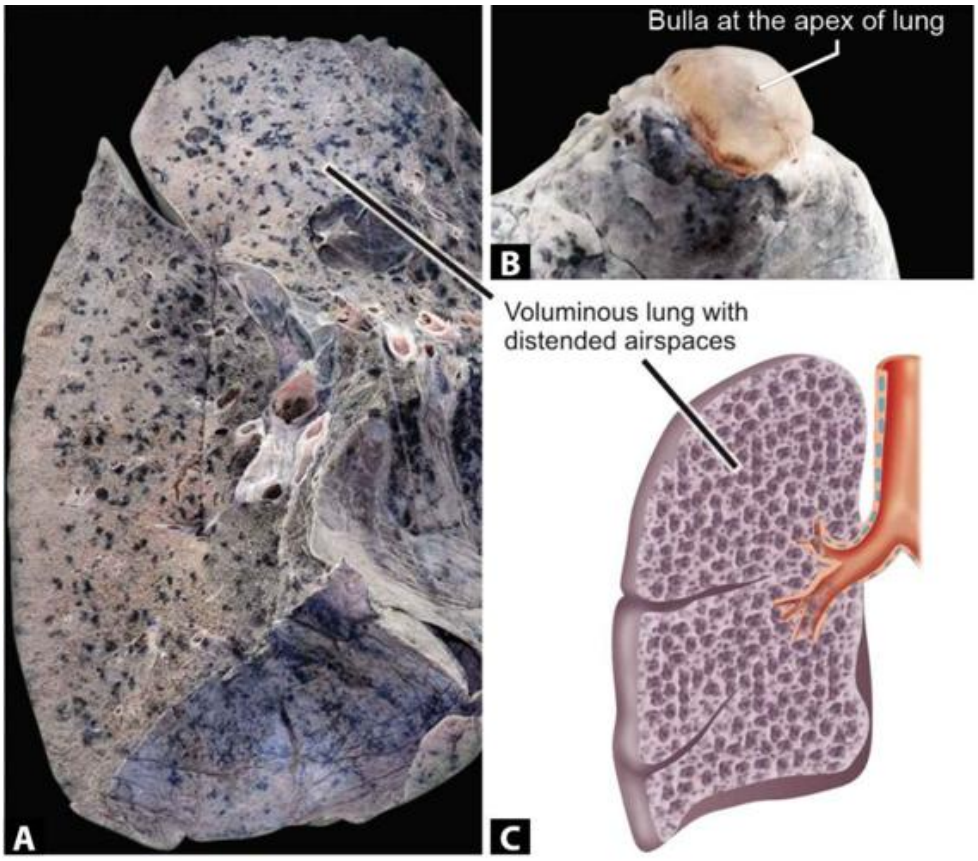
Clinical examination: **Barrel-shaped chest**, dyspnea with prolonged expiration, and breathes through pursed lips. Expiratory airflow limitation is helpful in diagnosis.

Complications: (i) **Cor pulmonale,** (ii) **congestive heart failure** due to secondary pulmonary hypertension and pneumothorax.

Cause of death: (i) Respiratory acidosis and coma, (ii) right-sided heart failure and (iii) massive collapse of the lungs secondary to pneumothorax.

Other Forms of Emphysema

- **Compensatory hyperinflation: It is characterized by dilation of alveoli without destruction of septal walls**. It develops as a compensatory response to loss of lung substance elsewhere (e.g., removal of a diseased lung or lobe).
- **Bullous emphysema (Fig. 17.3B):** The air**spaces>1 cm in diameter** in the distended state are known as blebs or bullae. They can occur in any type of emphysema. They **may rupture and produce pneumothorax**.



Figs. 17.3A to C: Voluminous lung in emphysema; (A) Cut section shows voluminous lung; (B) Part of lung bulla on the apex of lung; (C) Cut section (diagrammatic).

- **Interstitial emphysema:** It is characterized by the **entry of air into the connective tissue stroma** of the lung, mediastinum, or subcutaneous tissue. It may result from alveolar tears in pulmonary emphysema, which occurs during coughing.

Centriacinar emphysema:
- Involves proximal parts of acini (spares alveoli)
- Severe in upper lobes
- Smokers
- Seen as most common type clinically

Panacinar emphysema:
- Involves all the airspaces beyond terminal bronchiole (entire acinus)
- More common and severe in lower lobes
- Associated with α1-antitrypsin deficiency

Panacinar emphysema: Prefix “pan” refers to the entire acinus but not to the entire lung.

Distal acinar (paraseptal) emphysema: Involves distal air spaces and are prone to spontaneous

rupture → pneumothorax.

Irregular (scar or cicatricial) emphysema:
- Acinus is irregularly involved
- Associated with scars/fibrosis
- Histologically most common type

Bulla: Distended air spaces more than 1 cm in diameter

CHRONIC BRONCHITIS

Definition: Chronic bronchitis is defined clinically as **persistent cough with sputum** production (chronic productive cough) **for at least 3 months in at least 2 consecutive years**, in the absence of any other identifiable cause. It is defined on clinical basis in contrast to emphysema which is defined on the basis of morphologic and radiologic features.

Etiology

- **Cigarette smoking:** It is the most important risk factor.
- **Air pollutants:** Sulfur dioxide, nitrogen dioxide.
- **Toxic industrial inhalants**: Occupational dust exposure.
- **Respiratory tract infection**: It may initiate and promote chronic bronchitis.

Pathogenesis

Q. Discuss the pathogenesis of chronic bronchitis.

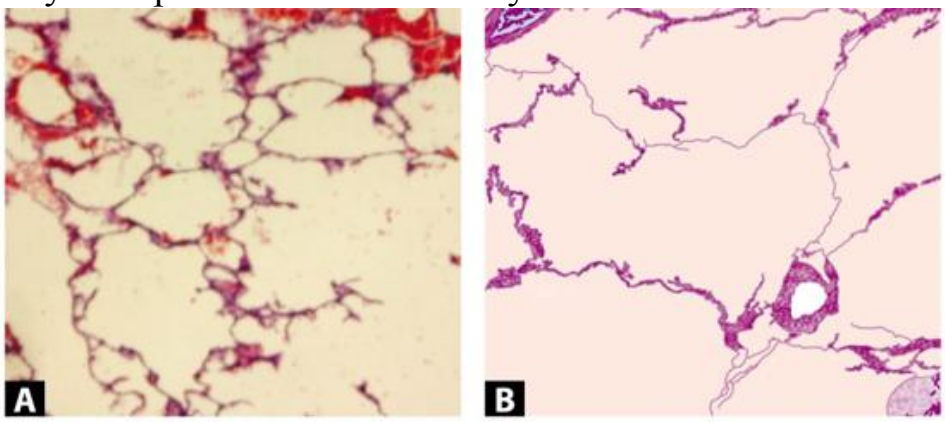
- **Irritation by inhaled air pollutants:** The initiating factor is irritation of large airways mucosa by inhaled air pollutants, such as **tobacco smoke** (90% of patients are smokers), **dust**, **cotton**, **sulfur dioxide**, **nitrogen dioxide** and **silica**. Irritants cause inflammation → infiltration by CD8+ T lymphocytes, macrophages and neutrophils.
- **Hypersecretion of mucus**:
  - **Hyperplasia/hypertrophy of the submucosal glands** in **large airways (trachea and bronchi)**: Develops in response to inhaled **environmental irritants** and **proteases released from neutrophils** (e.g., elastase, cathepsin histamine and IL-13). This leads to **hypersecretion of mucus**.
  - **Marked increase of goblet cells in small airways (small bronchi and bronchioles): They produce excessive mucus** → mucus

plugging of bronchial lumen → inflammation and fibrosis of bronchial wall → **leads to airway obstruction**.

Both the submucosal gland hypertrophy and the increase in goblet cells are thought to be protective metaplastic response against air pollutants (e.g., tobacco smoke, sulfur dioxide and nitrogen dioxide).

Role of Inflammation

Inhalants producing chronic bronchitis cause damage to cells and cause acute as well as chronic inflammation (neutrophils, lymphocytes and macrophages). Chronic inflammation and accompanying fibrosis involving small airways can produce chronic airway obstruction.



Figs. 17.4A and B: Microscopy of emphysema with destruction of alveolar walls forming large alveoli. (A) H & E; (B) Diagrammatic.

Role of Infection

- Cigarette smoke predisposes to infection in several ways.
- **Infection may be secondary** rather than primary and probably important in maintaining and producing acute exacerbations.
- **Infection may cause direct damage to airway epithelium** and interferes with ciliary action of the respiratory epithelium → **defective clearance of bacteria** by leukocytes.

MORPHOLOGY

Gross

Airways (large and small) changes include:

- **Mucous membrane:** It shows **hyperemia**, **swelling and edema**. It appears dusky red.

- **Lumen:** They are filled with excessive **mucus or mucopurulent** secretion.

Microscopy (Fig. 17.5)

Major characteristic features are as follows:

- **Hyperplasia and hypertrophy of mucus-secreting glands in the submucosa:** This results in **shift of type of gland from normal mixed seromucus to pure mucus** in the trachea and bronchi → results in **hypersecretion of mucus**.

Q. Write a short note on Reid index and its importance.

- **Reid index**: It is the **ratio of the thickness of the mucus gland layer to** the **thickness of the wall** between the base of the surface epithelium and the inner limit of the cartilage plates. It is useful for detecting the increase in the size and number of the mucus glands. Reid index (normally 0.4) is increased in chronic bronchitis.
- **Chronic inflammation of the airways** with predominant infiltration by lymphocytes.
- **Surface epithelium**: May show **mild increase in the number of goblet cells** and focus of **squamous metaplasia**.
- **Marked narrowing of bronchioles:** It is due to:
  - **Excess mucus in the airways.**
  - **Thickening of the bronchial wall** due to hyperplasia of mucus glands, edema, inflammation, and fibrosis.
  - In severe cases, there may be obliteration of lumen due to fibrosis (**bronchiolitis obliterans**).

Clinical Features

Usually affects **middle-aged men** who are **heavy smokers**.

- **Early symptoms**: **Persistent productive cough** (sputum) for many years.
- **Later stage**:
  - **Dyspnea on exertion**
  - **Blue bloaters**: Patients develop **hypercapnia, hypoxemia, and mild cyanosis**. Such patients are called ‘blue bloaters’.
  - **Pink puffers**: These patients have more emphysema than bronchial obstruction. Therefore, they **hyperventilate to produce** a relatively **normal blood gas profile**.

Complications

- Progression to **chronic obstructive pulmonary disease** (COPD).
- Long-standing cases lead to **cor pulmonale** with cardiac failure.

- May develop squamous **metaplasia and dysplasia** of the respiratory epithelium → a rich soil for cancerous transformation.

Chronic bronchitis: Persistent productive cough for at least 3 months in at least two consecutive years, in the absence of any other identifiable cause.

Chronic bronchitis: Cigarette smoking is the most important etiological risk factor.

Chronic bronchitis: Microscopically shows:
- Hyperplasia of submucosal glands
- Increase in goblet cells
- Squamous metaplasia
- Fibrosis of bronchiolar walls

Chronic bronchitis: Reid index (normal 0.4) is increased.

Reid index: Ratio of mucus gland layer thickness to the thickness of wall between epithelium and cartilage.

Chronic bronchitis:
- Blue bloater
- Retain carbon dioxide
- Develop cyanosis

Emphysema:
- Pink puffer
- Blow off carbon dioxide

ASTHMA

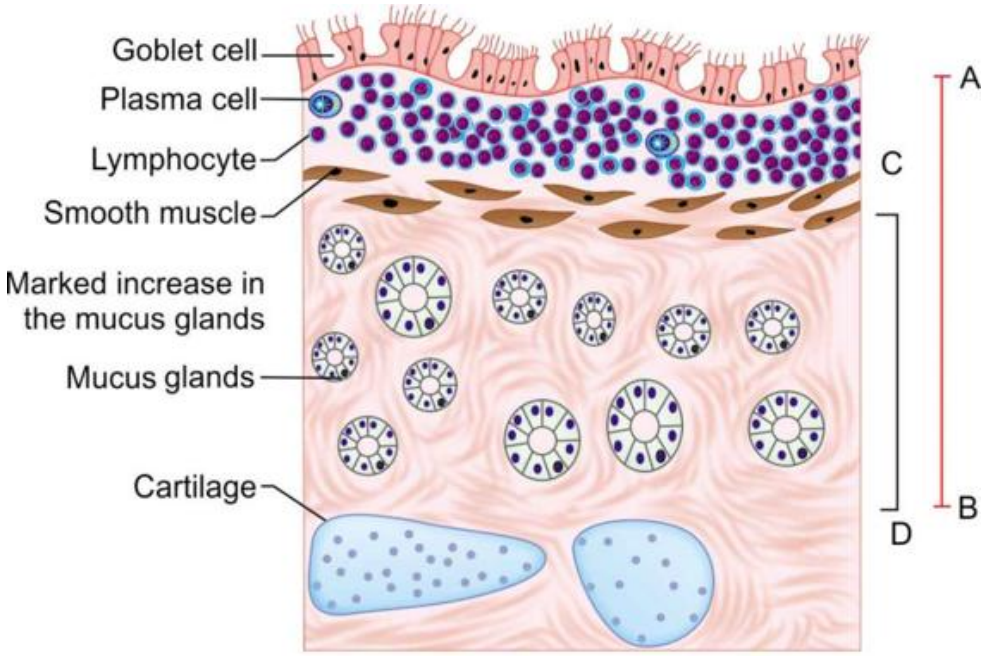


Fig. 17.5: Microscopic features of chronic bronchitis. The bronchial submucosa is markedly expanded by hyperplasia of submucosal glands and constitutes more than 50% of the thickness of the bronchial wall. The Reid index equals the maximum thickness of the bronchial mucus glands internal to the cartilage (C to D) divided by the bronchial wall thickness (A to B).

Q. Classify bronchial asthma. Discuss the pathogenesis of atopic bronchial asthma. Add a note on the sputum findings in bronchial asthma.

Definition: Asthma is a **chronic inflammatory disorder** of the bronchial tree (airways) in which **breathing is periodically rendered difficult** by **widespread narrowing of the bronchi** (reversible bronchoconstriction). It is **clinically characterized by recurrent episodes of wheezing, breathlessness, tightness of the chest and cough**.

Asthma is common, but is relatively rare cause of death. It usually develops at night and/or in the early morning.

Characteristics

- **Episodes of bronchoconstriction—partly reversible**, either spontaneously or after treatment.
- **Inflammation of the bronchial walls**.

- **Increased mucus secretion.**

Classification (Box 17.1)

Q. Classify bronchial asthma.

BOX 17.1: Classification of asthma.

According to type of antigen:
1. **Atopic** (allergic/extrinsic)
2. **Non-atopic/intrinsic** (without evidence of allergen sensitization)

According to the agents or events that trigger bronchoconstriction:
1. Seasonal
2. Exercise-induced
3. Drug-induced (e.g., aspirin)
4. Occupational asthma
5. Asthmatic bronchitis in smokers

Common Types of Asthma

Atopic Asthma

- **Most common type** of asthma that usually begins in **childhood**.
- **Family history of asthma or allergic diseases** (e.g., eczema, urticaria, or hay fever) is common.
- Classic example of **type I IgE-mediated hypersensitivity reaction**.
- Exogenous substances (**allergens**) causing asthma **can be recognized**.
- **Triggering environmental allergens**: It includes **dusts**, **pollens**, animal dander and foods.
- **Skin test** with the causative allergen **results in an immediate wheal-and-flare reaction**.

Non-atopic Asthma

- **No causative exogenous factors** can be identified and it does not show allergen sensitization.
- **Skin tests** are usually **negative**.
- **Family history** of asthma is **less common**.
- **Triggering events**:
  - **Respiratory infections due to viruses** (e.g., rhinovirus, parainfluenza virus).

- **Inhaled air pollutants** (e.g., smoke, fumes).

- It may be **due to hyperirritability of the bronchial tree**.

Drug-induced Asthma

Many drugs may trigger asthma (e.g., aspirin-sensitive asthma). Aspirin inhibits cyclooxygenase pathway of arachidonic acid metabolism → produces leukotrienes (bronchoconstrictor) → causes asthma.

Occupational Asthma

Triggering occupational agents:

- **Fumes** (epoxy resins, plastics).
- **Organic and chemical dusts** (wood, cotton, platinum).
- **Gases** (toluene).
- **Other chemicals** (formaldehyde, penicillin products).

Etiology

Q. Discuss the pathogenesis of asthma.

Endogenous Risk Factors

- **Genetic predisposition:** Major etiological factor in atopic asthma is genetic predisposition to **type I hypersensitivity (atopy) reaction** and exposure to environmental trigger. One of the **susceptibility locus is on the chromosome 5 (5q)** → several genes involved in regulation of IgE synthesis and mast cell and eosinophil growth and differentiation.
- **Airway hyperresponsiveness**: It is an abnormality in which there is **excessive tendency for airways to contract** (bronchoconstrictor) too easily in response to multiple inhaled triggers that usually does not have any effect on normal individuals.
- **Gender**: More common in boys than girls and, after puberty, women slightly more commonly than men.
- **Age:** Most cases **begin before the age of 25 years**.

Environmental Risk Factors: Asthma Triggers

Several stimuli trigger asthmatic attacks:

- **Inhaled allergens:** These include house dust, mites and house hold pets. They may cause allergic sensitization and asthma in early childhood.
- **Upper respiratory tract viral infections** (e.g., rhinovirus, respiratory syncytial virus and coronavirus).
- **Air pollution** (e.g., sulfur dioxide, ozone and diesel particulates). Indoor pollutants, such as exposure to nitrogen oxides from cooking stove.
- **Passive smoking**: It may trigger asthma.
- **Drugs** (e.g., β-adrenergic blockers and aspirin).

Hygiene Hypothesis

It proposes that individuals with **lack of infections in early childhood** are more prone to asthma than children brought up on farms who are exposed to a high level of endotoxin. Intestinal parasite infection may also be associated with a decreased risk of asthma.

Pathogenesis of Atopic Asthma (Figs. 17.6A and B)

Q. Discuss the pathogenesis of atopic bronchial asthma.

Major Etiological Factors in Atopic Asthma

- **Genetic predisposition** to **type I hypersensitivity** (atopy).
- Acute and chronic **airway inflammation.**
- Hyperresponse bronchi to various **environmental antigens (allergens)**. These include respiratory infections, airborne irritanats, cold air, stress, exercise, etc.

Inflammation involves many cell types and inflammatory mediators. **Genetic predisposition** with susceptibility genes makes individuals prone to develop **strong T_H2 reactions against environmental antigens** (allergens).

- **T_H2 cells secrete cytokines:** These cytokines promote allergic inflammation and **stimulate B cells to produce IgE** and other antibodies. The cytokines include:
  - **IL-4: Stimulates** the **production of IgE**.
  - **IL-5: Activates eosinophils.**

- **IL-13: Stimulates mucus secretion** from bronchial submucosal glands and **promotes IgE production by B cells**.

Steps in Pathogenesis

Sensitization

- **Genetically predisposed** individuals are sensitized against many **environmental antigens** (allergens).
- The allergen is taken by antigen presenting cells (macrophage/dendritic cell) and **elicits a T_H2-dominated response**. This promotes **production of IgE by B cells**.
- IgE produced during sensitization **coats bronchial submucosal mast cells**. IL-5 secreted by T_H2 cells and mast cells **recruit eosinophils**. Allergen and IL-13 stimulates bronchial submucosal glands to secrete mucus.

On re-exposure

The allergens binds to IgE bound to the bronchial submucosal mast cells → activates mast cells → immediately **release** bronchoconstrictor **mediators from the mast cell granules**. Mast cells releases **preformed mediators and produce cytokines** → responsible for the early-phase (immediate hypersensitivity) reaction and the late-phase reaction.

- **Early reaction:** It is characterized by **bronchoconstriction** (histamine, prostaglandin D2 and leukotrienes LTC4, D4 and E4 and reflux vagal nerve stimulation), **increased mucus production**, and **vasodilation with increased vascular permeability** (causes edema). These are produced either directly by the **mediators** released from mast cells **or** through **stimulation of vagal receptors** in the subepithelium.
- **Late-phase reaction:** It is characterized by:
  - **Inflammation:** Recruitment of leukocytes (eosinophils, neutrophils, basophils, lymphocytes and monocytes) takes place by fresh mediators released by mast cells, epithelial cells and leukocytes. Example, (i) eotaxin—produced by airway epithelial cells, is a potent chemoattractant and activator of eosinophils, (ii) chemokines promote recruitment of more T_H2 cells, and (iii) major basic protein of

eosinophils causes damage to epithelial cells and more constriction of airway.

- **Airway remodeling** (*refer* page 501).

Refer type I hypersensitivity reactions on pages 147-9.

MORPHOLOGY

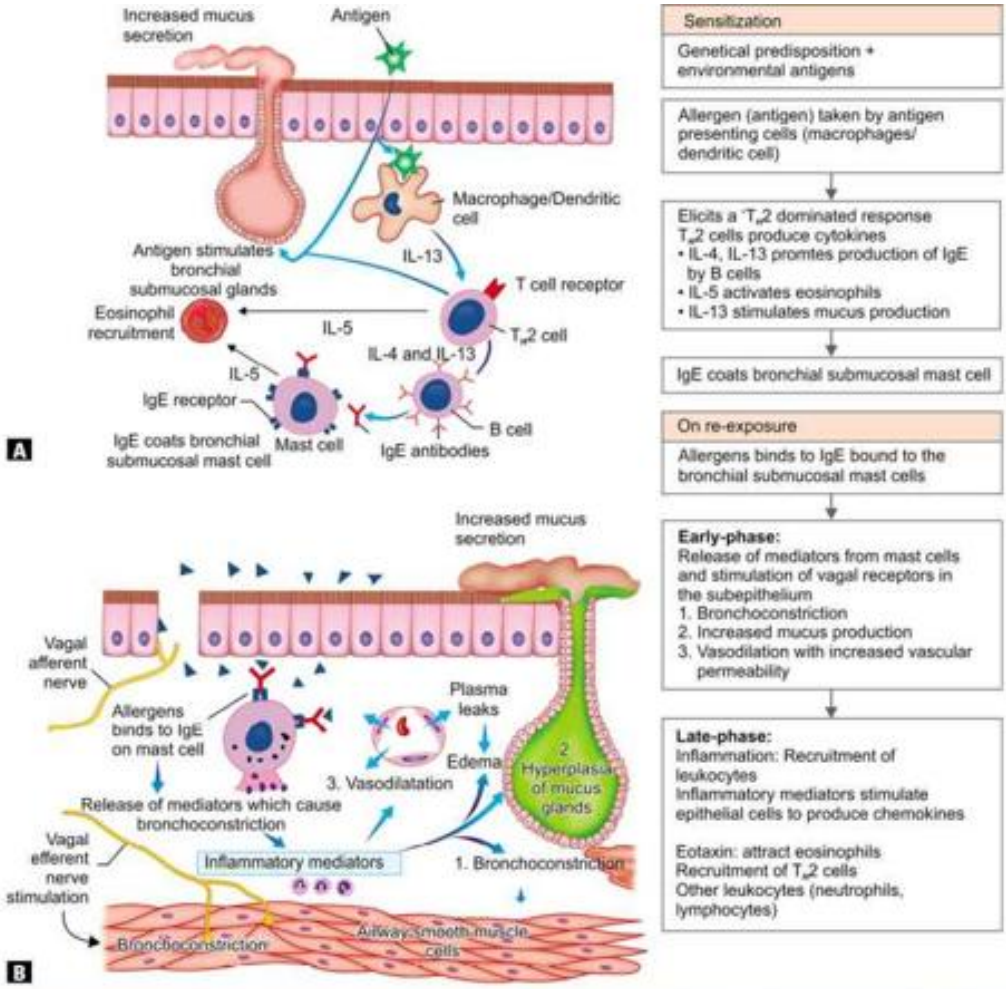
Gross

- **Airways** (bronchi and bronchioles) are **occluded by plugs** of thick, tenacious mucus.
- In patients who die due to status asthmaticus, the lungs are greatly distended due to overinflation, and shows small areas of atelectasis (collapse).

Microscopy

Major processes which contribute to the narrowing of airways are as follows:

1. **Increased amount of mucus forms mucus plugs** and are seen in the bronchioles or bronchi. **Mucus plug contains desquamated epithelial cells and eosinophils and forms spiral-shaped cast of the airways** and are called **Curschmann spirals**. These may be found in the sputum.



Asthma: Disorder of the bronchial tree character ized by reversible bronchoconstriction in response to various stimuli.

Extrinsic asthma:
- Family history
- Type I hypersensitivity reaction

Intrinsic asthma:
- No family history
- Normal serum IgE levels
- Non-immune

Atopic asthma: Caused by T_H2 and IgE-mediated type I hypersensitivity reaction to environmental antigens known as allergens.

Atopic asthma: T_H2 cells secrete cytokines IL-4, IL-5 and IL-13 that are important mediators.

IL-13 gene polymorphism: Strongly associated with bronchial asthma.

Atopic asthma: Two main steps namely sensitization and reactions (early and late).

Asthma: Eosinophils are the most important inflammatory cells in most of the types of asthma.

Asthma: Airway damage caused by major basic protein secreted by eosinophils.

Charcot–Leyden crystals: Crystalloid derived from an eosinophil lysophospholipase binding protein called galectin-10.

Curschmann spirals: Mucus plug containing desquamated epithelial cells and eosinophils and forms spiral-shaped cast of the airways.

Three Cs of sputum findings in asthma:
1. Curschmann spirals
2. Charcot–Leyden crystals
3. Creola bodies

Figs. 17.6A and B: Pathogenesis of asthma: (A) Sensitization: Inhaled allergens (antigen) are taken up by antigen presenting cells (macrophage/dendritic cell) and elicit a T_H2-dominated response favoring IgE production and eosinophil recruitment; (B) On re-exposure to antigen (allergen), they bind to IgE bound on mast cells and release preformed mediators from mast cells. These mediators, either directly or via vagal stimulation produce bronchospasm, increased vascular permeability, and mucus production.

2. **Inflammation: Numerous eosinophils** are found in the walls of the the bronchi and bronchioles. **Charcot–Leyden crystals**, which are **crystalloid derived from an eosinophil lysophospholipase** binding protein called galectin-10 are also found. There is also edema and congestion of the airways.
3. **Airway remodeling:** It is the **group of structural changes in the bronchial wall due to repeated bouts of inflammation** and consists of:
   - An **increase in size and number** (hypertrophy/hyperplasia) of the **submucosal glands.**
   - **Hypertrophy and/or hyperplasia of the bronchial wall smooth muscle.**
   - **Increased vascularity.**
   - **Deposition of subepithelial collagen** accompanied by fibrosis and thickening of the basement membrane.

Clinical Course

- Acute asthmatic attack usually lasts **up to several hours**.
- In some **mild degree of chest tightness, dyspnea, wheezing and cough** with or without sputum production, may constantly be present.
- **Between the asthmatic attacks**, patients may be **asymptomatic**.
- **Status asthmaticus:** It is the **most severe form of asthma** in which the severe acute paroxysm persists for days and even weeks. The **bronchoconstriction does not respond to the drugs**. It may cause **severe airflow obstruction leading to severe cyanosis and even death**.

Diagnosis

- Demonstration of an increase in airflow obstruction (from baseline levels).
- Difficulty with exhalation (prolonged expiration, wheeze).
- **Elevated eosinophil count** in the peripheral blood.

Q. Add a note on sputum findings in bronchial asthma.

- **Sputum:** It is viscous and yellow and is **rich in eosinophils**. Microscopy:
  - **Charcot–Leyden crystals** are mainly seen in atopic asthma. They have the shape of a pair of **long, narrow, six-sided pyramids placed base-to-base**.
  - **Curschmann spirals** are **corkscrew-shaped twist of condensed mucus several centimeters long** that is usually **surrounded by an elongated mass of clear or opalescent material**.

- **Creola bodies** are **compact clusters or strips of columnar epithelial cells** shed from the bronchus.

BRONCHIECTASIS

CC: Define and describe the etiology, types, pathogenesis, stages, morphology and complications of bronchiectasis.

Q. Define bronchiectasis. Discuss the etiopathogenesis, morphology (gross and microscopy) and complications of bronchiectasis.

Definition: Bronchiectasis is a disorder characterized by **irreversible** (permanent), **abnormal dilation of bronchi and bronchioles**. Bronchiectasis may be either a focal (localized) or a diffuse (generalized) process.

Etiology

Q. Discuss the etiopathogenesis of bronchiectasis.

The dilatation of bronchi and bronchioles is caused by **destruction of the muscle and elastic tissue of bronchial wall**. It represents a secondary disorder as the end stage of many unrelated disorders. It may be divided into obstructive and nonobstructive (postinflammatory).

Nonobstructive (Postinflammatory) Bronchiectasis

It is **usually of diffuse type** characterized by dilatation throughout the lung.

Postinfectious

It may be the result or associated with **chronic necrotizing infections**.

- **Bacteria** (*Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas*)
- **Viruses** [adenovirus, influenza virus, human immunodeficiency virus (HIV)].

- **Fungi** (*Aspergillus* species).

Focal nonobstructive bronchiectasis usually develops as a **complication of childhood infections, such as measles and pertussis**.

Genetic Causes

Q. Write a short note on Kartagener's syndrome.

- **Kartagener or immotile cilia syndrome (primary ciliary dyskinesia):** It is an **autosomal recessive** syndrome with **lack of ciliary function** (due to absence of inner or outer dynein arms of cilia) and causes retention of secretions and recurrent infections. It comprises of **sinusitis**, **bronchiectasis**, **dextrocardia** (with or without situs inversus) and **male infertility** (sperm dysmotility).
- **Young's syndrome**: Patients develop bronchiectasis, sinusitis and obstructive azoospermia. The cause is not known.
- **Other dyskinetic ciliary syndromes:** These have deficient cilia throughout the body and include radial spoke deficiency (Sturgess syndrome) and absence of the central doublet of cilia.

Immunodeficiency

Patients with hypogammaglobulinemia, HIV infection, bronchiolitis obliterans after lung transplantation have increased susceptibility to infections → localized or diffuse bronchiectasis.

Autoimmune and Immune-mediated Diseases

- Autoimmune diseases (e.g., rheumatoid arthritis, Sjögren's syndrome, systemic lupus erythematosus, inflammatory bowel disease).
- Immune-mediated disease (e.g., allergic bronchopulmonary aspergillosis).

Obstructive Bronchiectasis

It is **localized** to the obstructed segment of the lung.

Airway/Bronchial Obstruction

Partial or total obstruction of the bronchial lumen: It may be caused by tumor, foreign body aspiration and mucus plugs. It may also develop in patients with atopic asthma and chronic bronchitis.

Cystic Fibrosis

Major respiratory diseases in cystic fibrosis (CF) are sinusitis and bronchiectasis.

Pathogenesis (Flowchart 17.1)

It is **complex process**. Both **obstruction and chronic persistent infection** are the two major etiological conditions seem to be necessary for the development of bronchiectasis.

1. **Obstruction:** It **impairs clearing mechanisms** of the lung → results in **accumulation of secretions** distal to the obstruction → **leads to secondary infection** → inflammation → weakens and dilates airway.
2. **Chronic persistent necrotizing infection:** Chronic infection in the bronchi or bronchioles → increased bronchial secretion → **obstruction** of airways by secretions → inflammation and fibrosis of the airway walls → weakening and dilatation of airways.

Bronchiectasis associated with cystic fibrosis: It shows the **importance of both infection and obstruction.** In cystic fibrosis there is defect in the mucociliary action and there is accumulation of thick viscid secretions → leads to obstruction of the airways → favors microbial infections. With repeated infections the walls of airway are damaged and lead to dilatation of bronchi and bronchioles.

In patients with asthma and cystic fibrosis hypersensitivity reaction to the fungus may cause allergic bronchopulmonary aspergillosis. This may predispose to proximal bronchiectasis.

MORPHOLOGY

Q. Write short note on gross and microscopic pathology of bronchiectasis.

Classification

A. According to the shape of the bronchial dilation (Figs. 17.7A to D):

1. **Saccular (cystic) bronchiectasis**: It is characterized by markedly dilated bronchi which end blindly in dilated sacs.
2. **Cylindrical (tubular) bronchiectasis:** It shows uniform and moderate dilation.
3. **Fusiform:** Characterized by fusiform dilatation.
4. **Varicose bronchiectasis:** It shows irregular dilations and constrictions. They resemble varicose veins when seen by radiologic bronchography.

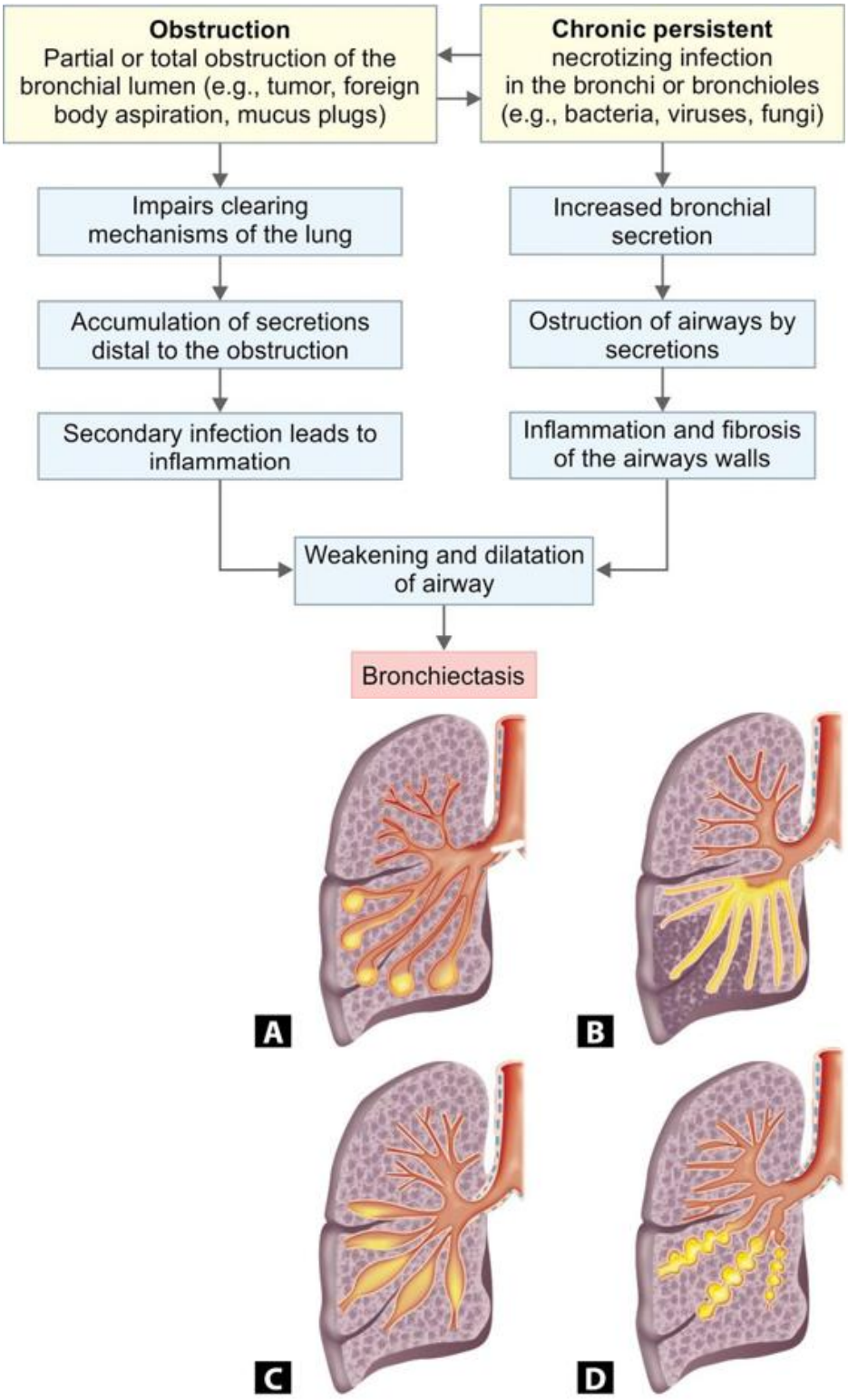
B. According to the extent of involvement:

1. **Generalized:** It is **usually bilateral** and commonly affects the **lower lobes**. **Left lobe is more commonly involved than the right**. It is most severe in the more distal bronchi and bronchioles.
2. **Localized**: It is **restricted to a single segment of the lung** and usually occurs in association with obstruction (tumors or aspiration of foreign bodies).

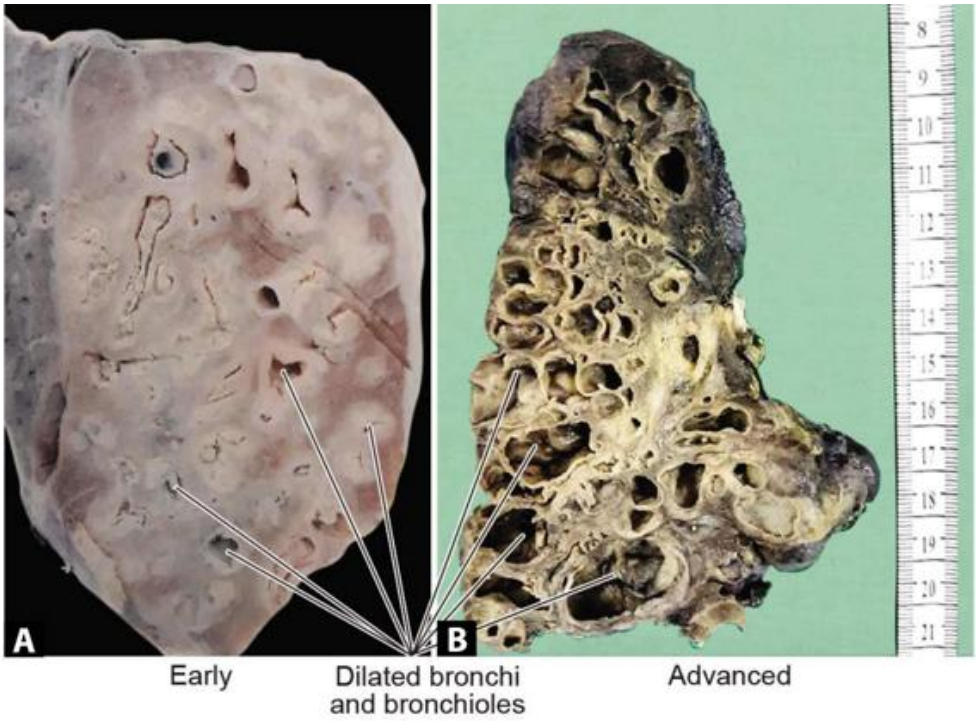
Gross (Figs. 17.8A and B)

- **Bronchi and bronchioles** are **dilated** and **can be traced almost up to the pleural surfaces.**
- **Extent of dilatation:** It may be up to four times normal lumen/size.
- **Cut surface**: The **dilated bronchi and bronchioles** appear as saccular, cylindrical or varicose (depending on the type). The **wall of the bronchi is thickened** by fibrosis. The **lumen is filled with thick**, **mucopurulent secretions** and the mucosal surface is congested.

Flowchart 17.1: Pathogenesis of bronchiectasis.



Figs. 17.7A to D: Morphological types of bronchiectasis: (A) Saccular; (B) Cylindrical type; (C) Fusiform type; (D) Varicose type.



Figs. 17.8A and B: Gross appearance (cut section) of bronchiectasis with dilated bronchi and bronchioles.

Microscopy (Figs. 17.9A and B)

Varies with the activity and chronicity of the disease.

Bronchial Wall

- **Dilatation of bronchi and bronchioles** and destruction of their wall due to severe inflammation. If severe → may lead to lung abscess.
- **Dense infiltration of chronic inflammatory cells:** It mainly consists of **lymphocytes and plasma cells** within the **walls of the bronchi and bronchioles**. Sometimes, prominent lymphoid follicles may be seen.
- **Fibrosis of the bronchial and bronchiolar wall:** It occurs in the chronic cases→ may result in partial or complete obliteration of bronchiolar lumens.

Mucosal Changes

- **Desquamation of the lining epithelium** and areas of ulceration are seen.
- **Lining columnar cells** may show **pseudostratification** and squamous **metaplasia in the surrounding epithelium**.

Microbes: Culture of content from the involved bronchi **may show mixed microbes**. These include staphylococci, streptococci, pneumococci, enteric organisms and (particularly in children) *Haemophilus influenzae* and *Pseudomonas aeruginosa*.

In allergic bronchopulmonary aspergillosis, fungal hyphae can be seen on special stains.

Clinical Course

- **Severe persistent productive cough**: It is the most common clinical feature.
- **Sputum is foul-smelling**, sometimes bloody. In severe cases, the patient presents with dyspnea and orthopnea.
- Symptoms are precipitated by upper respiratory tract infections.
- **Paroxysms of cough** occur **when the patient rises in the morning**. This is because, the changes in position lead to drainage of collections of pus and secretions into the bronchi.

Bronchiectasis: Characterized by irreversible, abnormal dilation of bronchi and bronchioles.

Reversible bronchial dilation may be seen in viral and bacterial pneumonia.

Kartagener or immotile cilia syndrome:
- Autosomal recessive
- Lack of ciliary function
- Sinusitis, bronchiectasis, dextrocardia (with or without situs inversus) and male infertility (sperm dysmotility)

Bronchiectasis:
- Obstruction
- Chronic persistent necrotizing infection

Normal lung: Bronchioles cannot be traced by ordinary gross dissection beyond a point 2–3 cm from the pleural surfaces.

Bronchiectasis: Dilated airways can be traced up to the pleura.

Q. Write short note on complications of bronchiectasis.

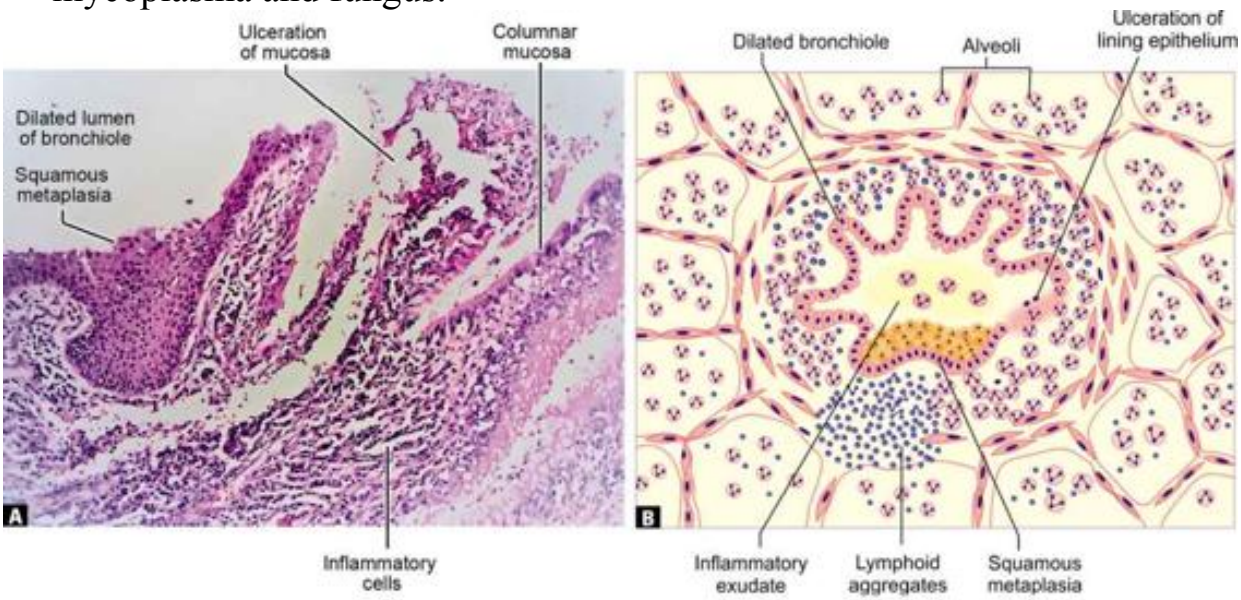
Complications:
- Lung abscess
- Pneumonia
- Empyema
- Septicemia
- Meningitis, osteomyelitis
- Metastatic abscesses (e.g., brain)
- Cor pulmonale, aspergilloma, hemoptysis, respiratory failure
- Generalized amyloidosis.

PULMONARY INFECTIONS

Respiratory tract infections are the most frequent infections.

Causes

- **Upper respiratory tract infections**: Majority are caused by viruses (common cold, pharyngitis).
- **Lower respiratory tract infections**: It may be due to bacteria, virus, mycoplasma and fungus.



Microscopy of bronchiectasis: Bronchi and bronchioles show

- Dilatation
- Desquamation of lining epithelium
- Fibrosis
- Dense chronic inflammation
- Squamous metaplasia

Figs. 17.9A and B: Microscopic features of bronchiectasis: (A) H&E; (B) Diagrammatic.

PNEUMONIA

CC: Define and describe the etiology, types, pathogenesis, stages, morphology and complications of pneumonia.

Definition: Pneumonia is defined as **infection of the lung parenchyma**. It causes the alveoli to be filled with inflammatory exudates and usually results in consolidation (solidification) of lung.

Etiology

Q. Etiology and pathogenesis of lobar pneumonia.

Factors Favoring Development of Pneumonia

- **Lowered systemic resistance of the host**: It may be due to
  - **Chronic diseases.**
  - **Immunological deficiency:**
    - Defects in innate immunity and humoral immunodeficiency.
    - Defects in cell-mediated immunity (congenital and acquired).
  - **Treatment with immunosuppressive agents**.
  - **Leukopenia**.
- **Impaired local defense mechanisms**
  - **Loss or suppression of the cough reflex**, e.g., **coma**, **anesthesia**, **drugs**, **chest pain**, or neuromuscular disorders. It may lead to aspiration of gastric contents into the lung.
  - **Damage or injury to the mucociliary apparatus**: It may be due to **cigarette smoke, viral diseases, inhalation of hot or corrosive gases**, or genetic defects of ciliary function (e.g., the immotile cilia syndrome).
  - **Accumulation of secretions:** Cystic fibrosis and bronchial obstruction.
  - **Interference with the phagocytic or bactericidal action of alveolar macrophages**: It may be due to **alcohol intoxication, tobacco smoke, anoxia, or oxygen intoxication**.
  - **Pulmonary congestion and edema**.

Portal of Entry

The causative agents may enter the lung parenchyma by following routes:

- Through respiratory tract.
- Hematogenous spread.
- Local spread.

Classification of Pneumonia (Box 17.2)

BOX 17.2: Classification of pneumonia.

1. Classification depending on the anatomic distribution
   - Lobar pneumonia
   - Bronchopneumonia
   - Interstitial pneumonia
2. Etiological classification

- Primary
- Secondary
- Suppurative

3. Clinical setting in which the infection occurs (if no pathogen can be isolated)
   - Community-acquired acute pneumonia
   - Community-acquired atypical pneumonia
   - Nosocomial pneumonia or hospital-acquired pneumonia
   - Pneumonia in immunocompromised host
   - Healthcare associated pneumonia

Most common cause of atypical pneumonia: Mycoplasma pneumonia
Nosocomial pneumonia: Commonest cause is *Staphylococcus aureus*.

Pneumonia:
- Lowered systemic resistance of host
- Impaired local defense mechanism

COMMUNITY-ACQUIRED ACUTE PNEUMONIAS

Definition: Community-acquired pneumonia includes infectious pneumonia in patients living independently in the community.

Causative Microorganisms (Table 17.1)

These may be bacteria or virus. Bacterial infection of the lung parenchyma **causes the alveoli to be filled with an inflammatory exudate and produces consolidation** (solidification) of the pulmonary parenchyma.

Predisposing Conditions

- **Extremes of age.**
- **Chronic diseases,** e.g., congestive heart failure, COPD and diabetes.
- **Congenital or acquired immune deficiencies.**
- **Decreased or absent splenic function,** e.g., sickle cell disease or post-splenectomy (risk for infection with encapsulated bacteria).

Pathogenesis

Various mechanisms by which lung is infected are as follows:

- **Aspiration of oropharyngeal contents:** Most frequent.

- **Inhalation of aerosolized droplets:** Second most frequent.
- **Bloodstream infection:** Less common.

Classification of Bacterial Pneumonia

Q. Classify pneumonia. Discuss in detail the etiopathogenesis, morphology and complications of lobar pneumonia.

Depending on the anatomic distribution, bacterial pneumonia can be divided into lobular bronchopneumonia and lobar pneumonia.

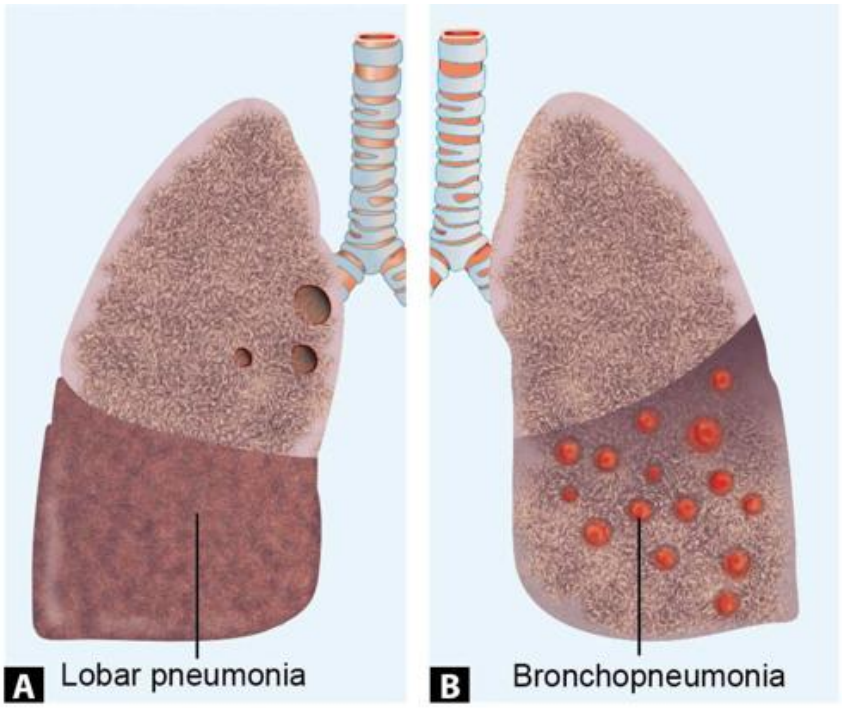
- **Lobar pneumonia:** It is defined as fibrinosuppurative **consolidation of a large portion of a lobe or of an entire lobe** of the lung **(Fig. 17.10A)**.
- **Bronchopneumonia:** It is characterized by **patchy** (scattered solid foci) **area consolidation in the same or several lobes** of the lung **(Fig. 17.10B)**.

These two anatomic patterns may overlap in the same lung. The same organisms may produce either pattern, which depends on the susceptibility of the patient.

Community-acquired pneumonia: Commonest cause is *Streptococcus pneumoniae.*

TABLE 17.1: Causative agents and salient features of community-acquired acute pneumonia.

Microorganism	***Features***
Streptococcus pneumoniae or Pneumococcus	Most common cause
Haemophilus influenzae	Most common bacterial cause in COPD
Moraxella catarrhalis	Elderly
Staphylococcus aureus	Secondary bacterial pneumonia following viral respiratory illnesses
Enterobacteriaceae (Klebsiella pneumoniae)	In debilitated and malnourished people
Pseudomonas aeruginosa	Common in patients with neutropenia
Legionella pneumophila	Organ transplant recipients



Figs. 17.10A and B: (A) Lobar pneumonia, anatomically involves whole or part of a lobe; (B) Bronchopneumonia, anatomically shows patchy area of consolidation.

Bacterial pneumonia: From the clinical standpoint, it is very important to identify the causative agent and determine the extent of disease.

LOBAR PNEUMONIA

Q. Stages, gross and microscopic features of lung in lobar pneumonia.

Lobar pneumonia is characterized by **diffuse inflammation** affecting the **part or entire lobe**, **usually the lower lobes (Fig. 17.10A)**.

MORPHOLOGICAL STAGES

The inflammatory response is classically divided into four stages: Congestion, red hepatization, gray hepatization and resolution.

Congestion

This stage lasts for less than 24 hours.

- **Gross:** The involved lobe of the lung is **heavy**, **boggy and red**. A **blood-stained frothy fluid oozes from the cut surface.**
- **Microscopy:** It is characterized by:
  - **Dilatation and congestion of capillaries** in the alveolar walls.
  - The air spaces in the **alveoli are filled with pale eosinophilic fluid with few neutrophils**, **red cells and numerous bacteria.**

Red Hepatization

It lasts for 2–3 days.

- **Gross: The lobe appears distinctly red**, **firm and airless**. **The firm consistency of the affected lobe of the lung resembles that of the liver**, **this stage has been aptly termed red hepatization**. **The pleura may show serofibrinous pleurisy**. **Cut section shows red and granular appearance.**
- **Microscopy (Figs. 17.11A and B):**
  - **Alveoli** show **exudate** and interlacing strands of fibrin (formed from clotted fibrinogen present in fluid exudate)
  - **Numerous neutrophils** and red cells are found in the fibrin meshwork.

Gray Hepatization

Q. Write short note on gray hepatization.

Gray hepatization of lungs is seen on 5–7 days.

- **Gross (Fig. 17.12A): The affected lobe gradually loses its red color and assumes gray appearance**. **Cut surface shows gray**, **dry and granular appearance.**
- **Microscopy (Figs. 17.12B and C):**
  - **Progressive disintegration of red cells.**
  - **Fibrinosuppurative exudate in the alveoli** in which **neutrophils are replaced by macrophages.**
  - There is a **clear space between the alveolar wall and the exudate** due to contraction of the fibrin thread (formed from fibrinogen in the exudate) present in the exudate.

Resolution

Resolution occurs by **liquefaction** of the previously solid, fibrinous constituents of the exudate in the air spaces. The fibrinous exudate within the air spaces undergoes progressive liquefaction to produce granular and semifluid debris. The liquefaction is due to fibrinolytic enzymes liberated from neutrophils. The liquefied material is removed, partly by expectoration, but mainly ingested by macrophages and drained through lymphatics. If not removed, it may become organized by ingrowth of capillaries and fibroblasts into the exudate.

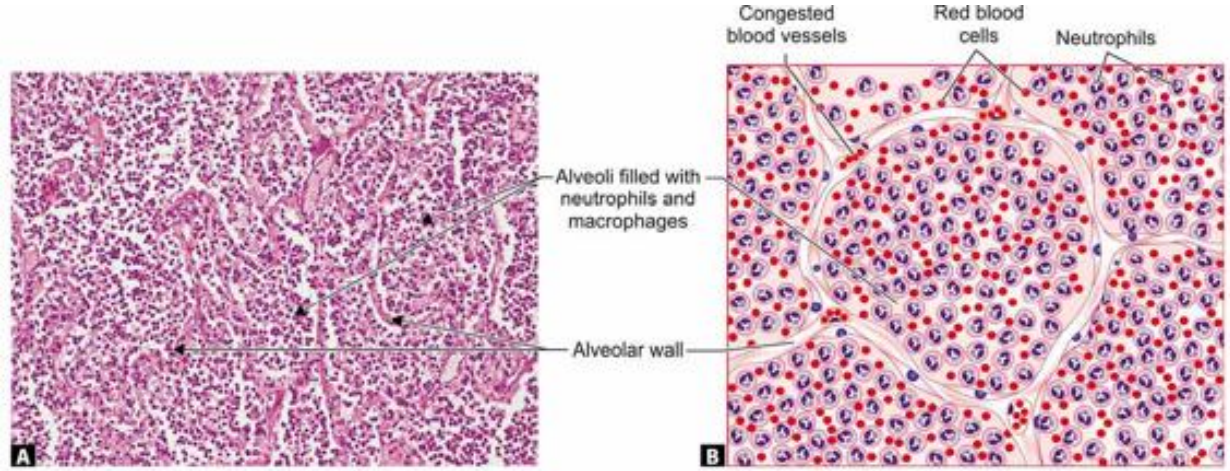
- **Gross:** The **affected lobe becomes more crepitant** as the air spaces reopen. Cut section appears frothy.
- **Microscopy:** The alveolar space shows granular material and semifluid debris.

BRONCHOPNEUMONIA

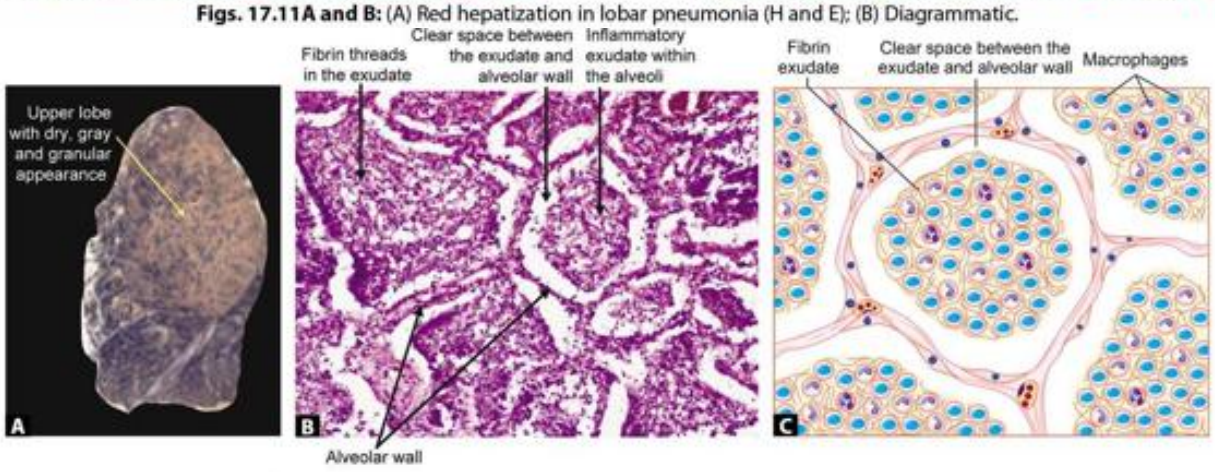
Q. Write short note on bronchopneumonia.

Gross (Fig. 17.10B)

- Bronchopneumonia is characterized by **widespread** focal/**patchy areas** of **acute suppurative inflammation.**
- They are **centered on bronchioles and bronchi** with subsequent spread to surrounding alveoli. The involved alveoli show **consolidation.**



Figs. 17.11A and B: (A) Red hepatization in lobar pneumonia (H and E); (B) Diagrammatic.



Figs. 17.12A to C: Gray hepatization of lung. (A) Gross appearance; (B) (H&E); (C) Microscopic appearance (diagrammatic).

- The **consolidated areas are larger and more numerous in lower lobes** (because of the tendency of secretions to gravitate into the lower lobes) and **frequently bilateral.**
- **Cut section**: It appears slightly **raised above the surface of the surrounding lung** and measures several millimeters in diameter. They are **dry**, **solid**, **granular**, **gray-red to yellow**, **and poorly delimited at their margins**.

Microscopy

The bronchi, bronchioles and adjacent alveolar spaces are filled with exudate rich in neutrophils **(Fig. 17.13)**.

Complications of Pneumonia

Q. Write short note on complications of pneumonia.

- **Lung abscess:** It may develop with extensive tissue destruction and necrosis.
- **Organization**: Delayed and incomplete resolution can cause **ingrowth of granulation tissue into the alveolar exudate.** The intra-alveolar plugs of granulation tissue are known as organizing pneumonia. Gradually, **increased alveolar fibrosis leads to a shrunken and firm lobe and is called as carnification**.
- **Spread of infection to the pleural cavity**: It may result in:
  - **Pleuritis** (inflammation of the pleura) due to extension of inflammation from lung. It may resolve or more often undergoes organization, and fibrous thickening or permanent adhesions.
  - **Pleural effusion** (fluid in the pleural space) is common and usually resolves.
  - **Pyothorax (pus in the pleural space):** It results from an infection of a pleural effusion and may heal with extensive fibrosis.
  - **Empyema:** It is a loculated collection of pus (intrapleural fibrinosuppurative reaction) with fibrous walls and may result from the persistence of pyothorax.
- **Bacteremic dissemination (bacteremia):** It **can cause:**
  - **Endocarditis (heart valves).**
  - **Pericarditis (pericardium).**
  - **Meningitis (meninges).**
  - **Suppurative arthritis (joint).**
  - **Metastatic abscesses in kidneys or spleen**.

Clinical Features

- **Sudden onset of high fever**, **chills and cough** with **mucopurulent sputum**.
- Fibrinosuppurative pleuritis causes pleuritic pain and accompanied by pleural friction rub.

- Radiography: Involved lobe appears radiopaque in lobar pneumonia and focal opacities in bronchopneumonia.

Differences between lobar pneumonia and bronchopneumonia are depicted in **Table 17.2**.

Q. A 63-year-old male complaints of fever with chills and rigors, fatigue, increasingly severe productive cough, with rusty sputum for the last 5 days. On examination, creptitations and rhonchi were heard on lower lobe of left lung. He was treated in the hospital and was completely recovered.

(a) What is the probable diagnosis? (Ans: Lobar pneumonia)

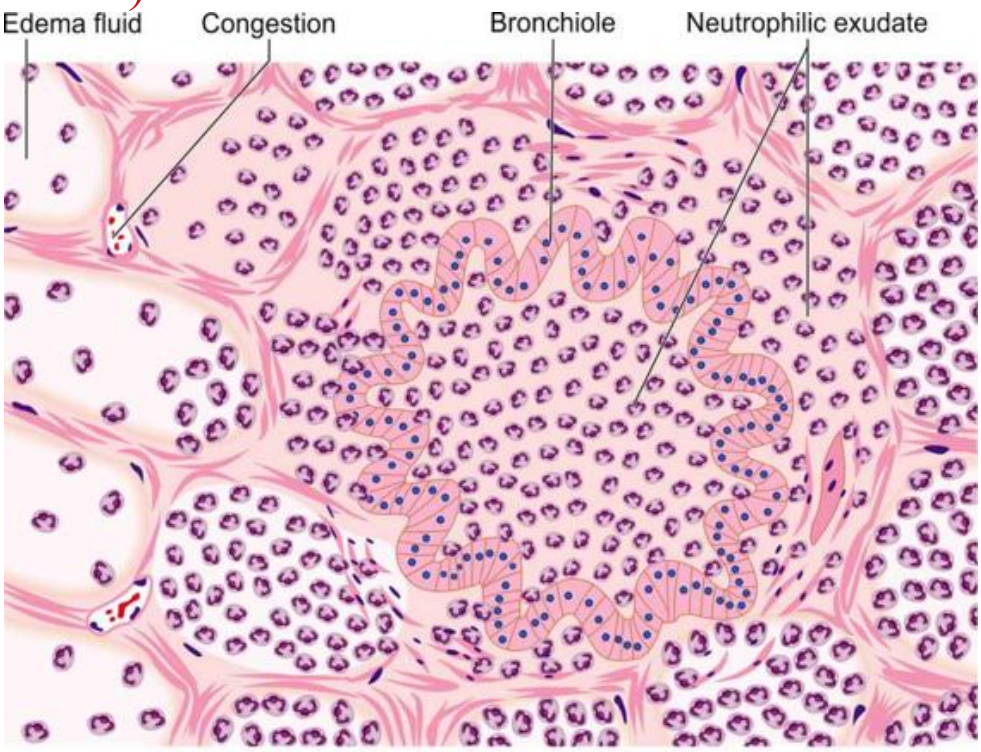


Fig. 17.13: Bronchopneumonia characterized by infiltration of bronchi, bronchioles and adjacent alveoli by acute inflammatory exudate.

Q. Differences between lobar pneumonia and bronchopneumonia.

TABLE 17.2: Differences between lobar pneumonia and bronchopneumonia.

Feature	***Lobar pneumonia***	***Bronchopneumonia***
Definition	Diffuse inflammation affecting the part or the entire lobe/s (usually lower lobes)	Widespread focal/patchy area of acute suppurative infection of terminal bronchioles extending into adjoining alveoli
Age group affected	More common in adults	Commonly develops at extremes of age (infants and old age)
Predisposing factors	Usually develops in healthy individuals	Usually accompany pre-existing diseases (e.g., chronic debility influenza)
Common causative organisms	Pneumococci, *Klebsiella pneumoniae* staphylococci, streptococci	Staphylococci, streptococci, Pseudomonas, *Haemophilus influenzae*
Pathologic features	Usually passes through four stages, namely (i) congestion, (ii) red hepatization, (iii) gray hepatization and (iv) resolution	Patchy consolidation. Bronchi, bronchioles and adjacent alveolar spaces are filled with exudate rich in neutrophils
Blood findings	Neutrophilic leukocytosis and positive blood culture	Neutrophilic leukocytosis, positive blood culture
X-ray	Shows consolidation	Shows mottled focal opacities
Complications	Less common and include pleural effusion, empyema, lung abscess	Bronchiectasis and complications similar to lobar pneumonia
Response to treatment	Better	Variable
Prognosis	Good	Poor

(b) Which is the most common causative bacteria? (refer Table 17.1).

(c) Describe the gross and microscopic features of the lung in various stages of this condition (refer page 506).

(d) What are the likely complications? (***refer*** page 507).

Lobar pneumonia: Most common cause is *Streptococcus pneumoniae* or *Pneumococcus*

Lobar pneumonia: Four stages
1. Congestion
2. Red hepatization
3. Gray hepatization
4. Resolution

Bronchopneumonia: Acute bronchitis with extension into adjacent alveoli.

Bronchopneumonia: Usually
- Bilateral basal location due to gravitation of secretions
- Affects extremes of age (infants or old)
- X-ray chest—patchy opacification of the lobe

Complications of pneumonia:
- Lung abscess
- Organization
- Spread of infection to the pleura
- Bacteremic dissemination

X-ray chest: Gold standard for diagnosing pneumonia.

Positive Gram stain on sputum: More useful than culture.

Pneumocystis Pneumoniae

Q. Write a short answer on Pneumocystis pneumoniae.

- Pneumocystis pneumonia is an opportunistic fungal infection of the lungs by inhalation of *Pneumocystis jirovecii.*
- It usually infects neonates and immunosuppressed individuals (HIV/AIDS, immunosuppression by chemotherapy for organ transplant and tumors, malnutrition, agammaglobulinemia, etc.). In HIV, *Pneumocystis pneumoniae* usually develops when the $CD4^+$ count is <200 cells/mm^3.

MORPHOLOGY

Gross

The affected parts of the lung are consolidated, dry and gray.

Microscopy

- Interstitial pneumonitis with thickening and mononuclear infiltration of the alveolar walls.
- Alveoli contain **pink frothy fluid** with the organisms. On staining by **Grocott's methenamine-silver (GMS) stain,** they appear as oval or crescentic cysts, about 5 µm in diameter and **surrounded by numerous tiny black dotlike organism.** *P. jirovecii* are demonstrable in the frothy fluid.
- No significant inflammatory reaction in the air spaces.

Clinical Course

- Rapid onset of dyspnea, tachycardia, cyanosis and non-productive cough. Chest X-ray shows diffuse alveolar and interstitial infiltrate.
- If untreated, death occurs in one or two weeks.

Community-acquired Viral Pneumonia

Q. Write a short notes on community-acquired pneumonia.

Etiology

Viruses that cause pneumonia include influenza virus types A and B, the respiratory syncytial viruses, human metapneumovirus, adenovirus, rhinoviruses, rubeola and varicella viruses.

Pathogenesis

- Viruses can cause a mild upper respiratory tract infection or a more severe lower respiratory tract infection.
- **Predisposing factors** for extension of the infection into the lower respiratory tract include extremes of age, malnutrition, alcoholism and underlying debilitating illnesses.

- **Damage to respiratory lining cells:** These viruses have affinity to attach and enter respiratory lining cells. Viral replication leads to cytopathic changes, leading to death of cells and secondary inflammation.
- **Secondary bacterial infection:** The damage produced by virus impairs the local pulmonary defensive forces (e.g., mucociliary clearance) and predispose to superinfection by bacteria. Thus, secondary bacterial infection usually becomes more serious than the viral infection itself.

MORPHOLOGY

Gross

Lung may show either patchy or involvement of entire lobes bilaterally or unilaterally. The affected region appears red-blue and congested. Pleuritis or pleural effusions are infrequent.

Microscopy

Depends on the severity of the disease.

- **Interstitial inflammatory reaction:** Involving the walls of the alveoli is the **predominant feature**. The alveolar septa shows widening, edema and mononuclear inflammatory infiltrate composed of lymphocytes, macrophages, and occasionally plasma cells. In acute cases neutrophils may also be seen.
- The alveoli may show intra-alveolar proteinaceous material and a cellular exudate. If patient develops ARDS, pink hyaline membranes may form lining the alveolar walls.
- Eradication of the viral infection results in resolution and return of the normal lung architecture.
- If there is superimposed bacterial infection, ulcerative bronchitis, bronchiolitis and bacterial pneumonia may develop.

Clinical Course

Extremely varied. Many present as severe upper respiratory tract infections with fever, headache, muscle aches and pains in the legs. Cough may be absent. Usually viral infections are mild and resolve spontaneously without any sequelae. However, interstitial viral pneumonias may assume epidemic proportions, and can lead to morbidity and mortality.

HOSPITAL-ACQUIRED PNEUMONIA

Definition: Hospital-acquired pneumonias are pulmonary infections acquired in the course of a hospital stay.

Etiology

Predisposing factors: Severe underlying disease, immunosuppression, prolonged antibiotic therapy, patients on mechanical ventilation or invasive access devices, such as intravascular catheters. The hospital-acquired pneumonias are serious and may be life-threatening.

The common causative organisms causing hospital-acquired pneumonia are shown in **Box 17.3.**

BOX 17.3: Various etiological agents causing hospital-acquired pneumonia.

Gram-negative bacteria, Enterobacteriaceae (*Klebsiella* species *Serratia marcescens*, *Escherichia coli*) and *Pseudomonas* species.
Staphylococcus aureus (penicillin resistant).

Atypical pneumonia:
- Interstitial pneumonia
- No signs of consolidation

LUNG ABSCESS

CC: Describe the etiology, gross and microscopic appearance and complications of lung abscess.

Q. Write short essay/note on lung abscess.

Definition: Lung (pulmonary) abscess is a **local suppurative process within the lung**. It is characterized by **accumulation of pus accompanied by the destruction of lung tissue**.

Etiology and Pathogenesis

Causative Organisms

- Any pathogen can produce lung abscess.
- It is usually due to aerobic and anaerobic organisms normally found in the oral cavity (gram-negative organisms).
- Mixed infections occur when lung abscess develops due to inhalation of foreign material.

- **Causative agents:** Anaerobic bacteria (extremely common), with or without mixed aerobic infection, *Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pyogenes* and type 3 *Pneumococcus* (uncommon).

Mechanism

Lung abscess may be **primary or secondary**.

A. Secondary Lung Abscess

Develops as a complication of several conditions.

- **Complication of necrotizing pneumonia**: The microbes usually associated are as follows: *S. aureus*, *K. pneumoniae*, and the type 3 *Pneumococcus*.
- **Septic embolism**: The source of embolus may be from thrombophlebitis in any part of the systemic venous circulation or from the vegetations of infective bacterial endocarditis on the right side of the heart. The embolus may be trapped in the lung causing multiple pyemic abscesses.
- **Bronchial obstruction**: By a **bronchial cancer** (primary or secondary) or foreign body (postobstructive pneumonia) → secondary infection.
- **Miscellaneous:**
  - Direct penetrating trauma to the lungs.
  - Spread of infections from a neighboring organs (e.g., suppuration in the esophagus, spine, subphrenic space, or pleural cavity).

B. Primary Cryptogenic Lung Abscesses

These type of lung abscess have no apparent cause and most of them develop as consequence of **aspiration of infected material**. Bacteria responsible are usually the mixed anaerobes found in the oropharynx.

- **Aspiration of infective material:** From **infected nasal sinuses or tonsils,** periodontitis or gingivitis may occur during alcoholic stupor, general anesthesia, coma, head injury or neurological disease causing loss of consciousness.
- **Aspiration of gastric contents.**

MORPHOLOGY

Gross (Fig. 17.14)

- **Site and number:**
  - Lung abscess may develop in **any part of the lung** and may be **single or multiple**.
  - Abscess due to **aspiration** are more common on the **right** (because of the more vertical right main bronchus) and are most often **single**.
  - Abscesses that complicate **pneumonia or bronchiectasis** are usually **multiple**, **basal**, **and diffusely scattered**.
  - **Septic emboli** and pyemic abscesses are **multiple** and may affect **any region** of the lungs.
- **Size: Vary from few millimeters to large cavities** of 5–6 cm.

- **Content:**
  - Abscess cavity may contain **suppurative debris**.
  - If **communication** with an air passage → the abscess may be partially drained to produce an **air-containing cavity**.
  - **Continued infection** may result in → **gangrene of the lung**.

Microscopy

- **Cardinal feature: It is characterized by suppurative destruction of the lung parenchyma within the central area of cavitation.**
- Abscess shows **numerous polymorphonuclear leukocytes** and variable numbers of **macrophages**. The abscess is **surrounded by fibrous wall infiltrated by inflammatory cells**.

Clinical Course

- Similar to bronchiectasis and is characterized by **cough**, **fever**, and **large amounts of foul-smelling purulent or sanguineous sputum**.
- **Fever**, **chest pain, and weight loss** are common.
- **Clubbing of the fingers and toes** may appear. Lung abscess in older individuals may be associated with carcinoma.

Complications

- **Extension of the infection into the pleural cavity** leading to empyema/pneumothorax/bronchopleural fistula.
- **Hemorrhage into the abscess cavity**.

- **Septic emboli** may cause **brain abscesses or meningitis**.
- **Secondary amyloidosis** (type AA).

Lung abscess:
- Local suppurative process within the lung.
- Commonest etiological agent is anaerobic bacteria.
- May be primary or secondary.
- Most common cause is aspiration.

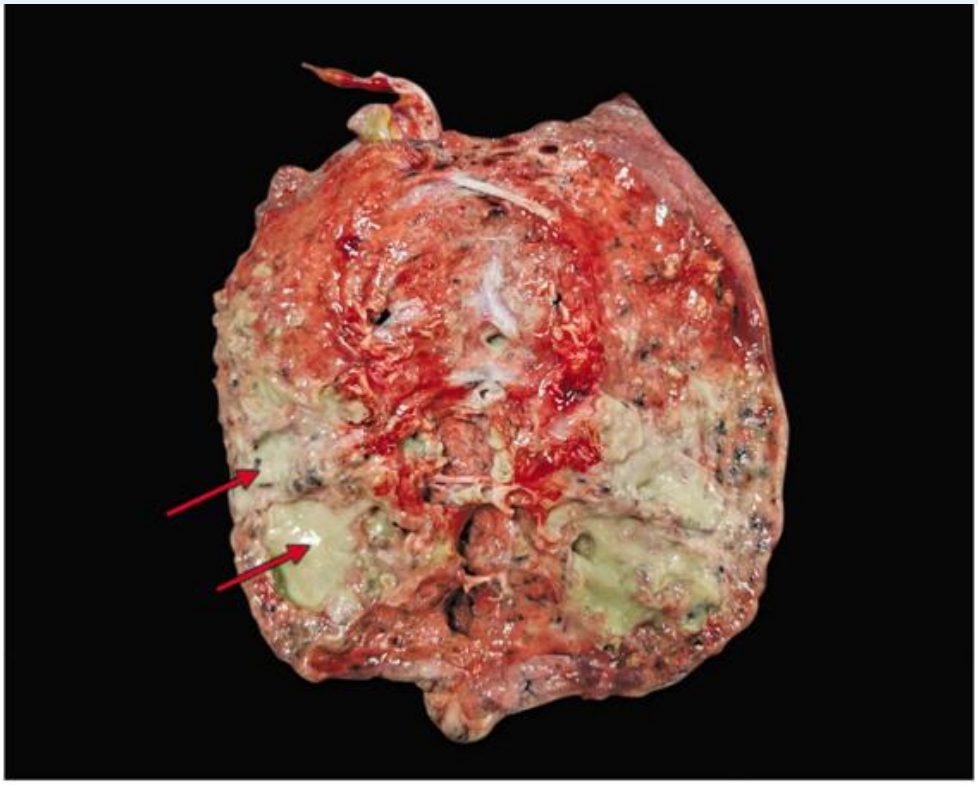


Fig. 17.14: Lung with suppurative abscess (arrows).

TUBERCULOSIS

CC: Define and describe the etiology, types, pathogenesis, stages, morphology microscopic appearance and complications of tuberculosis.

Definition: Tuberculosis (also called Koch's disease) is a **communicable, chronic granulomatous disease** caused by ***Mycobacterium tuberculosis***.

- Most cases of tuberculosis are caused by ***Mycobacterium tuberculosis hominins*** (human strain). The source of infection is patients with **active open case of tuberculosis**.

- **Oropharyngeal and intestinal tuberculosis** can be caused by drinking milk contaminated with ***M. bovis*** (bovine strain) from infected cows. Routine pasteurization has almost eliminated this source of infection.
- ***M. avium*** **and intracellulare**, which are nonpathogenic to normal individuals but cause infection **in patients with AIDS**.

Characteristics of Mycobacteria

- It is an aerobic, slender, rod-shaped bacteria measuring 2–10 μm in length.
- It has a lipid coat which makes it difficult to stain, but **once stained resists decolorization by acids and alcohol**. Hence, it is termed as acid-fast bacilli (AFB), because once stained by carbol fuchsin (present in **Ziehl–Neelsen stain**), it is not decolorized by acid and alcohol.

Epidemiology

- Tuberculosis is common in India. Incidence of tuberculosis is high wherever there is **poverty**, **overcrowding and chronic debilitating illness**.
- Diseases that are associated with increased risk: Diabetes mellitus, Hodgkin lymphoma, malnutrition, immunosuppression, alcoholism, chronic lung disease (e.g., silicosis) and chronic renal failure.
- **HIV is the most important risk factor**.

Infection versus Disease

Infection with *M. tuberculosis* is to be differentiated from disease.

- **Infection**: Tuberculous infection **indicates the presence of organisms in a person**, which **may or may not cause symptomatic disease**.
- **Disease**: Active tuberculosis refers to subset of **tuberculous infections manifested by destructive, symptomatic disease**.

Mode of Transmission

Inhalation

It is the **most common mode** of transmission.

- **Source of organisms** is from an **active open case of tuberculosis** to a susceptible host.
- Tubercle bacilli will be expelled while coughing, sneezing that creates aerosolized respiratory droplets. When an individual inhales the droplets,

it lodges in the lung and causes infection.

Ingestion

- Tuberculosis may be transmitted by drinking nonpasteurized milk from infected cows contaminated with *Mycobacterium bovis*.
- **Site of infection:** *Mycobacterium bovis* causes oropharyngeal and intestinal tuberculosis.
- Eradication of tuberculous herds with tuberculosis and **pasteurization** has almost **eliminated this mode of transmission** of tuberculosis. Nowadays, the ingestion mode of transmission occurs when a patient with open case of tuberculosis swallows the infected sputum which results in tuberculosis of intestine.

Inoculation

It is extremely rare mode of transmission. May develop during postmortem examination, while cuts resulting from handling tuberculous infected organs.

Tuberculin (Mantoux) Test

- Infection by mycobacteria → development of delayed hypersensitivity to *M. tuberculosis* antigens → which is detected by the tuberculin skin test.
- About 2–4 weeks after infection, **intracutaneous injection of 0.1 mL of purified protein derivative** (PPD) of *M. tuberculosis* produces a **visible and palpable induration of 10 mm diameter or more** at the site of injection of PPD. The induration peaks in 48–72 hours.
- **Significance:**
  - **Positive tuberculin test: It indicates T cell–mediated immunity** to mycobacterial antigens.
  - **False-negative reactions:** It is seen in certain viral infections, sarcoidosis, malnutrition, Hodgkin lymphoma, immunosuppression and overwhelming active tuberculous disease.
  - **False-positive reactions:** It is seen in infection by **atypical mycobacteria** or prior **vaccination with BCG** (*Bacillus Calmette–Guerin*).

Pathogenesis

Q. Write a short essay/note on pathogenesis of tuberculosis.

Immunity and hypersensitivity: Infection with tubercle bacillus results in two simultaneous immunological responses.

- Cell-mediated immunity.
- Type IV hypersensitivity reaction.

The effector cells for both cell-mediated immunity and hypersensitivity reaction are **T_H1** cells.

- **Cell-mediated immunity:** Tuberculosis developing first time in an immunocompetent individual depends on the cell-mediated immunity.
- **Type IV hypersensitivity reactions:** Develops to mycobacterial antigens and is **responsible for the tissue destruction, such as caseating granulomas and cavitation**. Appearance of type IV hypersensitivity reaction also signals the development of protective immunity.

Mechanism of Granuloma Formation in Tuberculosis (Figs. 17.15A and B)

Tuberculosis is a chronic granulomatous disease, caused by *M. tuberculosis*. Lung being commonly involved in tuberculosis, the pathogenesis is considered with respect to pulmonary tuberculosis.

Infection before Activation of Cell-mediated Immunity (*refer* page 155 and Fig. 6.10)

Initial infection with *Mycobacterium tuberculosis* **in a nonsensitized individual** is called **primary tuberculosis**. The following sequence of events occur:

Phagocytosis of mycobacteria by macrophages:

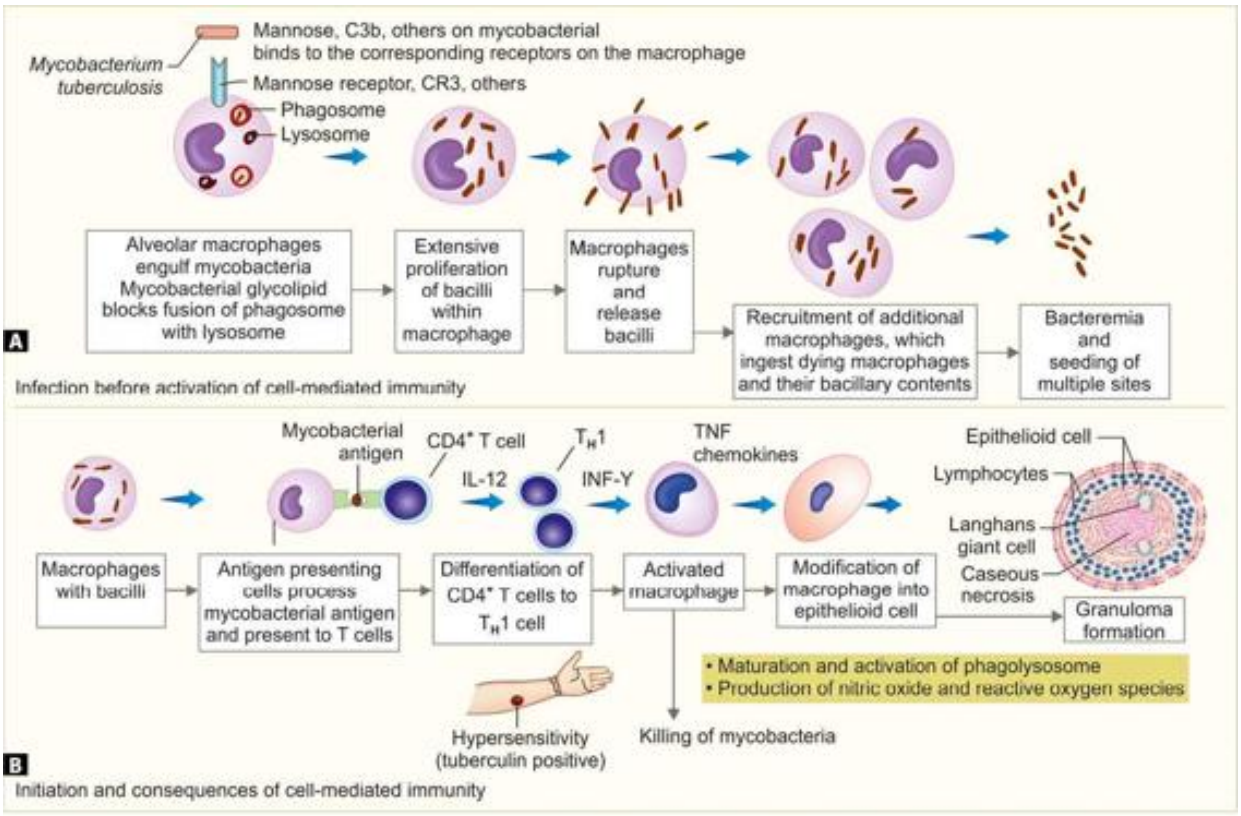
First time, when the **virulent tubercle bacilli** are deposited in the tissue, they primarily **infect macrophages**. In the lung, they **undergo endocytosis** into the alveolar macrophages through **macrophage receptors.** These receptors include:

- **Mannose receptors** bind lipoarabinomannan (LAM), a glycolipid in the cell wall of tubercle bacilli.
- **Complement receptors** (C3b receptor) which bind mycobacteria opsonized by C3b.

Proliferation of mycobacteria withinmacrophages:

- Tubercle **bacilli proliferate freely** within the phagosome of the macrophage by **blocking fusion of the phagosome and lysosome**. It **may result in bacteremia** and seeding of tubercle bacilli at many sites. This bacteremia may be either asymptomatic or may have a mild flu-like illness.
- **Genetic factor**: **NRAMP1 is a transmembrane protein** (a product of *NRAMP1* gene) **inhibits microbial growth**. In individuals with **polymorphisms in the *NRAMP1* (n**atural **r**esistance-**a**ssociated **m**acrophage **p**rotein 1**) gene**, **tuberculosis may progress due to the absence of an effective immune response**.

Initiation and Consequences ofCell-mediated Immunity



Figs. 17.15A and B: Sequence of events in primary tuberculosis of lung: (A) Infection before activation of cell-mediated immunity; (B) Initiation and consequences of cell-mediated immunity.

Cell-mediated immunity develops about 3 weeks after exposure.

Presentation of antigen to CD4⁺ T-cells:

Antigen-presenting cells (APCs which includes macrophages, dendritic cells) process the mycobacterial antigen and present it to naïve $CD4^+$ T cells. Processed antigen reaches regional lymph nodes.

Differentiation of CD4⁺ T-cells into T-helper 1 (T_H1) cell:

It is due to the action of IL-12 secreted by macrophages. **Role of T_H1 cells**:

- **T_H1 cells** produce **IFN-γ** which has several functions.
  - **Activation of macrophages:** IFN-γ activates macrophages to contain the *M. tuberculosis* infection. These macrophages become bactericidal and secrete TNF → which promotes recruitment of more monocytes → **differentiation into epithelioid (epithelium-like) cells**.

- **Stimulates formation of the phagolysosome** in infected macrophages → exposes the bacteria to acidic environment.
- **Stimulates nitric oxide synthase** → to produce nitric oxide (NO) → NO + other oxidants → create reactive nitrogen intermediates → antibactericidal.
- **Generation of reactive oxygen species** → antibacterial.
- Mobilizes antimicrobial peptides (defensins) against the mycobacteria.
- Stimulates autophagy (destroys damaged organelles and intracellular *M. tuberculosis*).

- **T_H1** is also responsible for the **formation of granulomas and caseous necrosis**.

Granulomatous inflammation:

- **Transformation of macrophages into epithelioid cells:** Activated macrophages are transformed into epithelioid cells. Some of these "epithelioid cells" may fuse to form giant cells.
- **Granuloma formation**: A microscopic aggregates of epithelioid cells, surrounded by a rim of lymphocytes, is referred as a granuloma and this pattern of inflammation, known as **granulomatous inflammation**. **Hypersensitivity reaction results in tissue destruction → caseation and cavitation**.

Primary Tuberculosis

Q. Write short note on primary tuberculosis.

Definition: Initial infection that occurs on first exposure to the organism **in an unsensitized** (previously unexposed) **individual** is known as primary tuberculosis.

- About 5% of newly infected people develop clinically significant disease.
- **Source** of the organism is always **exogenous**.

MORPHOLOGY

Sites of primary tuberculosis: Lung, intestine, tonsil and skin (very rare).

Q. Write short note on Ghon focus.

Lung

It is the **most common site** of primary tuberculosis.

Ghon Lesion

Following inhalation, tubercle bacilli gets deposited in the distal airspaces.

- **Site of deposit**: Usually **lower part of the upper lobe or upper part of the lower lobe near the pleural surface** (subpleural).
- **Ghon focus (Figs. 17.16A and B):** About 2–4 weeks after the infection, a circumscribed **gray-white area of** about **1–1.5 cm develops in the lung** known as the Ghon focus→ the center of which undergoes caseous necrosis.
- **Regional lymphadenitis**: Tubercle bacilli (free or within macrophages) are carried along the lymphatics to the regional draining nodes → which often show caseous necrosis.

Ghon Complex

It is the combination of subpleural parenchymal lung lesion (**Ghon focus**) and **regional lymph node** involvement.

Q. Write short note on Ghon complex.

Fate of Ghon Complex

- **Healing:** In **majority** (about 95%), cell-mediated immunity controls the infection and primary tuberculosis **heals**. The **hallmark of healing is fibrosis** and Ghon complex undergoes progressive fibrosis, followed by radiologically detectable **calcification** (Ranke complex), and very rarely ossification.
- **Spread:** During the first few weeks **lymphatic and hematogenous dissemination** occurs to other organs or parts of the body.

Other Sites of Primary Complex

- **Intestine:** Primary focus in the small intestine (usually ileal region) along with mesenteric lymphadenitis.
- **Tonsils:** Primary focus in the pharynx and tonsil with cervical lymph node enlargement.
- **Skin**: Primary focus in the skin along with regional lymph node involvement.

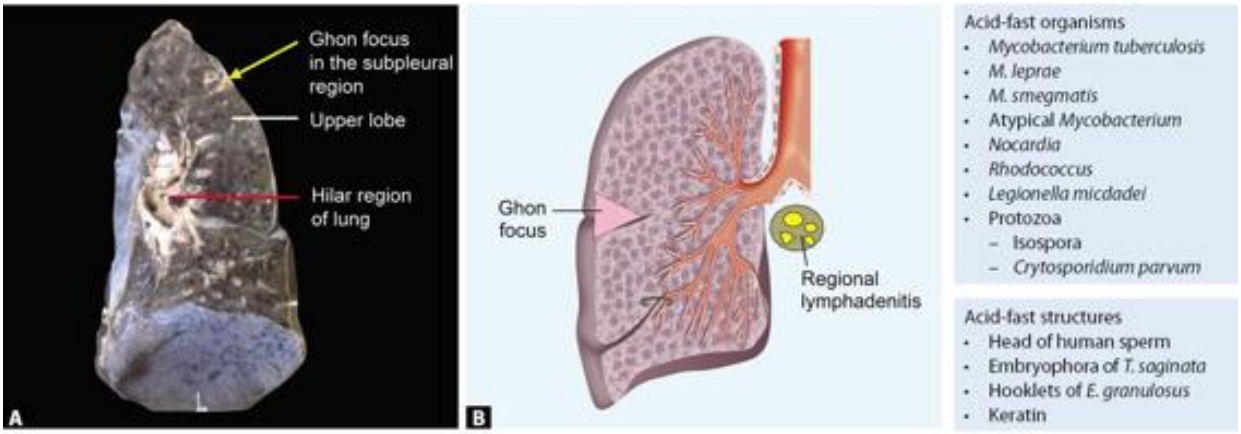
Microscopy (Figs. 17.17A and B)

Granuloma in tuberculosis is called as tubercle. Tubercle may show central area of caseous necrosis (caseating granuloma/soft tubercle) or may not show caseation (noncaseating tubercles/hard tubercle). Individual tubercles are identified only by microscopic examination. When multiple granulomas coalesce → they may be grossly identified.

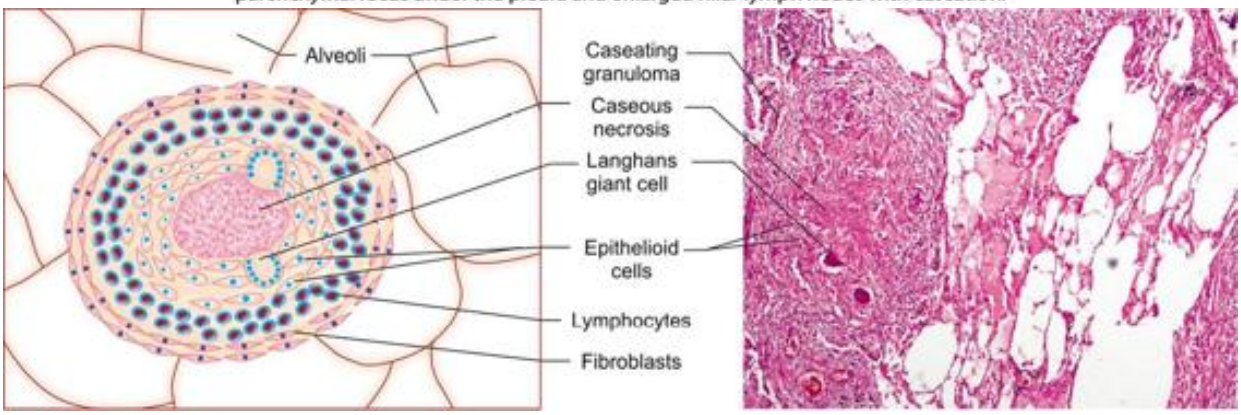
Caseating granuloma consists of:

- **Central area of caseous necrosis**

- **Surrounded by epithelioid cells** (modified macrophages), some of which may fuse to form multinucleate giant cells. The **giant cells may be Langhans** type (nuclei arranged in horse-shoe pattern) **or foreign body type** (nuclei in the center).
- The epithelioid cells are **surrounded by rim of lymphocytes.**
- These granulomas are usually enclosed within fibroblasts.



Figs. 17.16A and B: (A) Gross appearance of primary pulmonary tuberculosis; (B) Diagrammatic. Ghon complex consists of gray-white parenchymal focus under the pleura and enlarged hilar lymph nodes with caseation.



A

B

Figs. 17.17A and B: Microscopic appearance of tuberculous lung. (A) Diagrammatic; (B) Photomicrograph. Central area of caseation surrounded by epithelioid and multinucleated giant cells.

Secondary Tuberculosis

Q. Describe the morphological types of secondary tuberculosis.

(Synonyms: Post-primary tuberculosis, reactivation tuberculosis)

Definition: Tuberculosis developing in a previously sensitized individual is known as secondary

tuberculosis.

- It may follow shortly or many years after primary tuberculosis, usually when host resistance decreases.
- **Source of infection**:
  - Most common source is **reactivation of a latent infection.**
  - Rarely exogenous reinfection.
- Any location may be involved in secondary tuberculosis, but the lungs are by far the most common site.

Q. Morphology of secondary tuberculosis.

MORPHOLOGY

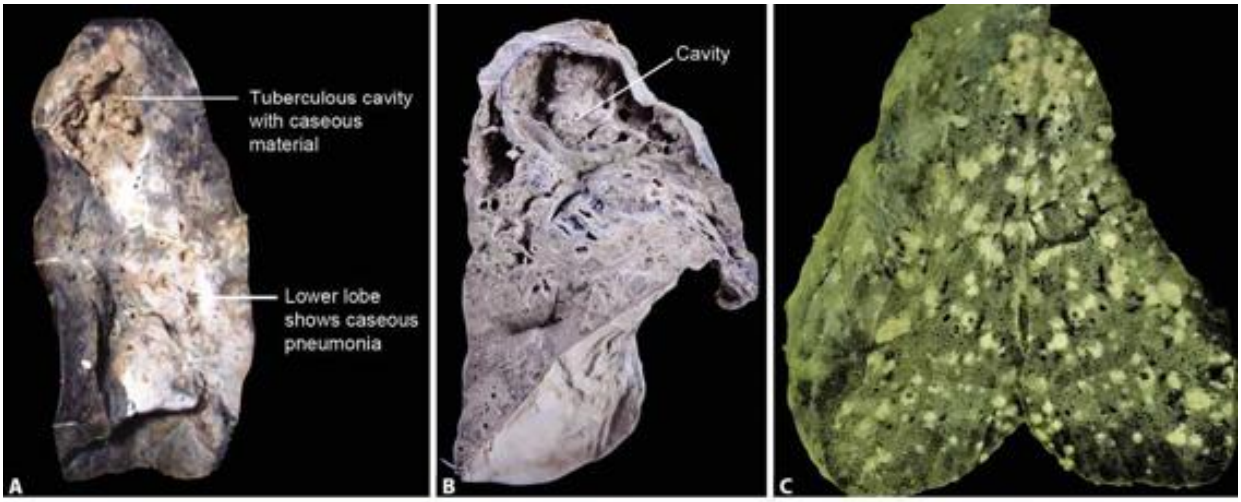
Gross (Figs. 17.18A and B)

- **Site:** In the lungs, secondary tuberculosis usually involves the **apex of the upper lobes** of one or both lungs, **within 1–2 cm of the apical pleura**.
- **Appearance: Initially-small focus (<2 cm in diameter) of consolidation**, sharply circumscribed, firm and **gray-white to yellow** in color.
- **Regional lymph nodes involvement is less prominent** than in primary tuberculosis.

Microscopy

Active lesions show **caseating granulomas** and **acid-fast stain** often shows **tubercle bacilli**.

Natural history and various stages of tuberculosis are depicted in **Figure 17.19**.



Assman focus: Infraclavicular lesion of tuberculosis.

Rich focus: Tuberculous granuloma occurring on the cortex of the brain that ruptures into the subarachnoid space.

Weigart (Wigard) focus: Healed foci of TB in the intima of blood vessels.

Simon focus: Healed calcified caseous nodule of tuberculosis at the apex of lung.

Simmond focus: Healed foci of TB in the liver.

Figs. 17.18A to C: Secondary pulmonary tuberculosis. (A) Early; (B) Advanced. The lung shows gray-white areas of caseation with a cavity in the upper lobe; (C) Miliary tuberculosis of the lung. The cut surface shows numerous gray-white granulomas.

Fate of Secondary Tuberculosis (Fig. 17.20)

Healing

In immunocompetent individuals, localized, apical, focus may **heal with fibrosis and calcification,** rarely ossification.

Progress

It may occur along several different pathways.

Progressive pulmonary tuberculosis

It occurs mainly in the elderly and immunosuppressed. Apical lesion may expand into surrounding lung and may erode into bronchi and vessels.

- **Erosion into bronchi:** It leads to release of the central area of caseous necrosis → resulting in a ragged, irregular **apical cavity (Figs. 17.18A and B)** surrounded by fibrous tissue. This produces an **important source of infection**, because when the patient coughs, sputum contains bacteria.
- **Erosion of blood vessels:** May results in hemoptysis. With prompt treatment, it may undergo healing by fibrosis.

Spread of infection

If the treatment is inadequate or if host defenses are impaired, the infection may spread via: (i) airways, (ii) lymphatics or (iii) blood vessels.

- **Local/direct spread:** Tuberculosis can directly spread to the surrounding tissue. In the lung, local spread to the pleura leads to serous **pleural effusions**, **tuberculous empyema**, or **obliterative fibrous pleuritis**.
- **Spread through bronchi/airways:** It may produce tuberculous pneumonia. It can spread to other parts of same lung as well as opposite lung.
  - **Intestinal tuberculosis (*refer* Fig. 18.23):** In the past, intestinal tuberculosis resulted by the drinking of contaminated milk and unpasteurized milk, now it is rare. Nowadays caused by the **swallowing of coughed-up infective material** in patients with open case of advanced pulmonary tuberculosis. **Mainly develops in the ileum.**
- **Spread along mucosal lining:** Spread through lymphatic channels or along the mucosal lining from mycobacteria present in the expectorated infectious material may lead to **endobronchial**, **endotracheal** and **laryngeal tuberculosis**.
- **Lymphatic spread:** Spread through lymphatic channels mainly reach regional lymph nodes. It may also cause disseminated disease.
  - **Miliary pulmonary disease:** It is the disseminated form of tuberculosis. When the dissemination occurs only to the lungs, it is called miliary pulmonary disease **(Fig. 17.18C)**.
    - **Mechanism of development**: Tubercle bacilli draining through lymphatics → enter the venous blood → to the right venous side of the heart → through pulmonary artery → to the lung.

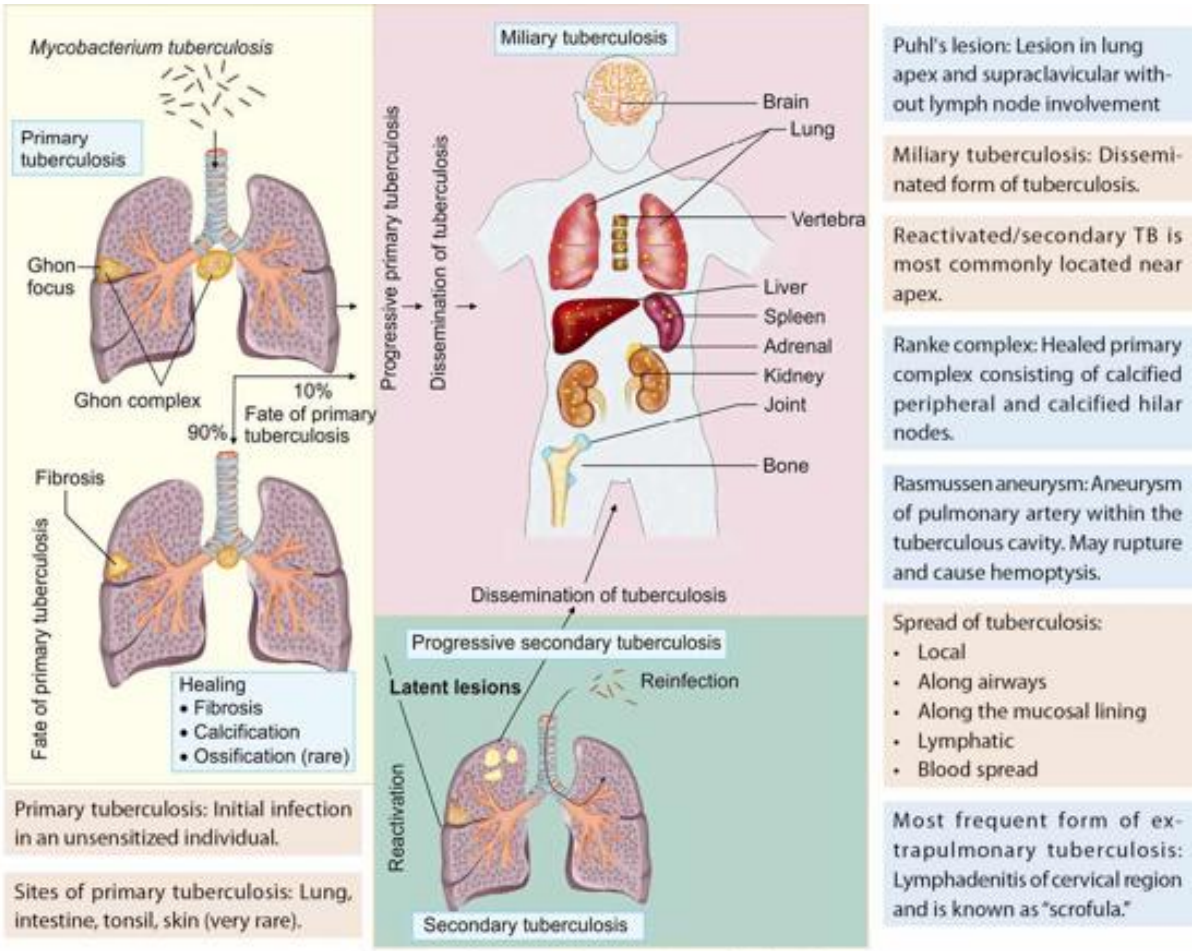


Fig. 17.19: Natural history and various stages of tuberculosis.

- **Lesions: Multiple**, **small**, **yellow**, **nodular** lesions in the lung parenchyma of both the lungs. Each lesion is either microscopic or small, visible (2 mm) foci of **yellow-white** consolidation → **resemble to millet seeds, hence named "miliary"**.

- **Lymphadenitis**: It is most frequent presentation of extrapulmonary tuberculosis, and usually occurs in the cervical region (scrofula).
  - HIV-negative individuals: Unifocal and localized.

- HIV-positive individuals: Multifocal and systemic disease.

- **Spread via blood vessels:**
  - **Systemic miliary tuberculosis** occurs when tubercle bacilli disseminate through the systemic arterial system. Miliary tuberculosis most commonly involves liver, bone marrow, spleen, adrenals, meninges, kidneys, fallopian tubes and epididymis. But no organ is exempt and can involve any organ.
  - **Isolated-organ tuberculosis:** Dissemination of tubercle bacilli through blood may seed any organ or tissue → resulting in isolated organ tuberculosis. Commonly involved organs are as follows:
    - Meninges (tuberculous meningitis).
    - Kidneys (renal tuberculosis).
    - Adrenals.
    - Bones (osteomyelitis): When it involves the vertebrae, the disease is referred to as **Pott disease**. Paraspinal "cold" abscesses may track along tissue planes and present as an abdominal or pelvic mass.
    - Fallopian tubes (salpingitis), epididymis (epididymitis).

Complications of Secondary Tuberculosis

- **Hemoptysis** due to erosion into small pulmonary arteries adjacent to the tuberculous cavity.
- **Bronchopleural fistula:** It may develop when a tuberculous cavity in the subpleural region ruptures into the pleural space. It may lead to tuberculous empyema and pneumothorax.

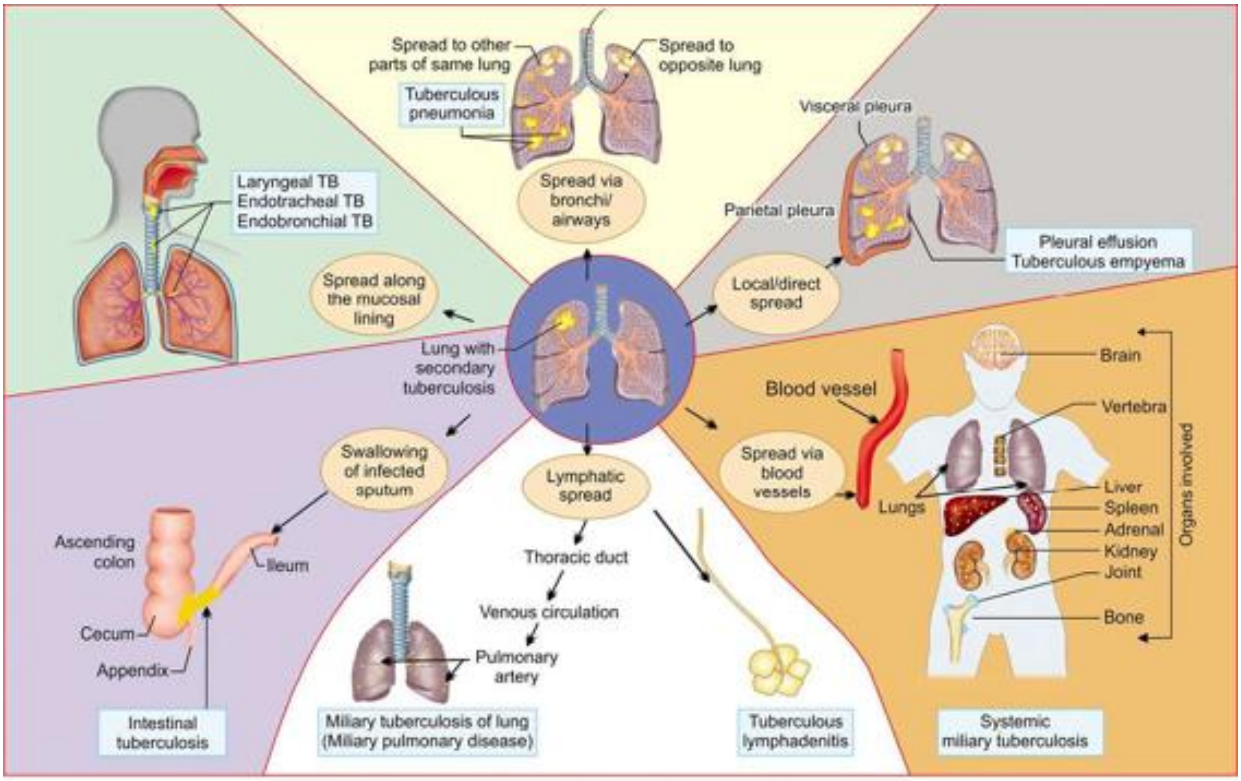


Fig. 17.20: Progress and complications of secondary tuberculosis of lung.

- **Aspergilloma:** It is a fungal mass produced due to superinfection of a persistent open cavity with *Aspergillus*. It may fill the entire cavity and form a fungal ball.

Clinical Features

- Localized secondary tuberculosis may remain asymptomatic.
- **Systemic symptoms of pulmonary tuberculosis**: Probably due to cytokines released by activated macrophages (e.g., TNF and IL-1).
  - **Nonspecific**: Malaise, anorexia, weight loss and fever.
  - **Low-grade fever:** It is **remittent** (appearing late each afternoon and then subsiding-commonly known as **evening rise of temperature**), and **night sweats**.
  - **Sputum:** It is first mucoid and later purulent.
  - **Hemoptysis:** It is present in 50% of cases of pulmonary tuberculosis.
  - **Pleuritic pain:** Due to infection to the pleural surfaces.
- **Extrapulmonary manifestations** depend on the organ/system involved.

Patients with HIV infection have increased risk of tuberculosis.

Diagnosis: Pulmonary Disease

Q. Write short note on diagnosis of tuberculosis.

- Based on the **history**, **physical and radiographic** findings.
- Identification of **acid-fast tubercle bacilli in the sputum**.
- **Culture of the sputum:** Conventional cultures require up to 10 weeks. Culture is the gold standard.
- **PCR amplification** of *M. tuberculosis:* DNA is the rapid method of diagnosis. PCR can detect as few as 10 organisms in clinical specimens.
- Cartridge based nucleic acid amplification test (CB-NAAT,) is an automated cartridge-based molecular technique which not only detects *Mycobacterium tuberculosis* but also rifampicin resistance within two hours. This has been endorsed by WHO as an initial diagnostic test for tuberculosis.

Prognosis: Generally good if infections are localized to the lungs. All stages of HIV infection are associated with an increased risk of tuberculosis.

Main difference between primary and secondary tuberculosis of lung are presented in **Table 17.3**.

Q. A 56-year-old male has a 4 month history of fever (evening rise of temperature), night sweats, weight loss and chronic productive cough with hemoptysis on and off. Sputum examined for the causative agent was positive. His X-ray chest showed right upper lobe cavitation. He was treated and completely recovered of his illness within 6 months.

(a) What is the probable diagnosis? (Secondary tuberculosis with cavitation)

(b) What is the morphological appearance of right lung in this case? (refer page 515).

(c) What are the investigations that would aid in reaching a diagnosis? (refer pages 517-8).

(d) What are the methods of spread of this lesion? (refer pages 515-7).

Tuberculosis: Communicable, chronic granulomatous disease caused by *Mycobacterium tuberculosis*.

Acid-fastness of the *Mycobacterium* is due to mycolic acid.

Zeihl–Neelsen stain: Acid-fast bacilli (AFB) stain for demonstration of mycobacteria.

Active tuberculosis: Develops only in few individuals who get infection.

In most, primary tuberculosis is asymptomatic but when immune defenses are lowered, the infection may produce potentially life-threatening disease.

Mode of transmission of tuberculosis:
- Inhalation
- Ingestion
- Inoculation (very rarely)

Open case of tuberculosis is a patient with tuberculosis in which cavitatory lung lesion is communicated to the external environment through the patient's airways.

Pulmonary tuberculosis: Spread by droplet infection.

Tuberculosis: Reservoir of infection is patient with active tuberculosis.

Tuberculin test positivity indicates: Good cell-mediated immunity:

Positive Mantoux test indicates that the individual is exposed to mycobacterial antigen. It does not differentiate between infection and disease.

In tuberculosis, immunity (resistant to infection) and hypersensitivity are two different manifestations of same mechanism.

Tuberculosis: Usually affect lungs, but any organ may be involved.

The granuloma in tuberculosis is known as tubercle.

Cord factor is a virulent factor for *Mycobacterium tuberculosis.*

Mycobacterial glycolipid (lipoarabinomannan) blocks the fusion of phagosome with lysosome in the alveolar macrophage.

Tuberculosis: T_H1 cells produce IFN-γ and plays a crucial role in the development of granulomatous lesion.

Epithelioid granuloma is caused by: Helper T-cells.

Primary tuberculosis: Ghon complex consists of Ghon lesion in the lung with regional lymphadenitis. The term Ghon focus is restricted only to primary tuberculosis of lung. In other organs, it is called as primary focus.

Caseating granuloma: Central area of caseous necrosis surrounded by epithelioid cells, lymphocytes and Langhans type of giant cells. Epithelioid cells are modified macrophages. Immunocompromised patients usually do not form the characteristic granulomas.

TABLE 17.3: Main difference between primary and secondary tuberculosis of lung.

Characteristics	*Primary TB*	*Secondary TB*
Type of infection	Initial infection in an unsensitized individual	Infection in a previously sensitized individual
Source of infection	Exogenous	Usually endogenous activation of latent infection
Site of lung involved	Lower part of upper lobe or upper part of lower lobe and subpleural	Apex and posterior segments of the upper lobe and superior segments of lower lobe
Regional lymph node involvement	Significant and prominent	Not prominent
Sputum for AFB	Rarely positive	Commonly positive
Cavity formation	Rare	Common
Healing	Mainly by calcification	By fibrosis
Extrapulmonary complications	Very common	Lesion usually localized to lung

Tuberculosis of lung: Hemoptysis is the beginning of end or end of the beginning: William Boyd.

Clinical features of tuberculosis:
- Drenching night sweats
- Fever
- Weight loss

Bronchial artery is the source of hemoptysis in tuberculosis

Most common cause of drug-resistant tuberculosis is previous antituberculous treatment (ATT).

Pott's disease: Tuberculosis of vertebrae.

Lung: Granuloma with necrosis is seen in:
- Tuberculosis
- Histoplasmosis
- Wegener's granulomatosis
- Cryptococcosis

SARCOIDOSIS

Definition: Sarcoidosis is a **multisystem disease of unknown cause** characterized by the presence of **noncaseating granulomas** in **many tissues and organs**.

- **Organs involved:** Sarcoidosis can affect every organ of the body. **It most commonly** affects the **lung** and the **lymph nodes** in the **mediastinum**

and hilar regions. Other organs commonly affected are the liver, skin and eye.

- **Age and gender:** Most patients are young (from 20 to 40 years of age) and more common in women than in men.

Etiology

Exact cause of sarcoidosis is **not known**. Factors that play a role in its pathogenesis are as follows: (i) Environmental, (ii) genetic and (iii) immunological.

- **Environmental factors:** Environmental agents (infectious and noninfectious) have been suggested as possible causes. Infectious inciting agent includes: Mycobacteria (both *Mycobacterium tuberculosis* and nontuberculous mycobacteria), *Propionibacterium acnes*, *Borrelia burgdorferi,* viruses, fungi, spirochetes and *Rickettsia*.
- **Genetic factors:** Certain specific HLA genotypes (e.g., HLA-A1 and HLA-B8) are associated with an increased risk for developing sarcoidosis.
- **Immunological factors:** They are important in pathogenesis. The most likely etiology is an environmental agent (infectious or noninfectious) that triggers cell-mediated immune response to an unidentified antigen in a genetically susceptible host.

Pathogenesis

The granuloma is the pathologic hallmark of sarcoidosis. A characteristic feature of sarcoidosis is the local accumulation of inflammatory cells. The sarcoid granulomas develop as a **cell-mediated response to an unidentified antigen.** The sequences of events are as follows:

- An unknown antigen is taken up by antigen-presenting cells (APCs).
- APC present the unknown antigen to the $CD4^+$ helper T cells. This results in antigen-driven proliferation of $CD4^+$ helper T cells and accumulation of activated monocytes.
- $CD4^+$ helper T cell release several cytokines, such as:
  - Interleukin (IL)-2 results in T-cell expansion.
  - Interferon- γ (IFN-γ) causes macrophage activation.
- Local increase of several cytokines (IL-8, TNF, macrophage inflammatory protein 1α) favors recruitment of additional T cells and monocytes and contributes to the formation of granulomas.

Systemic Immunological Abnormalities

Patients with sarcoidosis may also show:

- **Anergy to common skin test antigens,** such as tuberculosis purified protein derivative (PPD) or as *Candida.*
- **Polyclonal hypergammaglobulinemia** (another manifestation of helper T-cell dysregulation).

MORPHOLOGY

Noncaseating Granulomas (Fig. 17.21)

- The involved tissues show the classical well-formed **noncaseating granulomas.**
- They are composed of an **aggregate of epithelioid cells and lymphocytes** (mainly $CD4^+$ T-cell). Epithelioid cells are modified macrophages and characteristically have abundant eosinophilic cytoplasm and vesicular nuclei.
- Langhans or foreign body-type giant cells may also be seen.
- **Central necrosis is not seen.**

Other findings: Two other microscopic findings of sarcoidosis include:

- **Schaumann bodies:** These are laminated concretions of calcium and proteins.
- **Asteroid bodies:** These are stellate inclusions within giant cells in about 60% of the granulomas.

They are not pathognomonic because they can be seen in other conditions.

Fate of Granuloma

- The granulomas may resolve completely.
- As the lesion become chronic, proliferation of collagen forms concentric laminated layer giving them an onion-skin appearance and later may form nodular hyaline scars.

Diagnosis

- **Involvement of two or more organs is necessary**.
- **Histologic diagnosis** is made **after exclusion** of organisms that are known to be associated with granuloma formation. Noncaseating (hard) granuloma is not specific for sarcoidosis, and may be seen in (i)

mycobacterial infections, (ii) fungal infections, (iii) malignancy and (iv) environmental agents (e.g., beryllium).

Lung

The **lungs** are most commonly involved.

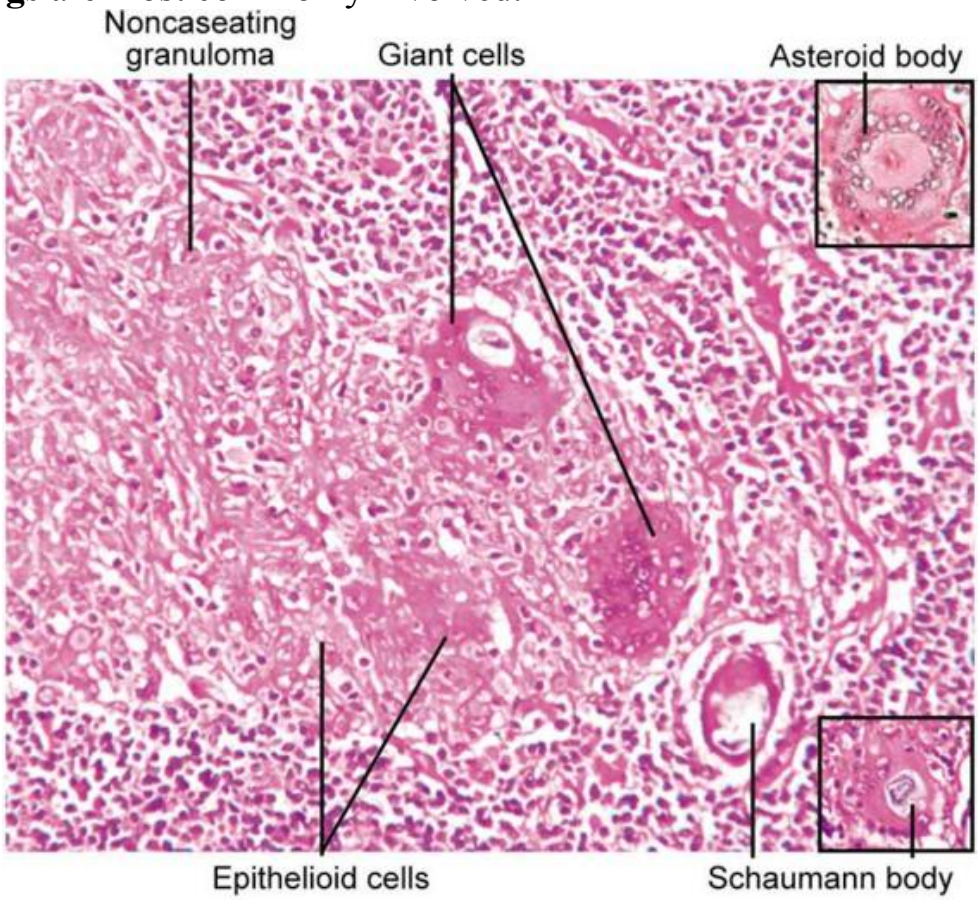


Fig. 17.21: Microscopic appearance of sarcoidosis showing non-caseating granuloma composed of epithelioid cells surrounded by lymphocytes and giant cells. Inset shows giant cell with asteroid body (upper right) and Schaumann body (lower right).

MORPHOLOGY

Gross

No gross changes in early stage. In advanced cases the coalescence of granulomas produces **small palpable or visual nodules** measuring 1–2 cm.

Microscopy

The **granulomas** are found **in the pulmonary interstitium** along the **lymphatics, around bronchi and blood vessels**, although alveolar lesions are also seen. The lesions in the lung may heal with fibrosis and hyalinization are often found.

Lymph Nodes

They are involved in almost all cases, particularly the hilar and mediastinal nodes. Lymph nodes are enlarged, discrete, and sometimes calcified.

Clinical Features

- Variable clinical manifestations.
- Insidious onset of respiratory symptoms (shortness of breath, cough, chest pain, hemoptysis) or of constitutional signs and symptoms (fever, fatigue, weight loss, anorexia, night sweats).
- May be detected on **routine chest films as bilateral hilar lymphadenopathy** or may present with peripheral lymphadenopathy, skin lesions, eye involvement, splenomegaly, or hepatomegaly.

Prognosis

Variable and is good for the majority of patients.

Recovery with resolution of the disease: Occurs either spontaneously or induced by steroid therapy in about **65–70% of patients.**

Chronic or progressive course: It may lead to permanent loss of some lung function or some permanent visual impairment in about 25% of patients.

Death: Less than 3% of patients may die due to cardiac or pulmonary involvement.

Sarcoidosis: Raised interleukin (IL)-2 results in T-cell expansion.

Sarcoidosis: Anergy to skin test antigens, such as tuberculosis purified protein derivative (PPD) or as *Candida.*

Sarcoidosis: Noncaseating granuloma. Schaumann bodies-laminated concretions of calcium and proteins. Asteroid bodies which are stellate (star shaped) inclusions within giant cells.

Sarcoidosis: Polyclonal hypergammaglobulinemia.

Sarcoidosis: Diagnosis is by exclusion of other granulomatous diseases. Kveim test can be used for diagnosis of sarcoidosis.

Sarcoidosis: (1) Increased ACE and (2) Hypercalcemia due to hypervitaminosis D.

ACUTE LUNG INJURY AND ACUTE RESPIRATORY DISTRESS SYNDROME (DIFFUSE ALVEOLAR DAMAGE)

Q. Write short essay/note on acute lung injury/ARDS/diffuse alveolar damage.

Definition: Acute lung injury (ALI) is also termed as noncardiogenic pulmonary edema characterized by: **(1) abrupt onset of significant hypoxemia and (2) bilateral pulmonary infiltrates in the absence of cardiac failure.**

- **Acute respiratory distress syndrome (ARDS) is a severe form of ALI.**
- ARDS as well as ALI has inflammation associated increase in pulmonary vascular permeability, edema and death of epithelial cells. Histologically they show ***diffuse alveolar damage*** **(DAD).**

Etiology

ALI is triggered by diverse conditions **(Box 17.4)**.

Pathogenesis

ALI/ARDS starts when there is injury to pneumocytes and pulmonary endothelium and creates a viscous cycle of inflammation and pulmonary damage.

- **Endothelial cell injury/activation:** It is an important early event.
- **Adhesion and extravasation of neutrophils:** Neutrophils adhere to the activated endothelium and migrate into the interstitium and the alveoli. In the alveoli, neutrophils release inflammatory mediators, proteases, reactive oxygen species, and cytokines. This further recruits leukocytes, causing further damage to endothelium. This cycle of inflammation and endothelial damage is the basic mechanism of ALI/ARDS.
- **Formation of hyaline membrane:** Endothelial activation and injury causes leakage of fluid from pulmonary capillaries into the interstitial tissue of lung. Loss of type I pneumocytes permits fluid to enter the alveolar spaces. Injury to type II alveolar pneumocytes produces surfactant abnormalities which compromises the alveolar gas exchange.

The protein-rich (derived from leaked plasma) edema fluid and debris from dead alveolar epithelial cells form hyaline membranes which is characteristic of ALI/ARDS.

- **Resolution of injury:** If the patient survives the acute phase, macrophages remove intra-alveolar debris and release fibrogenic cytokines [e.g., transforming growth factor β (TGF-β) and platelet derived growth factor (PDGF)]. These cytokines stimulate fibroblast growth and collagen deposition in the alveolar walls resulting in fibrosis of alveolar walls. Bronchiolar stem cells replace pneumocytes and uninjured capillary endothelium replace the endothelial cells.

MORPHOLOGY

Gross

Lungs are heavy, firm, red and boggy.

Microscopy

Exudative Stage

- Congestion, interstitial and intra-alveolar edema, accumulation of inflammatory cells, fibrin deposition and **diffuse alveolar damage.** Interstitial and intra-alveolar edema is prominent by the first day.
- **Hyaline membranes:** The alveolar walls become lined with waxy hyaline membranes which appear by the second day and are the **most conspicuous morphologic feature**. Alveolar hyaline membranes are eosinophilic and glassy. They consist of precipitated fibrin-rich plasma proteins in the edema fluid mixed with the cytoplasmic, nuclear and lipid debris from sloughed necrotic epithelial cells.

Organizing Stage

In this stage, type II pneumocytes proliferate, and granulation tissue grows in the alveolar walls and into the alveolar spaces. In majority, the granulation tissue resolves with only minimal functional impairment.

Clinical Course

Patients who develop ALI are usually admitted to the hospital for their predisposing conditions. Presenting features include severe dyspnea and tachypnea, increasing cyanosis and hypoxemia, respiratory failure, and the appearance of diffuse bilateral infiltrates on chest X-ray.

ATELECTASIS (COLLAPSE)

Q. Write short note on atelectasis.

Definition: Atelectasis is the term used for either incomplete expansion of the lungs (neonatal atelectasis) or to the **collapse of previously expanded/inflated lung** (acquired atelectasis). It produces relatively airless areas in the lung parenchyma.

Main Types of Acquired Atelectasis

- **Resorption atelectasis:** Develops due to complete obstruction of an airway. Causes include obstruction due to excessive secretions (e.g., mucus plugs) or exudates within smaller bronchi (e.g., bronchial asthma, chronic bronchitis, bronchiectasis and postoperative states), aspiration of foreign bodies, and fragments of bronchial tumors (rare). Air in the dependent alveoli distal to obstruction gets resorbed and causes collapse of alveoli. There is reduced lung volume which shifts the mediastinum toward the atelectatic lung.
- **Compression atelectasis:** Develops due to **compression of lung from external aspect.** Causes include **accumulation of significant volumes of fluid** (transudate, exudate or blood) or **air** (pneumothorax) **in the pleural cavity** and compression by tumor. In this type of atelectasis, the mediastinum is shifted away from the affected lung.

BOX 17.4: Etiological factors of acute respiratory distress syndrome.

- **Infection**
  - Sepsis (e.g., gram-negative septicemia)
  - Lung infections (viral, *Mycoplasma,* and *Pneumocystis* pneumonia, miliary tuberculosis)
  - Aspiration of gastric contents
- **Physical injury**
  - Mechanical trauma
  - Fat embolism
  - Lung contusions
  - Burns
  - Near-drowning
  - Ionizing radiation
- **Inhalation of toxic gases and chemicals**
- **Chemical injury**
  - Overdose of heroin, methadone, barbiturate
  - Acetylsalicylic acid
  - Cytotoxic drugs
- **Hematological conditions**
  - Transfusion associated lung injury (TRALI)

- Disseminated intravascular coagulation (DIC)

- **Pancreatitis**
- **Uremia**
- **Hypersensitivity reactions**

- **Contraction atelectasis** develops when **focal or generalized pulmonary or pleural fibrosis prevents full expansion of the lung.**

Significance

- Significant atelectasis **reduces oxygenation** and **predisposes to infection.**
- In long-standing atelectasis, the collapsed area of lung becomes fibrotic and bronchi dilate, in part owing to infection distal to the obstruction—**bronchiectasis.**
- It is usually reversible disorder except contraction atelectasis.

PNEUMOCONIOSES

CC: Define and describe the etiology, types, exposure, environmental influence, pathogenesis, stages, morphology, microscopic appearance and complications of occupational lung disease.

Q. Define and classify pneumoconiosis. Discuss the pathology and complications of asbestosis.

Definition: Pneumoconioses are defined as **lung diseases produced by organic as well as inorganic particulates and chemical fumes and vapors**.

Originally, the term pneumoconiosis (or dust diseases of the lung) was used for non-neoplastic lung reaction to inhalation of mineral dusts. Few disease caused by mineral dusts is shown in **Table 17.4**.

Pathogenesis

Only a small percentage of exposed people develop pneumoconioses, and indicates a genetic predisposition. The development of pneumoconiosis depends on:

- **Amount of dust retained in the lung and airways:** It depends on the concentration of dust in air, the duration of exposure, and the efficiency of host clearance mechanisms.

- **Size, shape, and floating capacity of the particles**: **Most dangerous particles range in size from 1–5 µm in diameter** → may reach the terminal small airways and air sacs and settle in their linings.
- **Solubility and physiochemical reactivity of the particles**: It influenced by their size.
  - **Smaller particles** which are soluble in pulmonary fluids can reach toxic levels rapidly and **produce acute lung injury**.
  - **Larger particles** which are not soluble persist within the lung parenchyma for years → produce pneumoconioses (e.g., silicosis).
- **Additional effects of other irritants,** e.g., accompanying **tobacco smoking → worsens the effects of all inhaled mineral dusts**.

Coal Workers' Pneumoconiosis

Definition: Coal workers' pneumoconiosis (CWP) is the **parenchymal lung disease caused by inhalation of carbon particles** (coal mine dust).

Pathogenesis

The pathogenesis of CWP is not completely known. In most cases, carbon dust itself is the major culprit. However, contaminating silica in the coal dust can also contribute to the development of coal workers' pneumoconiosis.

TABLE 17.4: Most common lung diseases caused by mineral dusts.

Causative agent	*Disease*	*Occupation/exposure*
Coal dust	Simple coal worker's pneumoconiosis (macules and nodules)	Coal mining
	Complicated coal worker's pneumoconiosis: Progressive massive fibrosis (PMF)	
Silica	Silicosis	Sandblasting, stone cuttings
Asbestos	Asbestosis	Mining, fabrication, and installation and removal of insulation
	Pleural plaques	
	Mesothelioma	
	Carcinoma of the lung, larynx, stomach, colon	

MORPHOLOGY

Group of lung diseases caused by coal mine dust is commonly referred to as **black lung**. Lung lesions due to progressive accumulation of carbon particles can be divided into three stages.

1. Pulmonary Asymptomatic Anthracosis

- It is a harmless coal-induced lesion in lungs of coal miners and also seen in persons residing in urban areas and tobacco smokers.
- Inhaled carbon pigment is engulfed by macrophages present in the alveoli or interstitium of lung → accumulate in the connective tissue → also carried by the lymphatics to the regional lymph nodes.

2. Simple CWP with Little/No Pulmonary Dysfunction

Gross

- **Coal macule:** Characteristic **nonpalpable lesion** that measure **1–2 mm in diameter**.
- **Coal nodule: Larger, but less than 2 cm, palpable, round or irregular and firm lesions**.
- Both are mainly seen in the **upper lobes and upper zones of the lower lobes**, adjacent to respiratory bronchioles. As the lesion progresses, dilation of adjacent alveoli may produce **centrilobular emphysema**.

Microscopy

- **Coal macule**: It consists of **focal collections of** many **carbon-laden macrophages** surrounding respiratory bronchioles.
- **Coal nodule**: It consists of carbon-laden macrophages and **small amounts of collagen and reticulin fibers**.

3. Complicated CWP (Progressive Massive Fibrosis—PMF)

Develops in individuals with simple CWP and may have impaired respiratory function.

Gross (Fig. 17.22)

Lung shows **dark**, **multiple**, **black scars larger than 2 cm** (sometimes up to 10 cm in greatest diameter).

Microscopy

Shows **carbon pigment**, carbon-laden macrophages, **dense collagen** and **disrupted lung's architecture**.

Clinical Course

CWP is usually associated with minimal loss of lung function. However, in less than 10%, PMF develops → leads to pulmonary dysfunction, pulmonary hypertension and cor pulmonale.

Silicosis

Q. Write a note on silicosis.

Definition: Silicosis is a **parenchymal lung disease** associated with **inhalation of crystalline silicon dioxide** (silica).

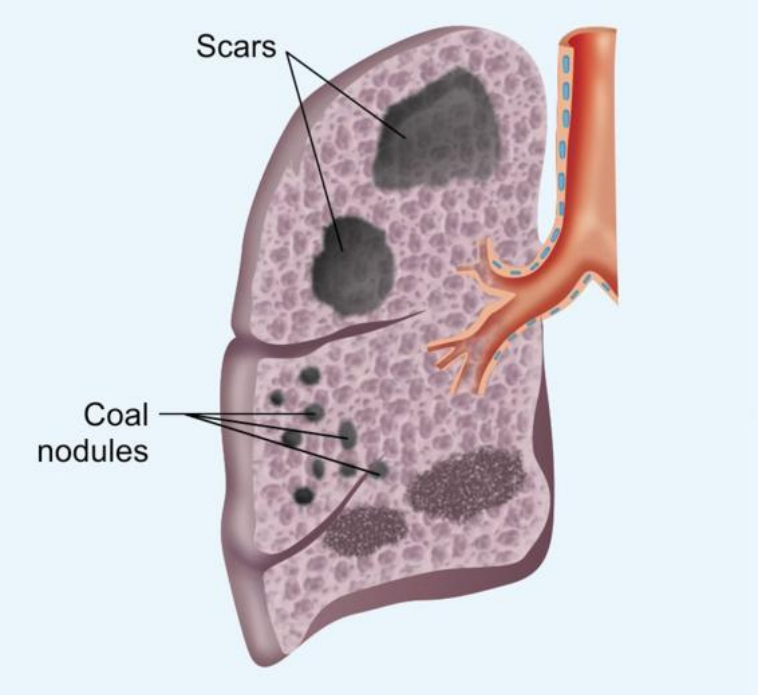
- Most prevalent slowly progressive pneumoconiosis and usually present many years after exposure.
- Crystalline silicon dioxide is abundant and found in the earth's crust and is used in a variety of industrial applications.
- **Susceptible individuals**: Sandblasters, stone cutting, polishing and sharpening of metals, ceramic manufacturing, foundry work, tunneling through rock with high quartz content and the cleaning of boilers.

Pathogenesis

Forms of silica: It occurs in both **crystalline** and **amorphous** forms. The crystalline forms include quartz, cristobalite, and tridymite are much more toxic and fibrogenic.

Steps

- **Inhalation**: Of all the crystalline forms, **quartz is most common cause of silicosis**. These particles reach lung by inhalation.
- **Interaction with epithelial cells and macrophages**: Silica is cytotoxic to alveolar macrophages. After inhalation, the particles reach the alveoli → **interact with alveolar epithelial cells** → ingested by alveolar macrophages → activates macrophages to **release of mediators** (IL-1, TNF, fibronectin, lipid mediators, oxygen-derived free radicals, and fibrogenic cytokines) → **stimulate fibroblast proliferation and collagen formation.**



Caplan syndrome: Coexistence of rheumatoid arthritis with pneumoconiosis.

Fig. 17.22: Complicated coal worker's pneumoconiosis showing two large black scars in the upper part of lung.

MORPHOLOGY

Chronic silicosis occurs in **two forms**, namely, **simple** form and **progressive massive fibrosis**. Simple silicosis is not associated with significant respiratory dysfunction**.**

Gross

Simple Sodular Silicosis (Fig. 17.23A)

Represents early stage.

- Shows **tiny**, **barely palpable**, **discrete** pale to blackened (if coal dust is also present) **silicotic nodules** (usually 2–4 mm) in the upper zones.
- **Hilar lymph nodes** may become enlarged. The periphery of the lymph node may show thin rim of calcification and are seen radiographically as **eggshell calcification** (i.e., calcium deposition surrounding a zone lacking calcification).

Progressive Massive Fibrosis (Fig. 17.23B)

As the disease progresses, expansion and coalescence of lesions may produce progressive massive fibrosis.

- Progressive massive fibrosis is defined radiologically as nodular masses of more than 2 cm diameter in a background of simple silicosis.

- The smaller silicotic nodules coalesce to form **large lesions,** which range from 5 to 10 cm in size. These lesions are **hard** and some of them may undergo central softening and cavitation. These changes may be due to superimposed tuberculosis or to ischemia.
- Fibrotic lesions may also develop in the hilar lymph nodes and pleura.

Microscopy

- **Silicotic nodule (Fig. 17.24):**
  - It has a characteristic **whorled appearance**.
  - **Center:** It consists of **concentric layers of hyalinized acellular collagen** surrounded by a **dense cellular capsule** of more condensed collagen.
  - **Periphery:** Shows **aggregates of mononuclear cells**, mostly **macrophages, lymphocytes and fibroblasts**.
- **Polarized light microscopy** may show **birefringent silica particles** in the center of silicotic nodule.

Clinical Course

- In simple nodular silicosis, lung functions are either normal or only moderately affected.
- Chest radiographs typically show a fine nodularity in the upper zones of the lung.
- The patients with progressive massive fibrosis develop shortness of breath.
- The patients have **increased susceptibility to lung infections, such as *Mycobacterium tuberculosis*, atypical mycobacteria and fungi.**
- The crystalline silica is also carcinogenic.

Asbestosis and Asbestos-related Diseases

Q. Write a note on asbestosis.

Definition: Asbestosis is defined as interstitial fibrosis of the lung caused by exposure to **asbestos dust**. It does not include asbestos-induced pleural diseases and carcinoma of lung that are found in asbestos-exposed workers.

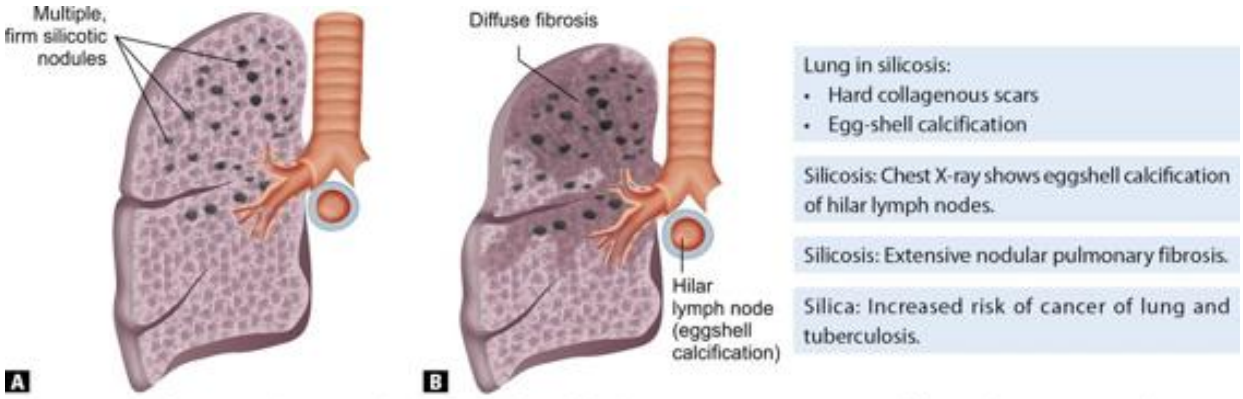
Asbestos Use and Exposure

Q. Write a note on asbestos-related diseases.

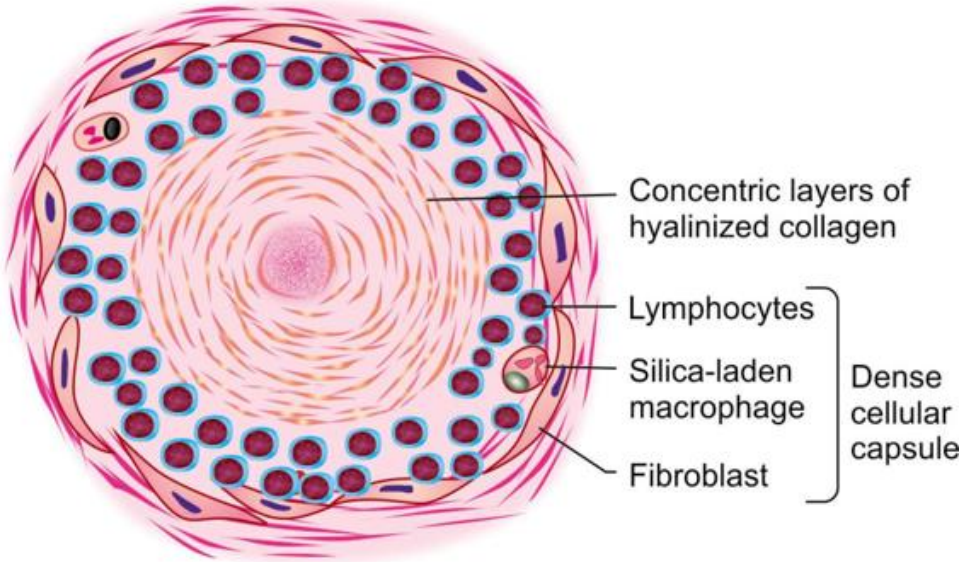
- Asbestos is a family of crystalline hydrated silicates that form fibers.
- They have unique physical-chemical properties that make them effective for insulation, reinforcing materials, and friction products.

- Asbestos is used for fireproofing, in heat and sound insulations and for strengthening plastics and cement.

Asbestos Types



Figs. 17.23A and B: Gross changes in silicosis. (A) Simple nodular silicosis; (B) Progressive massive fibrosis shows scarring at the upper lobe.



Asbestos is a generic term used for naturally occurring fibrous silicates. Two major forms are: **Serpentine and amphibole**.

1. Serpentine Form

- **Chrysotile** (white asbestos) is the **most important** serpentine forms of **asbestos used in industry**.
- Chrysotile **consists of long, curly, flexible structure** → are likely to become impacted in the upper respiratory passages and removed by the mucociliary action.

- They are less readily carried to the lungs and even if trapped in the lungs, they fragment into short particles and easily ingested by macrophages.
- They are also **more soluble than amphiboles**.

2. Amphibole Form

- The main amphibole forms are **crocidolite (blue asbestos) and amosite (brown asbestos)**.
- Amphibole form **consists of straight, rigid, brittle fibers** that may align themselves in the airstream and remain stable in the lung.
- They neither fragment nor are soluble and thus reach deeper into the lungs, where they can penetrate epithelial cells and reach the interstitium.
- Though they are not commonly used, they are **more pathogenic than chrysotiles** particularly with respect to induction of malignant pleural tumors (mesotheliomas).

Pathogenesis

Fibrogenic Effect

Inhaled asbestos fibers reach the air spaces → macrophages (alveolar and interstitial) attempt to ingest and clear the fibers → **activates the macrophages → release chemotactic factors and fibrogenic mediators**. **Both amphibole and serpentine forms are fibrogenic**. The initial injury occurs at bifurcations of small airways and ducts, where the asbestos fibers land and penetrate. Chronic exposure and deposition of asbestos fibers lead to persistent release of fibrogenic mediators. **The end result is generalized interstitial pulmonary inflammation and interstitial fibrosis**.

Oncogenic Effect

Asbestos can act both as a tumor **initiator and as promoter**.

- **Reactive free radicals**: These are produced by asbestos fibers → oncogenic.
- **Potentially toxic chemicals**: Inhaled toxic chemical are adsorbed onto the asbestos fibers → oncogenic. For example, the adsorption of carcinogens in **tobacco smoke** onto asbestos fibers → **increases the risk of lung carcinoma in asbestos workers**.

MORPHOLOGY

Gross (Fig. 17.25A)

- **Sites:** Lesions **begin** in the **lower lobes and subpleura.**
- **Early stages:** Interstitial fibrosis may not be apparent on gross examination. Begins as interstitial **fibrosis around respiratory bronchioles and alveolar ducts** and extends to involve adjacent alveolar sacs and alveoli. The lesions are **termed mineral dust-induced small airways disease.**
- **Later stages:** The **interstitial fibrosis becomes more extensive and may involve the entire lung**. The fibrosis may **destroy the normal architecture of the lung** to produce **dilated airspaces (cystic spaces) surrounded by** thick **fibrous walls** → produces **honeycombed** appearance to the involved regions. The lungs become small.

Microscopy

Asbestos Bodies (Fig. 17.25B)

- The asbestos fibers after reaching the finer bronchioles and alveoli may form → asbestos bodies.
- They **consist of translucent center of asbestos fibers coated with a film of proteins rich in iron.**
- Asbestos bodies appear as **golden brown**, **fusiform or beaded rods.**
- The coating is thickest at the ends of the fibers, giving a **dumb-bell appearance.**
- They form when macrophages attempt to phagocytose asbestos fibers; the iron is probably derived from phagocyte ferritin.

Ferruginous Bodies

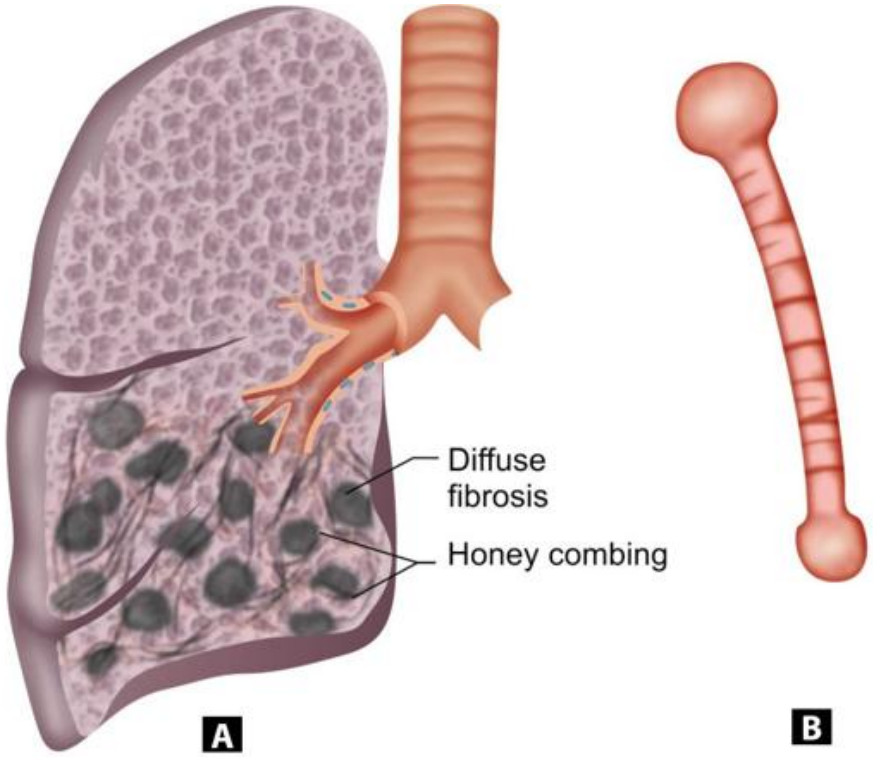
Other inorganic particulates/fibers (not asbestos) may also become coated with similar iron-protein complexes → called as **ferruginous bodies.**

Fibrosis of the Lung

Lung shows **diffuse pulmonary interstitial fibrosis** with multiple **asbestos bodies.**

Complications: Pulmonary hypertension and cor pulmonale.

Fig. 17.24: Silicotic nodule is composed of concentric whorls of dense, sparsely cellular collagen. The edge of the nodule shows silica-laden macrophages and lymphocytes.



Figs. 17.25A and B: (A) Asbestosis of lung showing honeycomb appearance in the lower lobe; (B) Asbestos body is a ferruginous body, golden brown, beaded and having dumb-bell shape.

Pleural Plaques

They are most **common manifestation of asbestos exposure**.

MORPHOLOGY

Gross

Pleural plaques appear as **pearly white; well-circumscribed plaques** (may be over 10 cm in diameter). **Usually bilateral** and most frequent on the **anterior and posterolateral aspects of the parietal pleura** and **over the domes of the diaphragm**.

Microscopy

- It consists of **acellular, dense, hyalinized fibrous tissue**, with many **slit-like spaces** arranged in a parallel fashion (**basket-weave pattern**). May show calcification.
- They **neither show asbestos bodies nor** do they **progress to mesotheliomas**.

Lung Cancer and Mesothelioma

The workers exposed to asbestos may develop lung carcinomas and mesotheliomas (pleural and peritoneal). Concomitant cigarette smoking markedly increases the risk of lung carcinoma but not that of mesothelioma.

Clinical Features of Silicosis

- Initially patients present with **dyspnea on exertion**, but **later** it is present **even at rest**.
- Usually it is accompanied by productive cough.
- Chest X-rays show irregular linear densities, particularly in both lower lobes.
- As **the disease progresses, a honeycomb pattern** develops → may progress to respiratory failure, cor pulmonale, and death.
- Pleural plaques are usually asymptomatic and are visualized on radiographs as circumscribed densities.

Pneumoconioses: Lung diseases produced by organic as well as inorganic particulates and chemical fumes and vapors.

Pneumoconiosis: Most dangerous particles causing pneumoconiosis: 1–5 μm. Particle <0.5 μm reach the alveoli.

Coal workers' pneumoconiosis: Due to inhalation of carbon (anthracotic) pigment. Not associated with risk of cancer or tuberculosis. Dust cells are alveolar macrophages with anthracotic (carbon) pigment.

Silicosis:
- Nodular fibrosing disease due to inhalation of silica
- Most common chronic occupational disease in the world
- Crystalline forms of silica are most toxic and fibrogenic
- Involves upper lobes of the lung
- Opacities in the lung-contain collagen and quartz
- Polarizing microscopy-birefringent silica particles

Asbestosis: Interstitial fibrosis of the lung caused by exposure to asbestos dust. No increased risk of tuberculosis.

Asbestos-related diseases:
- Asbestosis
- Localized fibrous plaques
- Pleural effusions
- Carcinoma of lung
- Malignant mesotheliomas
- Laryngeal carcinomas.

Asbestos: (1) Fibrogenic and (2) Oncogenic.

Asbestos: Exposure to increasing doses are associated with a higher incidence of all asbestos-related diseases except mesothelioma, which is only associated with amphibole exposure.

Asbestosis: Common site is:
- Lower lobe of lung
- Subpleura

Asbestosis: Asbestos bodies (asbestos fibers coated by iron), Ferruginous bodies.

Asbestos body: Iron containing proteinaceous material coating asbestos fiber. Hallmark of asbestosis.

Ferruginous body: Iron protein complex coating other inorganic particles, such as talc, mica and glass in the lung (not asbestos fiber).

Benign pleural plaques: Most common lesions in asbestosis.

Asbestos-related disease: Lung cancer is the most common.

Asbestosis: Mesothelioma arises from the pleura.

Bagassosis is lung disease caused by Sugarcane.

Byssinosis: Interstitial lung disease caused by organic dust and is associated with hypersensitive pneumonitis.

LUNG CARCINOMAS

CC: Define and describe the etiology, types, exposure, genetics, environmental influence, pathogenesis, stages, morphology, microscopic appearance, metastases and complications of tumors of the lung and pleura.

Q. Classify lung tumors. Discuss the pathogenesis, morphology and clinical features including paraneoplastic syndromes of squamous cell carcinoma of lung.

Q. Discuss the pathology of adenocarcinoma of lung.

Q. Discuss the etiopathogenesis and morphology of bronchogenic carcinoma.

Q. Discuss the etiopathogenesis of lung carcinoma and write the morphology of neuroendocrine tumors (small cell carcinoma).

- Carcinoma of the lung is the **most common cause of cancer death**. This is mainly due to the carcinogenic effects of **cigarette smoke**.
- In the past, the term bronchogenic carcinoma was used for primary lung cancer, to indicate the origin from the bronchi. Now it is known that about 5% of primary lung cancers do not arise from bronchus. Hence, the term lung cancer is used.
- **Age and gender**: Mostly found between **50 and 80 years** of age. More common in males, but there is a recent increase in females due to increased smoking among females.

Etiology and Pathogenesis

Q. Discuss etiopathogenesis of carcinoma of lung.

Tobacco Smoking

Major carcinogens in tobacco smoke (Table 17.5): These include both initiators and promoters (such as phenol derivatives).

- **Polycyclic aromatic hydrocarbons:** Benzo[*a*]pyrene, dibenzanthracene.
- **Radioactive elements and other contaminants**: Arsenic, nickel, cadmium, molds and vinyl chloride.

There is strong (i) statistical, (ii) epidemiological, (iii) clinical and (iv) experimental evidence that tobacco smoking is the most important cause of cancer of lung. Smoking also multiplies the risk of other carcinogenic influences, such as asbestos and uranium. The **strongest association** of smoking is with **squamous cell and small cell carcinoma**.

Statistical Evidence

About **90% of lung carcinomas occur in smokers** and it depends on:

- Amount of daily smoking.
- Tendency to inhale.
- Duration of the smoking habit.

The **risk increases with number of cigarettes smoked** and is **proportional to the duration of smoking**. Compared to nonsmokers, the smokers have a 10 times and heavy smokers (more than 40 cigarettes per day for several years) 60 times more risk of lung cancer. Females are more susceptible to tobacco carcinogens than males. Cessation of smoking for 10 years reduces risk but not to the level in nonsmokers. However, only 11% of heavy smokers develop lung cancer, which indicates that genetic factors are involved. **Pipe and cigar smokers have lower incidence than cigarette smokers**.

Epidemiologic Studies

Cigarette smoking is associated with not only **lung cancer** but also carcinoma of the **mouth, pharynx, larynx, esophagus, pancreas, uterine cervix, kidney and urinary bladder** (*refer* page 203).

Clinical Evidence

Microscopic changes in the lining epithelium of the respiratory tract in habitual smokers also revealed the carcinogenic effect of smoking. There is **a positive relation between the intensity of exposure to cigarette smoke and the histological changes in the respiratory epithelium**. The sequence of changes is: Basal cell hyperplasia → squamous metaplasia → dysplasia → carcinoma in situ → invasive carcinoma.

Experimental Work

Few animals developed bronchioloalveolar carcinomas after exposure to tobacco smoke.

Industrial Hazards

- **Ionizing radiation:** High-dose of ionizing radiation is carcinogenic. Increased incidence of lung cancer was reported among survivors of the Hiroshima and Nagasaki atomic bomb blasts. **Radon** is a radioactive gas

and indoor air pollution by radon **is associated with increased lung cancer in miners exposed to high concentrations.**

TABLE 17.5: Selected tobacco smoke constituents and its effect.

Constituent in tobacco smoke	*Effect*
• Tar • Polycyclic aromatic hydrocarbons • Nitrosamine • Benzopyrene	Carcinogenesis
• Nicotine • Phenol	Tumor promotion

- **Uranium:** It is **weakly radioactive** and uranium miners (both nonsmokers and smokers) have higher incidence of lung cancer than in the general population.
- **Asbestos:** Exposure **increases the risk** and risk increases when associated with smoking. It develops after a latent period of about 10–30 years.
- **Other carcinogens:** Chloromethyl ethers, chromium, nickel, vinyl chloride, polyaromatic hydrocarbons, cadmium, formaldehyde and dioxin.

Others

- **Atmospheric pollution:** The risk of **lung cancers is higher in urban areas** than rural areas, suggesting role of air pollution. Major air pollutants include polycyclic hydrocarbons from fossil fuels and motor vehicle exhaust especially diesel smoke.
- **Diet: Vitamin A deficiency** → leads to squamous metaplasia → increased **susceptibility to cancer**. Folate, carotenoid-rich fruits and vegetables, vitamin E and beta-carotene are associated with reduced risk of lung cancer.
- **Idiopathic pulmonary fibrosis:** It is associated with increased risk of lung cancer.
- **Heredity:** Epidemiological studies have shown lung cancers in close relatives. First-degree relatives of patients with lung cancer have a two- to six-fold increase in the risk for lung cancer. Various susceptibility genes associated with lung cancers have been identified.
- **Genetic predisposition:** Genetic polymorphisms involving cytochrome P-450 gene CYP1A1 → increased capacity to metabolize procarcinogens

present in the cigarette smoke → increased risk of lung cancer.

Clinical subgroups of lung cancer: Until recently, lung carcinoma was divided into two clinical subgroups on the basis of likelihood of metastases and response to therapies.

- **Small cell lung carcinomas** (SCLC) (**highly metastatic, high response to chemotherapy**).
- **Non-small cell lung carcinomas** (NSCLC) **(less metastatic, less responsive)**. This group includes adenocarcinoma, squamous and large cell carcinoma, and large cell neuroendocrine carcinomas. However, presently therapies are available that target specific oncoproteins found in a subset of NSCLC (adenocarcinomas and squamous cell carcinomas).

Molecular Genetics

Most lung cancers develop as **multistep** process by accumulation of oncogenic "driver" mutations. This causes neoplastic transformation of pulmonary epithelial cells from a premalignant lesion to frank cancer after a number of years. Exposures to **various carcinogens produce sequential genetic alterations and epigenetic changes** that result in the loss of normal control of cell growth which eventually produce neoplasms.

Squamous Cell Carcinoma

Strongly associated with smoking and genetic aberrations include:

- **Inactivation of tumor suppressor gene:** Chromosome deletions involves tumor suppressor loci and involve 3p, *9p* (site of the *CDKN2A* gene), and *17p* (site of the *TP53* gene). They occur during early stages in tumor evolution. Loss of retinoblastoma (RB) tumor suppressor is found in 15% of squamous cell carcinomas. The cyclin-dependent kinase inhibitor gene *CDKN2A* is inactivated and its protein product, *p16*, is lost in 65% of tumors.
- **Activation of oncogenes:** These are found both in squamous cell carcinoma and adenocarcinomas.
  - **Amplification of *FGFR1* and *MET*:** Many squamous cell carcinomas have amplification of *FGFR1*, a gene encoding the fibroblast growth factor receptor tyrosine kinase.

- **Mutations** [e.g., *EGFR* (tyrosine kinase domain of this gene), *KRAS*, *BRAF* and *ERBB2*. *EGFR* mutations are more common in adenocarcinomas in nonsmokers].
- **Translocations** (e.g., *ALK*, *ROS1* and *RET)*.

Neuroendocrine Tumors (Small Cell Carcinoma)

Strongest association with smoking and has many molecular features of squamous cell carcinoma. This includes:

- **Inactivation of tumor suppressor gene:**
  - Loss-of-function mutations involving *TP53* (75–90% of tumors).
  - *RB* (almost 100% of tumors).
  - Chromosome *3p* deletions.
- **Activation of oncogenes: Amplification of genes of the *MYC*** family occurs in 10–40% of small cell carcinomas but is rare in other types.

Adenocarcinoma

It shows oncogenic gain-of-function mutations involving components of growth factor receptor signaling pathways. All are found in a minority of tumors and include:

Activation of oncogenes

- **Gain-of-function mutations** in multiple genes encoding receptor tyrosine kinases, e.g., *EGFR, BRAF, ERBB2/HER2, ALK, ROS, MET* and *RET*, which are also mutated in other types of cancer.

BOX 17.5: WHO classification (2015 abridged) of lung tumors.

Epithelial tumors

- Squamous cell carcinoma
- Adenocarcinoma
  - Preinvasive lesions: Atypical adenomatous hyperplasia, adenocarcinoma in situ (AIS: non-mucinous/mucinous)
  - Minimally invasive adenocarcinoma (MIA: non-mucinous, mucinous)
  - Invasive: Lepidic, acinar; papillary, micropapillary, solid (according to predominant pattern)
- Neuroendocrine tumors
  - Small-cell carcinoma: Combined small cell carcinoma
  - Large-cell neuroendocrine carcinoma: Combined large-cell neuroendocrine carcinoma
  - Carcinoid tumors: Typical, atypical

- Large-cell carcinoma
- Mixed carcinomas
  - Adenosquamous carcinoma
  - Combined small cell carcinoma
- Carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements and giant cell carcinoma
- Salivary gland type tumors
- Benign tumors: Squamous papilloma, adenoma

Mesenchymal tumors

- Pulmonary hamartoma
- Chondroma
- PEComatous tumors

Lymphohistiocytic tumors

Tumors of ectopic origin

Metastatic tumors

- ***ALK* receptor alterations:** Activating rearrangements of the *ALK* gene by fusion with *EML4* gene is observed in 3–7% of adenocarcinoma of lung.

- **Mutations in the *KRAS* gene:** Tumors without tyrosine kinase gene mutations often have mutations in the *KRAS* gene, which is involved in growth factor signaling pathways of receptor tyrosine kinases.

Classification (Box 17.5)

Q. Write short note on classification of lung cancer.

MORPHOLOGY

Q. Write short note on morphology of lung cancer.

Site (Figs. 17.27A to C)

- **Central (hilar):** Lung cancers arising in and around the hilus of the lung are the most common. About 75% arise from first, second, and third-order bronchi.
- **Peripheral:** Lung cancers may also arise in the periphery of the lung from the alveolar septal cells or terminal bronchioles. They are **usually adenocarcinomas, including adenocarcinoma in situ**.

Squamous Cell Carcinomas

Preneoplastic Lesions

They **may occur** in the bronchial epithelium and include **squamous metaplasia or dysplasia**, which then transforms to **carcinoma in situ**. They often precede for years and may progress to invasive squamous cell carcinoma. Sequential changes during the pathogenesis of lung cancer are shown in **Figures 17.26A to F**.

Squamous cell carcinoma is **most common** in **men** and is most **strongly related to cigarette smoking**.

MORPHOLOGY

Gross (Figs. 17.27A to C)

- **Site and pattern of growth:** Most tumors arise in the **central portion of the lung** from the major bronchi (main to segmental bronchus).
  - **Peribronchial growth:** In which the tumor appears as ulcerated lesions in the bronchi that penetrates the wall of the involved bronchus and infiltrate along the peribronchial tissue into the adjacent carina or mediastinum.
  - **Intraprenchymal growth:** In this pattern, the tumor grows like a cauliflower-like intraparenchymal mass which pushes the surrounding lung substance.
  - **Exophytic growth:** It starts as endobronchial tumor projecting into the lumen of the bronchi producing an intraluminal mass. As it enlarges, it obstructs the involved bronchus leading to atelectasis and infection distal to the obstruction.
- **Color and consistency**: **Gray-white** in color and **firm to hard** in consistency.
- **Cut section:** It may show focal **areas of hemorrhage or necrosis**. It may produce red or yellow-white mottling and softening. The central necrotic foci may form cavity.

Microscopy (Figs. 17.28A and B)

The characteristic features of squamous cell differentiation are:

- **Keratinization**:
  - **Epithelial keratin pearls** or
  - **Individual cell keratinization.**
- **Intercellular bridges**: They are seen as slender gaps between adjacent cells.

Grades

On the basis of the amount of keratinization graded as:

1. **Well differentiated**: These tumors show keratinization in the form of **keratin/squamous pearls**. They **appear as small round nests of brightly eosinophilic aggregates of keratin surrounded by concentric (onion skin) layers of squamous cells.**

2. **Moderately differentiated**: These tumors show **individual cell keratinization**. This appear as cells with intensely eosinophilic, glassy and dense cytoplasm.
3. **Poorly differentiated**: These tumors may show only intercellular bridges without any foci of keratinization. **Mitotic activity is higher** in these tumors.

Adenocarcinoma

Adenocarcinoma in situ (AIS)

Once known as broncioloalveolar carcinoma (BAC-obsolete) has distinct gross, microscopic, and clinical features. It is a preinvasive form of adenocarcinoma.

MORPHOLOGY

Gross

- **Occurs in the terminal bronchioloalveolar regions** of the lung.
- Seen at the **peripheral portions of the lung** as a **single nodule (**coin lesion) measuring 3 cm or less.

Microscopy (Figs. 17.29A and B)

- Shows a **pure bronchioloalveolar growth pattern** with **no evidence of stromal**, **vascular**, **or pleural invasion**.
- Characteristically, they **grow along pre-existing alveolar walls without destruction of alveolar architecture**. This growth pattern is known as **lepidic**, where the neoplastic cells resemble butterflies sitting on a fence.

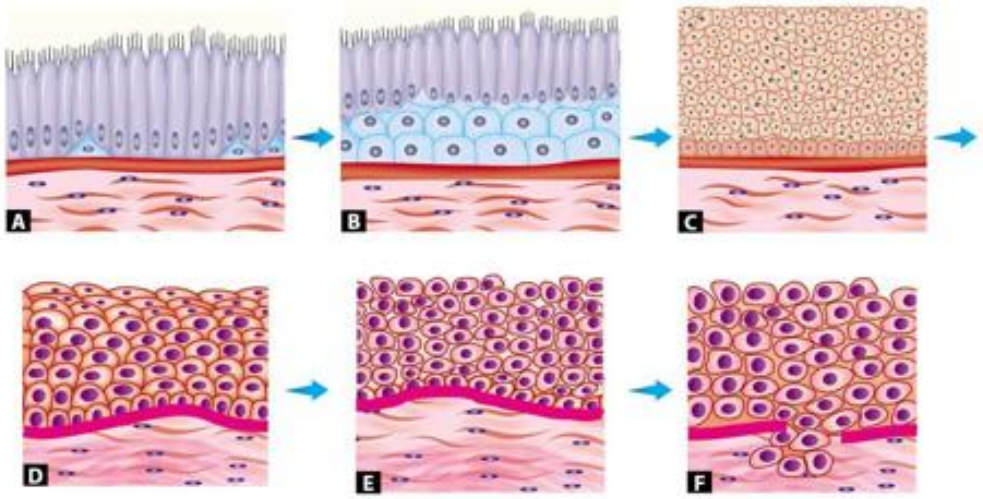
Subtypes

- **Nonmucinous**: It consists of columnar, peg-shaped, or cuboidal cells.
- **Mucinous:** It consists of distinctive, tall, columnar cells with cytoplasmic and intra-alveolar mucin, growing along the alveolar septa.
- **Mixed:** Mixture of both nonmucinous and mucinous type.

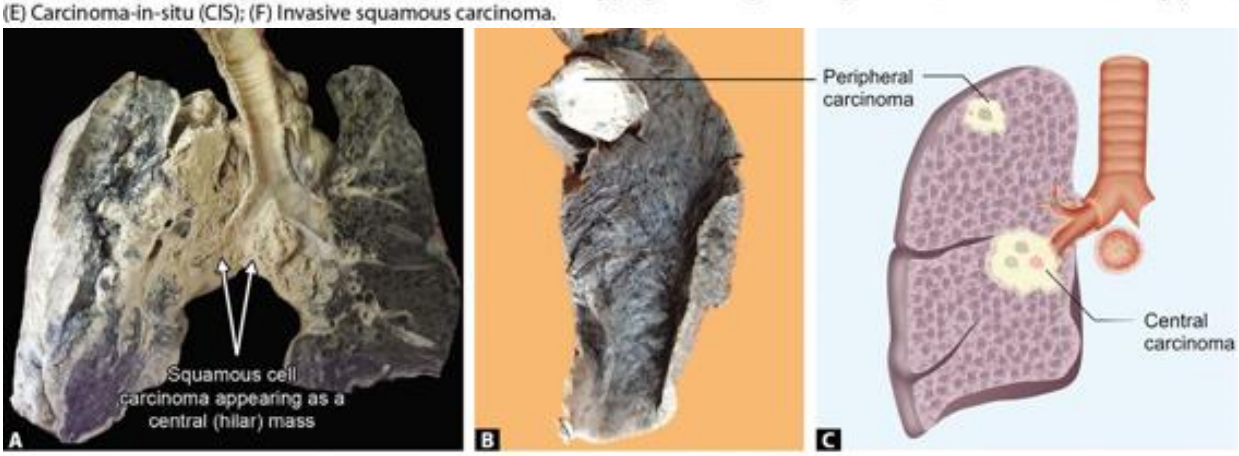
Minimally Invasive Adenocarcinoma

In an adenocarcinoma showing lepidic growth, presence of a minimal invasion does not adversely affect prognosis. This category of adenocarcinoma is termed as minimally invasive adenocarcinoma (MIA, formerly bronchioloalveolar carcinoma—obsolete) and has the same favorable prognosis as AIS. MIA is defined as a lepidic—predominant

adenocarcinoma tumor as in AIS, but with foci of invasion less than or equal to 5 mm in maximal dimension and without any pleural or lymphovascular invasion and necrosis. At the site of tumor there may be pleural fibrosis and subpleural scars. These scars represent desmoplastic response to the tumor. These tumors are single and discrete and usually non-mucinous.



Figs. 17.26A to F: Sequential changes during the pathogenesis of lung cancer. (A) Normal epithelium; (B) Basal cell (or reserve cell) hyperplasia; (C) Squamous metaplasia; (D) Dysplasia (characterized by the presence of disordered squamous epithelium, with loss of nuclear polarity, nuclear hyperchromasia and pleomorphism) may progress through the stages of mild, moderate and severe dysplasia; (E) Carcinoma-in-situ (CIS); (F) Invasive squamous carcinoma.



Figs. 17.27A to C: Gross types of carcinoma lung namely, central and peripheral: (A) Squamous cell carcinoma appearing as a central (hilar) mass which has invaded the contiguous lung parenchyma; (B) Peripheral carcinoma of lung; (C) Diagrammatic.

Invasive Adenocarcinoma

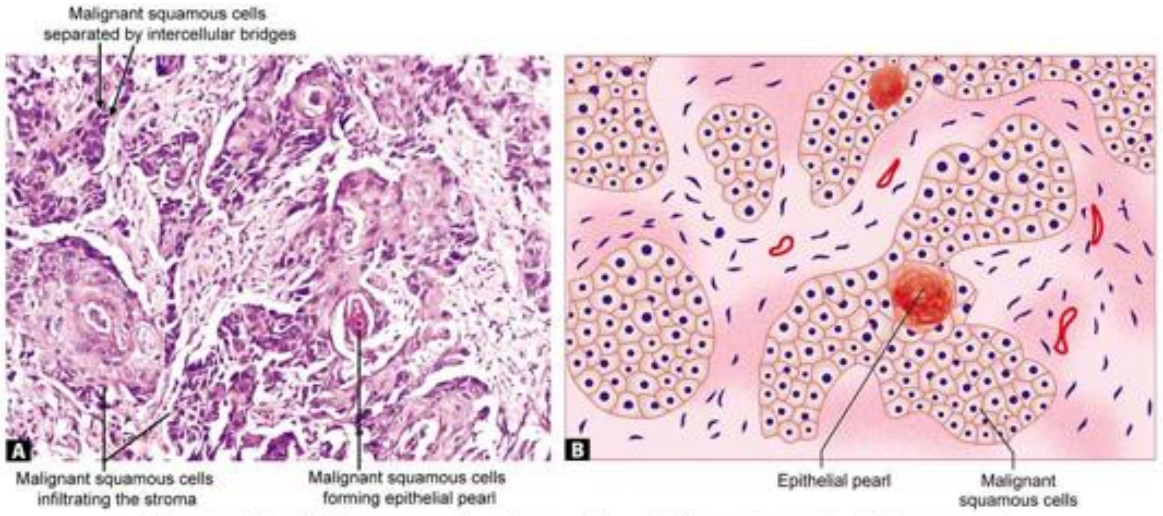
Adenocarcinoma is an invasive malignant epithelial tumor in which tumor cells show glandular differentiation or mucin production. Most **common type of carcinoma in women and nonsmokers**. They are classified based

on the predominant growth pattern namely: lepidic, acinar, papillary, solid and micropapillary.

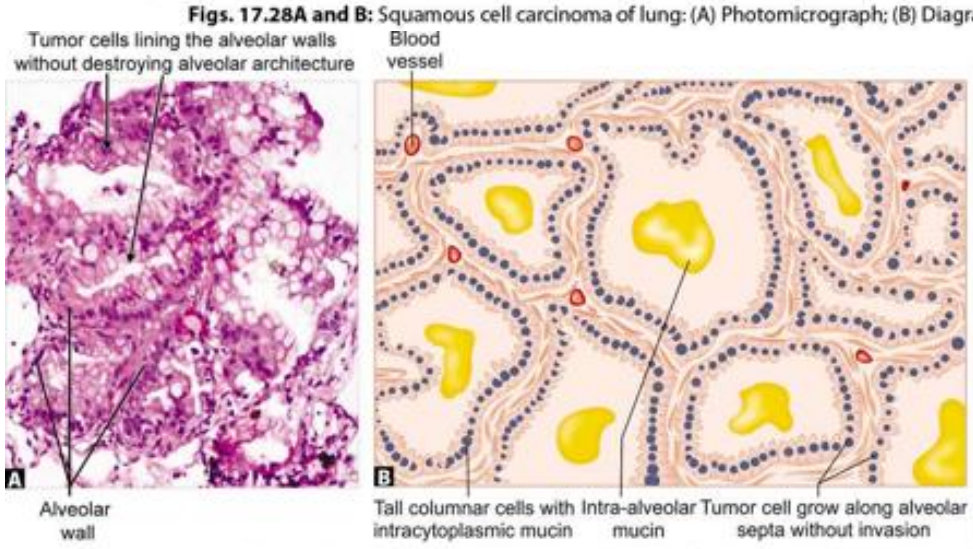
MORPHOLOGY

Gross

- **Site:** Usually more **peripherally located** (in contrast to squamous cell carcinoma), and **tend to be smaller.**
- **Appearance**: They appear as **irregular masses** ranging from 2 to 5 cm in diameter.
- **Cut section**: Nonmucinous tumors appear **grayish white** mucinous tumors may appear glistening or gelatinous (depending on the amount of mucus).



Figs. 17.28A and B: Squamous cell carcinoma of lung: (A) Photomicrograph; (B) Diagrammatic.



Adenocarcinoma in situ:
- Tumor 3 cm or less in diameter
- Lepidic growth pattern
- No stromal invasion.

Adenocarcinoma of lung: Precursor lesion may be atypical adenomatous hyperplasia (AAH).

Adenocarcinoma of lung:
- Overall most common type of cancer
- Most common lung cancer in non-smokers and females

Figs. 17.29A and B: Adenocarcinoma in situ: (A) Photomicrograph; (B) Diagrammatic.

Microscopy

Most adenocarcinomas show mixtures of lepidic, acinar, papillary, micropapillary and solid patterns: (i) **lepidic**—predominant tumors show prominent lepidic growth pattern, (ii) **acinar**—predominant tumor pattern, consists of regular glands lined by cuboidal or columnar cells, (iii) **papillary** adenocarcinomas show a single cell layer covering a fibrovascular connective tissue core, (iv) **micropapillary** carcinomas consist of small papillary tufts of tumor cells without fibrovascular core, (v) **solid** adenocarcinomas with mucus formation are poorly differentiated tumors.

Mucinous adenocarcinomas consist of tall columnar cells with apical cytoplasmic mucin. They usually spread aerogenously and form satellite tumors. Grossly, they may appear as solitary nodule or as multiple nodules, or an entire lobe may be consolidated by tumor (resembles lobar pneumonia).

Majority of tumors are **positive for thyroid transcription factor-1 (TTF-1).**

Spread: They **grow more slowly than squamous cell carcinomas but metastasize widely and earlier.**

Neuroendocrine Tumors

Neoplasms of neuroendocrine cells in the lung include: (i) low-grade carcinoids; (ii) the highly aggressive small cell carcinoma and (iii) large cell neuroendocrine carcinoma of the lung.

Small Cell Carcinoma

Q. Write a short note on small cell carcinoma of lung.

- Previously known as oat cell carcinoma.
- **Highly malignant** epithelial tumor **with neuroendocrine features** and is **strongly associated with cigarette smoking**. The male-to-female ratio is 2:1.

MORPHOLOGY

Gross

- **Site**: It may arise not only in **major bronchi (central)** but also in the **peripheral portion** of the lung.
- **Cut section**: It is **soft**, **friable and white,** and shows **extensive hemorrhage and necrosis**.

Light Microscopy (Figs. 17.30A and B)

- **Characteristics of tumor cells:**
  - **Size and shape: Small round to fusiform**. The small cell is termed as an oat cell. There is no absolute size for the tumor cells, but a useful rule of thumb is that their diameter/size is less than three times the diameter of a small resting lymphocyte (a size of about 25 microns).
  - **Cytoplasm: Scanty**, faintly stained with ill-defined cell borders.
  - **Nucleus:** It is hyperchromatic with **finely granular chromatin** (**salt and pepper pattern**) and **nuclear molding (better seen in cytology specimens)** is prominent.
  - **Nucleolus:** Absent or **inconspicuous.**
  - **Mitotic count: High** with an average of 60–70 mitosis per 10 high-power fields.
  - **Cells are fragile** and show fragmentation and **crush artifact** in biopsy specimens.
- **Growth pattern**: The tumor cells grow in **sheets or clusters**. The stroma is delicate, vascular and scanty.
- **Necrosis: Common and extensive**. Blood vessel wall may stain blue due to deposition of DNA from necrotic tumor cells (**Azzopardi effect**).

Electron Microscopy

The tumor cell shows **membrane-bound neurosecretory granules** similar to the neuroendocrine cells.

- **Origin of tumor:** Small cell carcinoma arises from neuroendocrine progenitor cells present in the lining bronchial epithelium. The evidences for this include: (i) presence of neurosecretory granules, (ii) expression of neuroendocrine markers (e.g., chromogranin, synaptophysin, and CD57), and (iii) secretion of hormones (e.g., parathormone-related protein which produces paraneoplastic hypercalcemia) by some tumors. **Small cell carcinoma is one of the most common lung cancers associated with ectopic hormone production and paraneoplastic syndromes.** Immunohistochemistry shows high positivity of the antiapoptotic protein *BCL2* in 90% of tumors.
- **Behavior:** All small cell carcinomas are **high grade** and are most aggressive lung tumors. They **widely metastasize** and are **incurable by surgery** but are **markedly sensitive to chemotherapy**.
- Combined small cell carcinoma is a variant in which small cell carcinoma is mixed with other nonsmall cell tumors (e.g., large cell neuroendocrine carcinoma).

Large Cell Carcinoma

- Large cell carcinoma is an **undifferentiated malignant epithelial tumor** of lung.
- The tumor cells do not have the characteristic of small-cell, glandular or squamous cell carcinoma.
- They probably represent so undifferentiated squamous cell carcinomas and adenocarcinomas that cannot be identified by light microscopy. Its diagnosis is by exclusion.
- Some large cell carcinomas are called large cell neuroendocrine carcinoma. They grow like carcinoid tumors, i.e., organoid pattern, trabecular growth, peripheral palisading of cells and rosette formation. They also show neuroendocrine differentiation by immunohistochemistry or ultrastructure.

MORPHOLOGY

Gross

It arises more frequently in the **periphery of the lung**. It forms a spherical tumor with well-defined borders. Cut section shows bulging, fleshy, homogeneous, rather sarcomatous appearance.

Microscopy (Fig. 17.31)

Tumor **cells are large with moderate amount of cytoplasm, large nuclei, prominent nucleoli and vesicular chromatin.**

Combined Carcinoma

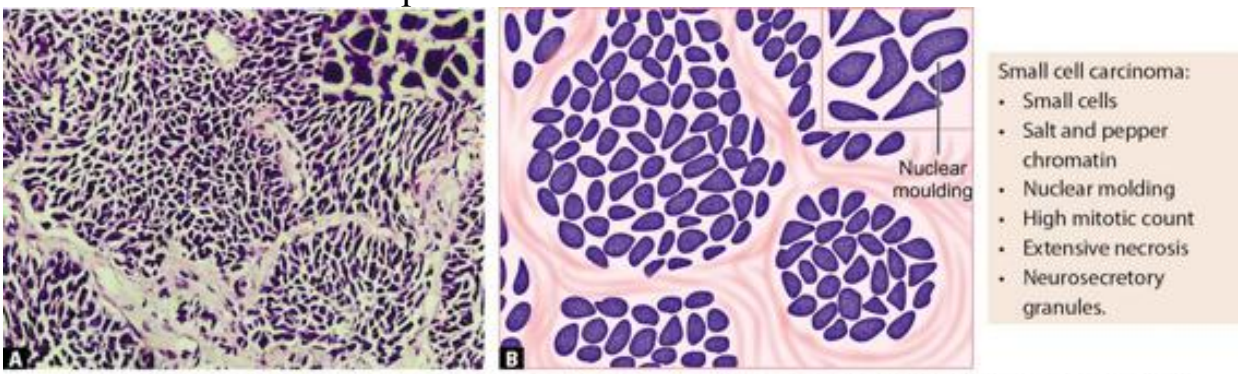
They constitute about 10% of all lung carcinomas. Microscopically they have combined histology of two or more of the above histological types, e.g., squamous cell carcinoma and adenocarcinoma or small-cell and squamous cell carcinoma.

Carcinoid Tumors

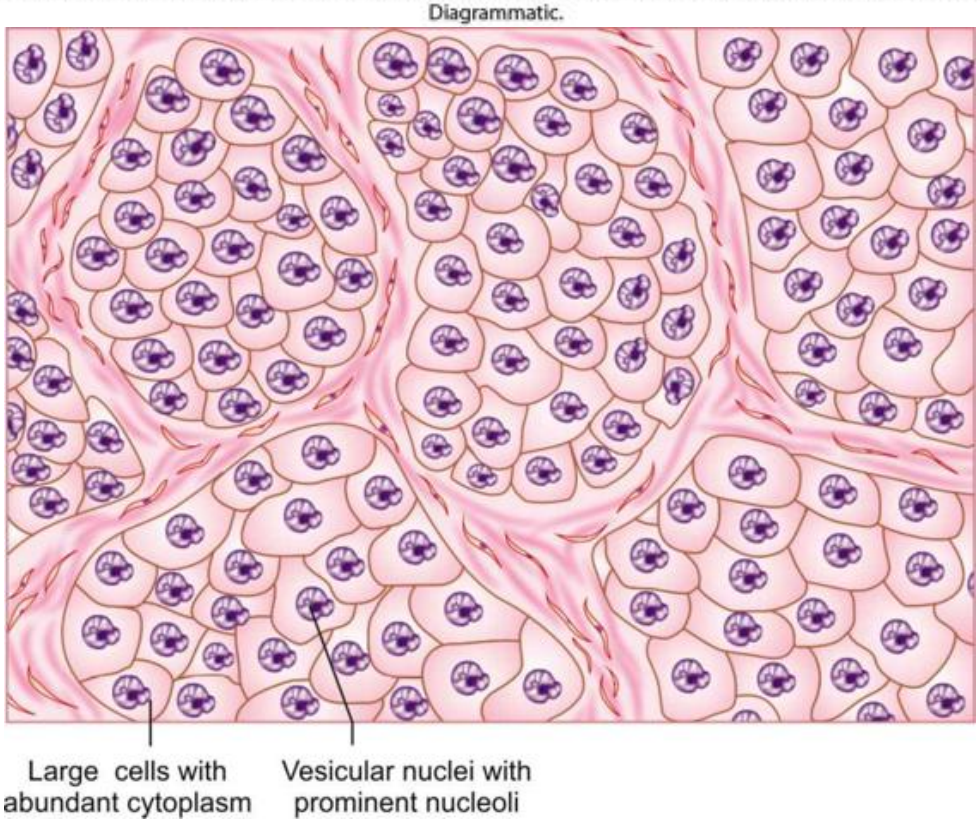
Q. Write a short note on bronchial carcinoid.

- Carcinoid tumors are low-grade malignant epithelial neoplasms that arise from the resident neuroendocrine cells normally in the bronchial epithelium.

- Age and gender: Most common below 40 years of age and both males and females are equally affected. There is no relation with cigarette smoking and about 20–40% of patients are nonsmokers.



Figs. 17.30A and B: Microscopic appearance of small cell carcinoma. Inset shows nuclear molding. (A) Photomicrograph; (B) Diagrammatic.



MORPHOLOGY

Gross

Carcinoids may arise centrally or may be peripheral.

- **Central tumors:** Usually they present as fingerlike or spherical polypoid masses in the mainstem bronchi covered by an intact mucosa. They are small and measure about 3.0 cm diameter. They project into the bronchial lumen as an endobronchial lesion. Some

may penetrate the bronchial wall into the peribronchial tissue, producing the so-called collar-button lesion.

- **Peripheral tumors:** They are solid and nodular.

Microscopy

It shows organoid, trabecular, palisading, ribbon, or rosette-like arrangements of cells separated by a delicate fibrovascular stroma. Similar to carcinoids of the gastrointestinal tract (*refer* **Fig. 18.28**), the individual tumor cells are uniform, regular with uniform round nuclei with finely granular chromatin. The cytoplasm is moderate, eosinophilic and finely granular. Depending on the microscopic appearance they are subclassified into:

1. **Typical carcinoids:** These tumors have less than two mitoses per 10 high-power fields and lack necrosis.
2. **Atypical carcinoids:** They differ from typical carcinoids by (i) increased mitoses (2–10 mitoses per 10 high-power fields), (ii) foci of necrosis, (iii) increased pleomorphism, (iv) increased cellularity, and (v) tendency to grow in a disorganized fashion and invade lymphatics.

Electron microscopy: Tumor cells contain dense-core granules.

Immunohistochemistry: Shows positivity for serotonin, neuron-specific enolase, bombesin, calcitonin, or other peptides.

Clinical Features

Carcinoid tumors grow slowly and about 50% of patients are asymptomatic at presentation. They are often discovered incidentally.

If a patient is symptomatic, symptoms are due to:

- **Intraluminal growth:** Persistent cough, hemoptysis, secondary infections, dyspnea, bronchiectasis, emphysema, and atelectasis.

Fig. 17.31: Large cell carcinoma of lung consisting of large tumor cells with moderate amount of cytoplasm, large nuclei with vesicular chromatin and prominent nucleoli.

- **Metastasis and secretion of vasoactive amines:** Majority neither metastasize nor have secretory activity and follow a relatively benign course. In about 10% of cases, they may produce classic carcinoid syndrome, characterized by intermittent attacks of diarrhea, flushing and cyanosis.

Differences between small cell lung carcinoma and non–small cell lung carcinoma (adenocarcinoma and squamous cell carcinoma) are summarized in **Table 17.6**.

Secondary Changes due to Tumor

They **occur in the lung distal to the point of bronchial involvement.**

- **Obstruction** of the **lumen of a major bronchus**.
  - **Partial** obstruction → may cause **focal emphysema**.
  - **Total** obstruction → may lead to **atelectasis** (collapse of the lung) distal to obstruction.
- **Suppuration: Due to impaired drainage** of the airways.
  - **Severe suppurative or ulcerative bronchitis or bronchiectasis**.
  - **Pulmonary abscesses.**
- **Compression or invasion of the superior vena cava**: It produces marked dilation of the veins of the head, neck, and arms and cyanosis → results in a characteristic clinical complex known as **superior vena cava syndrome**.

Spread of Tumor (Fig. 17.32)

Q. Write short note on mode of spread of lung cancer.

Local Spread (Table 17.7)

- Extension to the **pleural surface**, pleural cavity (**pleuritis)** or into the pericardium (pericarditis**)** associated with **effusions**.

TABLE 17.6: Differences between small cell lung carcinoma and non-small cell lung carcinoma (adenocarcinoma and squamous cell carcinoma).

Features	***Small cell lung carcinoma***	***Non-small cell lung carcinoma***
Microscopy • Cytoplasm • Nuclear features • Pattern/architecture	Scant Small, hyperchromatic nuclei with fine chromatin pattern; nucleoli indistinct Diffuse sheets of cells	Abundant Pleomorphic nuclei with coarse chromatin pattern; nucleoli often prominent Glandular or squamous architecture
Neuroendocrine markers Dense core granules on electron microscopy; expression of chromogranin, synaptophysin and CD56	Present	Absent

TABLE 17.6: Differences between small cell lung carcinoma and non-small cell lung carcinoma (adenocarcinoma and squamous cell carcinoma).

Features	***Small cell lung carcinoma***	***Non-small cell lung carcinoma***
Epithelial markers • Epithelial membrane antigen, carcinoembryonic antigen and cytokeratin intermediate filaments • Mucin • Peptide hormone production	Present Absent Adrenocorticotropic hormone, anti-diuretic hormone, gastrin-releasing peptide, calcitonin	Present Present in adenocarcinomas Parathyroid hormone-related peptide (PTH-rp) in squamous cell carcinoma
Abnormalities of tumor suppressor gene *3p* deletions *RB* mutations *TP53* mutations *p16/CDKN2A* mutations	>90% ~90% >90% ~10%	>80% ~20% >50% >50%
Abnormalities of oncogene *KRAS* mutations *EGFR* mutations *ALK* rearrangements	Rare Absent Absent	~30% (adenocarcinomas) ~ 20% (adenocarcinomas, nonsmokers, women) 4–6% adenocarcinomas, nonsmokers, often have signet ring morphology
Response to chemotherapy and radiotherapy	Often complete response but invariably recur	Incomplete

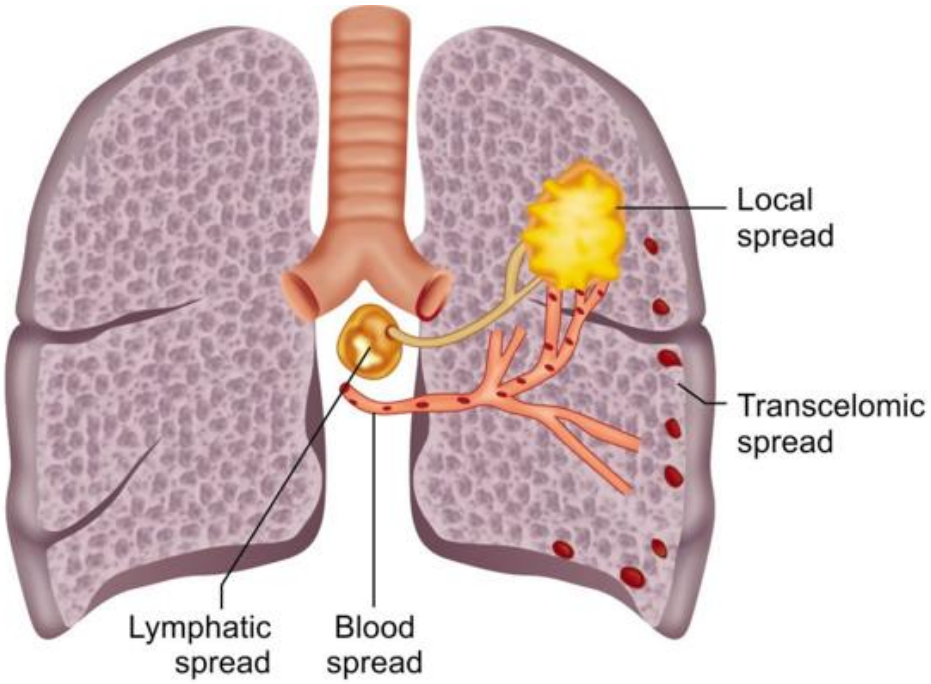


Fig. 17.32: Various routes of spread of lung cancer.

- **Tumor at the apex of lung** may invade the **neural structures around the trachea** and the **cervical sympathetic plexus**. They may produce **severe pain in the distribution of the ulnar nerve** and **Horner syndrome** (enophthalmos, ptosis, miosis, and anhidrosis) on the same side as the tumor. **Such tumors are** also called as **Pancoast tumors**.

Lymphatic Spread

Tumor may spread into the **tracheal**, **bronchial**, **hilar and mediastinal** nodes. It may also spread to lymph nodes in the neck (scalene node) and clavicular region. Involvement of supraclavicular node (**Virchow node**) may call attention to primary.

Hematogenous Spread

Common sites of metastases are to **adrenals (in more than 50%), liver, brain and bone**.

Staging: TNM system is used for staging cancer.

Clinical Course

- Lung cancer is insidious in onset.
- Major symptoms include cough, weight loss, chest pain and dyspnea.

- Local effects of lung cancer and their pathologic bases are listed in **Table 17.7**.
- The tumor may also present with symptoms due to metastasis.

Investigations

- **Radiologic examination** of the chest.
- **Cytologic examination** of sputum, bronchial washings or brushings or fine needle aspiration or bronchoscopy biopsy for demonstration of malignanccy.

Prognosis: Poor for most patients with lung carcinoma. In general, the adenocarcinoma and squamous cell carcinomas remain localized and have slightly better prognosis than undifferentiated carcinomas.

Paraneoplastic Syndromes

Q. Write a short note on paraneoplastic syndromes associated with lung cancer (hormones eleborated) or write short note on paraneoplastic syndromes produced by oat cell carcinoma of lung.

Lung cancer may occasionally be associated with paraneoplastic syndromes. They are summarized in **Table 17.8**.

Any histologic types of tumors may produce any one of the hormones. **Usually, ACTH and ADH are produced by small cell carcinomas, whereas hypercalcemia is mostly observed in squamous cell carcinomas.**

Other Systemic Manifestations

- Lambert–Eaton myasthenic syndrome: It is characterized by muscle weakness due to auto-antibodies directed against the neuronal calcium channel.

TABLE 17.7: Local effects of lung cancer.

Pathological basis	*Local effects*
• Obstruction of airway by tumor	Pneumonia, abscess, atelectasis, focal emphysema
• Local spread into pleura	Pleuritis and effusion
• Local spread to pericardium	Pericarditis, effusion, tamponade
• Compression of SVC by tumor	Superior vena cava syndrome
Invasion of	
• Recurrent laryngeal nerve	Hoarseness
• Phrenic nerve	Diaphragm paralysis
• Sympathetic ganglia	Horner syndrome
• Esophagus	Dysphagia
• Chest wall	Destruction of rib

Small cell carcinoma of lung:
- Cushing syndrome (due to ACTH)
- Hyponatremia (due to ADH)

TABLE 17.8: Paraneoplastic syndromes in lung cancer.

Elaborated hormones orhormone-like factors	***Effects***
Antidiuretic hormone (ADH)	Hyponatremia
Adrenocorticotropic hormone (ACTH)	Cushing syndrome
Parathormone, parathyroid hormone-related peptide	Hypercalcemia
Calcitonin	Hypocalcemia
Gonadotropins	Gynecomastia
Serotonin and bradykinin	Carcinoid syndrome

- Peripheral neuropathy: It is usually purely sensory.
- Dermatologic abnormalities: Acanthosis nigricans.
- Hematologic abnormalities: Leukemoid reactions.

- Hypertrophic pulmonary osteoarthropathy: It is associated with clubbing of the fingers.

Q. A 55-year-old male complains of persistent productive cough of1 year duration. He had episodes of intermittent blood in the sputum for the last 2 months. He also complains of progressive loss of weight and appetite for 1 month and occasional right-sided chest pain. He was a chronic smoker, smoking about 20 cigarettes/day for the last 25 years. On examination he was pale and there was clubbing of fingers. On inspection of the right half of chest is not moving equally with respiration. On auscultation, breath sounds absent on right side. He was transferred to oncology ward for further management.

(a) What is the probable diagnosis? (Ans; Bronchogenic carcinoma)

(b) What are etiological factors responsible for this condition? (refer pages 527-9).

(c) Discuss the morphological features of the lesion in the lung (refer pages 529-33).

(d) What are the different methods of investigation that are helpful for the diagnosis? (refer page 535).

Lung cancer: Cigarette smoking is the most common cause.

Lung cancer: Nonsmokers usually develop adenocarcinoma.

Second hand smoke or environmental tobacco smoke and passive smoking also carry a small risk of lung cancers.

Tobacco smoking in lung cancer: Evidence
- Stastistical
- Epidemiological
- Clinical
- Experimental

Precursor epithelial lesions in the lung:
- Squamous dysplasia and carcinoma in situ
- Atypical adenomatous hyperplasia
- Adenocarcinoma in situ
- Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

Centrally (hilar) located lung cancer: Squamous cell carcinoma and small cell carcinoma.

Peripherally located lung cancer: Adenocarcinoma.

Squamous cell carcinoma of lung:
- Most common lung cancer in smokers
- Most common lung cancer in males

Grades of squamous cell carcinoma: Well differentiated; Moderately differentiated; Poorly differentiated.

Lung cancer: Tumor cells may be detected in the sputum by exfoliative cytology. It can also be diagnosed by cytological examination of bronchoalveolar lavage fluid, or fine-needle aspiration.

Small cell carcinoma of lung:
- May be central or peripheral
- Most commonly associated with paraneoplastic syndromes.

Small cell carcinoma of lung: There is no known preinvasive phase or carcinoma in situ.

Small cell carcinoma:
- Common in smokers
- High expression of *Bcl-2* gene

Small cell carcinoma: Azzopardi effect—blood vessel stain blue due to DNA deposits from necrotic tumor cells.

SVC syndrome: Most commonly caused by small cell lung cancer.

Horner syndrome: Enophthalmos, lid lag (ptosis), miosis and anhydrosis on the same side of lung cancer. Caused due to compression of sympathetic nerve plexus by apical tumor of the lung called as Pancoast tumors.

Most common site of metastasis in lung carcinoma: Adrenal.

Diagnosis of bronchogenic carcinoma:

- Sputum cytology
- Fiberoptic bronchoscopy
- Bronchial lavage, washings and brushings
- Radiological examination of chest
- FNAC
- Bronchoscopic biopsy

Squamous cell carcinoma of lung: Hypercalcemia due to parathormone or parathyroid related peptide.

Lambert–Eaton myasthenic syndrome: Muscle weakness due to autoantibodies directed against the neuronal calcium channel.

METASTATIC TUMORS

Q. Write short note on metastatic tumors of lung.

- The lung is the most common site for metastasis.
- Both carcinomas and sarcomas from any site may spread to the lungs via the blood or lymphatics or by direct invasion.
- Local spread into the lungs occur in carcinoma of esophagus, carcinomas and mediastinal lymphomas.



Fig. 17.33: Lung with multiple secondaries.

MORPHOLOGY

The pattern of metastatic growth is variable.

- **Multiple nodules (Fig. 17.33):** Usually they appear as multiple **discrete nodules, distributed throughout all lobes**, more being at the periphery. Radiologically, when large nodules are seen in the lungs, they are known as **cannonball metastases**.
- **Other patterns:** They may also present as solitary nodule, endobronchial, pleural, pneumonic consolidation, or mixtures of any of these patterns.

Sources of Metastases to Lung

- **Common:** Carcinoma of **gastrointestinal tract**, **breast**, **thyroid**, **kidney**, **pancreas and liver.**
- **Other tumors:** Osteogenic sarcoma, neuroblastoma, Wilms tumor, melanoma, lymphomas and leukemias.

The microscopic appearance of metastases usually resembles that of the primary tumor.

Metastasis/secondaries: Most common malignant tumor of the lung.

PLEURAL TUMOR

The pleura may be affected by primary or secondary tumors. Secondary metastatic tumors are more frequent than the primary tumors. The most common primary neoplasms metastasizing to the pleura are from the lung and breast. These metastatic tumors usually produce a serous or serosanguineous effusion containing neoplastic cells. Hence, careful cytologic examination of the sediment prepared from pleural effusion (*refer* page 404) is of diagnostic value.

Solitary Fibrous Tumor

Solitary fibrous tumor **of the pleura** is an uncommon localized a soft-tissue tumor arising in the pleura and less commonly in the lung. They arise from submesothelial connective tissue (not mesothelium) and are usually attached to the pleural surface by a pedicle. Similar tumors can occur on any mesothelial surface, including the mediastinum, peritoneum, pericardium, liver and tunica vaginalis. Most of these tumors are benign, but a small percentage is malignant.

Etiology: It is not associated with asbestos exposure. It may be associated with a cryptic inversion of chromosome 12 involving the genes *NAB2* and *STAT6*. This rearrangement creates a *NAB2-STAT6* fusion gene that is unique to solitary fibrous tumor.

MORPHOLOGY

Gross: It may be small (1–2 cm in diameter) in size or may attain very large size (some reach 40 cm and may weigh up to 3800 g). Cut surface is gray-white, with a nodular, whorled or lobulated appearance. Occasional cysts filled with viscid fluid may be present.

Microscopy: The most commonly it has the "patternless pattern" composed of disorderly or randomly arranged mixtures of fibroblast-like cells and connective tissue.

Rarely, this tumor may be malignant. Microscopic features suggesting malignancy include increased cellularity, pleomorphism, necrosis, mitotic activity (more than 4 mitoses per 10 high-power fields) and large size (>10 cm).

Immunohistochemistry: The tumor cells are $CD34^{+}$ and keratin-negative. This helps in distinguishing solitary fibrous tumors from malignant mesotheliomas (which are keratin-positive CD34 –ve).

Malignant Mesothelioma

CC: Define and describe the etiology, types, exposure, genetics, environmental influence, pathogenesis, morphology, microscopic appearance and complications of mesothelioma.

Q. Write a short note on malignant mesothelioma.

Malignant mesothelioma is a rare malignant tumor of mesothelial cells.

Sites: Mesotheliomas can arise in the **pleura** (most common), **peritoneum, pericardium,** tunica vaginalis of the testis and genital tract (benign adenomatoid tumor). **Thoracic mesothelioma** arises from either the visceral or the parietal pleura.

Etiology

Q. Write a short note on etiology of malignant mesothelioma.

- Exposure to **asbestos**.
- Homozygous **deletion of the tumor suppressor gene *CDKN2A/INK4a*** involving chromosome *9p* (in about 80% of mesotheliomas).

MORPHOLOGY

Gross

- Malignant pleural mesothelioma is a **diffuse lesion** that arises either from the visceral or parietal pleura. It usually **spreads widely in the pleural space** and is associated with extensive pleural effusion and direct invasion of thoracic structures.
- It appears as thick layer of soft, gelatinous, grayish-pink tumor tissue that often encase and compress the affected lung. It may also extend into fissures and interlobar septa, a distribution often termed as a "pleural rind".

Microscopy

Q. Write a short note on microscopy of malignant mesothelioma.

Types

1. **Epithelioid type (60%):** Consists of **cuboidal, columnar, or flattened cells** forming **tubular, glandular or papillary** structures resembling adenocarcinoma. Immunohistochemistry is essential for differentiating mesothelioma from adenocarcinoma of lung. Most mesotheliomas are strongly positive for keratin proteins, calretinin, Wilms tumor 1 (WT-1), cytokeratin 5/6, and D2-40.

2. **Mesenchymal (sarcomatoid) type (20%):** Consists of **spindle cells** similar in appearance to a fibrosarcoma. They have lower expression of many of the markers described under epithelioid type mentioned above.
3. **Mixed (biphasic) type (20%):** Consists of both epithelioid and sarcomatoid patterns.

Clinical Course

- Average age is 60 years.
- Chest pain, dyspnea, recurrent pleural effusions and nonspecific symptoms, such as weight loss and malaise.

Spread

- Direct local invasion of the lung.
- Lymphatic spread to the hilar lymph nodes.
- Hematogenous spread to the liver, bones, peritoneum and adrenals.

Prognosis is poor and 50% die within 12 months of diagnosis.

CHAPTER
18

Disorders of Oral Cavities and Gastrointestinal Tract

ORAL CAVITY

PRECANCEROUS LESIONS OF ORAL CAVITY

Q. Write short note on premalignant lesions of the oral cavity.

Leukoplakia and Erythroplakia

Q. Write short note on definition and morphology of leukoplakia.

Leukoplakia

Definition: It is defined as a **white patch or plaque, not less than 5 mm in diameter**, that cannot be removed (scraped off) by rubbing and cannot be classified as any other diagnosable disease.

If any white patches in the oral cavity can be given a specific diagnosis, it is not a leukoplakia.

- **Sites:** May be seen **anywhere in the oral cavity**. Most common sites are buccal mucosa, floor of the mouth, ventral surface of the tongue, palate, and gingiva.
- **Number:** It may be **single or multiple.**
- **Appearance: White patches or plaques** having **sharply defined borders**. Surface may be smooth or wrinkled.

Erythroplakia

- **Less common** and appears as a **red, velvety area within the oral cavity**.
- It remains in level with or slightly depressed in relation to the surrounding mucosa.
- **Microscopically**, the **epithelium is atypical** and has an **increased risk of malignant transformation** than leukoplakia.

Intermediate forms, which have the characteristics of both leukoplakia and erythroplakia, are termed **speckled leukoerythroplakia**.

Features of Leukoplakia and Erythroplakia

Q. List the differences between leukoplakia and erythroplakia.

Age and Gender

Both leukoplakia and erythroplakia may be found in adults of any age, but they are usually seen between **40 to 70 years**. Male to female ratio is 2:1.

Etiology

Both have **multifactorial** origin and are associated with use of **tobacco** (cigarettes, pipes, cigars, and chewing tobacco).

MICROSCOPY

Leukoplakia

- **Surface stratified squamous epithelium** show a spectrum of changes ranging from **hyperkeratosis and acanthosis** to lesions with **variable degree of dysplastic changes** (including carcinoma in situ).
- **Subepithelial region** shows **inflammatory infiltrate of lymphocytes and macrophages**, the intensity of which is proportional to the degree of dysplasia.

Erythroplakia

- **Epithelium shows erosions with dysplasia, carcinoma in situ, or frank carcinoma.**
- **Subepithelial region** shows **intense inflammatory reaction** and **vascular dilatation,** which is **responsible for the reddish clinical appearance**.

Hairy Leukoplakia

- Hairy leukoplakia is a **distinctive oral lesion** and is **seen in immunocompromised patients.**
- About 80% of patients with hairy leukoplakia are infected with the human immunodeficiency virus (**HIV**) and remaining 20% are seen in patients who are immunocompromised for other reasons (e.g., cancer therapy or transplant immunosuppression).

Etiology: It is due to **Epstein-Barr virus (EBV) infection**.

Site: Located mostly on the **lateral edges/border of the tongue**.

MORPHOLOGY

- **Gross: White, confluent patches of fluffy ("hairy") thickenings**, which cannot be scraped off.
- **Microscopy:** It shows **hyperparakeratosis and acanthosis** with "**balloon cells**" in the **upper spinous layer** of the epithelium.

Leukoplakia: White mucosal patch or plaque that can undergo malignant transformation. Until otherwise proved by microscopic examination, all leukoplakias must be considered precancerous.

Erythroplakia: Risk of malignant transformation is more than that of leukoplakia.

Leukoplakia/erythroplakia: Biopsy to rule out dysplasia or carcinoma.

Toluidine blue:
- Basic metachromatic dye with high affinity for acidic components and stains tissues rich in DNA and RNA.
- Stain used to diagnose premalignant lesion (dysplasia) of oral cavity (e.g., lip).
- Detects efficiently and rapidly mitotic figures in paraffin embedded tissues.

SQUAMOUS CELL CARCINOMA

CC: Describe the etiology, pathogenesis, pathology and clinical features of oral cancers.

In India, oral cavity cancer is the most common malignant tumor.

Etiology

Q. Write short note on etiology of squamous cell carcinoma of oral cavity.

Squamous cell carcinoma (SCC) of oral cavity is **multifactorial disease** usually seen in middle-aged men.

Risk Factors

1. **Tobacco products and smoking:** Any irritating smoked product increases the risk for tumors of the oral cavity. Nicotine in tobacco and other tobacco leaf components cause cancer. **Carcinogens in tobacco can act as initiators, as well as promoters.** Risk increases with amount and duration of tobacco use. Tobacco may be used either for **smoking** or as **smokeless tobacco**.
   - **Smoking:** It may be in the form of cigarette, beedi, cigar, or pipe smoking, or reverse smoking (smoking a cheroot with the burning end inside the mouth is practiced in certain regions of India). Regular marijuana use has also been associated with oropharyngeal cancer.
   - **Smokeless tobacco:** It is in the form of **betel quid**/pan that contains several ingredients such as areca nut, slaked lime, and tobacco, which are wrapped in a betel leaf. It is commonly used in India and Southeast Asia, and is associated with marked increase in oral cancer. **Betel quid appears to be the major carcinogen**. However, it may also be related to slaked lime and the areca nut. Other methods of tobacco consumption include snuff dipping and tobacco chewing.

Oral cancers are found on the **buccal and gingival** surfaces in the sites **where tobacco products** are held in **contact** with the mucosa for long periods.

2. **Alcohol consumption**: It is another important etiologic factor and act synergistically with tobacco as either a cocarcinogen (increasing the risk) or a promoter (decreasing the lag time). The risk of oral SCC is magnified in individuals who smoke as well as consume alcohol.
3. **Other risk factors:**
   - Radiation exposure and solar actinic radiation (sunlight).
   - Welding, metal refining, diesel exhaust, wood stove, and asbestos exposure.

- *Chronic irritation of the mucosa:* It may be due to ill-fitting dentures, jagged teeth, or chronic infections.
- Vitamin A deficiency and immunosuppression.
- Poor nutrition.

Role of Oncogenic HPV Virus Infection

High-risk HPV types 16 and 18 and, less commonly low-risk HPV types 6 and 11 have been found in oral carcinomas.

Inherited Genomic Instability

Family history of head and neck cancer is a risk factor, and is thought to be due to inherited genomic instability.

Pathogenesis

The development of squamous cell carcinoma is a **multi-step process**. It involves sequential activation of oncogenes and inactivation of tumor suppressor genes in a clonal population of cells.

- **Inactivation of the *p16* gene** (about 80% of the cases) → stratified squamous epithelium undergoes hyperplasia/hyperkeratosis.
- **Mutation of the *p53* tumor suppressor gene** is associated with progression of hyperplasia/hyperkeratosis to dysplasia.
- **Amplification and overexpression of the** *cyclin D1 gene* constitutively activates cell cycle progression.

MORPHOLOGY

Site

Anywhere in the oral cavity. Common sites are **lower lip, the ventral surface of the tongue, floor of the mouth**, buccal mucosa, soft palate, and gingiva.

Gross

- **Early stages**: It appears either as raised, firm, pearly plaques or as irregular, roughened, or verrucous areas of mucosal thickening. May be superimposed on a leukoplakia or erythroplakia.
- **Later**: It may appear as **ulcerated and protruding gray white** masses with **irregular and indurated** (rolled) **borders.**

Microscopy

Squamous cell carcinomas **(Figs. 18.1A and B)** range from well-differentiated keratinizing neoplasms to poorly differentiated/anaplastic tumors. However, the histological grading does not correlate with behavior (*refer* page 868 for general microscopic description of squamous cell carcinoma).

Spread

- **Local:** Tissue involved depends on the primary site.
- **Lymph node:** The involved site of lymph node depends on the location of the primary tumor. The more anterior the tumor, more is the spread to the cervical nodes. Carcinomas of the base of the tongue and oropharynx metastasize to the deep retropharyngeal lymph nodes.
- **Blood spread:** It spreads to lungs, liver, and bones.

Verrucous Carcinoma

Verrucous carcinoma (Ackerman tumor) is **a variant of well-differentiated squamous cell carcinoma**.

Site: Most common in the buccal mucosa and lower gingiva.

Age and gender: Most are elderly males.

Etiology: Tobacco, especially chewing or snuff dipping and HPV infection.

MORPHOLOGY

Gross: Large, fungating, soft papillary growth.

Microscopy (Fig. 18.1C): The surface epithelium shows hyperkeratosis, acanthosis, and benign-appearing papillomatosis. Rete pegs are swollen and voluminous and extend into the deeper tissues.

Spread: May infiltrate the soft tissues of the cheek, mandible or maxilla, and invade perineural spaces. **Regional lymph node metastases are exceedingly rare**, and **distant metastases have not been reported**.

Oral cancer: Majority are squamous cell carcinomas.

Most common site of cancer of oral cavity:

- Tongue > lip.
- In India: Buccal mucosa > anterior tongue > lower alveolus.

Most common type of cancer in India: Carcinoma of oral cavity.

Squamous cell carcinoma of oral cavity: Risk factors include tobacco and alcohol use. It may also be associated with HPV infection.

Patients with head and neck squamous cell carcinoma are at increased risk for second primary malignancy. Typically in the aerodigestive tract (e.g., lung, esophagus).

Risk factors associated with cancer of the head and neck.

- Tobacco products, smoking and areca nut/pan masala
- Alcohol consumption
- Human papillomavirus
- Plummer–Vinson syndrome
- Inherited genomic instability
- Poor nutrition

Field cancerization: Phenomenon of susceptibility of the oral mucosa for multiple primary cancers.

Verrucous carcinoma (Ackerman tumor):

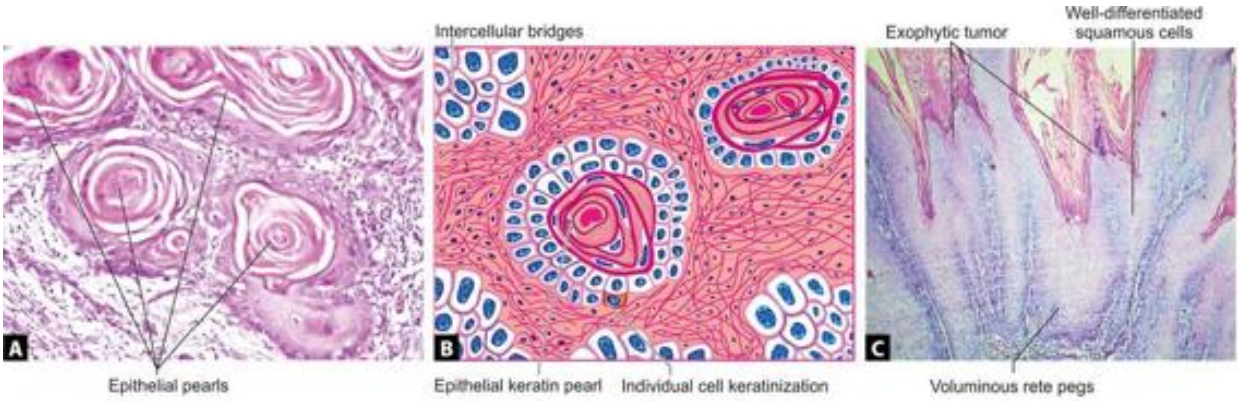
- Distinct variant of well-differentiated squamous cell carcinoma
- Mainly in elderly patients
- Warty appearance
- Pushing infiltrative margins
- Lymphatic spread rare

SALIVARY GLAND NEOPLASMS

Salivary gland neoplasms are relatively uncommon.

Incidence

- Parotid gland—65–80% and ~15–30% parotid tumors are malignant.
- Submandibular gland—10% and ~40% are malignant.
- Minor salivary glands including the sublingual glands— 10%. About 50% of minor salivary gland and 70–90% of sublingual tumors are malignant.



Figs. 18.1A to C: Well differentiated squamous cell carcinoma microscopic appearance (A, H&E and B, diagrammatic); (C) Verrucous carcinoma showing exophytic growth, swollen and voluminous rete pegs extending into the deeper tissues.

TABLE 18.1: Classification of tumors of the salivary glands.

Benign	***Malignant***
Pleomorphic adenoma (mixed tumor)	Mucoepidermoid carcinoma
Warthin tumor	Adenocarcinoma
Oncocytoma	Acinic cell carcinoma
Basal cell adenoma	Adenoid cystic carcinoma
Cystadenoma	Malignant mixed tumor

- Malignancy in salivary gland is inversely proportional to the size of the gland.

Classification of Salivary Glands Tumors (Table 18.1)

Q. Classify salivary gland tumors.

- **Age:** Benign tumors occur in the fifth to seventh decades of life. The malignant tumors appear later.
- **Sex: Slight female predominance**, except **Warthin tumor, which occur often in males than in females**.

Clinical Presentation

- Parotid gland neoplasms produce swellings in front of and below the ear.
- Both benign and malignant tumors range in size from 4–6 cm in diameter.
- Most tumors are mobile except advanced malignant tumor.

Parotid gland: Most common site for tumors of salivary gland.

PLEOMORPHIC ADENOMA

Q. Write short note on pleomorphic adenoma.

Pleomorphic adenoma is a **most common benign tumor of the salivary glands,** characterized by an **admixture of epithelial and stromal elements**, and is also called **mixed tumors**. In pathology, the term "pleomorphic" is used to indicate nuclear variation (size and shape of nuclei) in neoplasm. However, the term pleomorphic adenoma is used because of variable cell type seen in this lesion.

Age: It occurs usually during **third to fifth decade** of life (middle-age).

Sex: It is most frequent in **females**.

Site

- **Major salivary gland:** Common site and constitute about 60% of tumors in the parotid. Usually arise in the **superficial lobe of the parotid**. Less common in the submandibular glands and very rare in the sublingual gland.
- **Minor salivary gland:** Its involvement is relatively rare.

Etiology

Not known. Exposure to radiation increases the risk.

- **Nature:** It is a benign tumor.
- **Cell of origin:** Histogenesis uncertain. **It was called as mixed tumor**, because of the mixture of epithelial and mesenchymal components. However, it is now considered that the tumor neoplastic cells (epithelial and those which appear mesenchymal), are of either **myoepithelial or ductal reserve cell origin.**

MORPHOLOGY

Q. Write short note on gross and microscopy of pleomorphic adenoma.

Gross

- **Shape**: Round.
- **Size:** Ranges from **2.5 to 6 cm in diameter**.
- **Consistency**: It depends on the relative amount of epithelial cells and stroma. **Usually rubbery**, resilient mass with a **bosselated surface.**
- **Well-circumscribed/capsulated:** In some, the capsule is not fully developed, and small extensions can be seen protruding into the surrounding salivary gland. This makes enucleation of the tumor difficult.
- **Cut surface (Fig. 18.2):** It shows **gray-white** with **myxoid and blue glistening, translucent chondroid** (cartilage-like).

Microscopy (Figs. 18.3A and B)

Characteristic feature is the **pleomorphic appearance**. Neoplastic cells show varying **mixture of epithelial tissue** component intermingled with **cells showing mesenchymal differentiation.**

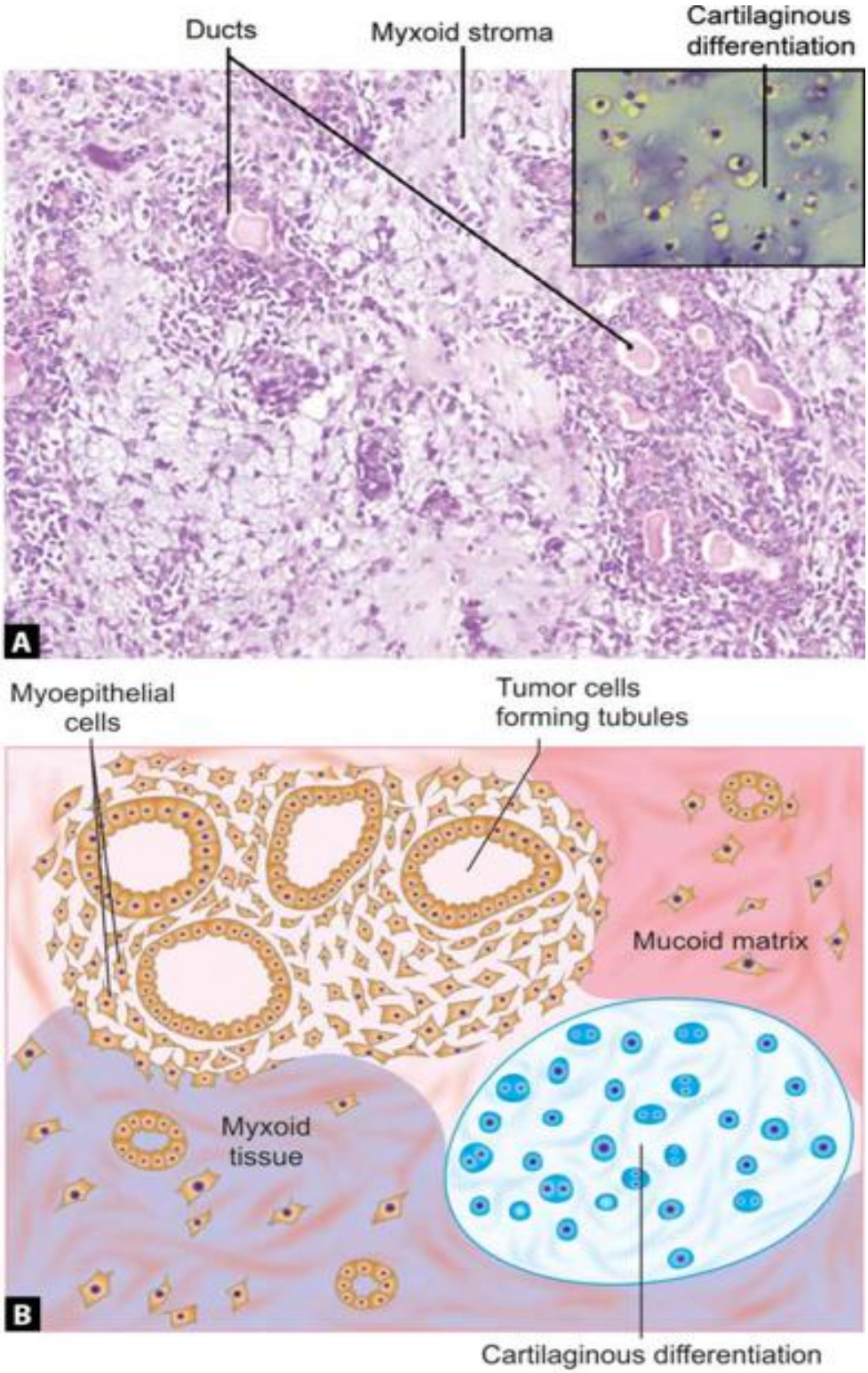
Epithelial Element

This component consists of **ductal cells and myoepithelial cells**.

- **Epithelial elements** are arranged in the form of **ducts, acini and irregular tubules**. The ducts are lined by both epithelial (cuboidal to columnar) cells and **surrounded by myoepithelial**



Fig. 18.2: Pleomorphic adenoma of salivary gland. Cut section shows well-circumscribed gray-white tumor with blue glistening area.



Figs. 18.3A and B: Mixed tumor (pleomorphic adenoma) of the parotid, gland (A, H&E and B, diagrammatic) shows epithelial cells forming ducts surrounded by myxoid stroma and cartilaginous differentiation (inset A).

components (a layer of deeply chromatic, small myoepithelial cells).

- May show **strands or sheets** of plasmacytoid or spindled **myoepithelial cells**.
- Islands of well-differentiated squamous epithelium may also be seen.

Mesenchymal-like Elements

The epithelial elements are dispersed within a varying amount of mesenchyme-like background of loose **myxoid tissue, islands of hyaline, chondroid (cartilaginous), and mucoid matrix**.

Clinical Features

- Pleomorphic adenomas present as **painless, slow-growing, mobile, discrete** tumors in the **parotid or submandibular** areas or in the buccal cavity.
- The tumors tend to protrude focally from the main tumor into adjacent tissues.
- Failure to recognize these minute protrusions at surgery is responsible for recurrence of these tumors.

Carcinoma Ex Pleomorphic Adenoma

- Rarely, a carcinoma may arise in pleomorphic adenomas—referred to as a **carcinoma ex pleomorphic adenoma or a malignant mixed tumor**.
- Incidence of carcinoma increases with the duration of the tumor.
- **Microscopy**: These tumors show poorly differentiated adenocarcinoma or undifferentiated carcinoma in an otherwise benign pleomorphic adenoma.

Pleomorphic adenoma: Most common benign tumor of the salivary glands.

Pleomorphic adenoma:

- Most common tumor of major salivary gland mainly parotid
- Less common in submandibular and sublingual gland
- Rare in minor glands

Pleomorphic adenoma: Benign salivary gland tumor composed of mixture of:

- **Epithelial components**
- **Mesenchymal-like components**

In significant number of these tumors, there is chromosomal rearrangements involving

- *PLAG1* (gene encoding a transcription factor). This upregulates the expression of a many genes which increase cell growth (e.g., growth factor receptor signaling pathways).
- Pleomorphic adenoma: Most common site is parotid tail (superficial lobe).

Pleomorphic adenoma: Encapsulated but pseudopodia (finger-like projections) into the surrounding gland.

Pleomorphic adenoma: Slow growing tumor.

Pleomorphic adenoma: Unicentric but recurrences are multicentric.

WARTHIN TUMOR

Q. Write short note on Warthin tumor.

Warthin tumor (**papillary cystadenoma lymphomatosum, adenolymphoma**) is a **benign** and the **second most common** salivary gland neoplasm.

- **Site:** It almost **exclusively** arises in the **parotid gland**.
- **Sex:** It is the only salivary glands tumor that is more **common in males** than in females.
- **Age:** It usually occur between **fifth to seventh** decades of life.
- **Predisposing factor: Smokers** have eight times the risk of nonsmokers.

MORPHOLOGY

Gross

- **Shape:** It is round to oval, **encapsulated masses**.
- **Size:** It ranges from **2 to 5 cm** in diameter.
- Usually arises in the **superficial parotid gland** and are readily palpable.
- About 10% are multifocal and 10% bilateral.
- **Cut section**: It is **pale gray** tumor punctuated by narrow cystic or cleft-like spaces filled with a mucinous or serous secretion or even resemble used (dark) motor oil.

Microscopy (Figs. 18.4 and 18.5)

Tumors consist of cystic glandular spaces embedded in dense lymphoid stromal tissue.

1. Cystic Spaces

- Cystic glandular spaces show **papillary or polypoid projections**.
- **Cystic spaces** are lined by a distinctive **double layer of neoplastic epithelial cells** consisting of:

- **Surface superficial layer of columnar cells** with **abundant, finely granular, eosinophilic cytoplasm** (oncocytes). Oncocytes have **abundant mitochondria** → responsible for the **granular appearance** to the cytoplasm.
- **Second layer** below the superficial layer consisting of **cuboidal to polygonal cells**.

2. Lymphoid Stromal Tissue

Cystic spaces are embedded in a dense lymphoid stroma which **closely resembles a normal lymph node.** The lymphoid tissue is prominent with germinal centers and is composed of B and few T cells**.**

Histogenesis: Warthin tumor may represent an aberrant incorporation of lymphoid tissue in the parotids.

Behavior: It is **benign**, and about 2% may recur.

Warthin tumor: Exclusively arises in the parotid gland.

Warthin tumor:
- Most common in males
- Fifth to seventh decade
- Associated with smoking

Warthin tumor: Second most common benign tumor of the parotid gland.

Warthin tumor:
- 10% multifocal
- 10% bilateral
- Never involves facial nerve

Warthin tumor: Consists of
- **Epithelial and**
- **Lymphoid elements**

Warthin tumor:
- Cystic spaces are lined by a distinctive double layer of neoplastic epithelial cells
- Lymphoid stroma

MUCOEPIDERMOID CARCINOMA

Most common primary malignant tumor of the salivary glands.

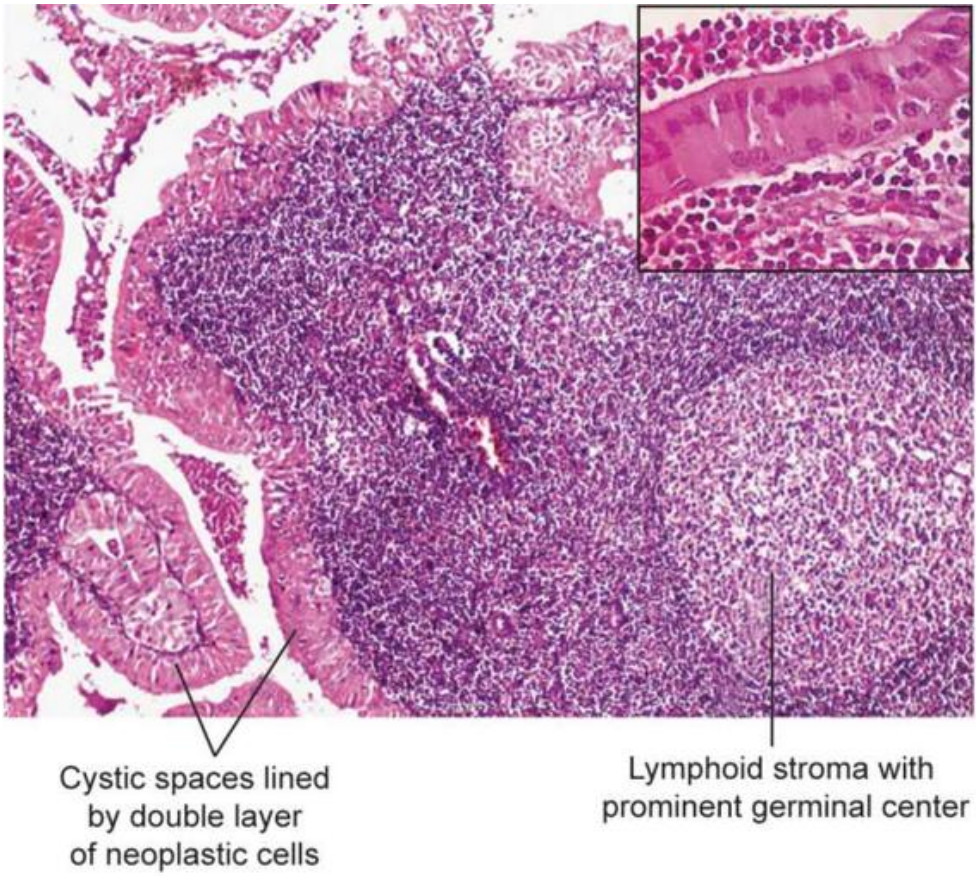


Fig. 18.4: Warthin tumor showing epithelial and lymphoid elements. Note the follicular germinal center in the center. The cystic spaces are lined by double layer of eosinophilic epithelial cells (inset) separated by reactive lymphoid stroma.

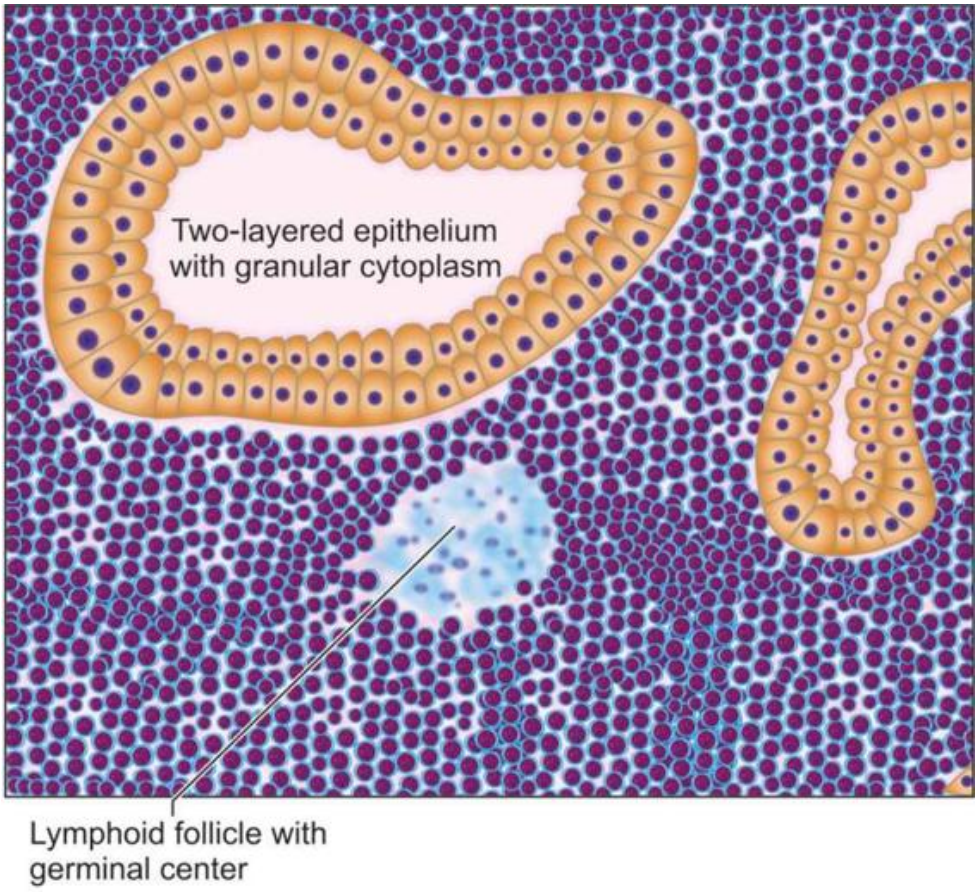


Fig. 18.5: Warthin tumor showing epithelial and lymphoid elements (diagrammatic).

Incidence

- Constitute about 15% of all salivary gland tumors.
- Occur mainly (60–70%) in the parotids.
- Account for a major fraction of the minor salivary gland tumors.

Etiology

In about one-third of cases, it is associated with a distinctive balanced (11;19) (q21;p13) chromosomal translocation. This creates a fusion gene composed of portions of the *CRTC1* (formerly known as *MECT1*) and *MAML2* genes.

The translocation results in activation of the Notch pathway.

MORPHOLOGY

Gross

- **Size:** It can grow as large as 8 cm in diameter.
- **Appear circumscribed**, but do not show well-defined capsules and are often infiltrative at the margins.
- **Cut section:** It is **pale and gray-white** and frequently contain small, mucin-containing **cysts**.

Microscopy (Fig. 18.6)

Composed of variable mixtures of:

- Squamous cells
- Mucus-secreting cells
- Intermediate cells.

These tumor cells are arranged in cords, sheets, or cystic structures. The intermediate cells have squamous features, with small to large mucus-filled vacuoles. The mucus stains positive with mucin stains. The tumor cells may appear regular and benign or highly anaplastic and malignant.

Grading: These tumors are graded as low, intermediate or high grade. Clinical features and prognosis depends on the grade of the neoplasm.

Mucoepidermoid carcinoma is the most common malignant tumor:

- Primary malignant tumor of salivary gland
- Tumor of salivary gland in children

Tumors that spread perineurally:

- Adenoid cystic carcinoma
- Cholangiocarcinoma
- Carcinoma of pancreas
- Adenocarcinoma of prostate
- Carcinoma of gallbladder

GASTROINTESTINAL TRACT

ESOPHAGUS

Lacerations: Mallory–Weiss Tears

Q. Write short note on Mallory-Weiss syndrome.

Mallory–Weiss tears are **nontransmural longitudinal tear** in the esophagus at the **gastroesophageal region**.
Etiology: Associated with **severe/vigorous retching** or **vomiting** secondary to acute alcohol intoxication or vigorous coughing.

MORPHOLOGY

- **Site of tears:** Usually at the **gastroesophageal junction** but may also be located in the proximal gastric mucosa.
- **Appearance of tear:** Superficial, **longitudinal oriented lacerations** of **variable length**.

Clinical features: Hematemesis. Usually, do not require surgical intervention, and healing is rapid and complete.

Barrett Esophagus

Q. Write short note on Barrett esophagus.

Barrett esophagus is characterized by **intestinal metaplasia** within the squamous mucosa of esophagus occurring as a **complication to chronic gastroesophageal reflux** (GERD).

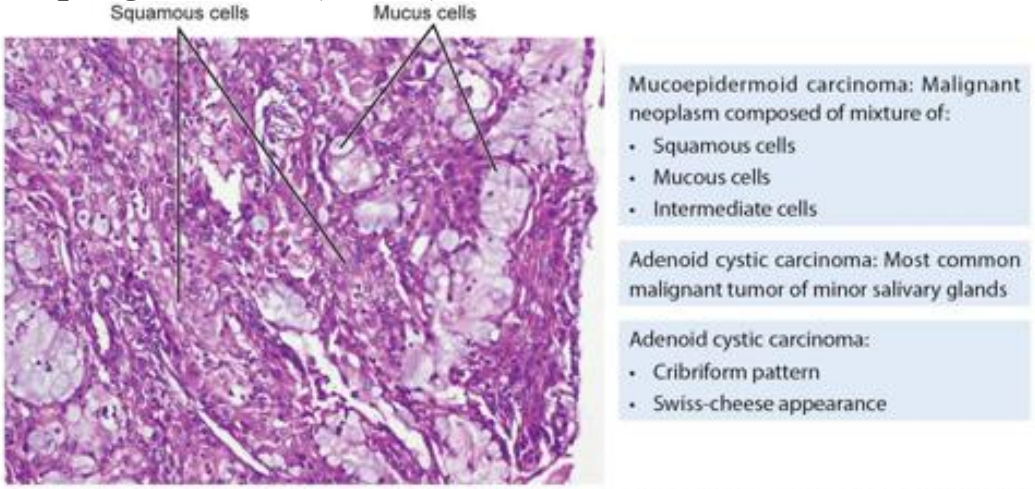


Fig. 18.6: Mucoepidermoid carcinoma composed of squamous cells as well as clear vacuolated cells containing mucin.

Incidence: Occur in ~10% of patients with symptomatic GERD.
Gender and age: Males, usually between **40 and 60 years of age**.

MORPHOLOGY

Gross

Appears as **one or several** tongues or **patches of red, velvety mucosa** extending **from gastroesophageal junction upwards into esophagus**.

Microscopy (Fig. 18.7)

- **Intestinal metaplasia:** Normal **squamous lining** of the lower esophagus is replaced by **columnar mucosa** (columnar lined esophagus; CLO) containing **areas of intestinal metaplasia**. **Goblet cells** with distinct mucous vacuoles are seen in the region of **intestinal metaplasia,** are **necessary for diagnosis**. **Intestinal metaplasia** is an important **risk factor** for the development of **adenocarcinoma.**
- **Dysplasia** (low grade or high grade): It may be seen in the metaplastic epithelium.

Esophagus:
- Normal length of esophagus: 25–30 cm
- Only Auerbach plexus and no Meissner's plexus
- Lacks a serosal layer

Causes of hematemesis:
1. Mallory–Weiss syndrome
2. Esophageal varices
3. Reflux esophagitis
4. Esophageal ulcers

Mallory–Weiss syndrome: Longitudinal mucosal tear of distal esophagus.

Barrett esophagus: Develops in ~10% of patients with symptomatic GERD.

ESOPHAGEAL CANCER

Morphologic types: Squamous cell carcinoma and adenocarcinoma.

Squamous Cell Carcinoma

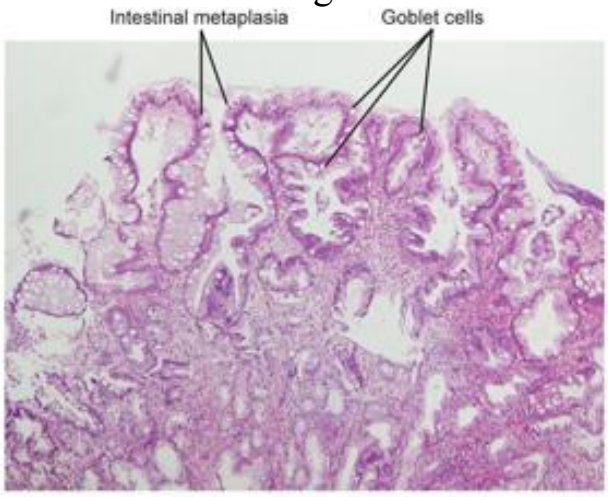
Malignant neoplasm of the esophagus showing squamous differentiation.
Age and gender: Adults over age 45, M:F = 4:1.

Etiology

Q. Write short note on etiology of esophageal cancer.

Risk Factors

- High **alcohol** intake
- **Tobacco smoking**
- **Nutrition:** Poverty, **diet deficient in vitamins** and certain trace elements, **polycyclic hydrocarbons**, **nitrosamines**, fungal toxins in pickled vegetables foods, etc.
- **Hot beverages:** Frequent consumption of burning-hot beverages, which causes thermal injury.
- **Others:** Caustic esophageal injury, **Plummer-Vinson syndrome**, **achalasia cardia**, celiac disease and **previous radiotherapy** to the mediastinum. **Human papillomavirus (HPV)** infection in high-risk areas but not in low-risk regions.



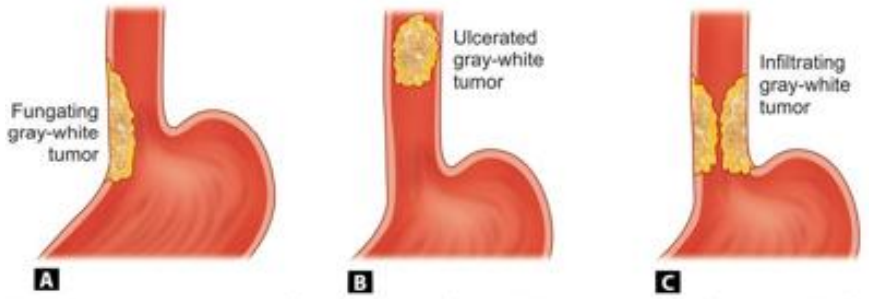
Barrett esophagus: Replacement of squamous epithelium by intestinal type of **epithelium with goblet cells** (columnar metaplasia)

Barrett esophagus:
- Premalignant condition
- Increased risk of esophageal adenocarcinoma

Complications of Barrett esophagus:
- High (midesophageal) and long strictures
- Chronic peptic ulceration (Barrett ulcer)
- Risk of esophageal adenocarcinoma

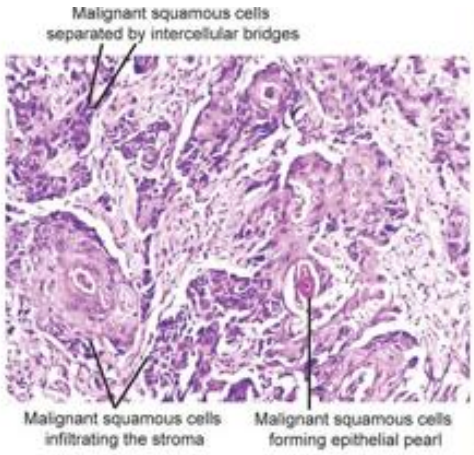
Goblet cells: Characterized by the presence of distinct mucous vacuoles

Fig. 18.7: Barrett esophagus with intestinal metaplasia containing goblet cells.



Gross types of esophageal cancer:
Three patterns
1. Fungating
2. Ulcerative
3. Infiltrating

Figs. 18.8A to C: Gross types of carcinoma esophagus: (A) Fungating or exophytic type; (B) Ulcerative type; (C) Infiltrative/stenotic type.



Squamous cell carcinoma: Grades
- Well-differentiated
- Moderately differentiated
- Poorly differentiated

Esophagus:
Lymphatic channels in the lamina propria are unique to the esophagus.
Dense submucosal lymphatic plexus facilitates early dissemination of esophageal malignancy

Most common benign tumor of esophagus: Leiomyoma

H. pylori: CagA positive strain
Protective for adenocarcinoma of esophagus
Can lead to squamous cell carcinoma

Fig. 18.9: Microscopic appearance of well-differentiated squamous cell carcinoma.

MORPHOLOGY

Gross (Figs. 18.8A to C)

- **Location:** Most common in the **middle-third of esophagus**.
- **Patterns of growth**:
  1. **Fungating or exophytic:** Tumor protrude into and obstruct the lumen.
  2. **Ulcerative:** Growth with elevated ulcer edges.
  3. **Infiltrating, or stenotic:** Least common, predominantly intramural.

Mixed gross growth patterns also occur.

Microscopy (Fig. 18.9)

Malignant squamous cell with varying degree of differentiation (well to poor differentiated type) (*refer* page 868 for general microscopic description of squamous cell carcinoma).

Spread

- **Local spread:** Into respiratory tree, aorta, mediastinum and pericardium.
- **Lymphatic spread:** Into regional lymph nodes.
  - Cancers in the upper third to → cervical lymph nodes
  - Middle third to → mediastinal, paratracheal and tracheobronchial nodes
  - Lower third to → gastric and celiac nodes.
- **Hematogenous spread**: Occurs late; to liver, lungs, adrenals, kidneys and bones.

Clinical features: Insidious in onset. Dysphagia, odynophagia (pain on swallowing) and obstruction.

Adenocarcinoma

Q. Write a short note on adenocarcinoma of esophagus.

Carcinoma displaying glandular differentiation and usually arises in a background of Barrett esophagus and long-standing gastroesophageal reflux diseases (GERD).

Etiology

Risk factors include dysplasia, tobacco use, obesity and prior radiation therapy. Risk is reduced by diets rich in fresh fruits and vegetables.

Pathogenesis

Progression of Barrett esophagus to adenocarcinoma develops in a stepwise pattern with genetic and epigenetic changes.

- **Genetic changes** include chromosomal abnormalities and mutation or overexpression of *p53*, amplification of *ERBB-2,* cyclin D1, cyclin E genes, etc.
- **Epigenetic changes** include silencing by hypermethy-lation of p16/INK4a.

MORPHOLOGY

Gross

- **Site:** Usually in the distal third of the esophagus.
- **Appearance**: **Early lesions—flat or raised** patches → later **infiltrate diffusely or ulcerate**.

Microscopy

- It consists of malignant tumor with **intestinal-type morphology of cells forming glands**.
- Tumors most commonly produce mucin. Barrett esophagus may be present adjacent to the tumor.

Clinical features: Pain or difficulty in swallowing, chest pain, progressive weight loss, hematemesis, or vomiting.

Most common site of esophageal cancer: Middle-third of esophagus.

Most common type of esophageal cancer in India is squamous cell carcinoma.

Squamous cell carcinoma of esophagus: Most common risk factors
- Alcohol use
- Tobacco use

Plummer–Vinson syndrome:
- Iron deficiency anemia
- Esophageal webs
- Glossitis

Squamous cell carcinoma of esophagus: Most common site is middle third.

Carcinoma esophagus: Most common symptom—dysphagia > weight loss.

Investigation of choice in esophageal cancer: Endoscopy and biopsy.

Adenocarcinoma of esophagus: Usually arises in a background of:
- Barrett esophagus
- Long-standing GERD

Adenocarcinoma of esophagus: Risk factors
- GERD leading of Barrett esophagus
- Obesity
- Scleroderma
- Diet deficient in fruits and vegetables
- Diet rich in animal fat and cholesterol

Adenocarcinoma of esophagus: Most common site is lower/distal third.

Barium swallow in esophageal cancer: Rat tail appearance.

STOMACH

Various cells in the mucosa of the stomach and their secretions are presented in **Table 18.2**.

Definitions

- **Erosion:** Loss of the superficial epithelium which produces a small defect in the mucosa that is **limited to the lamina propria** (do not penetrate the muscularis mucosae).
- **Ulcer:** Break in the mucosal surface **more than 5 mm in size, with depth up to the submucosa** (i.e., they penetrate the muscularis mucosae). It leads to a local defect or excavation due to active inflammation. **Gastric ulcers (GUs) and duodenal ulcers** (DUs) may be acute or chronic.
- **Gastritis: Inflammation of the gastric mucosa** and is **usually a histological diagnosis**. The term gastropathy is used when inflammatory cells are rare or absent (hypertrophic gastropathy).
- **Gastropathy:** This term is used when cell injury and regeneration are present but inflammatory cells are rare or absent.

Classification of Gastritis (Box 18.1)

BOX 18.1: Various ways of classifying gastritis.

A. Depending on the inflammatory cells and duration
- Acute gastritis shows predominately acute inflammatory cells
- Chronic gastritis shows mononuclear cell (lymphocytes, plasma cells) infiltration
  - *Helicobacter pylori* gastritis
  - Autoimmune gastritis
  - Others

B. Depending on the segment of involved stomach
- Antral-predominant gastritis
- Corpus-predominant gastritis
- Pangastritis

C. Depending on the absence or presence of premalignant stages
- Nonatrophic
- Atrophic gastritis $\longrightarrow$ may progress to carcinoma

Ulcer: Break in the mucosal surface more than 5 mm in size, with depth to the submucosa.

TABLE 18.2: Various types of cells in stomach and their secretions.

Type of cell	*Secretions*
Parietal (oxyntic) cells	**Acid** from the proton pump, H^+-K^+ ATPase **Intrinsic factor** required for absorption of vitamin B_{12}
Chief (zymogenic) cells	**Proenzymes pepsinogen I and II** (activated to pepsin)
Foveolar cells	**Mucin** layer over the mucosal cells
Endocrine cells	**Gastrin in antrum** (G cells), **histamine** in the body

ACUTE GASTRITIS

Q. Discuss the etiology, pathogenesis and complications of acute gastritis.

Definition: Acute gastritis is a transient inflammation of gastric mucosa.

Etiology

- **Drugs:** Aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs) and other drugs (e.g., iron preparations).
- *H. pylori*
- Alcohol
- Chemicals
- Severe physiological stress (e.g., burns)
- Bile reflux (e.g., following gastric surgery)
- Viral infections (e.g., cytomegalovirus—CMV).

Pathogenesis

Acute (also chronic gastritis) can **develop following disruption of any of the protective mechanisms (***refer* pages 552-3).

- **Reduced synthesis of mucin and bicarbonate secretion (e.g., during old age)**
- NSAIDs → interfere with protection provided by prostaglandins or reduce bicarbonate secretion.
- **Gastric injury:** For example, in uremia and infection with urease-secreting ***H. pylori*** may be due to **inhibition of gastric bicarbonate transporters**.
- **Ingestion of chemicals** (e.g., acids or bases), either accidentally or as a suicide attempt → **direct injury to mucosal epithelium**. Direct cellular injury may also be caused by excessive alcohol consumption, NSAIDs, radiation therapy and chemotherapy.
- **Insufficient epithelial regeneration** following cancer chemotherapy.
- **Decreased oxygen supply** at high altitudes.

MORPHOLOGY

- **Mild acute gastritis**: No significant changes
- **Severe gastritis:** Severe mucosal damage, erosions and hemorrhage → termed **acute erosive hemorrhagic gastritis**.
- **Microscopy:** It shows, dense infiltration by neutrophils within the mucosa and purulent exudate in the lumen.

Clinical Features

It may be asymptomatic or cause variable degrees of epigastric pain, nausea and vomiting. When severe, it causes mucosal erosion, ulceration, hemorrhage, hematemesis and melena.

Gastritis: Presence of neutrophils above the basement membrane in direct contact with the epithelial cells indicates active inflammation.

MUCOSAL DISEASE RELATED TO STRESS

Mucosal disease related to stress develops in association with severe stress. These include severe trauma, extensive burns, intracranial disease, major surgery and any severe physiologic stress.

Types

- **Stress ulcers:** They occur with **shock, sepsis or severe trauma**.
- **Curling ulcers:** They develop in the proximal duodenum with **severe burns or trauma**.
- **Cushing ulcers:** They develop in the stomach, duodenum, and esophagus in patients with **intracranial disease**. Highly prone for perforation.

Pathogenesis

Mostly due to **local ischemia**.

- Ischemia due to systemic hypotension or reduced blood flow.
- **Ulcer associated with intracranial injury**: They are probably due to the direct stimulation of vagal nuclei → causes increased secretion of gastric acid.

- **Stress-induced splanchnic vasoconstriction** → hypoxia and reduced blood flow.
- Upregulation of inducible NO synthase and increased vasoconstrictor endothelin-1.

MORPHOLOGY

Gross

- **Size and shape:** It ranges from **shallow erosion to deep ulcers** up to the mucosa; measure less than 1 cm in diameter and round in shape.
- **Site: Anywhere** in the stomach.
- **Number**: Single or multiple (more common).
- **Characteristics of ulcer**: Margins and base are not indurated. Floor appears brown to black due to acid digestion of extravasated blood.

Microscopy

- Sharply demarcated, surrounded by normal mucosa
- **Acute inflammatory reaction**
- Complete healing occurs after the injurious agent/factors are removed.

Clinical Features

Most critically-ill patients in intensive care units show microscopic evidence of gastric mucosal damage.

Curling ulcer: Associated with severe burns.

Cushing ulcer: Associated with head injury/intracranial disease.

CHRONIC GASTRITIS

Q. Describe the pathogenesis of Helicobacter pylori gastritis. Add a note on morphology and complications of it.

Chronic gastritis is chronic inflammation of stomach associated with mucosal injury. Microscopically, there is an increase in inflammatory cells in the lamina propria.

Causes: Common cause of chronic gastritis is due to infection by *Helicobacter pylori*. Other causes include autoimmune gastritis and less common causes such as radiation injury, chronic bile reflux and mechanical injury (e.g., an indwelling nasogastric tube).

H. pylori Gastritis

Q. Write short essay/note on H. pylori gastritis.

Q. Write a short note on Helicobacter pylori.

Q. Write a short note on role of Helicobacter pylori in pathogenic mechanism of peptic/gastric ulcer.

- *H. pylori* are **short, spiral-shaped or curved (Figs. 18.10A to C) gram-negative bacilli** with multiple **flagella** at one end. They **reside on the mucin of the surface and neck regions of foveolar cells.** In gastric biopsies examined, they are found in all patients with duodenal ulcers and in majority with gastric ulcers or chronic gastritis.
- Acute *H. pylori* infection usually does not produce noticeable symptoms and produces symptoms when chronic gastritis develops. *H. pylori* can be demonstrated in about 90% of patients with chronic gastritis of the pyloric antrum.
- ***Predisposing factors*** for *H. pylori* infection includes poverty, overcrowding and limited education. Humans are the primary carriers of infection and transmission is mainly through the fecal-oral route.
- **Mode of spread: Oro-oral or feco-oral route** by either kissing or ingestion of contaminated vomitus.

Pathogenesis

- **Antral gastritis**: *Helicobacter* gastritis is **predominantly localized** and mostly affects the antrum causing **antral gastritis.** There may be **normal or increased acid production.** If inflammation is limited to the antrum, increased production of acid is likely to cause duodenal peptic ulcer.

Local production of gastrin may be increased without increased serum gastrin (hypergastrinemia).

- **Multifocal atrophic gastritis**: With advancing time, the antral gastritis may progress to involve the more proximal stomach (i.e., body and fundus) producing multifocal atrophic gastritis. This is characterized by **patchy mucosal atrophy, reduced parietal cell mass and acid secretion**, intestinal metaplasia, and has an **increased risk of developing adenocarcinoma of stomach**. Thus, there is an inverse relationship between duodenal ulcer and gastric adenocarcinoma. *H. pylori* do not invade and they are not found in the absence of foveolar cells or in association with intestinal-type epithelium. Thus, wherever there is intestinal metaplasia, *H. pylori* are not found.

Mechanism of Action

- **Flagella:** It makes them motile, allows it to burrow and live beneath the mucus layer above the epithelial surface.
- **Urease:** Produced by *H. pylori* releases ammonia (strong alkali) from endogenous urea, raises the local gastric pH and enhances bacterial survival. Raised local gastric pH acts on the antral G cells and releases gastrin → hypergastrinemia result in **hypersecretion of gastric acid.**
- **Adhesins**: It helps *H. pylori* to bind to the surface of foveolar cells of gastric epithelium.
- **Cytotoxins**: Products of two genes, namely—cytotoxin-associated gene A (*cagA*) and vacuolating agent (*vacA*) gene involved in the progression of the disease and cause gastritis, peptic ulceration and cancer.

Host factors: Play role in the outcome of *H. pylori* infection.

- **Increased expression of proinflammatory cytokines**: Normally, *H. pylori* do not invade the cells/tissues. It causes increased production of proinflammatory cytokines [e.g., interleukin-1β (IL-1β) and tumor necrosis factor (TNF)] by the mucosal epithelial cells.
- **Decreased expression of the anti-inflammatory cytokines**: For example, interleukin-10 (IL-10).

The above mechanism activates neutrophils and macrophages (inflammatory response in the gastric mucosa) and impairs mucosal defense→pangastritis, atrophy and gastric cancer.

Thus, development and course of *H. pylori* gastritis depends on interplay between gastroduodenal mucosal defenses, inflammatory responses and bacterial virulence factors.

Microscopy

- Presence of **intraepithelial neutrophils** and **subepithelial (superficial lamina propria) plasma cells** are **characteristics of *H. pylori* gastritis**. Neutrophils may accumulate in the lumen of gastric pits to produce pit abscesses.
- In **long-standing cases, gastritis may extend proximally to involve the body and fundus. Then the mucosa** can become **atrophic with loss of parietal and chief cells. Microscopically, it is accompanied by lymphoid aggregates**, some with germinal centers. These lymphoid aggregates represent an induced form of **mucosa-associated lymphoid tissue** or MALT, which **can transform into lymphoma**.

Demonstration of H. pylori

- Initially, *H. pylori* gastritis is limited to gastric antrum and later may extend to involve the body and fundus.
- *H. pylori* are concentrated within the superficial mucus covering the epithelial cells in the surface and neck regions.
- *H. pylori* have an affinity for gastric epithelium and are generally not found in association with intestinal metaplasia or duodenal epithelium. Biopsy of the antral region is preferred for evaluation of *H. pylori* gastritis.
- **Special stains for *H. pylori (Figs. 18.10A to C):*** Organisms can be easily demonstrated with special stains.
  - **Modified Giemsa stain and Diff–Quik** stain are popular, quick, cheap and easy to perform.
  - **Silver stains: Warthin–Starry, Genta stain**.
  - **Immunohistochemistry for *Helicobacter***.

Diagnostic Tests for H. pylori

- Demonstration of *H. pylori* in tissue **(remains the gold standard for detection of *H. pyori*)**
- Serologic test for antibodies to *H. pylori*
- Fecal bacterial detection
- Urea breath test based on the generation of ammonia by the bacterial urease
- **Gastric biopsy specimens:** Histological examination (*refer* special stains mentioned above), analysis by the rapid urease test, bacterial culture or bacterial DNA detection by PCR.

Peptic ulcer disease is a complication of chronic*H. pylori* gastritis.

H. pylori: Gastric antrum is the most common site of colonization.

H. pylori:
- Produces urease
- Noninvasive
- Colonizes in the mucosal layer
- Causes intestinal metaplasia—precursor of gastric cancer

Mechanism of action of *H. pylori:*

- Flagella
- Urease
- Its products (adhesive molecules, enzymes and cytotoxins)

H. pylori-induced gastritis: Due to the imbalance between gastroduodenal mucosal defenses and damaging forces.

Humans are the only known host for *H. pylori*.

H. pylori gastritis:
- Intraepithelial neutrophils
- Subepithelial (superficial lamina propria) plasma cells

H. pylori: Giemsa stain, Warthin-starry stain

H. pylori: Increased risk of
- Gastric adenocarcinoma
- Gastric MALT lymphoma

H. pylori: Biochemical reactions positive for
- Catalase
- Oxidase
- Urease.

H. pylori: Rapid urease test on gastric mucosal biopsy is 100% specific test.

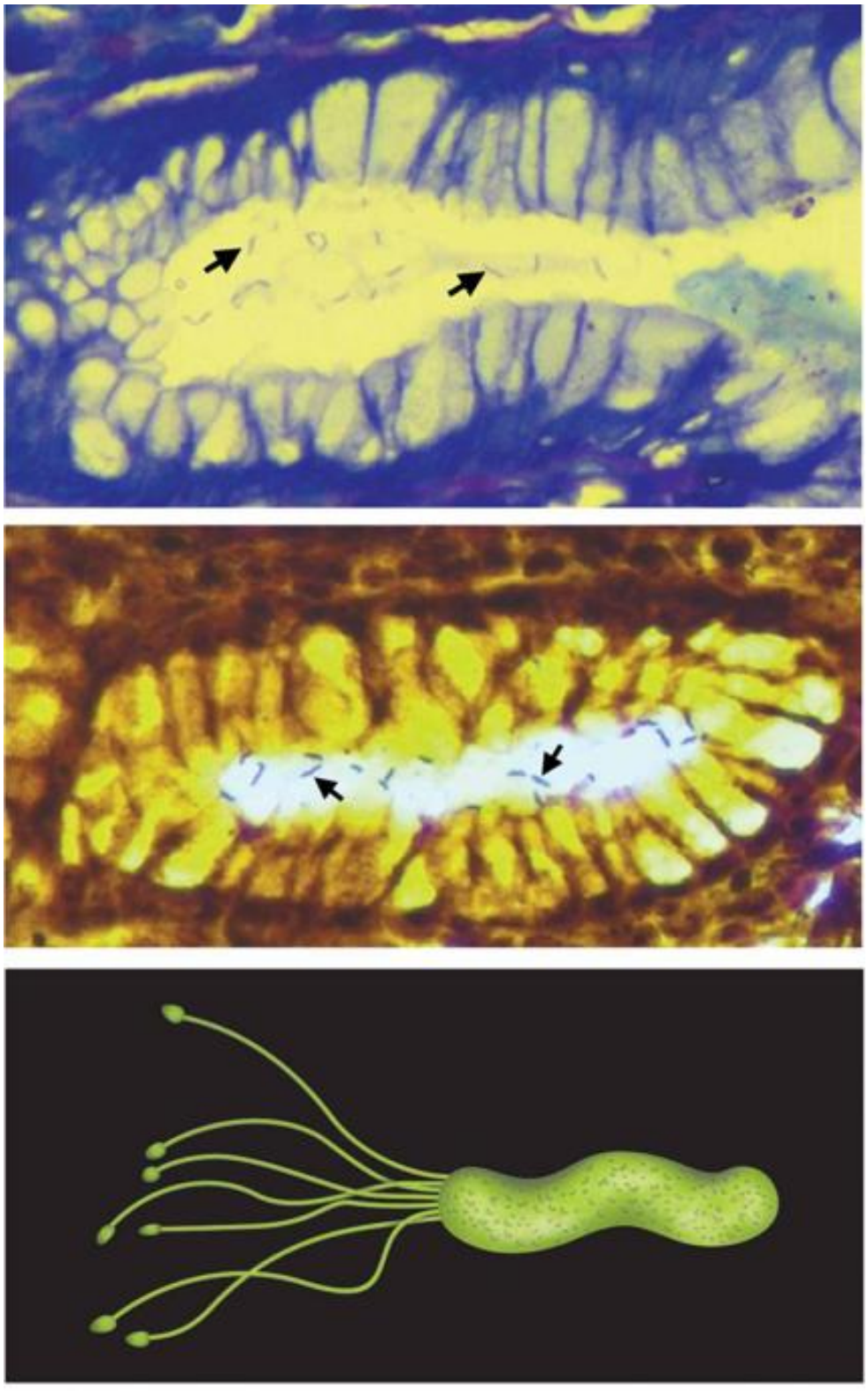
H. pylori: Grows well at 37°C in microaerophilic conditions. Culture media includes
- Skirrow's medium
- Chocolate medium.

PEPTIC ULCER DISEASE

CC: Describe the etiology, pathogenesis, pathology, microbiology, clinical and microscopic features of peptic ulcer disease.

Q. Define peptic ulcer.

Q. Discuss the pathogenesis of peptic ulcer.



Figs. 18.10A to C: Photomicrograph shows *H. pylori* (arrows) in the lumen of gastric glands: (A) Giemsa stain; (B) Warthin–Starry stain; (C) Diagrammatic.

A

B

C

Definition: Peptic ulcer is defined as a chronic mucosal ulceration/defect that penetrates the muscularis mucosae. It usually affects the duodenum (duodenal ulcer) or stomach (gastric ulcer).

Peptic ulcer disease (PUD) is one of the complications of chronic gastritis. It is **most often associated with** colonization with ***H. pylori*** and *H. pylori*-induced chronic gastritis (with hyperchlorhydria), NSAIDs, or cigarette smoking.

Normal Process in the Stomach

The gastric lumen pH is about 1 which is several times more than the pH of blood. Though it is needed for the digestion, sometimes, it can damage the gastric mucosa. There are several mechanisms which protect the gastric mucosa.

Two opposing sets of forces keep stomach in a normal state: A. damaging forces and B. Defensive forces **(Fig. 18.11)**.

Damaging Forces

These forces are capable of inducing mucosal injury and consists of two gastric secretory products: (1) hydrochloric acid and (2) pepsinogen.

Gastric Acidity

Hydrochloric acid plays **main role in digestion,** but it also **can damage the gastric mucosa**.

- Enzyme H^+/K^+ ATPase is responsible for producing the large concentration of H^+. Acid is secreted by the parietal cell located in the oxyntic gland.
- Parietal cell expresses receptors for many stimulants of acid secretion, including histamine (H2), gastrin (cholecystokinin B/gastrin receptor), and acetylcholine (muscarinic receptor, M3).

Peptic Enzymes

They **can also damage** the gastric mucosa.

- Pepsinogen, which is an inactive precursor of pepsin, is synthesized and secreted by the chief cell, found mainly in the gastric fundus.
- The acid environment within the stomach leads to conversion of pepsinogen to pepsin and provides the low pH (<2.0) required for activity of pepsin.
- Pepsin activity decreases as the pH increase; it is markedly decreased at a pH of 4 and inactivated at a pH of 7 and above. Many of the substances, which stimulate acid secretion, also stimulate pepsinogen release.

Defensive Forces

These are a three-level barrier composed of: (1) Pre-epithelial, (2) epithelial, and (3) subepithelial elements.

Peptic ulcer: Mucosal defect that is at least 0.5 cm in diameter penetrates the muscularis mucosae.

Stomach: Two opposing sets of forces

1. Damaging forces
2. Defensive forces

Stomach: Defensive forces in the stomach may be:

- Pre-epithelial
- Epithelial
- Subepithelial

Chief cells secrete: Pepsin and leptin.

Parietal cells secrete: Ghrelin, intrinsic factor, leptin and acid.

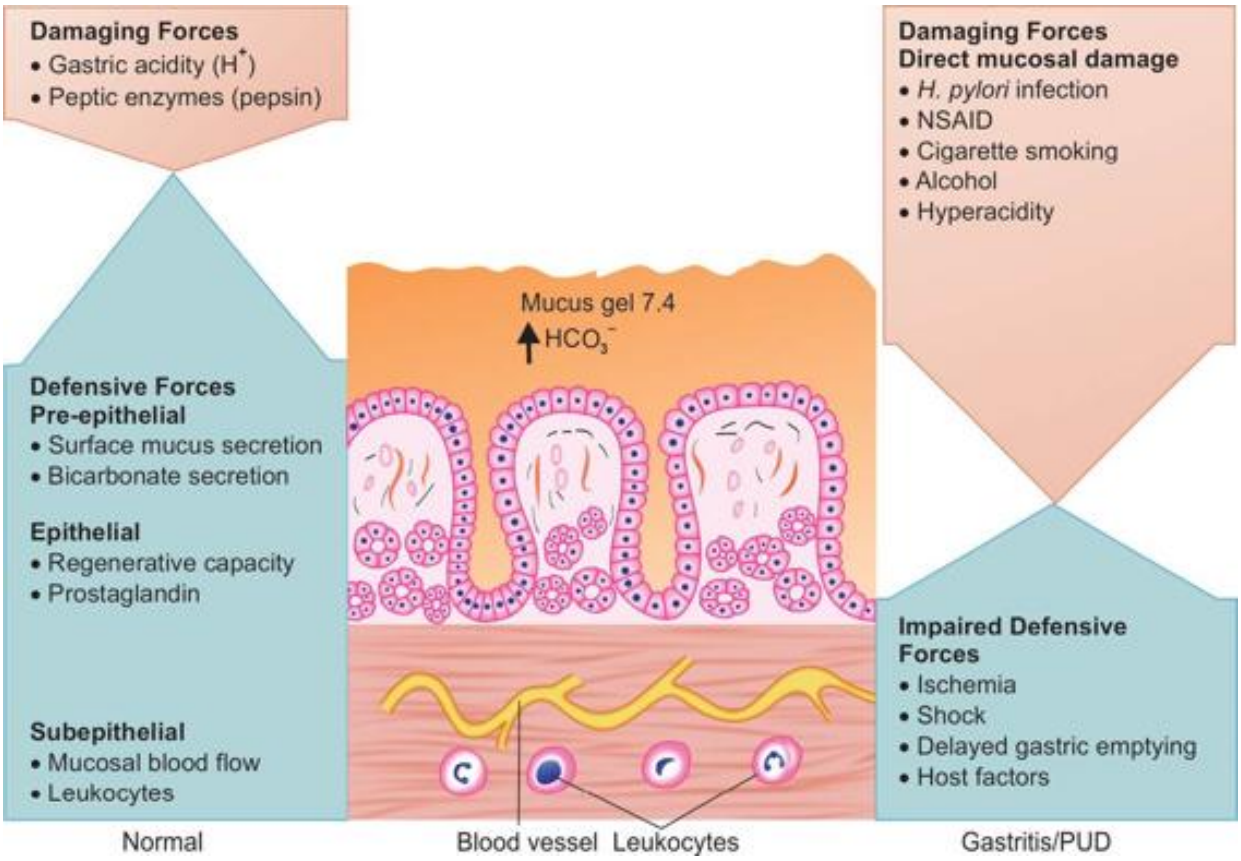


Fig. 18.11: Components involved in mucosal defense and repair in normal (left side) and in acute or chronic gastritis (right side). Gastric mucus barrier consists of viscid mucus (forms an unstirred layer between the epithelium and the gastric lumen) and bicarbonates.

Pre-epithelial Barrier

It is a mucus-bicarbonate layer of the stomach. It is formed by several factors produced by surface epithelial cells, such as: (a) production of mucus, (b) bicarbonate secretion, (c) epithelial cell ionic transporters that maintain intracellular pH and (d) intracellular tight junctions.

- **Surface mucus secretion**: Mucin is secreted by surface foveolar cells. Actions of mucus are:
  - Mucus layer **promotes formation of an "unstirred" protective layer** of fluid on the mucosa.
  - **Prevents the direct contact of large food particles to the epithelium**.
  - Impedes the diffusion of ions and molecules such as pepsin.
- **Bicarbonate secretion:** Surface epithelial cells secrete bicarbonate into the mucus → bicarbonate diffuses into the unstirred mucus → buffer the hydrogen ions entering from the luminal aspect. It results in a pH

gradient, ranging from 1 or 2 at the gastric luminal surface, and reaching to a neutrality of 6 to 7 along the epithelial cell surface.

Epithelial Barrier

It consists of surface epithelial cells that acts through (a) restitution of damaged gastric epithelial cells, (b) epithelial regeneration, (c) secretion of prostaglandins and (d) production of mucus (see above).

- **Restitution:** It is the process of restoration of a damaged region by the gastric epithelial cells and requires continuous blood flow and an alkaline pH in the surrounding environment.
- **Epithelial regeneration:** It is regulated by prostaglandins and growth factors such as EGF and TGF-α.
- **Secretion of prostaglandins:** Gastric mucosa secretes prostaglandin which plays a main role in gastric epithelial defense/repair. **Prostaglandins maintain mucosal blood flow and epithelial cell restitution.**

Subepithelial Barrier

Rich gastric mucosal blood flow: It provides (1) bicarbonate (HCO_3^-), which neutralizes the acid generated by parietal cells, (2) an adequate supply of nutrients and oxygen, and (3) removes toxic metabolic by-products.

Pathogenesis of PUD/Acute or Chronic Gastritis

Q. Discuss the role of H. pylori in gastric ulcer (refer page 550).

Components of the normal process involved in maintenance of balance of two opposite forces of gastric mucosa is discussed above. **The imbalances between mucosal defenses and damaging forces cause chronic gastritis** and also **PUD**.

PUD can occur due to direct mucosal injury or disruption of any of protective mechanisms.

Risk factors for peptic ulcer disease are listed in**Box 18.2**.

Direct Mucosal Injury/Increased Damage

- ***H. pylori* infection** (*refer* pages 550-1) is one of the **most important, common, primary cause** of PUD. *H. pylori* is detected from the gastric antrum in almost all patients with duodenal ulcers. It is associated with increased secretion of gastric acid and reduced duodenal secretion of bicarbonate.
- **Nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin**: It causes (1) **direct chemical irritation of mucosa, (**2) **suppresses mucosal prostaglandin synthesis** and (3) **reduces the bicarbonate secretion**.
- **Cigarette smoking: Impairs blood flow to the mucosa** and healing of mucosal damage.
- **Alcohol,** radiation therapy and chemotherapy: They cause direct injury to mucosal cells.
- **Ingestion of chemicals:** These include acids or bases and cause direct injury.
- **Gastric hyperacidity**: The causes of hyperacidity include *H. pylori* infection, parietal cell hyperplasia and Zollinger–Ellison syndrome (*refer* page 556).
- **Others**
  - **High-dose corticosteroids:** They suppress prostaglandin synthesis and impair healing.
  - Psychologic stress.
  - Duodenal gastric reflux.

Impaired Defense

- **Ischemia**: Decreased oxygen delivery (e.g., at high altitudes and shock)
- **Shock**
- **Delayed gastric emptying**
- **Host factors:** Reduced mucin synthesis in the elderly → increased susceptibility to gastritis.

BOX 18.2: Risk factors for peptic ulcer disease.

- *H. pylori* infection
- Cigarette smoking
- NSAIDs (non-steroidal anti-inflammatory drugs)

- Chronic obstructive pulmonary disease (COPD)
- Alcoholic cirrhosis
- Psychological stress
- Zollinger-Ellison syndrome
- Viral infection: CMV, herpes simplex virus

MORPHOLOGY

Q. Write short note on morphology of peptic ulcer.

Gross (Fig. 18.11)

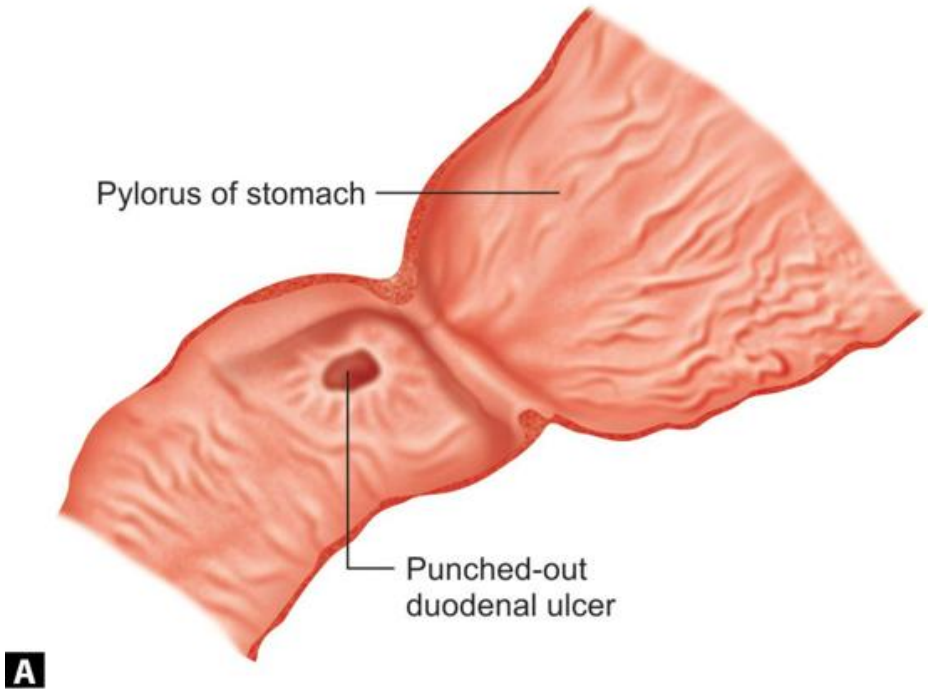
- **Sites of peptic ulcer:** They can develop in any portion of the GI tract exposed to acidic gastric juices.
  - **Duodenum: More common in the first portion of the duodenum (**anterior or posterior wall) within a few centimeters of the pyloric valve **than in the stomach**.
  - **Stomach: Lesser curvature** near the junction (transitional zone) of the body and antrum:
    - Proximal ulcers: Located in the body of the stomach.
    - Distal ulcers: Located in the antrum and angulus of the stomach.
  - **Gastroesophageal junction of esophagus**.
  - **Anastomotic site**: It can develop at the anastomotic site in patients who have undergone a distal gastric resection. Occur at margins of the gastroduodenal anastomosis/gastrojejunostomy (anastomotic ulcer). These are rare nowadays because of present efficient acid—suppressing therapies available for peptic ulcer.
  - **Multiple ulcers**: In the duodenum, stomach, and/or jejunum in Zollinger-Ellison syndrome.
  - **At metaplastic or heterotopic gastric mucosa**: For example, Meckel diverticulum within an ileum having ectopic gastric mucosa.
- **Number**: **Solitary** in more than 80% of patients, but may be more than one.
- **Size:** Lesions less than 0.3 cm in diameter are shallow and those larger than 0.6 cm are likely to be deeper ulcers.
- **Shape: Round to oval**, **sharply punched-out defect (Figs. 18.12A to C)**.
- **Margin:** Usually **in level with the surrounding mucosa**. The gastric mucosal folds can be traced up to the margins of ulcer and the radiating folds of mucosa from ulcer **(Figs. 18.12A to C)** appear like a spoke wheel. In contrast, **heaped-up margins are more characteristic of gastric cancers**.
- **Depth:** Varies.
- **Base:** It is **smooth and clean** as a result of peptic digestion of exudate.

CC (Show How): Describe and identify the microscopic features of peptic ulcer.

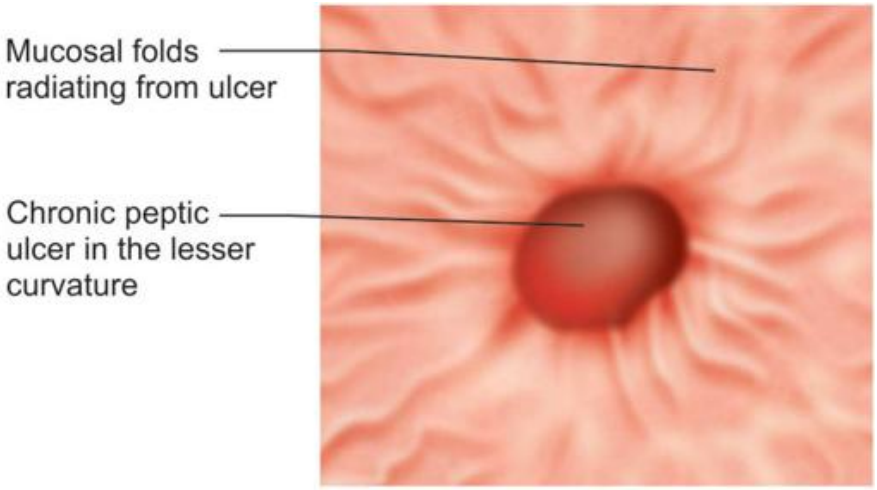
Microscopy (Figs. 18.13A and B)

Gastric and duodenal ulcers are microscopically similar. From the lumen outward **four layers** can be identified and are known as **Askanazy zones**.

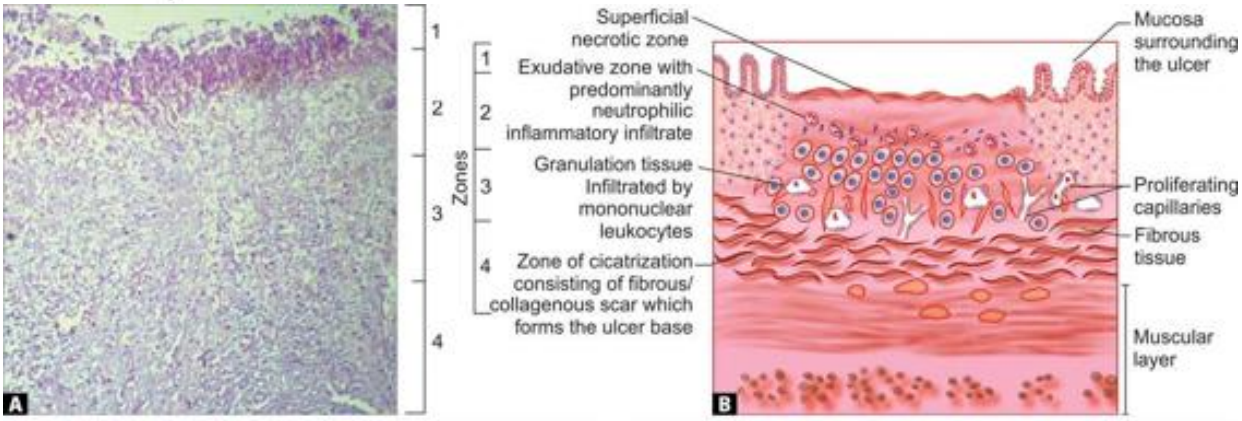
1. **Necrotic zone**: It is the most superficial zone.
2. **Superficial exudative zone**: It consists of **fibrinopurulent exudates with predominantly neutrophilic inflammatory infiltrate**.
3. **Granulation tissue zone**: It consists of granulation tissue infiltrated with mononuclear leukocytes.
4. **Zone of cicatrization:** It consists of **fibrous tissue or collagenous scar** which forms the base of the ulcer and may show chronic inflammatory cells.




B



Figs. 18.12A to C: Gross appearance of peptic ulcer: (A) Duodenal ulcer in the first part of duodenum showing characteristic sharp demarcation from the surrounding mucosa (diagrammatic); Peptic ulcer of stomach (B, specimen; C, diagrammatic) showing characteristic sharp demarcation from the surrounding mucosa. Note the gastric mucosal folds can be traced up to the margins of ulcer and the radiating folds of mucosa from ulcer appear like a spoke wheel.



Malignant transformation of peptic ulcers is very rare

PUD: Duodenal > gastric

Microscopy of peptic ulcer: From the lumen outward four layers can be identified and are known as Askanazy zones.

Autoimmune gastritis involves fundus and body of stomach.

Figs. 18.13A and B: Microscopic features of a peptic ulcer (A, photomicrograph and B, diagrammatic) showing four layers of chronic gastric ulcer.

Clinical Features

- Peptic ulcers are **chronic, recurring lesions with more morbidity than mortality**.

- **Age: Young adults** but are most often diagnosed in middle-aged to older adults.
- **Periodicity:** After a period of weeks to months of active disease, healing may occur with or without treatment.
- **Pain:** Epigastric burning or aching pain exacerbated by fasting and improved with alkali or food. It usually develops 1–3 hours after meals during the day and is worse during night between 11 PM and 2 AM.
- **Other symptoms:** These include nausea, vomiting, bloating, belching and significant weight loss.

Complications of Peptic Ulcer Disease

Q. Write a short note on complications of gastric ulcer.

- **Bleeding:** Most common complication of peptic ulcer. Chronic blood loss may lead to iron deficiency anemia. Severe bleeding may cause "coffee ground" vomitus or melena and may be life-threatening.
- **Perforation**: Develops in ~5% of patients and is the **most common complication of gastric ulcer**. It is most common from anterior aspect of first part of duodenum.
- **Pyloric obstruction (gastric outlet obstruction):** It is associated with ulcers in the pyloric region and occurs in ~ 10% of ulcer patients. It is secondary to either edema or scarring.
- **Rarely malignant transformation:** The dictum is that 'cancers ulcerate but ulcers rarely cancerate'**.**
- **Development of combined ulcers**: In the stomach and duodenum of the same patient.

Differences between gastric and duodenal peptic ulcer are given in **Table 18.3**.

Q. A 38-year-old male executive working in a public sector company complains of a long history of heart burn (dull pain in the midepigastric region) and dyspepsia for the last 1 year. The pain used to occur 2 to 3 hours after a meal and is relieved if he takes

antacids or eats more food. There is no weight loss. On enquiry, he has been consuming analgesic (about 1–2 tablets per week) for headache and consumes alcohol regularly. He underwent upper GI endoscopy and found to have lesion in the lesser curvature of stomach. He recovered of this illness after few days of treatment with drugs and modification life style. He felt better and discontinued treatment. Three weeks later, the epigastric pain recurs.

(a) What is the probable diagnosis? (Ans: Peptic ulcer disease)

(b) Describe the etiopathogenesis. What bacterial infection can be an etiological factor? (refer pages 552-3).

(c) Describe the gross and microscopy of the stomach in this disease (refer pages 554-5).

(d) Mention the complications (refer above).

(e) How is it different from a malignant lesion at the same location? (refer Table 18.6).

Prostaglandins protect gastric mucosa by:
- Increasing secretion of bicarbonate
- Increasing vascular perfusion
- Inhibition of acid secretion
- Promoting synthesis of mucin

PUD usually develops on a background of chronic gastritis.

Peptic ulcer disease: *H. pylori* infection and use of NSAID are the main causes.

H. pylori infection is associated with:

- ~90% of duodenal ulcers
- ~65% of gastric ulcers

TABLE 18.3: Differences between gastric and duodenal peptic ulcer.

Features	***Gastric ulcer***	***Duodenal ulcer***
Most common site	Along the lesser curvature	First part of duodenum
Incidence	Less common	More common
Age	Beyond 6th decade, M > F	Between 25 and 50 years, M > F
Association with *H. pylori* infection	Less common	Strong association
Acid level	Usually normal	High
Clinical features		
Relationship of pain to antacids	Relief of pain not consistent	Prompt relief of pain
Relationship of pain to food	Aggravates the pain	Relieves the pain
Night pain	Not observed	Common
Heart burn	Not common	Common
Hematemesis/melena	Hematemesis more common	Melena more common
Vomiting	Common	No vomiting
Weight loss	Present	Absent
Complication	Rarely undergo malignant change	No malignant change

Gastroduodenal artery is the source of bleeding in duodenal ulcer.
Left gastric artery is the source of bleeding in gastric ulcer.
Urea breath test is used to ensure the efficacy of the treatment for peptic ulcer disease caused by *H. pylori.*

NSAID and aspirin causes:
- Direct chemical irritation of mucosa
- Suppresses prostaglandin synthesis by mucosa
- Reduces the bicarbonate secretion

Chronic disorders associated with PUD:
- Cirrhosis
- Chronic pulmonary disease
- Chronic renal failure
- Alpha 1-antitrypsin deficiency

- Systemic mastocytosis
- Nephrolithiasis

Sites of peptic ulcer:
- Duodenum
- Stomach
- Esophagus
- Jejunum
- Gastric mucosa in Meckel diverticulum

Peptic ulcer: Most common sites
- Duodenal ulcer: First part of duodenum
- Gastric ulcer: Lesser curvature along the incisura angularis

Gastric ulcer: Size and location cannot differentiate benign and malignant ulcers.

Kissing ulcers: Peptic ulcers occurring at both posterior and anterior wall of the duodenum.

Peptic ulcer: Once a peptic ulcer, always a peptic ulcer patient.

PUD: Peptic ulcers in the duodenum never become malignant.
Small percentage of gastric ulcers may undergo malignant transformation.

ZOLLINGER–ELLISON SYNDROME (ZES)

Q. Write short note on Zollinger–Ellison syndrome.

Triad: (1) Hypergastrinemia caused by gastrin-secreting tumors (gastrinomas), (2) increased acid production, and (3) severe peptic ulcer disease.

- **Hypergastrinemia**: **Gastrinomas** are commonly seen in the small intestine or pancreas → increased gastrin secretion → leads to massive secretion of **acid and pepsin**.
- **Increased acid secretion** → inactivation of pancreatic enzymes and bile salts → leading to malabsorption and diarrhea. Stomach shows **increase in the number of parietal cells.**
- **Multiple and recurrent peptic ulcers** in the duodenum, stomach and/or jejunum.

ZES: Fasting serum gastrin level greater than 1000 pg/mL is diagnostic.

ZES: Caused by gastrin-secreting tumors (gastrinomas) → massive acid production → hyperacidity

→ single or multiple peptic ulcers (stomach, duodenum, and even jejunum).

GASTRIC ADENOCARCINOMA

CC: Describe and etiology and pathogenesis and pathologic features of carcinoma of the stomach.

Q. Discuss the etiopathogenesis, classification and morphology of gastric carcinoma.

Q. Classify gastric carcinoma. Describe the gross and microscopic features and spread of gastric carcinoma.

Adenocarcinoma is the most **common malignancy of the stomach**. It comprises ~ 90% of all gastric cancers.

Epidemiology

- More common in Japan and in males.
- Overall **decrease in the incidence** of gastric cancer is noted and may be due to:
  - Closely linked to decrease in *H. pylori* prevalence.
  - **Decreased consumption of dietary carcinogens**, such as (1) N-nitroso compounds and benzo[a]pyrene, (2) reduced use of salt and smoking food, (3) decreased use of preservatives due to easy availability of food refrigeration.
  - **Intake of green, leafy vegetables and citrus fruits** that contain antioxidants such as vitamin C, vitamin E and beta-carotene.

Though overall there is reduction of cancer of stomach, the cancer of the cardia region is on the rise. This may be due to Barrett esophagus, increased incidence of chronic GERD and obesity.

Etiology and Pathogenesis

Q. Write a short note on risk factors for carcinoma of stomach.

Gastric cancer is a multifactorial disease.

Risk Factors (Box 18.3)

Q. Discuss the etiology and pathogenesis of gastric carcinoma.

***Helicobacter pylori* infection:** It increases the risk by five- to six-fold. However, majority of individuals infected with *H. pylori* does not develop cancer. Possible mechanism of development of carcinoma stomach by *H. pylori* infection is presented in **Flowchart 18.1**.

Epstein–Barr virus (EBV): It is associated with about 10% of gastric adenocarcinomas. The exact role of EBV in the development of gastric adenocarcinomas is not known. Probably EBV infection preceded neoplastic transformation. *TP53* mutations are uncommon in EBV-positive gastric tumors, suggesting that the molecular pathogenesis of these cancers is distinct from that of other gastric adenocarcinomas. EBV-positive tumors tend to develop in the proximal stomach and most commonly is of diffuse type with a marked lymphocytic infiltrate.

Nitrites: High concentrations of nitrates are found in dried, smoked, and salted foods. The long-term ingestion of nitrates appears to be associated with a higher risk of gastric cancer. The nitrates present in the diet can be converted to carcinogenic nitrites by bacteria **(Box 18.4)**. The source of these bacteria may be exogenous such as ingestion of partially decayed foods (consumed especially by the lower socioeconomic classes) or infection by bacteria such as *Helicobacter pylori*. *H. pylori* cause chronic inflammatory atrophic gastritis, loss of gastric acidity, and favor growth of bacteria that convert nitrate into carcinogenic nitrite. Though the risk for gastric carcinoma is 6 times higher in individuals infected with *H. pylori*, it not known whether eradication of the bacteria in an individual already infected with *H. pylori* reduces the risk of gastric cancer.

BOX 18.3: Risk factors of gastric carcinoma.

A. Environmental factors

1. *H. pylori* infection (intestinal-type carcinoma and Epstein–Barr virus infection)

2. Dietary/nutritional: Nitrites derived from nitrates (water, preserved food), smoked foods (due to benzpyrene) and excess of salt (salted pickled vegetables, chili peppers), and deficiency of fresh fruit, vegetables, vitamins A and C, refrigeration
3. Low socioeconomic status: Low fat or protein consumption, high complex carbohydrate consumption
4. Rubber and coal workers

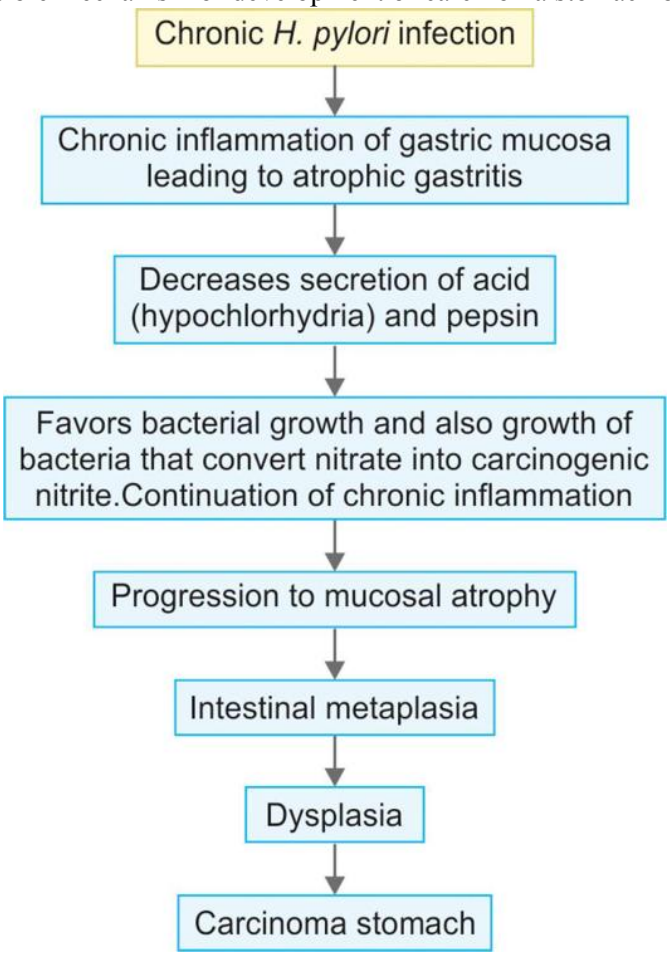
B. Host factors/predisposing conditions

1. Chronic gastritis (especially atrophic gastritis), pernicious anemia: Hypochlorhydria: Favors colonization with *H. pylori.* Intestinal metaplasia is a precursor lesion.
2. Partial gastrectomy: Favors bile reflux, alkaline intestinal fluid
3. Gastric dysplasia and adenomas more than 2 cm
4. Ménétrier's disease (hypertrophic gastropathy)
5. Barrett esophagus

C. Genetic factors

Blood group A, family history of gastric cancer, hereditary nonpolyposis colon cancer (HNPCC) syndrome, familial gastric carcinoma syndrome [germ line mutation in E-cadherin gene *(CDH1)*], Li–Fraumeni syndrome.

Flowchart 18.1: Possible mechanism of development of carcinoma stomach by *H. pylori* infection.



BOX 18.4: Nitrate-converting bacteria as a factor in the causation of

gastric cancer.

Exogenous sources of bacteria that convert nitrate into carcinogenic nitrite

- Diet contaminated by bacteria (responsible for higher incidence in low socioeconomic classes and contamination can be diminished by improved food preservation and refrigeration)
- Infection by *Helicobacter pylori*

Endogenous factors: They favors the growth of nitrate-converting bacteria in the stomach

- Atrophic gastritis and/or pernicious anemia
- Decreased gastric acidity
- Prior gastric surgery (antrectomy in which the acid-producing cells of the gastric antrum are removed)

Pathogenesis

Majority of gastric cancers have no hereditary basis. However, mutations detected in familial gastric cancer have helped in understanding the mechanisms of carcinogenesis in sporadic cases.

Familial Gastric Cancer

Germline loss-of-function mutations in the tumor suppressor gene ***CDH1*** is strongly associated with familial gastric cancer. Gene *CDH1* encodes the cell adhesion protein E-cadherin.

Sporadic Gastric Cancer

- **Sporadic diffuse gastric cancers** show **loss of E-cadherin** and is the key step in the development of diffuse gastric cancer. This may be by the following mechanisms:
  - **Loss-of-function mutations in the tumor suppressor gene *CDH1*** is also observed in about 50% of sporadic diffuse gastric tumors.
  - **Hypermethylation and silencing of the *CDH1* promoter** which decreases the E-cadherin expression.
  - ***BRCA2* mutations**: Increased risk of diffuse gastric cancer.

  CDH1 mutations are also common in sporadic and familial lobular carcinoma of the breast. These breast tumors are similar to diffuse gastric carcinoma infiltrate as single cells.
- **Sporadic intestinal-type gastric cancers**.

- **Mutations causing increased signaling via the Wnt pathway**: This may be due to loss of—function mutations in the adenomatous polyposis coli (APC) tumor suppressor gene and gain-of-function mutations in the gene encoding β-catenin.

- **Mutation of *TP53*** in majority of sporadic gastric cancers of both diffuse and intestinal type.
- **Loss-of-function mutations in other genes**: These include other tumor suppressor genes, genes involved in regulation of apoptosis (*BAX*), and cell cycle control (*CDKN2A*).

MORPHOLOGY

Q. Write short note on morphology of gastric carcinoma.

Site

- **Pylorus and antrum (50–60%)**
- Cardia (25%)
- Body and fundus (15–25%).

Lesser curvature is involved more often (in ~ 40%) than the greater curvature (~12%). Most favored site is the lesser curvature of the **antropyloric region.**

Classification of Gastric Carcinoma

Q. Classify gastric carcinoma.

There are several classifications of carcinoma of stomach. Classification is done according to histological features, depth of invasion, gross and histological subtype (microscopic appearance).

A. WHO Classification of Gastric Tumors

It is **based on the histological features** and is presented in **Table 18.4.**

Q. Write a short note on early gastric carcinoma.

B. Based on Depth of Invasion

Important morphological feature that decides the clinical outcome of gastric cancer is depth of invasion. According to this, gastric cancer can be divided into early gastric cancer and advanced gastric cancer.

1. Early gastric carcinoma (Fig. 18.14)

It is defined as an invasive **cancer that is limited to the mucosa and submucosa, with or without** perigastric **lymph node metastases.** It is not synonymous with carcinoma in situ (which is confined to the surface epithelial layer). Early gastric cancer is a **pathologic diagnosis** based on depth of invasion and is associated with better prognosis.

2. Advanced gastric carcinoma

It is a neoplasm that has **extended below the submucosa into the muscular wall.** All cancers may begin as "early" lesions, which develop into 'advanced' lesions. It is associated with poor prognosis. **Bormann classification** of growth patterns of advanced gastric carcinoma is presented in **Figures 18.15A to C**.

C. Based on the Macroscopic Growth Pattern (Fig. 18.14)

Three patterns are observed and are used for both early and advanced gastric cancers.

TABLE 18.4: WHO histologic classification (abridged) of gastric tumors.

Epithelial tumors	***Mesenchymal tumors***
Premalignant lesions: Intraepithelial neoplasia, adenoma	Leiomyoma
• Adenocarcinoma	• Schwannoma
• Papillary adenocarcinoma	• Granular cell tumor
• Tubular adenocarcinoma	• Leiomyosarcoma
• Mucinous adenocarcinoma	• Gastrointestinal stromal tumor (GIST)
• Signet-ring cell carcinoma	• Kaposi sarcoma
• Undifferentiated carcinoma	• Others
Adenosquamous carcinoma	*Malignant lymphoma*
Neuroendocrine neoplasms	
• Carcinoid tumor	

1. Type I (exophytic/polypoid/fungating)

It is a solid tumor **which projects/protrudes into the lumen** as a polypoid or nodular mass.

2. Type II (flat or depressed)

It is a **superficial, flat lesion** with no obvious tumor mass within the mucosa and may be slightly elevated or depressed. Three patterns are (1) elevated (type IIa), (2) flat (type IIb), and (3) depressed (type IIc).

3. *Type III (excavated)*

It is characterized by a **shallow or deeply erosive crater** in the wall of the stomach. An excavated malignant ulcer does not ordinarily occur alone but rather represents ulceration of type I or type II tumors.

- They may closely **resemble chronic peptic ulcers**. The **mucosa surrounding the ulcers appears firm, raised and nodular.**
- Characteristically, the **margins** of the ulcer are **heaped-up, beaded,** shaggy and irregular and the **base is ragged and necrotic**. This is in contrast to that of the benign peptic ulcer, which shows punched-out margins and a smooth base.

D. Lauren Classification Depending on the Histologic Subtype

According to this, gastric adenocarcinomas may be subdivided into two pathologically defined categories: Intestinal type and diffuse type. The *diffuse type* is characterized by absence of cell cohesion, so that individual cells infiltrate and thicken the stomach wall without forming a discrete mass.

1. *Intestinal type*

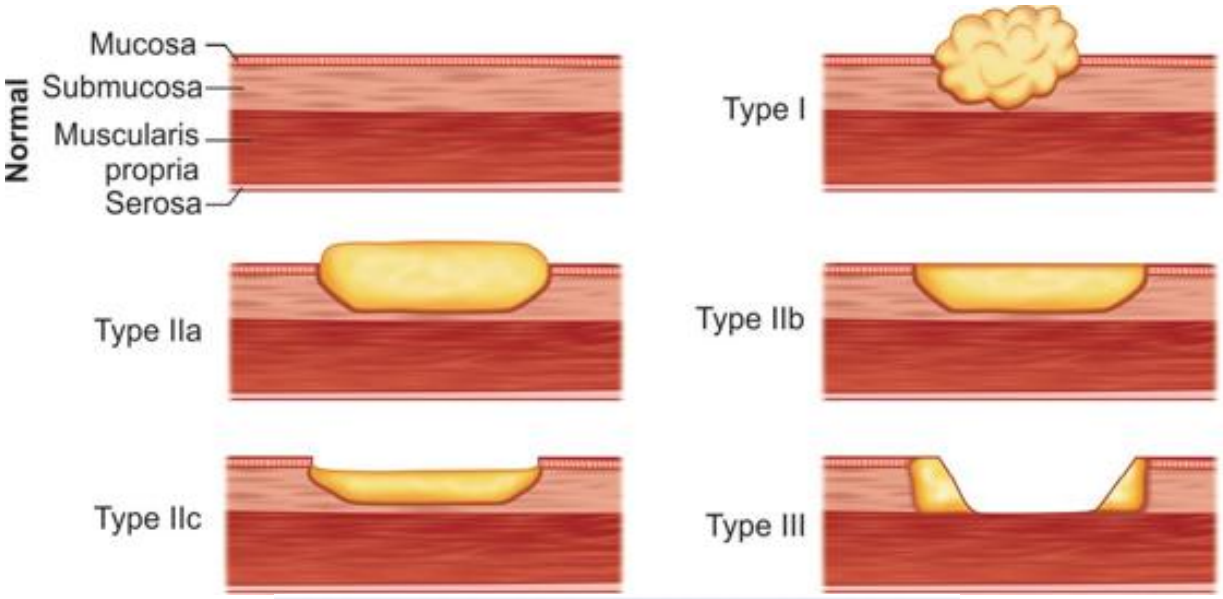
- **Gross:** It forms **polypoid bulky tumor (Fig. 18.15A) or** may be **ulcerated (Fig. 18.15B)**. More commonly, they appear in the antrum and lesser curvature of the stomach. They are often preceded by a prolonged precancerous process, often initiated by *H. pylori* infection.
- **Microscopy (Figs. 18.16A and B):** It consists of cohesive tumor cells that **form gland-like tubular structures**, resembling adenocarcinoma of colon. It probably **arises in areas of intestinal metaplasia**. The tumor cells show **apical mucin vacuoles**, and mucin may be present in the lumen of the glands

Q. Write a short note on linitis plastica.

2. *Diffuse or infiltrating gastric adenocarcinoma:*

The diffuse carcinomas occur more often in younger patients and carry a poorer prognosis.

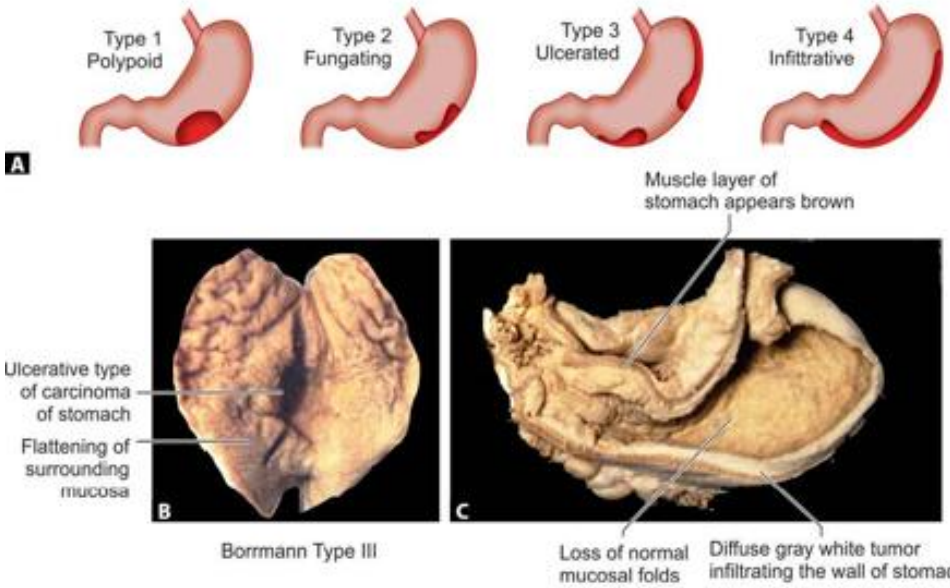
- **Gross:** Diffuse gastric cancer **infiltrates deeply into the stomach without forming obvious mass** lesions. It involves broad region or entire stomach (throughout the stomach including the cardia) with local rigidity of the wall. They elicit a **desmoplastic reaction** → produces diffuse **flattening of rugal folds** in the mucosa and rigid, widespread **thickening of the wall.** If the entire stomach is involved, it may become nondistensible (loss of distensibility of the stomach wall) and lumen is narrowed → producing **leather bottle appearance** termed **linitis plastica (Fig. 18.15C)**.



Early gastric carcinoma: Does not refer to the duration of the disease, its size, presence of symptoms and absence of metastases or curability.

Early gastric carcinoma: Tumor confined to the mucosa and submucosa irrespective of lymph node status.

Fig. 18.14: Japanese classification of early gastric cancer.

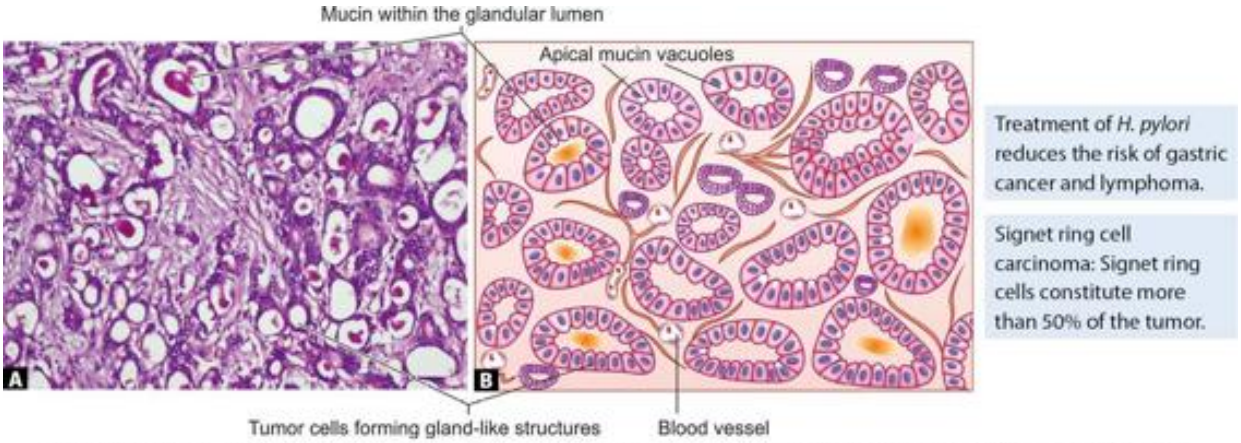


Sister Mary Joseph nodule/ node/sign:
Palpable nodule bulging into the umbilicus due to metastasis of cancer in the pelvis or abdomen.

Diffuse or infiltrating gastric cancer (linitis plastic): **Not** associated with *H. pylori*.

Linitis plastica (leather bottle stomach): Due to diffuse infiltrative growth that thickens the wall without forming a discrete mass.

Figs. 18.15A to C: (A) Borrmann classification of growth patterns of advanced gastric carcinoma. Gastrectomy specimen showing; (B) Excavated ulcerative type of carcinoma (Borrmann type 3); (C) Diffuse type of carcinoma stomach with leather bottle appearance (Bormann type 4).



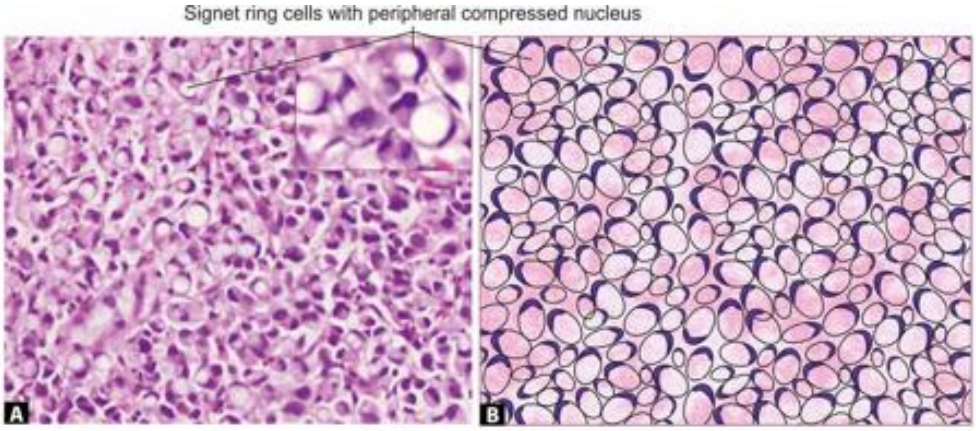
Figs. 18.16A and B: Microscopic appearance of intestinal type of gastric carcinoma: (A) Photomicrograph; (B) Diagrammatic.

Q. Write a short note on signet ring cell.

- **Microscopy:** It is composed of **discohesive** (cohesion is absent because of loss of E-cadherin) **tumor cells** which **do not form glands**. The tumor cells infiltrate and thicken the stomach wall without forming a discrete mass.
  - The **tumor cells contain abundant mucin** which expand the cytoplasm and push the nucleus to the periphery creating a **signet-ring appearance to the malignant cells (Figs. 18.17A and B)**.
  - If the signet-ring cells constitute **more than 50%** of the tumor, it is classified as **signet ring cell carcinoma**.

E. Molecular/Genomic Subtypes of Gastric Carcinomas

According to genomic profiling, gastric adenocarcinomas can be subdivided into four molecularly defined subgroups: (i) chromosomally unstable tumors (intestinal type, *TP53* mutation and RTK-RAS activation), (ii) genomically stable tumors (diffuse type, *CDH1, RHOA* mutations, *CLDN18–ARHGAP* fusion and cell adhesion), (iii) microsatellite unstable tumors (hypermutation, gastric- CpG-island methylator phenotype [CIMP], *MLH1* silencing and mitotic pathways), and (iv) Epstein-Barr virus (EBV) positive tumors (*PIK3CA* mutation, *PD-L1/2* overexpression, EBV-CIMP, *CDKN2A* silencing and immune cell signaling).



Figs. 18.17A and B: (A) Microscopic appearance of diffuse type of gastric carcinoma predominantly consisting of signet ring cells (signet ring carcinoma). Inset shows signet ring cells (upper right); (B) Diagrammatic appearance of signet ring carcinoma.

Spread of Carcinoma Stomach

Q. Write short note on modes of spread of gastric cancer (carcinoma stomach).

- **Local/direct spread:** It spreads into the muscularis and serosa of the stomach. It can invade into the duodenum, pancreas, liver, colon and retroperitoneum.
- **Lymphatic spread:** It spreads via the submucosal and subserosal lymphatics to both regional and distant lymph nodes. Pattern of lymph node involvement depends on the site of tumor **(Table 18.5)**.
  - Frequently metastasize to the **supraclavicular sentinel (Virchow) node** and **may be the first clinical manifestation of an occult neoplasm (Troisier's sign)**.
    - Left axillary lymph node—Irish node
  - Metastasize to the **periumbilical lymph nodes**: This forms a subcutaneous nodule → called **Sister Mary Joseph nodule** (after the nurse who noted this lesion as a marker of metastatic carcinoma) **(Fig. 18.18)**. This nodule represents metastasis from GI tract malignancies (in about 50% of cases) and includes carcinoma of stomach (most common), colon and pancreas. Other primary sites include carcinoma of ovary and uterus.
  - Metastasis to the **ovaries** is called **Krukenberg tumor**.
  - Metastasis to pouch of Douglas (Blumer shelf).

3. **Blood spread:** It can spread via the portal vein into **liver**. Other sites are **lungs** and **bones**.

Clinical Features

- **In high-risk areas, intestinal-type gastric cancer may arise from precursor lesions (e.g., flat dysplasia and adenomas.**
- **Advanced cancer:** It presents with **early satiety, bloating, distension and vomiting**. **The tumor frequently bleeds → causing iron-deficiency anemia**. Tumor in the pyloric region may present with **gastric outlet obstruction**.
- **Troisier's sign**: Metastatic lymph nodes may be palpable in the left supraclavicular fossa (Virchow's node, Troisier's sign).
- **Trousseau's sign:** Non-metastatic effects may be in the form of **migrating thrombophlebitis** (Trousseau's sign) and **deep venous thrombosis**. These are due to the effects of the tumor on thrombotic and hemostatic mechanisms.

Prognosis: It depends on the **depth of invasion, the extent of nodal and distant metastasis.**

Differences between benign and malignant ulcers of stomach are listed in **Table 18.6**.



Fig. 18.18: Sister Mary Joseph nodule which represents metastasis to periumbilical lymph node from GI tract cancers.

TABLE 18.5: Pattern of lymph node metastasis in gastric carcinoma.

Site of tumor	*Lymph nodes likely to be involved*
Proximal stomach and GE junction	Mediastinal and pericardial lymph nodes
Body of stomach	Greater and lesser curvature nodes near the tumor

TABLE 18.5: Pattern of lymph node metastasis in gastric carcinoma.	
Site of tumor	***Lymph nodes likely to be involved***
Tumors of distal stomach	Periduodenal, peripancreatic and porta hepatis nodes

Q. List the differences between benign and malignant ulcers of stomach.

TABLE 18.6: Differences between benign and malignant ulcers of stomach.

Characteristics	*Benign ulcer*	*Malignant ulcer*
Age	Usually in younger age	Older age
Duration of symptoms	Weeks to years	Weeks to months
Location/usual site	Lesser curvature of pylorus and antrum	Greater curvature of pylorus and antrum
Gross		
1. Size	Small	Large
2. Shape	Regular	Irregular
3. Surrounding mucosa	Mucosal rugae projects outwards (radiates) from the margins of the ulcer	Mucosal rugae stop far of the ulcer and shows flattening
4. Floor of the ulcer	Clean	Necrotic
5. Margins	Overhanging like spokes of wheel	Heaped up and everted
Acidity	Normal to low	May show achlorhydria

Q. A 55-year-old male laborer presents with early satiety, bloating, distension nausea, vomiting and

midepigastric pain for the last five months. He was treated by a local doctor for peptic ulcer disease without any relief. He has lost significant weight for the last 2 months. On examination, an epigastric mass was found and there was also enlargement of left supraclavicular lymph node. An abdominal CT scan showed gastric outlet obstruction. He underwent upper gastrointestinal endoscopy and found to have ulcerated 2 × 4 cm bulky mass in the lesser curvature of antrum at the pylorus. Biopsy taken from stomach confirmed the clinical diagnosis. He died within 3 years of diagnosis.

(a) What is the probable diagnosis? (Ans: Carcinoma of stomach)

(b) Describe the gross and microscopy of the stomach in this disease (refer pages 558-9).

(c) How does this disease spreads? (refer page 561).

Q. A 65-year-old female complains of 9 kg weight loss with severe nausea, vomiting and early satiety over the past 4 months. Upper gastrointestinal endoscopy reveals that the entire gastric mucosa is eroded and has an erythematous and leather bottle appearance. She underwent a total gastrectomy.

(a) What is your diagnosis? (Ans: Diffuse or infiltrating gastric adenocarcinoma). Give reasons for

your diagnosis (Ans: clinical features and leather bottle appearance)

(b) What are risk factors involved in the disease? (refer pages 557-8)

(c) Describe the gross appearances and microscopy of this lesion (refer pages 558-60).

(d) Enumerate the classification of tumors of this organ (refer pages 558-60).

(e) What is Krukenberg's tumor? (refer pages 561 and 765)

Antrum: Most common site for
- Gastric carcinoma
- Gastric lymphoma

Fundus: Most common site for
- Gastric carcinoma in pernicious anemia
- Diffuse type of gastric carcinoma

H. pylori increases the risk of gastric carcinoma by 5 to 6 fold. Infection with *H. pylori* is associated with distal intestinal type of gastric carcinoma and NOT with diffuse proximal carcinoma.

Gastric carcinoma is associated with blood group: A.

Risk factors for carcinoma stomach: Environmental, host and genetic.
Carcinoma of stomach: Alcohol is **NOT** a risk factor.
Decreased risk of carcinoma stomach with:
- Aspirin
- Diet rich in fresh fruit and vegetables
- Vitamin A and C
- Calcium, selenium, zinc and iron

Carcinoma stomach: Lesser curvature of pyloric and antral region are the most common sites.

Classification of carcinoma of stomach: Based on
- Depth of invasion
- Macroscopic growth pattern
- Histologic subtype

An ulcerative lesion on the greater curvature is more likely to be malignant.

Carcinoma of stomach: Intestinal type and diffuse type.

Gastric carcinoma: Virchow's left supraclavicular lymph node involvement due to metastasis (**Troisier's sign**).

Sister Mary Joseph nodule/node: Associated with multiple peritoneal metastases. Prognosis poor.

Krukenberg tumor: Metastasis of signet-ring carcinoma to both ovaries. Primary may be in the stomach, breast, pancreas and gallbladder.

Gastric carcinoma:

- Abdominal pain > weight loss are the most common symptom
- Hematemesis is very rare
- Troisier's sign: Left supraclavicular lymph node metastasis

Gastric carcinoma: Tumor markers (1) CEA, (2) CA-19.9; (3) CA-125, (4) CA72-4, (5) Beta-hCG.

GASTROINTESTINAL STROMAL TUMOR

- Gastrointestinal stromal tumor (GIST) is the **most common mesenchymal tumor of the abdomen**.
- **Most common location** of GIST is the **stomach**.

Epidemiology

- **Sex**: Slightly more common in males.
- Age: Peak age for GIST in the stomach is about 60 years.
- **Carney triad:** Nonhereditary syndrome seen in young females. Triad consists of (1) gastric GIST, (2) paraganglioma and (3) pulmonary chondroma.
- **Increased incidence** of GIST in patients with **neurofibromatosis type 1**.

Cell of origin: GISTs arise from the interstitial **cells of Cajal**, that are located in the muscularis propria and serve as **pacemaker cells for gut peristalsis**.

Pathogenesis

- **About 80%** of all GISTs have oncogenic, gain-of-function **mutations of the gene encoding the tyrosine kinase KIT** (receptor for stem cell factor).
- About 8% of GISTs have mutations that activate a related tyrosine kinase, platelet-derived growth factor receptor α (PDGFRA).
- Above mutations cause **constitutive activation** of **KIT or PDGFRA receptor tyrosine kinases** → produce intracellular signals → activate the RAS and PI3K/AKT pathways → **promote tumor cell proliferation and survival**.
- Few GISTs do not have mutated KIT or PDGFRA, but show mutations in other genes involved in these pathways (e.g., *NF1*, *BRAF*, *HRAS*, or *NRAS*).

MORPHOLOGY

Gross (Fig. 18.19A)

Size varies and may be quite large (up to 30 cm in diameter). Usually solitary, well-circumscribed, fleshy mass covered by intact or ulcerated mucosa, but can also project outward toward the serosa.

Microscopy (Fig. 18.19B)

Consists of thin elongated **spindle cells or** epithelial-appearing (**epithelioid)** cells **or mixtures** of these two types of cells.

Diagnostic Markers

GISTs and Cajal cells express **KIT** (also known as **CD117**) and CD34. Most **useful diagnostic marker is KIT (Fig. 18.19C)**, and immunohistochemically detected in 95% of gastric GISTs.

Clinical Features

- Symptoms may be due to mass effects.
- Mucosal ulceration can cause blood loss.
- **Investigations**: Best diagnosed with CT scan and PET scan.

Prognosis

Correlates with:

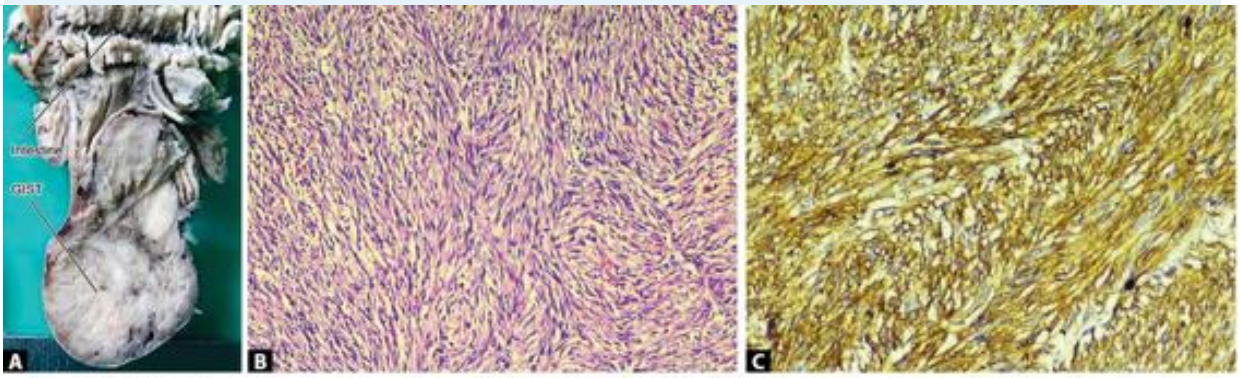
- **Tumor size**: Recurrence or metastasis is rare when size **is less than 5 cm**.
- **Mitotic index**: **Mitotically active** tumors **larger than 10 cm commonly metastasize or recur**.
- **Location**: **Gastric** GISTs is **less aggressive** than those of the small intestine.

GIST: Most common mesenchymal tumor of the abdomen. Most common location in the **stomach**. **They arise** from the interstitial **cells of Cajal (pacemaker cells for gut peristalsis)**. Most useful diagnostic marker is **KIT (CD117)** in 95% of cases.

Carney triad: Nonhereditary syndrome in young females.

- **Gastric GIST**
- **Paraganglioma**
- **Pulmonary chondroma.**

Imatinib, a tyrosine kinase inhibitor that inhibits KIT and PDGFRA, often useful in patients with unresectable, recurrent, or metastatic tumor.



Figs. 18.19A to C: Gastrointestinal stromal tumor: (A) Intestine with GIST; (B) GIST showing bundles and fascicles of spindle-shaped tumor cells; (C) Tumor cells show KIT (CD117) positivity.

MECKEL DIVERTICULUM

Q. Write short note on Meckel diverticulum.

Meckel diverticulum is the most common type of true diverticula of GI tract and occurs in the ileum.

Rule of 2s: It is used to remember characteristics of Meckel diverticulae.

- Occur in about **2**% of the **population**.
- Generally present within **2 feet** of the **ileocecal valve**.
- ~ **2 inches long** (varies from 2 to 15 cm).
- Two times common in **males** than in females.
- Most often **symptomatic** by age **2**.

MORPHOLOGY

- **Single** and always lies on the **antimesenteric** side of the bowel **(Fig. 18.20)**.
- **True diverticulum:** The wall consists of mucosa, submucosa and muscularis propria and has its own blood supply.
- Lining of **epithelium resembles** that of **normal small intestine**, but **ectopic pancreatic or gastric tissue is common**. **Heterotopic gastric mucosa** may lead to **peptic ulceration** of adjacent small intestinal mucosa and present with **bleeding, or perforation**.

Complications

- **Severe hemorrhage**: Develops if the diverticulum contains acid-secreting gastric epithelium with peptic ulceration → maroon-colored painless bleeding per rectum.
- **Meckel's diverticulitis***:* Develops secondary to peptic ulcerations or obstruction of the orifice of the diverticula and symptoms appear similar to acute appendicitis.
- **Intestinal obstruction**: Results from intussusception, volvulus, adhesions or the presence of a tumor or ectopic tissue or enteroliths.

Meckel diverticulitis: Symptoms mimic acute appendicitis.

Meckel diverticulum: Bleeding is the most common complication.

TYPHOID FEVER

- Typhoid fever (enteric) is an **acute systemic disease** caused by infection with *Salmonella typhi***.**
- Paratyphoid fever is clinically similar but milder disease caused by *Salmonella paratyphi*. **Enteric fever** is the general term, which includes **both typhoid and paratyphoid** fever.

Etiology

Causative agent: Enteric fevers are caused by ***Salmonella typhi* and *Salmonella paratyphi***. *Salmonella* are gram-negative, motile bacilli (rods). Boiling or chlorination of water and pasteurization of milk destroy the bacilli.

Source of infection: Humans are the only natural reservoir and includes:

- **Patients suffering from disease:** Infected urine, feces, or other secretions from patients.
- **Chronic carriers of typhoid fever**: *S. typhi* or *S. paratyphi* **colonizes in the gallbladder** or biliary tree → may be associated with gallstones and the chronic carrier state.

Mode of transmission: From **person-to-person contact**.

- **Ingestion** of **contaminated food** (especially dairy products) and shellfish or **contaminated water**.
- **Direct spread:** Rare by **finger-to-mouth contact** with feces, urine, or other secretions.

Incubation period: Usually 7–14 days.

Pathogenesis (Fig. 18.21)

Q. Write short note on pathogenesis of typhoid fever.

- The typhoid bacilli (*Salmonella*) are **ingested through contaminated food or water** → able to **survive in gastric acid of the stomach** → reach mucosa of small intestine.
- **Events during incubation period**:
  - During the initial asymptomatic period (about 2 weeks), the *Salmonella* **attach to the microvilli** and penetrate the **ileal mucosa** of the small intestine → reach lamina propria and submucosa.
  - *Salmonella* are **phagocytosed by the macrophages** (mononuclear phagocytes) present in the lymphoid tissue and **Peyer's patches** in

the submucosa of small intestine.

- *Salmonella* **multiply within the macrophages** and are **carried to** the **mesenteric lymph node** via lymphatics. They multiply in the lymph nodes and **via** the **thoracic duct** enter the **bloodstream** causing **transient bacteremia**.
- During bacteremia, the bacilli are **seeded in many organs.** They colonize reticuloendothelial tissues (**liver, gallbladder, spleen, bone marrow)**, where bacilli multiply further causing **massive bacteremia** (occurs towards the end of incubation period) → disease clinically manifests.

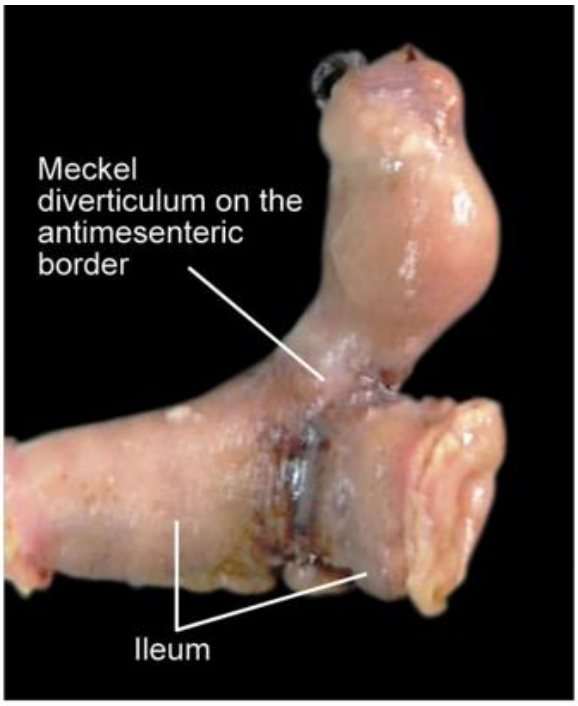


Fig. 18.20: Meckel diverticulum on the antimesenteric border.

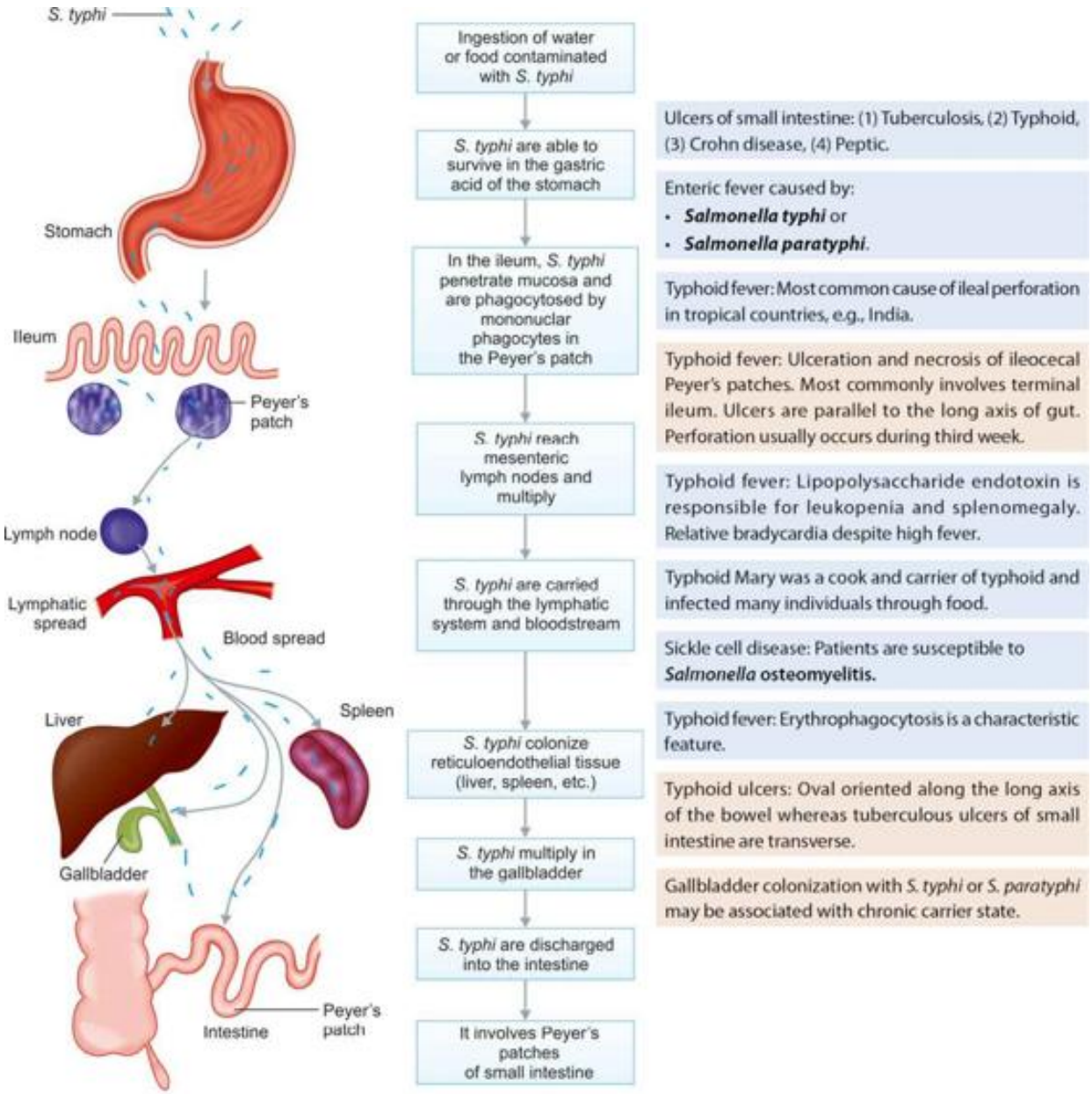


Fig. 18.21: Pathogenesis of typhoid fever.

- **Bile is a good culture medium for the typhoid bacillus**, and bacilli **multiply in the gallbladder** → bacilli are continuously shed through the bile into the → intestine.
- In the intestine, the bacilli are localized to the **Peyer's patches and lymphoid follicles** of the terminal ileum. They cause inflammation, plateau-like elevations of Peyer's patches and necrosis, which results in characteristic **oval typhoid ulcers.**

MORPHOLOGY

Q. Morphology of intestine in typhoid fever.

Lesions may be (1) intestinal and (2) extraintestinal.

Intestinal Lesions

Gross (Fig. 18.22)

Q. Write a short note on pathology of ileum in typhoid fever.

- **Site:** Most commonly involved is **terminal ileum**, but may be seen in the jejunum and colon.

- **Appearance**: Peyer's patches in the terminal ileum enlarge into sharply delineated, plateau-like elevations → shedding of mucosa produces → typhoid ulcers. **Characteristics of typhoid ulcer**:
  - **Number**: **Varies.**
  - **Orientation: Oval ulcers oriented along the long axis of the bowel** (tuberculous ulcers of small intestine are transverse).
  - **Base of the ulcer:** It is **black** due to sloughed mucosa.
  - **Margins: Slightly raised** due to inflammatory edema and cellular proliferation.
  - **No fibrosis:** Hence, narrowing of the intestinal lumen seldom occur in healed typhoid lesions.

Microscopy

- **Mucosa: Oval ulcers** over the Peyer's patches.
- **Lamina propria**:
  - **Macrophages** containing bacteria, red blood cells (erythrophagocytosis) and nuclear debris
  - **Lymphocytes and plasma cells**
  - **Neutrophils** within the superficial lamina propria.

Extraintestinal Lesions

- **Typhoid nodules:** Systemic dissemination of the bacilli leads to **formation of focal granulomas** termed **typhoid nodules.**

  These nodules are composed of **aggregates of macrophages** (typhoid cells) containing ingested bacilli, red blood cells and lymphocytes. They are **common in the lymph node, liver, bone marrow and spleen**.

- **Mesenteric lymph nodes**: They are enlarged due to accumulation of macrophages, which contains typhoid bacilli.
- **Liver:** It shows small, scattered foci of hepatocyte necrosis replaced by **typhoid nodules**.
- **Spleen:** It is enlarged and soft.
- **Abdominal muscles**: Zenker's degeneration (severe glassy or waxy hyaline degeneration or necrosis of skeletal muscles).
- **Gallbladder: Typhoid cholecystitis.**

Clinical Features

- Onset is gradual and patients present with anorexia, abdominal pain, bloating, nausea, vomiting and diarrhea.
- **Fever: Continuous rise in temperature** (step-ladder fever).
- **Rose spots:** These are small erythematous maculopapular lesions on the skin that fade on pressure, appear on the chest and abdomen and occur during second or third week.
- **Spleen:** It is soft and palpable and may be accompanied by hepatomegaly.

Complications

- ***Intestinal complications:*** It can be fatal.
  - **Perforations of ulcer**
  - **Hemorrhage from the ulcer**.
- ***Extraintestinal complications***: **Encephalopathy, meningitis, seizures, endocarditis, myocarditis, pneumonia and cholecystitis**. **Sickle cell disease → susceptible to *Salmonella* osteomyelitis**.
- ***Carrier state***: Persistence of bacilli in the **gallbladder or urinary tract** may result in passage of bacilli in the feces or urine → causes a '**carrier state'** → source of infection to others.

Laboratory Diagnosis

Isolation of Bacilli

- **Blood culture**: It is positive in **first week** of fever in 90% of patients and remains positive in second week till the fever subsides. Blood culture rapidly becomes negative on treatment with antibiotics.
- **Stool cultures:** It is almost as valuable as blood culture and becomes positive in the **second and third** weeks.

- **Urine culture**: It reveals the organism in approximately 25% of patients by **third week**.

Other Tests

- **Widal reaction:** Classic Widal test **measures antibodies against O and H antigens** of *S. typhi*, but **lacks sensitivity and specificity**. Widal test (immunological reactions) becomes positive from end of the first week till fourth week. There are many false-positive and occasional false-negative Widal reactions.
- **Other serologic tests**: They are available for the rapid diagnosis of typhoid fever with a higher sensitivity.
- **Total leukocyte count**: It shows **leukopenia with relative lymphocytosis**. Eosinophils are usually absent.

Enteric fever:
- Abdominal pain (hallmark symptom)
- Step-ladder fever
- Rose spots

Absolute lymphocytosis is NOT seen in enteric fever.

Enteric fever: (1) Blood culture; (2) Stool culture; (3) Urine culture; (4) Widal test, and (5) Total WBC count.

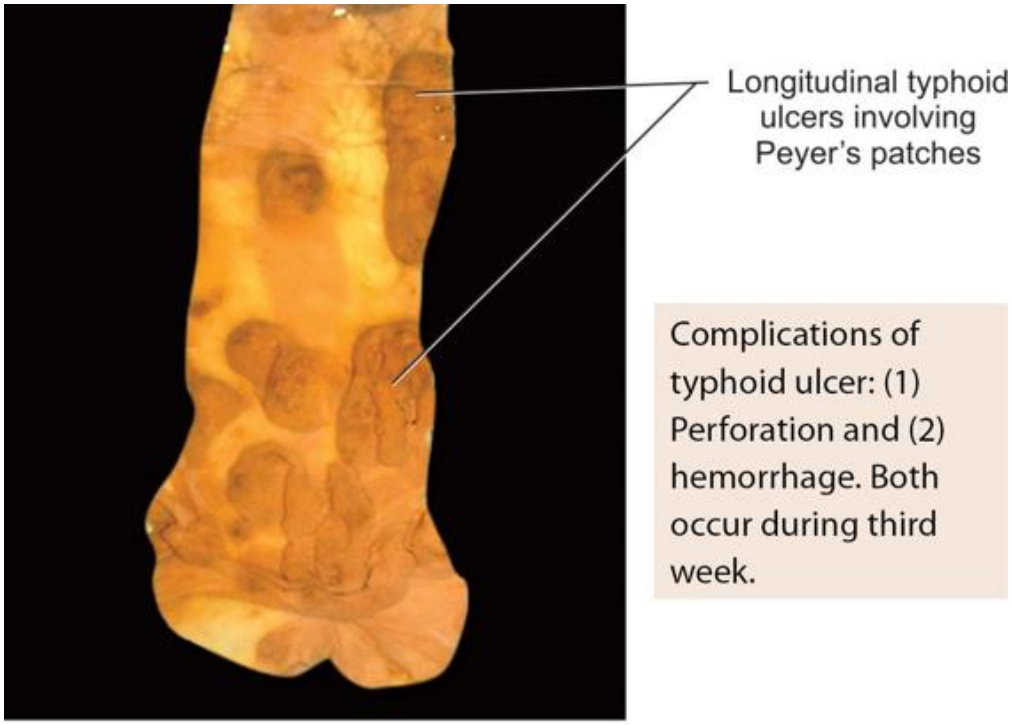


Fig. 18.22: Gross appearance of typhoid ulcers in the ileum of small intestine.

INTESTINAL TUBERCULOSIS

NC: Describe and etiology and pathogenesis and pathologic features of tuberculosis of the intestine.

Q. Enumerate the different ulcerative lesions of intestine. Describe the pathology of tuberculosis of intestine.

Q. Write a short note on intestinal tuberculosis.

Q. Write a short essay on the morphology of tuberculosis of small intestine.

There are three main forms of intestinal tuberculosis, namely:

1. Primary
2. Secondary
3. Hyperplastic cecal tuberculosis.

Primary Intestinal Tuberculosis

Uncommon in the developed countries, but **common in developing countries** including India.

Mode of Infection

- In the pre-pasteurization era, ingestion of cow's unpasteurized milk infected with *Mycobacterium bovis.*
- Nowadays due to eradication of tuberculosis in cattle and pasteurization of milk, all intestinal tuberculosis are caused by *Mycobacterium hominis*.

MORPHOLOGY

Predominant changes are in the **regional mesenteric lymph nodes without any significant intestinal lesion**.

Gross

- **Lymph nodes are enlarged, matted and caseous (tabes mesenterica)** → may heal by fibrosis and calcification.

Microscopy

- Primary complex shows a focus in the intestinal mucosa and mesenteric lymph nodes show typical **tuberculous granuloma** with caseation necrosis **(Fig. 18.23C)**.

Secondary Intestinal Tuberculosis

Mode of Infection

- Swallowing of sputum in patients with active pulmonary tuberculosis. The lesions are most **common in the terminal ileum**.

MORPHOLOGY

Gross (Figs. 18.23A and B)

- **Lesions of intestine are prominent than** that of **regional lymph nodes**.
- **Large ulcers** that is **transverse to the long axis of the bowel**. The surface of ulcers may be coated with caseous material. **Serosal surface may show visible tubercles (Fig. 18.23B)**.
- **Healed lesions** may show transverse **fibrous strictures** and intestinal obstruction.

Microscopy

Mucosa and submucosa show ulceration and typical granulomas of tuberculosis (Fig. 18.23C). The muscularis propria may show variable degree of fibrosis.

Hyperplastic Cecal Tuberculosis

Variant of secondary tuberculosis.

MORPHOLOGY

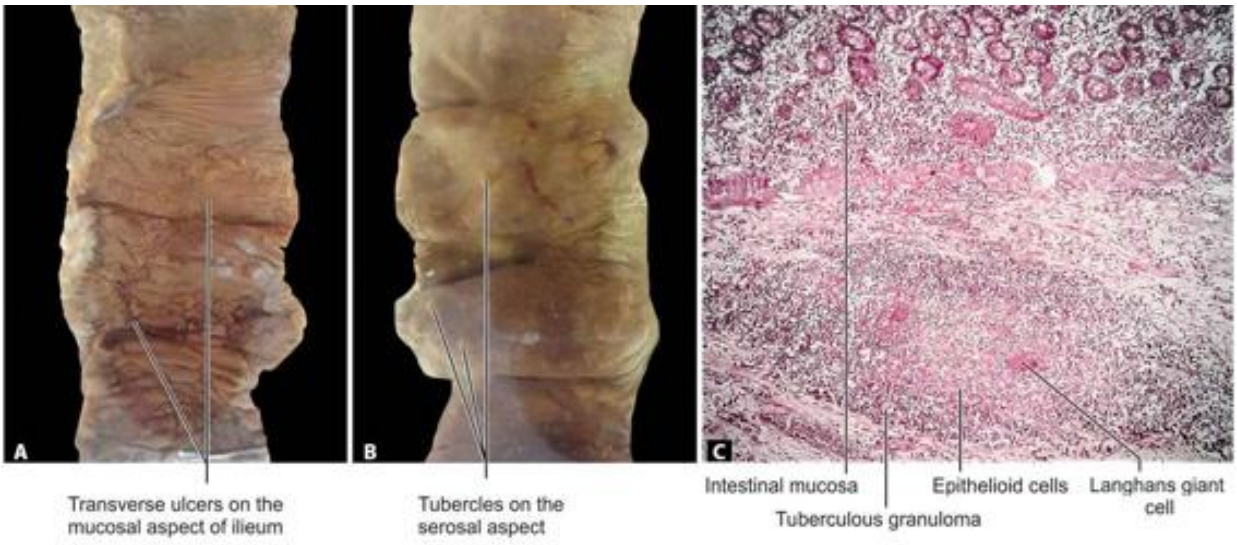
Gross

Lesions are seen mainly in **cecum and/or ascending colon → thickening of wall and mucosal ulceration**. **Clinically**, the lesion present as a palpable mass and may be **mistaken for carcinoma**.

Microscopy (Fig. 18.23C)

It shows **caseating tubercles** and should be **distinguished from Crohn disease in which granulomas are non-caseating**.

Differences between typhoid and tuberculous ulcers are listed in **Table 18.7**.



Figs. 18.23A to C: Ileum with tuberculous ulcer on the mucosal aspect: (A) Specimen; (B) Serosal aspect shows tubercles; (C) Microscopy of tuberculosis of intestine.

Q. List the differences between tuberculous and typhoid ulcer of intestine.

TABLE 18.7: Differences between typhoid and tuberculous ulcers.

Characteristics	*Typhoid ulcer*	*Tuberculous ulcer*

TABLE 18.7: Differences between typhoid and tuberculous ulcers.

Characteristics	*Typhoid ulcer*	*Tuberculous ulcer*
Site	Small intestine, involves Peyer's patches	Anywhere in the intestine but common in the ileocecal region
Orientation	**Longitudinal** to the long axis, because it involves Peyer's patch	**Transverse** to the long axis, because it spreads through lymphatics, which are arranged circumferentially
Microscopy	Macrophages and lymphocytes and plasma cells	Granulomas composed of central area of caseous necrosis, surrounded by epithelioid cells, Langhans giant cells and lymphocytes
Differential count	Neutropenia	Lymphocytosis
Intestinal complications		
Perforation	Common as it is an acute process associated with thinning of bowel wall	Not seen, because chronic inflammation results in healing by fibrosis
Hemorrhage	Common	Does not occur
Obstruction	Not seen	Stricture formation leads to obstruction
Extraintestinal	May develop carrier state	May develop tuberculous peritonitis

Tuberculous ulcer of intestine: Stricture formation can lead to obstruction.

Intestinal tuberculosis: Most common site is terminal ileum and ileocecal junction. Involved segment become thickened and serosal aspects shows tubercles.

SHIGELLOSIS-BACILLARY DYSENTERY

Bacillary dysentery is **a necrotizing infection** of the **distal small bowel and colon** caused by ***Shigella.***

Etiology

- *Shigella* causes bacillary dysentery and is one of the **most common causes of bloody diarrhea**.
- *Shigella* is an unencapsulated, nonmotile, facultative anaerobic gram-negative bacilli. They belong to the Enterobacteriaceae and are closely related to *enteroinvasive E. coli*.

- *Shigella* species that cause colitis are classified into four major subgroups, namely, *S. dysenteriae* (most virulent), *S. flexneri*, *S. boydii*, and *S. sonnei*.

Source of infection: **Humans** are the only natural reservoir.

Mode of transmission: By **ingestion** through fecal–oral route or via fecally contaminated water and food. It can be acquired by oral contact with any contaminated surface (e.g., clothing, towels, or skin surfaces).

Incubation period: It ranges from **1 to 3 days.**

Pathogenesis

- *Shigella* is the **most virulent** enteropathogens and ingestion of few (10–100 organisms) produce disease.
- After ingestion, *Shigella* reaches the stomach. They are r**esistant to the action of acid in the stomach**. Small intestinal infections do not occur unless the patient has disturbance in motility of intestine.
- **In the colon**, the bacteria penetrate the intestinal mucous epithelium and are **taken up by M or microfold epithelial cells** → proliferate inside the cytoplasm of these epithelial cells → penetrate into the lamina propria → phagocytosed by macrophages → *Shigella* induces apoptosis of macrophages → causes inflammatory reaction → loosens the intercellular barriers and damages surface epithelium → leading to superficial ulcers → allows entry of *Shigella* in the intestinal lumen to the colonocyte basolateral membrane.
- *Shigella* **produces a toxin** that has cytotoxic, neurotoxic, and enterotoxic effects. When inflammation is severe, ileus, toxic megacolon, gross hemorrhage and perforation may develop.

MORPHOLOGY

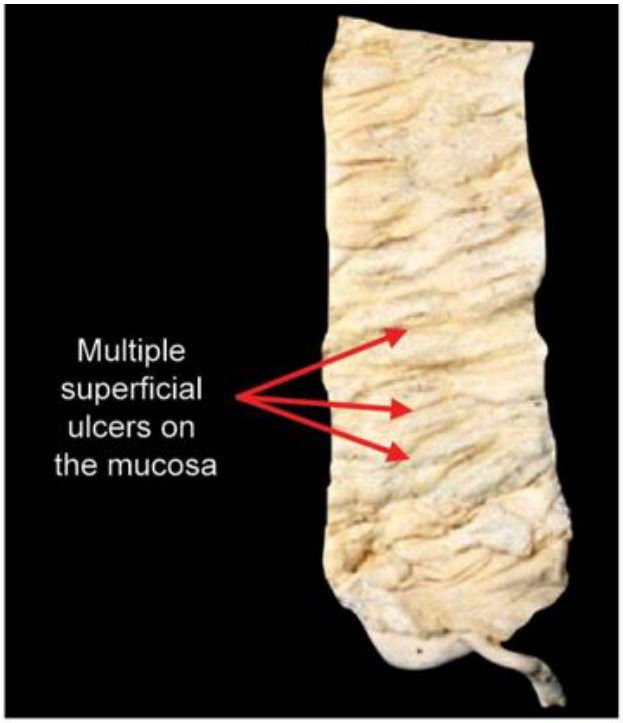
Gross (Fig. 18.24)

- **Site of lesions:** Most prominent in the **left colon** mainly in the **rectosigmoid area**. Mostly, the **lesions are continuous and diffuse**.
- **Characteristics of ulcer**: Mucosa shows **edema, ulceration** and appears **friable granular and hemorrhagic**. In severe infections, a gray mucopurulent exudate covers the mucosa. **Ulcers** appear first on the edges of mucosal folds, **perpendicular to the long axis of the colon**.

Microscopy

Changes are predominant in the **mucosa**.

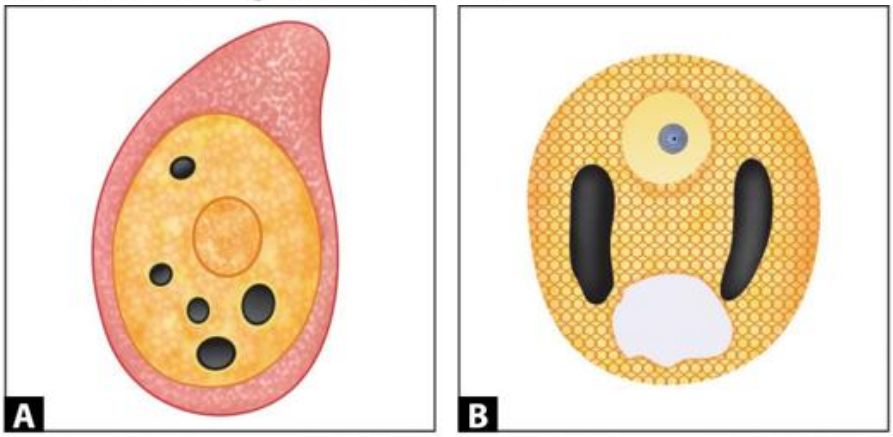
- **Early-stage:** It shows erosions and **small aphthous ulcers** (microulcers), with **infiltration by neutrophils** below the microulcers.
- **Advanced stage**:
  - **Necrosis of epithelial cell**s and the damaged **mucosa is covered by purulent exudate** (composed of detached epithelial cells, neutrophils and red blood cells).



Bacillary dysentery: Predominantly involves mucosa of left colon and rectosigmoid area.

Ulcers of large intestine:
- Bacillary
- Amebic
- Ulcerative colitis
- Crohn disease

Fig. 18.24: Bacillary dysentery. Specimen of cecum and ascending colon (with appendix). Mucosa shows multiple superficial ulceration with intervening normal mucosa.



Figs. 18.25A and B: (A) Trophozoite; (B) Cyst of *E. histolytica*.

- **Ulcers:** They are **superficial, or may extend down to the muscularis mucosae**. May show superficial crypt abscess, edema and mild plasma cell infiltrates.

Clinical Features

- Presents as diarrhea, fever, and abdominal pain.
- The initial watery diarrhea progresses to a dysenteric phase.
- Stool culture is required for confirmation of *Shigella* infection.

Complications

They are **uncommon**.

- **Reiter syndrome**: A triad of **sterile arthritis, urethritis and conjunctivitis**.
- **Hemolytic-uremic syndrome (HUS)**: It is **due to toxin** secreted by *Shigella* organisms.

Shigella: Most virulent enteropathogens which produce toxin that has cytotoxic, neurotoxic and enterotoxic effects.

Bacillary dysentery:
- Ulcers are continuous, diffuse and perpendicular to the long axis of the colon
- Most prominent in the left colon

Bacillary dysentery: Superficial ulcers with infiltration by neutrophils.

Bacillary dysentery: Most common cause of bloody diarrhea caused by *Shigella*.

Complications of bacillary dysentery:
- Reiter syndrome
- Hemolytic-uremic syndrome

AMEBIASIS

Q. Write short note on amebic ulcer of intestine.

Amebiasis is an **infection caused by protozoan *Entamoeba histolytica*** (named so because of its lytic actions on involved tissue).

Etiology

E. histolytica has three distinct stages:

1. **Trophozoite stage (Fig. 18.25A):** Amebic trophozoites are spherical or oval. They are seen in the stools of patients with acute symptoms. The cytoplasm of the trophozoites stain positively by PAS.
2. **Precyst stage**: In the colon, the trophozoite develops into a cyst through an intermediate form termed the precyst.
3. **Cyst stage (Fig. 18.25B): Amebic cysts are the infecting stage** and are found only in stools. They are spherical and have thick chitin walls and usually four nuclei.

Source of infection: **Humans** are the only known reservoir for *E. histolytica*. It is reproduced in the colon of infected individual and passes in the feces.

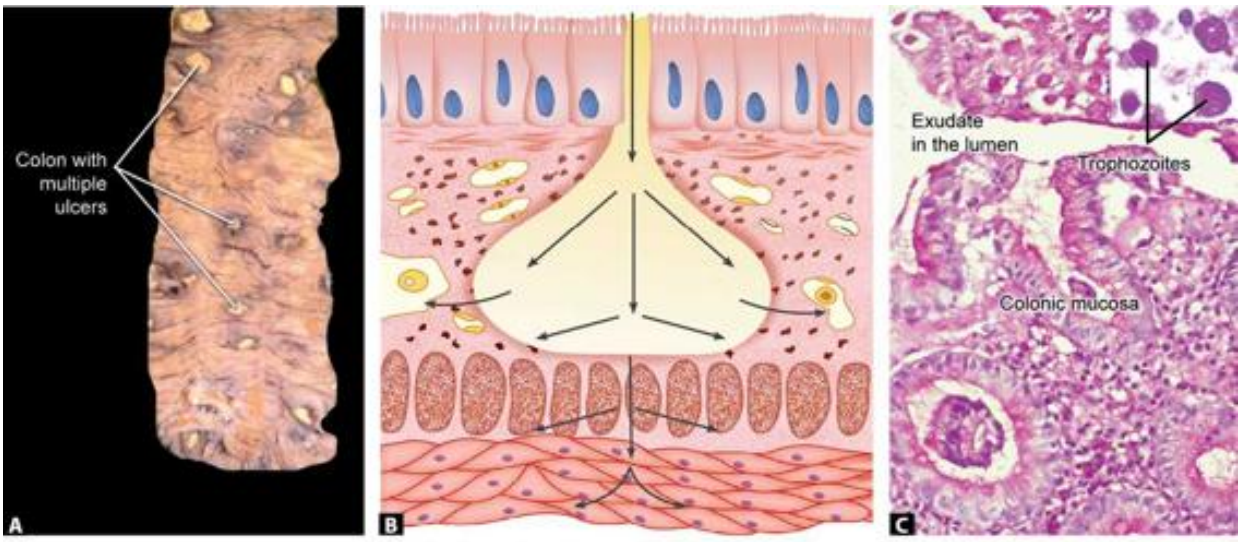
Mode of infection: It is **acquired by fecal–oral route** through ingestion of materials contaminated with human feces containing *E. histolytica*.

Incubation period: About 8–10 days.

Pathogenesis

The amebic cysts are passed in the stool and the **cysts can contaminate water, food, or fingers**.

- On ingestion, *E. histolytica* cysts traverse the stomach. They are **resistant to the action of gastric acid** and they pass to the colon.
- **Amebic cysts then colonize the epithelial surface of the colon and release trophozoites**. Each cyst containing four nuclei divides to form four small, immature trophozoites → which then matures to full size. In the colon, they feed on bacteria and human cells. They may colonize any part of the large intestine, but **most frequently in the cecum and ascending colon causing amebic colitis**. These patients pass both cysts and trophozoites in the stool.
- **Trophozoite attaches the colonic epithelial cell** and **invades crypts, and burrow laterally into the lamina propria**. The trophozoite kills the intestinal cell by producing a lytic protein that damages the cell membrane and creates a superficial ulcer. Recruitment of neutrophils causes tissue damage and creates a **flask-shaped ulcer** with a narrow neck and broad base.
- **Trophozoite may penetrate blood vessels and reach the liver** to produce **abscesses** in about 40% of patients with amebic dysentery.



Figs. 18.26A to C: (A) Gross appearance of multiple amebic ulcers in the colon; (B) Microscopically appear as flask-shaped amebic ulcer (diagrammatic); (C) Microscopic appearance of amebic ulcer (PAS stain) shows PAS-stained trophozoites of ameba in the exudate (inset).

MORPHOLOGY

Q. Write a short note on gross and microscopy of amebic ulcer intestine.

E. histolytica can produce intestinal or extraintestinal disease.

- **Intestinal disease:** It mainly involves the **colon** and ranges from **asymptomatic colonization to severe invasive infections** causing bloody dysentery.
- **Extraintestinal disease:** It can produce **amebic liver abscesses**.

Colon

- Amebic lesions start as small **foci of necrosis,** which progress to **ulcers**. The ulcer floor is **gray and necrotic**. The **chronic amebic ulcers** are described as **flask-shaped ulcer** with a narrow/bottle neck and broad base resembling a **flask (Figs. 18.26A and B)**.
- Trophozoites can be detected on the surface of the ulcer and in the exudate. The ulcers show infiltration by acute and chronic inflammatory cells. PAS stain shows trophozoites of ameba in the exudate **(Fig. 18.26C)**.
- **Ameboma:** It is a **rare complication** of amebiasis. The intestinal wall shows thickening due to inflammation (napkin-ring constriction) and may **resemble colon cancer**. Microscopically, i**t consists of granulation tissue, inflammatory cells, fibrosis and clusters of trophozoites.**

Clinical Features

Intestinal amebiasis: It may be asymptomatic or produce dysentery of varying severity. The incubation period for acute amebic colitis is about 8 to

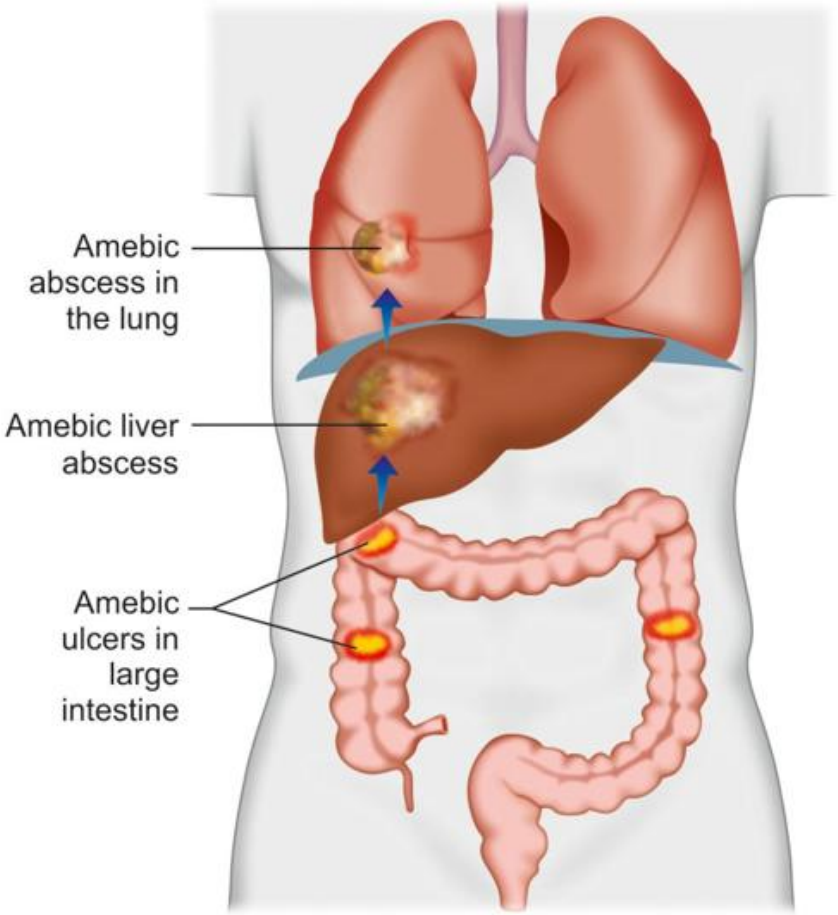
10 days. Amebic dysentery may present with **abdominal pain, bloody diarrhea, or weight loss**. Liquid stools (up to 25 a day) contain blood and mucus.

Amebic Liver Abscess (Fig. 18.27 andrefer page 633)

Q. Write a short note on complications of amebic ulcer of intestine.

It is a **major complication of intestinal amebiasis**.

- *E. histolytica* trophozoites **from the colon** may reach liver **through the portal circulation**.
- **Trophozoites kill hepatocytes** → produce **abscess**.
- Abscess cavity is filled with a dark brown, odorless, semisolid necrotic material, which resembles **anchovy paste (sauce) in color and consistency**. The size of amebic liver abscess may vary and can exceed 10 cm in diameter.
- **Spread of amebic liver abscess:**
  - **Local spread**: It may expand and rupture through the capsule of the liver → may directly **spread into the peritoneum, diaphragm, pleural cavity, lungs, or pericardium**.



Amebic liver abscess: Anchovy sauce pus.

Fig. 18.27: Complications of amebiasis (diagrammatic).

- **Hematogenous spread**: Rare → to the **brain** and kidneys → necrotic lesions.

Clinical features of amebic liver abscess: It may present with severe right upper quadrant pain, low-grade fever and weight loss. The diagnosis is usually made by radiologic or ultrasound demonstration of the abscess, in conjunction with serologic testing for antibodies to *E. histolytica*.

Amebiasis: Caused by *Entamoeba histolytica*.
E. histolytica: Three stages—(1) Trophozoite; (2) Precyst; (3) Cyst
Amebiasis: Most frequently involve cecum and ascending colon.
Amebic ulcers: Flask-shaped ulcers in large intestine.
E. histolytica: Trophozoite invades crypts and burrows laterally in the lamina propria to produce

flask-shaped ulcer.

CARCINOID TUMOR

Q. Write short note on carcinoid tumor.

Origin: Carcinoid tumors **arise from neuroendocrine organs** (e.g., endocrine pancreas) **and neuroendocrine-differentiated epithelial cells** of GI tract (e.g., **G-cells**). They were originally termed carcinoid, or "carcinoma-like," because these tumors were thought to be more indolent clinical course than GI carcinomas.

Now these tumors are properly termed as **well-differentiated neuroendocrine tumors.**

Sites of Carcinoid Tumors

- **GI tract**: Major site
  - **Small intestine** (more than 40%) mostly in appendix.
  - Stomach: Gastric carcinoids may be associated with endocrine cell hyperplasia, chronic atrophic gastritis and Zollinger–Ellison syndrome.
- **Tracheobronchial tree and lungs** (*refer* pages 533-4).

MORPHOLOGY (FIGS. 18.28A TO C)

Gross

- Carcinoids are **intramural or submucosal masses** → small polypoid lesions
- Mucosa covering the tumor may be intact or ulcerated
- **Yellow or tan color**
- **Very firm in consistency** due to intense desmoplastic reaction
- May invade the wall deeply to involve the mesentery.

Microscopy

- Composed of **uniform cells** → forming **islands, trabeculae, strands, glands, or sheets**.
- Cells have **scant, pink granular cytoplasm** and a **round to oval stippled nucleus**.
- Most tumors show **minimal pleomorphism, but rarely may show anaplasia, mitotic activity and necrosis.**

Immunohistochemical stains:

- Tumor cells are positive for endocrine granule markers, such as **synaptophysin** and **chromogranin A**.

Clinical Features

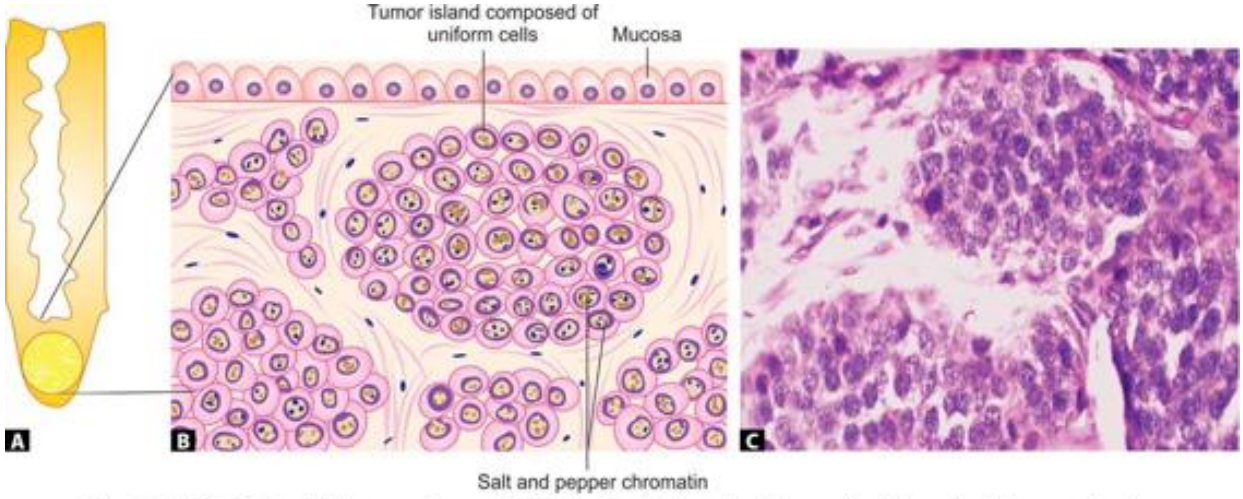
Age: Peak incidence in the **sixth decade**, but can occur at any age.

Symptoms depend on the hormones produced. For example, if the tumors produce **gastrin → may cause Zollinger-Ellison syndrome**. **Ileal tumors → may produce carcinoid syndrome**.

Carcinoid Syndrome (Fig. 18.29)

Q. Write short note on carcinoid syndrome. List its symptoms.

- Develops in less than **10% of patients.**
- Symptoms are **due to vasoactive substances** secreted by the tumor.
- **Carcinoid tumors confined to the intestine, secrete vasoactive substances, which are metabolized to inactive forms by the liver**.



Figs. 18.28A to C: Carcinoid tumor of appendix: (A) Longitudinal section of appendix with carcinoid tumor at the tip; (B) Microscopically shows uniform cells with round to oval stippled nuclei; (C) Photomicrograph of carcinoid tumor.

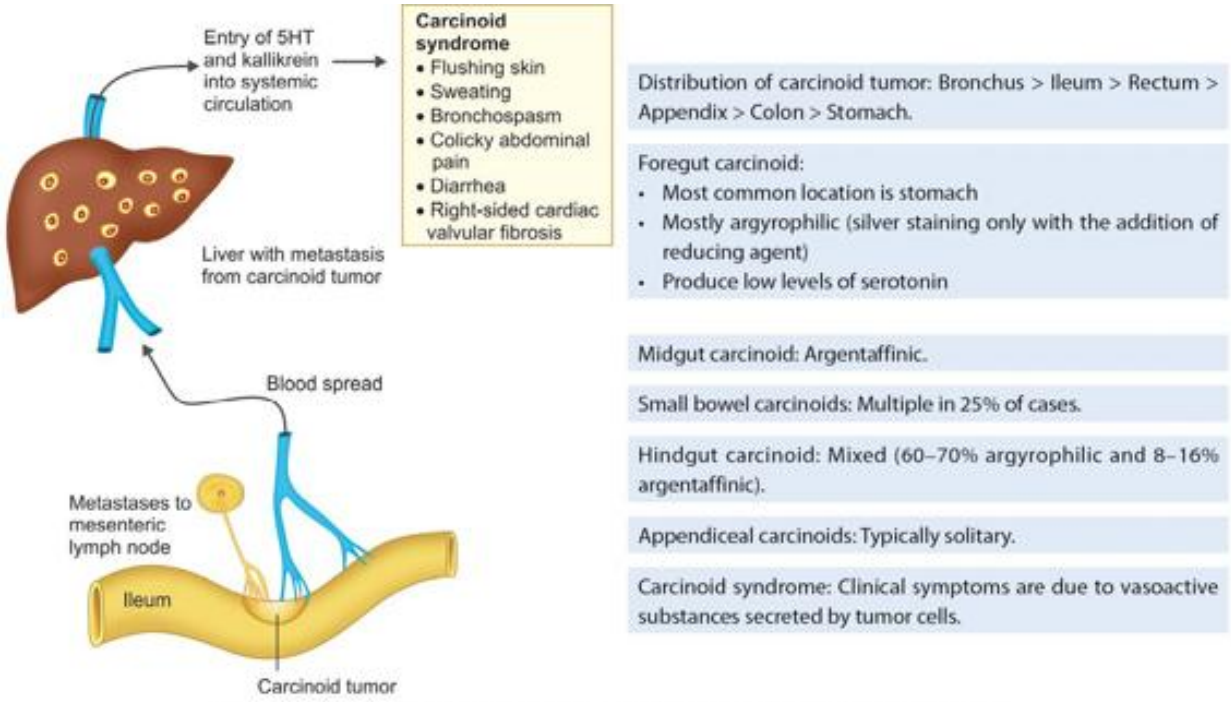


Fig. 18.29: Mechanism of development of carcinoid syndrome and its clinical features.

- **Carcinoid syndrome develops when tumors secrete hormones into a non-portal venous circulation**. Therefore, it is **strongly associated with metastatic disease**.
- **Clinical features: Flushing of skin, sweating, bronchospasm, colicky abdominal pain, diarrhea, and right-sided cardiac valvular fibrosis**.

Location is the most **important prognostic factor** for GI carcinoid tumors.

- **Foregut carcinoid tumors**
  - **Sites:** Stomach, duodenum and esophagus
  - Metastasis rare and are cured by resection.
- **Midgut carcinoid tumors**
  - **Sites:** Jejunum and ileum
  - May be multiple and aggressive
  - Deep local invasion, increased size and presence of necrosis and mitosis are associated with poor prognosis.
- **Hindgut carcinoids**
  - **Sites:**
    - Appendix: Usually seen at the tip and are less than 2 cm in diameter and are benign.
    - Rectal carcinoid: It tends to produce polypeptide hormones, but metastasis is uncommon.

Carcinoid (carcinoma-like): Term was used because they were thought to be more indolent than GIT

carcinomas.

Appendix: Most common site for carcinoid tumor.

Carcinoid tumors: Those located in
- Small intestine tend to be malignant
- Appendix almost always show benign course

Carcinoid tumors: Yellow in color.

Carcinoid tumor: Positive for synaptophysin and chromogranin A.

Carcinoid tumor: Malignant potential depends on
- Location (most important prognostic factor)
- Size
- Depth of invasion
- Growth pattern.

Malignant neuroendocrine tumors may produce carcinoid syndrome.

Carcinoid tumor syndrome: Develops with metastasis to liver/lung.

Carcinoid syndrome: Flushing, diarrhea, wheezing and sweating.

INFLAMMATORY BOWEL DISEASE

CC: Describe and etiology and pathogenesis and pathologic and distinguishing features of inflammatory bowel disease.

Q. What are inflammatory bowel diseases? Discuss the etiopathogenesis of inflammatory bowel disease.

- Inflammatory bowel disease (IBD) is an **immune-mediated chronic intestinal inflammatory condition**. It results from **inappropriate mucosal immune activation**.
- **Major types of IBD: (1) Ulcerative colitis (UC)** and (2) **Crohn disease (CD)**.

Epidemiology

- **Age of onset:** Both UC and CD occurs between **15 and 30 years** of age. A second peak is between the ages of **60 and 80 years**.
- **Sex:** Male to female ratio in:
  - Crohn disease is 1.1–1.8:1 (males more than female)
  - Ulcerative colitis is 1:1 (equal in males and females).
- **Prevalence** of IBD is **higher in urban than rural areas**, and **higher prevalence in high socioeconomic classes** than lower socioeconomic classes.
- **Cigarette smoking:** Risk of UC in smokers is 40% more than that of nonsmokers. Smoking is associated with a two-fold increased risk of CD.
- **Oral contraceptives** use: Increased risk of CD.
- **Appendectomy: It is protective against UC** but is associated with **a higher risk of CD**.

Etiology and Pathogenesis

Q. Describe the etiopathogenesis of inflammatory bowel disease.

Normally, homeostasis exists between the three major host compartments namely—(i) commensal microbiota, (ii) epithelial cells lining the intestines [intestinal epithelial cells (IECs)] and (iii) immune cells within the tissues. These three (microbiota, IECs, and immune cells) function together. They may be affected by environmental and genetic factors in a susceptible host and produce inflammatory bowel disease (IBD). The exact causes for IBD are not yet defined. Currently, IBD is considered to be due to an inappropriate host immune response to the endogenous (intestinal) commensal microbiota within the intestines, with or without some autoimmunity.

Genetic Factors

Genetic **predisposition/susceptibility** contributes to IBD.

Familial

IBD is **more common among relatives of patients with IBD** than in the general population. However, in ~85–90% of cases, IBD develops as sporadic disease.

- **Crohn disease:** Genetic factors **play a prominent role**. The concordance rate for monozygotic twins is about 50%.
- **Ulcerative colitis:** Genetic factors are **less prominent than in Crohn disease**. The concordance of monozygotic twins is only 16%. Concordance for dizygotic twins for both Crohn disease and ulcerative colitis is less than 10%.

Susceptibility Genes

- **Genes associated with innate immunity and autophagy** (e.g., *NOD2/CARD15, ATG16L1* and *IRGM*): **They respond to infection and clear the infective agents** such as bacteria, mycobacteria and viruses. *NOD2/CARD15* is the first Crohn disease susceptibility gene that was originally known as *NOD2* (nucleotide oligomerization binding domain 2) but subsequently renamed as *CARD15*. ***NOD2/CARD15* gene codes for a NOD2/CARD15 protein, which is required for normal recognition and response against microbes in the intestinal lumen**. Actions of *NOD2/CARD15* protein are:
  - **Prevents the entry of microbes** into the wall of the intestine
  - **Regulates innate immune responses**
  - **Prevents excessive immune response** against luminal microbes.
- **Defective *NOD2/CARD15*:** It is produced due to **mutations** and mutant forms that are less effective at recognizing and combating luminal microbes. Defective *NOD2*:
  - Allows the entry of luminal microbes into the lamina propria of the intestinal wall and stimulates inflammatory reactions.
- **Two other genes**, namely—***ATG16L1*** (autophagy-related 16-like) and ***IRGM*** (immunity-related GTPase M) are involved in the recognition and response to intracellular pathogens.

Commensal Microbiota

The **gut lumen** contains **abundant commensal bacteria.** This endogenous commensal microbiota in the intestines plays main role in the pathogenesis of IBD. Humans are born sterile and the commensal microbiota are acquired initially from the mother during passage through the birth canal and subsequently from environmental sources. Each individual possesses a unique combination of species and a stable species of bacteria is achieved by

3 years of age. This composition within individuals is likely persists into adult life and remains stable for several years.

Control of microbiota: The composition and function of the commensal intestinal microbiota is under the control of **host** (e.g., immune and epithelial responses), **environmental** (e.g., diet and antibiotics), and likely **genetic** (e.g., *NOD2*) factors.

Influence on epithelial and immune function of the host: The microbiota has major influences on the epithelial and immune function of the host through its structural components and metabolic activity. Many of the changes in the commensal microbiota occur as a result of the inflammation.

Modification (alteration) of intestinal microflora/microbiota:

- It **can be modified by diet and disease**. **IBD is associated with an alteration in the bacterial flora**.
- **Improved food storage conditions and decreased food contamination:** It has **reduced frequency of enteric infections** with 'clean' environment in the intestinal lumen → the **immune system may not be exposed to microorganisms** (pathogenic or non-pathogenic) → **inadequate development of regulatory processes** limit mucosal immune responses.

Inadequate immune regulation: The **'untrained' immune system** when exposed to the normal commensal bacterial antigens/infections → **inadequate immune regulation** → allows pathogens (which normally cause self-limited disease) to produce an **over aggressive immune response** and **uncontrolled chronic inflammatory response** in a genetically **susceptible individual.**

Dysfunction of Intestinal Epithelial Cell

Epithelial defects: A **variety of epithelial defects/dysfunction** can develop in IBD.

Impaired/defective mucosal barrier function: Defects in intestinal epithelial tight junction barrier function is observed in IBD. It **can activate innate and adaptive mucosal immunity and sensitize individuals to disease**.

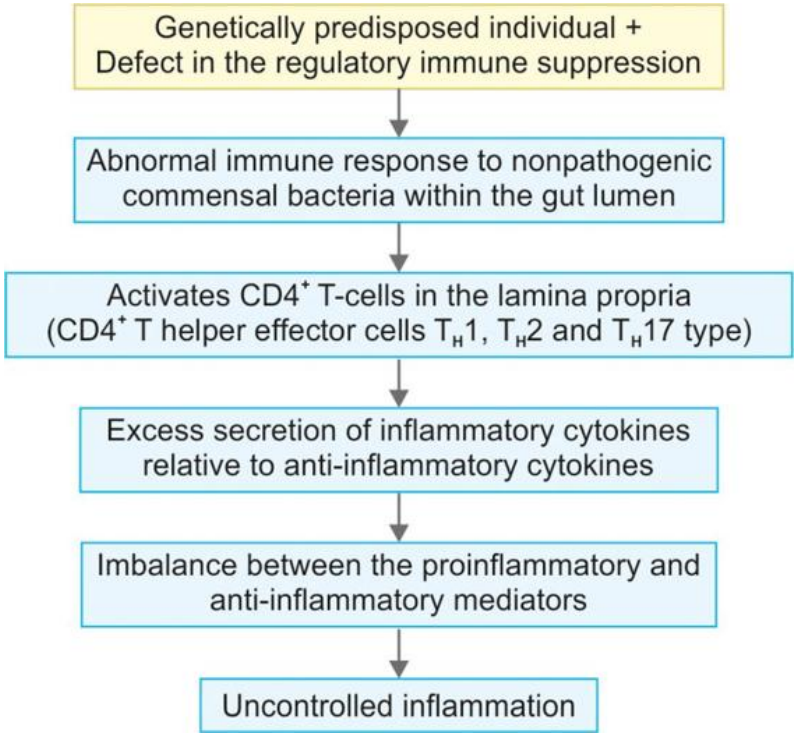
Abnormal intestinal defensins: Paneth cell granules contain **antimicrobial peptides termed defensins,** which normally protect the mucosa against adherent and invading bacteria. **Abnormal defensins** are found in Crohn disease patients carrying *ATG16L1* mutations.

Defective Immune Regulation

Normal regulatory immune response of gut mucosa: Normal uninflamed intestine contains a many immune cells in a unique state of activation, thereby the **gut is prevented from producing full immunologic/inflammatory response against the commensal microbiota and dietary antigens** in the intestinal lumen.

Defective mucosal immune responses (immune dysregulation) in IBD **(Flowchart 18.2)**: In IBD, the normal immune suppression of inflammation is altered and there is uncontrolled inflammation. There defective response in both **innate** (macrophage and neutrophil) and **acquired** (T- and B-cell) **immunity**.

Flowchart 18.2: Consequences of defective regulatory immune response in IBD.



Environmental Factors

It includes enteropathogens, antibiotics, diet, hygiene, psychological factors, and smoking.

Psychosocial factors: It can contribute to worsening of symptoms in IBD. Major life events (e.g., illness or death in the family, divorce or separation) are associated with an increase in symptoms such as pain, bowel dysfunction and bleeding.

Smoking: CD patients are more likely to be smokers and smoking exacerbates CD. In contrast, an increased risk of UC is observed in non- or ex-smokers.

Factors involved in the pathogenesis of IBD is depicted in **Figure 18.30**.

Hypothesis for Pathogenesis of IBD

The IBD is characterized by **abnormal mucosal immunological response** and shows lymphocytes, macrophages and other cells of the immune system infiltrating the lamina propria. However, the antigens that trigger the immune response are not yet identified. Whatever the antigenic trigger, **activated T-cells in the lamina propria are involved** in the pathogenesis of IBD.

Hypothesis (Fig. 18.31): One hypothesis signifies the role of intestinal microbiota, epithelial function and mucosal immunity in the pathogenesis of IBD. The various events are:

- **Transepithelial flux of luminal bacterial components** antigens within the intestinal microbiota **pass between leaky epithelial cells** or enter the lamina propria through ulcerated mucosa. It activates both innate and adaptive immune responses.
- **Processing of bacterial antigens**: **Antigens** from the lumen of the bowel are **transported by M (microfold) and dendritic cells**. **Antigen-presenting cells** (APC) in the lamina propria **process** these **bacterial antigens** and **present to CD4$^+$ T helper (T_H) cells**.
- **T-cell activation and differentiation**: $CD4^+$ T-cells undergo differentiation **CD4$^+$ T helper (T_H)** cells.
- **Differentiation of CD4$^+$ T-cells:** Cytokines secreted by APCs causes differentiation of $CD4^+$ T-cells into three major types of $CD4^+$ T helper effector cells, namely—(i) T_H1 type by IL-12; (ii) T_H2 type by IL-4 ; (iii) T_H17 type by IL-23.
  - **T_H1 cells** secretes interferon gamma (IFNγ), which is the most powerful cytokine that activates macrophages; and also tumor necrosis factor (TNF). In a **genetically susceptible individual**, the release of **TNF** and other immune-mediated signals **increases the tight junction permeability of epithelial cells**. This causes further **increase in the entry of bacterial components** into the lamina propria. These events may result in a **self-amplifying cycle** which

may be sufficient to initiate IBD. They continue the inflammatory process with activation of non-immune cells and release of other important cytokines, e.g., interleukin (IL)-12, IL- 23, IL-1 and IL-6.

- **T_H2 cells** secretes IL-4, IL-5, IL-13 and IL-17. IL-13 **induces superficial inflammation of mucosa**.
- **T_H17 cells** secrete IL-17, IL-21 and are responsible for neutrophilic recruitment. **IL-23** secreted by APCs is essential for the **development and maintenance of T_H17 cells**.

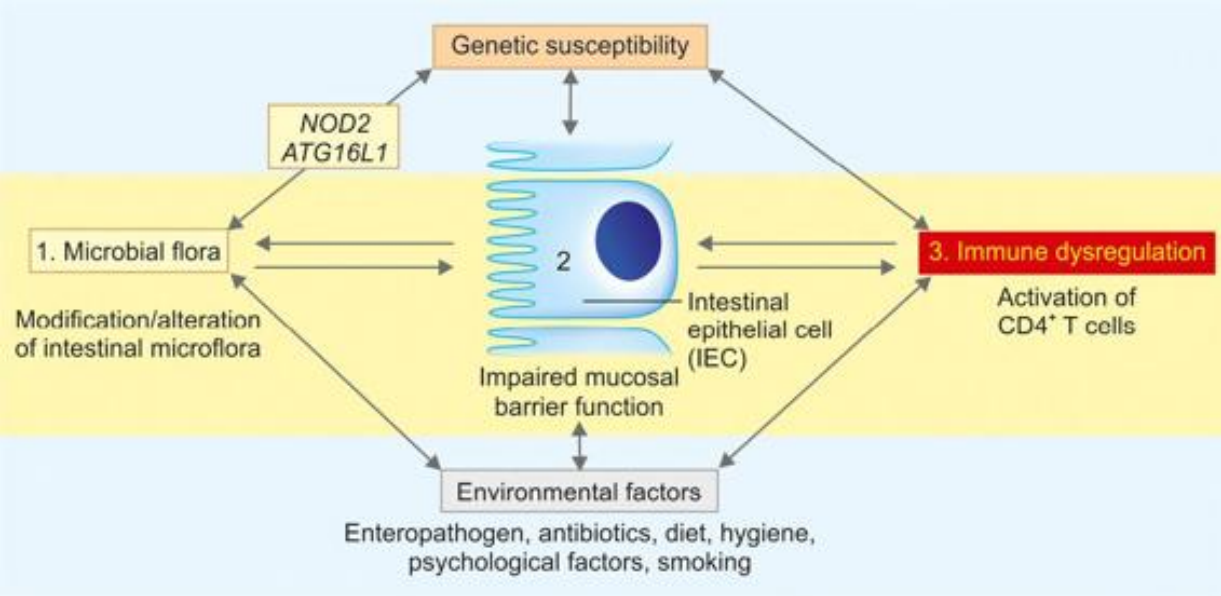


Fig. 18.30: Factors involved in the pathogenesis of inflammatory bowel disease (IBD). IBD results from dysregulation of the tridirectional relationship between the (1) commensal flora (microbiota), (2) intestinal epithelial cells (IECs), and (3) mucosal immune system. This results in chronic inflammation. Each of these three factors can be affected by genetic and environmental factors.

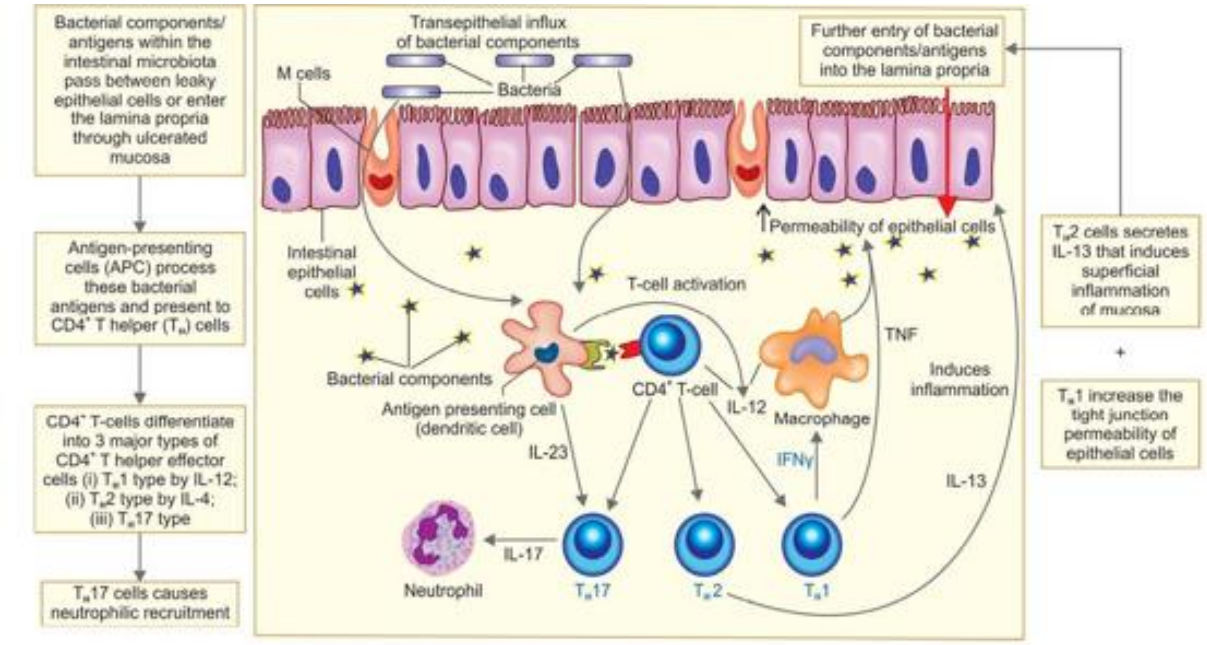


Fig. 18.31: Hypothesis for pathogenesis of inflammatory bowel disease (IBD).

The above mentioned pathways occur in all normal individuals exposed to an inflammatory insult. These processes are self-limiting in healthy subjects.

IBD: Immune-mediated chronic intestinal inflammatory condition.

IBD: (1) Ulcerative colitis and (2) Crohn disease.

Etiology of IBD: Genetic factors; Environmental factors; Host factors.

IBD susceptibility genes: *NOD2/CARD15, ATG16L1, IRGM.*

NOD2/CARD15 is on chromosome 16q, also known as IDB-1 locus.

Environmental factors in IBD: Intestinal microflora, smoking.

Smoking: Strong exogenous risk factor for development of CD, Partly relieves symptoms in UC.

Host factors in IBD: Epithelial defects, defective mucosal immune response.

IBD: Imbalance between proinflammatory and anti-inflammatory mediators → uncontrolled inflammation.

Crohn disease: Abnormal defensins in patients with *ATG16L1* mutations.

IBD: Three types of T_H T-cells involved—1. T_H1 T-cell; 2. T_H2 T-cell;
3. T_H17 T-cell.

In genetically susceptible host, combination of defects in host interactions with intestinal

microbiota, intestinal epithelial dysfunction and aberrant mucosal immune responses trigger IBD.

Polymorphism of IL-23 receptor is protective in IBD.

CROHN DISEASE

Q. Enumerate the different ulcerative lesions of intestine. Discuss the etiopathogenesis, gross and microscopic features of Crohn disease. What are its complications?

Crohn disease (regional enteritis) is a **chronic multifocal relapsing and remitting, progressive inflammatory bowel disease** of unknown cause that **can involve any portion of the gastrointestinal tract**.

MORPHOLOGY

Q. Write short note on morphological features of Crohn disease.

Gross

- **Sites (Fig. 18.32A):** It can involve any area of the GI tract **from mouth to anus**.
  - **Most commonly** in the **terminal ileum, ileocecal valve** and **cecum (Fig. 18.32B)**.
  - Small intestine alone in ~40%; small intestine and colon in ~30% and only colon in ~30%.
- **Number of lesions**: Usually **multiple** and each lesion is **sharply demarcated** from intervening normal bowel → giving rise to **characteristic skip lesions.** It **helps in differentiating it from ulcerative colitis**.
- **Mucosal lesions (Fig. 18.33A):**
  - **Aphthous ulcer** is the **earliest lesion. Multiple lesions may coalesce** longitudinally to form → elongated, linear or **serpentine** (snake-like)/train track/ rake **ulcers oriented along the axis of the bowel.**
  - **Later** the ulcer become deeper and form linear clefts or **fissures** → may extend deeply **to become fistula tracts or sites of perforation**.
  - Mucosa shows edema and loss of the normal mucosal fold.
  - Ulcers may fuse longitudinally and transversely. The **islands of normal mucosa between the ulcers** show edema and produce a **cobblestone** appearance **(Fig. 18.33B)**.
- **Intestinal wall (Fig. 18.32B):** In the **involved region, it** shows **fibrotic thickening** and is **rubbery**. The **lumen is narrowed**

and strictures are common. It appears **like a hosepipe** → produces a characteristic radiological **'string sign'**.

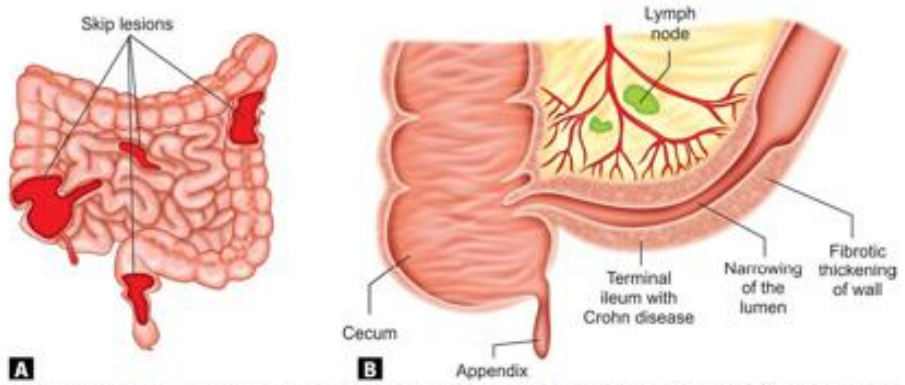
- **External surface/serosa**: It appears red, opaque, hyperemic and covered with serosal exudate producing **serositis.** When the disease shows extensive transmural involvement, the subserosal fat is often firm and contracted over areas of involvement. This may encircle around the antimesenteric serosal surface producing a pattern known as **creeping fat (Fig. 18.33C)**.
- **Adhesion:** Involved loops of bowel may adhere to each other.

Microscopy (Figs. 18.34A and B)

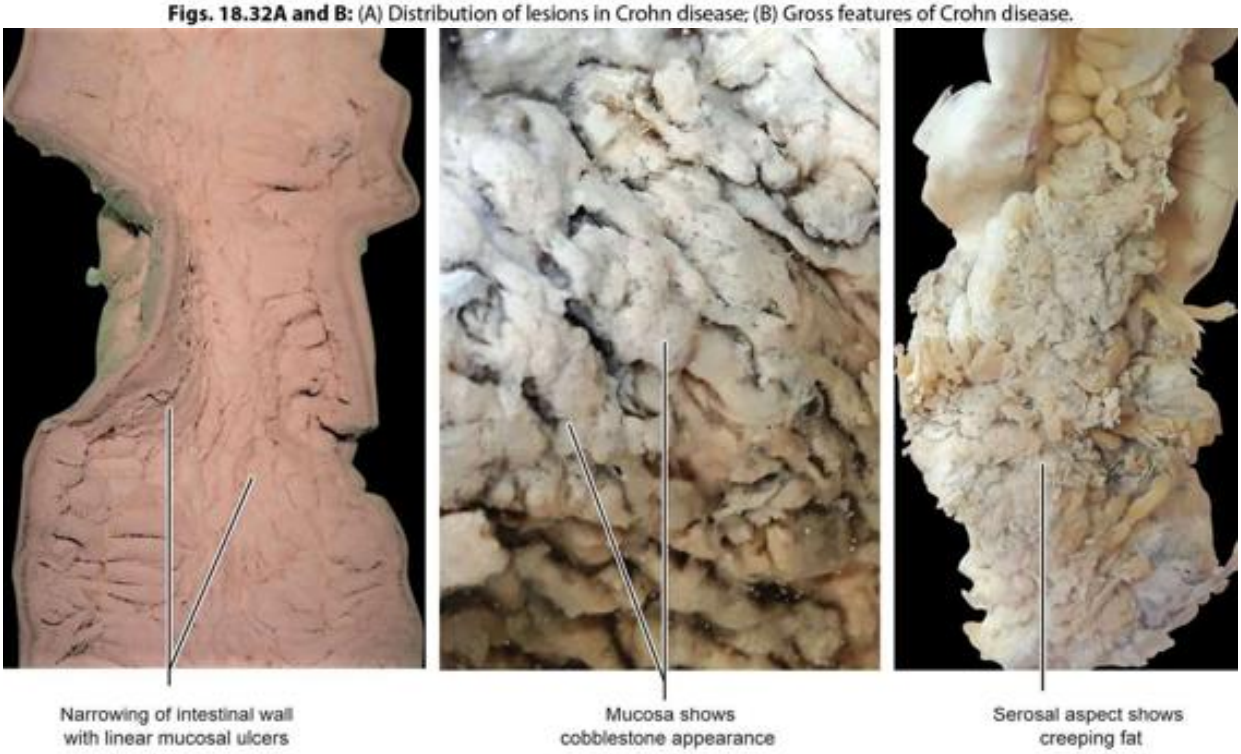
Q. Describe the gross and microscopy of Crohn disease.

Crohn disease is a **chronic inflammatory process**.

- **Mucosal ulcers and inflammation:** They appear as small, superficial ulcers called as **aphthous ulcers**. During early phases, the inflammation may be limited to the mucosa and submucosa. Both mucosa and submucosa shows edema and an increase in the number of lymphocytes, plasma cells and macrophages.
- **Crypt abscesses:** These are characterized by the presence of numerous/**clusters of neutrophils within a crypt**. These neutrophils may infiltrate and damage crypt epithelium and are often associated with crypt destruction.
- **Distortion of mucosal crypt architecture:** Normally, crypts are straight and parallel to each other. Repeated cycles of crypt destruction and regeneration lead to **bizarre branching shapes and unusual orientations to one another** → referred as distortion of mucosal crypt.
- **Epithelial metaplasia:** It develops as a consequence of chronic injury.
  - **Pseudopyloric metaplasia:** It is characterized by the occurrence of **glands, which appear like** those in the **gastric antrum** may be seen in the involved segment of the intestine.
  - **Paneth cell metaplasia:** It may appear in region with metaplasia (Paneth cells are normally absent in the left colon).
- **Noncaseating granulomas (Figs. 18.34A to C):** It is a **hallmark of Crohn disease**. They are **mostly seen in the submucosa** (but may be located at any level of the bowel wall) and found in ~35% of cases. These granulomas when present, are typically poorly formed and consist of focal aggregates of epithelioid cells, surrounded by a thin rim of lymphocytes. Multinucleated giant cells may also be present. Granulomas also may be found in mesenteric lymph nodes.
- **Transmural inflammation:** It is characterized by **chronic inflammation involving all layers of the intestinal wall** and may show **lymphoid aggregates** in the submucosa or subserosa.



Figs. 18.32A and B: (A) Distribution of lesions in Crohn disease; (B) Gross features of Crohn disease.

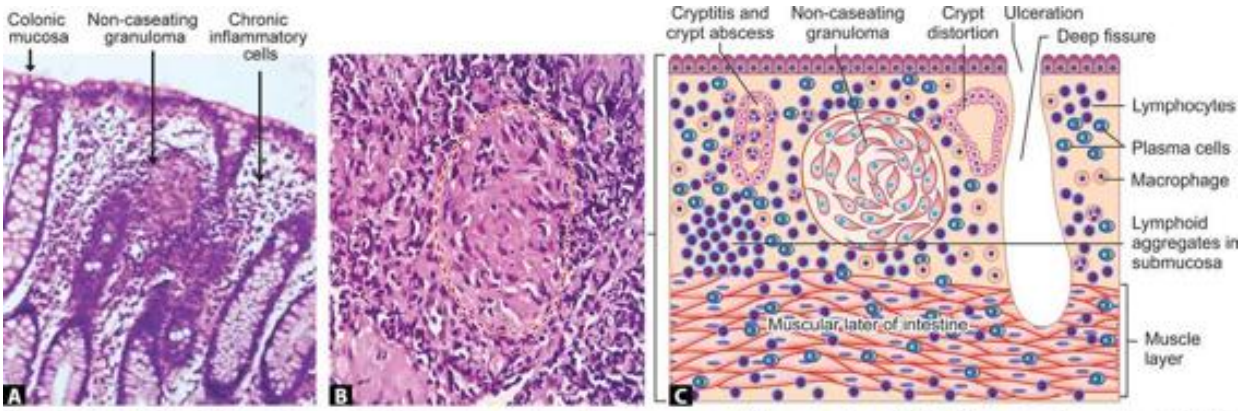


Figs. 18.33A to C: (A) Small-intestinal stricture with linear mucosal ulcers and thickened intestinal wall; (B) Mucosa showing cobblestone appearance; (C) Creeping fat pattern on serosal aspect of small intestine.

A

B

C



Figs. 18.34A to C: (A) Photomicrograph of Crohn disease showing one non-caseating granuloma; (B) High power of granuloma (dotted outline); (C) Microscopic features of Crohn disease (diagrammatic).

Clinical Features

Intestinal Manifestations

- Intermittent attacks of **mild diarrhea, fever** and **abdominal pain.**
- About 20% present with acute right lower quadrant pain, fever and bloody diarrhea that may mimic acute appendicitis or bowel perforation.
- Periods of active disease are usually interrupted by asymptomatic periods. Disease may be re-activated by external triggers, such as physical or emotional stress, specific diets and cigarette smoking.

Extraintestinal Manifestations

These include **uveitis, migratory polyarthritis, sacroiliitis, ankylosing spondylitis, erythema nodosum and clubbing of the finger**.

Laboratory Findings

ESR and CRP are raised. In severe disease, there may be hypoalbuminemia, anemia and leukocytosis. Barium study may show string sign. Histopathological examination of endoscopic biopsy will show microscopic features of Crohn disease.

Complications

Q. Write short note on complications of Crohn disease.

- **Iron-deficiency anemia**: It may develop in patients with colonic disease.
- **Malabsorption:** Extensive involvement of the small intestine may result in loss of protein, hypoalbuminemia and malabsorption.
- **Stricture formation:** It may occur in the terminal ileum.
- **Fistula formation:** It may form between loops of intestine and surrounding structures such as urinary bladder, vagina and abdominal or perianal skin. Perforations and peritoneal abscesses are common.
- **Acute complications:** Perforation and hemorrhage.
- **Development of carcinoma:** It is rare and risk of carcinoma colon is increased in patients with long-standing colonic disease.
- **Systemic amyloidosis:** It is rare.

Crohn disease: Chronic progressive inflammatory bowel disease. Commonly in the terminal ileum, but can involve any portion of the gastrointestinal tract.

Crohn disease: Radiologically characteristic string sign is due to only a trickle of contrast medium passing through the narrowed affected segment.

Crohn disease: Two major characteristic features
1. Transmural inflammation (inflammation involving all layers of bowel)
2. Skip lesions (inflamed segments separated by normal intestine)

Crohn disease: Granulomas in the skin may form nodules and are called (misleading) as metastatic Crohn disease.

Mnemonic for Crohn disease: SISTER IN CCF
S—Skip lesions
I—Ileum is the most commonly involved
S—String sign on radiology
T—Transmural inflammation
E—Extensive fibrosis
R—Rectum usually spared
I—Intermittent attacks with colicky abdominal pain
N—Noncaseating granuloma
C—Cobblestone appearance on mucosa
C—Creeping fat on serosa

F—Fistula may develop

Crohn disease: Fistulas may penetrate the bowel into other organs (e.g., bladder, uterus, vagina and skin). Lesions in the distal rectum and anus may produce perianal fistulas.

ULCERATIVE COLITIS

Q. Mention inflammatory bowel diseases. Discuss the etiopathogenesis, distinctive morphological features and complications of ulcerative colitis. Mention the differences between ulcerative colitis and Crohn disease.

Ulcerative colitis (UC) is a **severe, chronic crypt destructive**, **ulcerating inflammatory** bowel disease of unknown cause. It is **limited to the colon and rectum** and inflammation **involves** only the **mucosa and submucosa** of the intestinal wall. It is clinically associated with exacerbations and remissions of bloody diarrhea.

MORPHOLOGY

Q. Write short note on morphology of ulcerative colitis.

Gross (Figs. 18.35A and B)

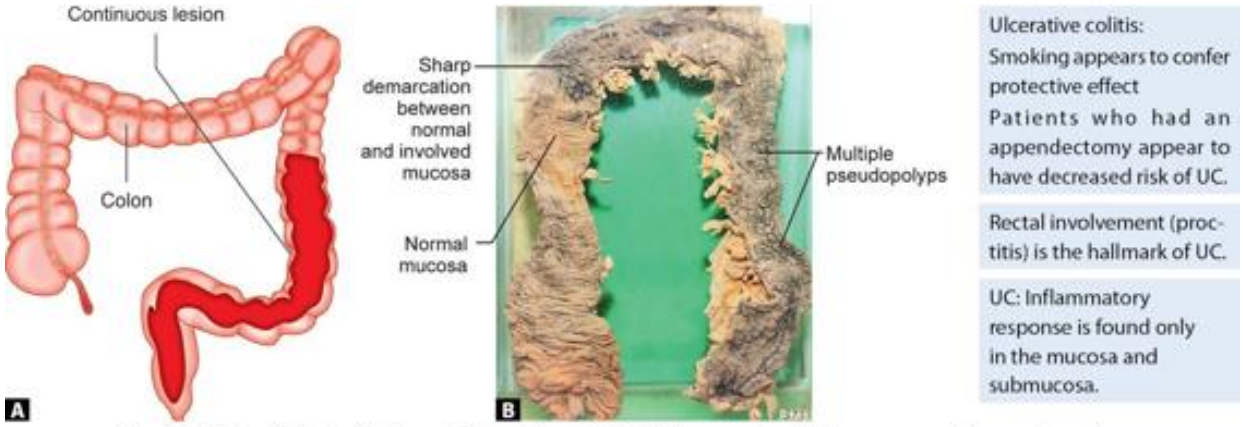
Site: Ulcerative colitis is a **diffuse disease limited to colon and rectum**. Usually involves the rectum and extends proximally for a variable distance (continuous lesion) to involve part or the entire colon. **Skip lesions are not seen** in UC.
Terminology used depending on the region involved is shown in **Table 18.8**.

- **Mucosa** may appear **slightly red** and **fine granular (resembles sandpaper) or smooth**. It is **frequently covered with a yellowish exudate** and bleeds easily. **As the disease progresses**, mucosal folds are lost → **mucosal atrophy**.
- **Small**, **superficial erosions or broad-based ulcers** may develop **later. Ulcers are aligned along the long axis** of the colon but do not appear like serpentine ulcers of Crohn disease.
- **Pseudopolyps (inflammatory polyps):** They develop in long-standing disease. **Isolated islands of regenerating mucosa** may **bulge into the lumen** of the bowel to produce small elevations → termed **pseudopolyps (Fig. 18.35B)**.
- **Inflammatory process** is **diffuse** (without skip lesion) and **limited to the mucosa** and **superficial submucosa**.
- **Bowel wall is not thickened, the serosa is normal, and strictures do not develop**.
- **In severe cases**, inflammation may damage the muscularis propria and disturb neuromuscular function → lead to **colonic dilation** and **toxic megacolon** → can undergo perforation.

Microscopy (Box 18.5 and Figs. 18.36A and B)

Microscopic features of ulcerative colitis are similar to colonic Crohn disease. These include inflammatory infiltrates, crypt abscesses, crypt distortion, and pseudopyloric epithelial metaplasia. However, **the inflammatory process is diffuse and usually seen in the mucosa and superficial submucosa and granulomas are not found**.

- **Inflammatory infiltrate**
  - **Mucosal shows** the inflammation limited to the mucosa and submucosa. Both mucosa and submucosa shows edema and an increase in the number of lymphocytes, plasma cells and macrophages.



Figs. 18.35A and B: Gross features of ulcerative colitis: (A) Diagrammatic; (B) Transverse and descending colon showing numerous pseudopolyps.

- **Changes in the crypt: Neutrophils** are the **hallmark of acute disease**. They are located in the epithelium of crypts and damage the crypt epithelium giving rise to **cryptitis. Neutrophils within crypt epithelium is termed as cryptitis.** Clusters of neutrophils are seen within a dilated crypt and are known as **crypt abscesses (Fig. 18.36)** and are **usually associated with destruction of crypts**. The term chronic active colitis is used when acute activity is superimposed on a background of chronic changes.

- **Histological hallmarks of chronicity: The regenerating epithelium is immature and as such does not contain mucin(mucin depletion).**
  - **Distortion of crypt architecture:** It is **due to repeated destruction and regeneration of crypt**. The crypts may **appear shortened, tortuous with bizarre branching** (bifid) and **decreased** in number. Often a gap is seen between the crypt bases and the muscularis mucosae.
  - **Chronic inflammatory infiltrate:** A chronic inflammatory infiltrate of **lymphocytes**, **plasma cells** and **macrophages** are seen **in the mucosa and superficial submucosa**. **Distortion of crypt architecture and increased mucosal chronic inflammation** are the two characteristic microscopic findings of UC.
  - **Basal plasmacytosis** and multiple basal lymphoid aggregates. Plasma cells extend into the muscularis mucosae.
- **Mucosal atrophy:** It occurs in **late stages.** The mucosa surface appears smooth, flat without any normal folds**.**
- **Paneth cell metaplasia:** It is common, which may occur in the left colon (where Paneth cells are normally not found).
- **Loss of goblet cells and mucin depletion:** The regenerating epithelium is immature and does not contain mucin, and is called as mucin depletion. (Typical mature goblet cell contain mucin).
- **Dysplastic changes in epithelium: It may appear and may progress to carcinoma.**

Mnemonic for ulcerative colitis: COLD TIP
C—Cryptitis, crypt abscess
O—Originates in the rectum
L—Limited to colonic mucosa and submucosa
D—Distortion of crypts and depletion of mucin
T—Toxic megacolon
I—Increased risk of colonic cancer compared to Crohn
P—Pseudopolyps

Clinical Features

- Occurs mainly in young adults.
- **Triggering event:** Infectious enteritis and psychological stress.
- UC is a relapsing disorder with **exacerbations and remissions**.

Intestinal Manifestations

Presents with **attacks of bloody diarrhea** with mucoid material, **lower abdominal pain** and cramps.

Extraintestinal Manifestations

- **Migratory polyarthritis, sacroiliitis, ankylosing spondylitis**.
- Inflammation of eye (mostly **uveitis**).

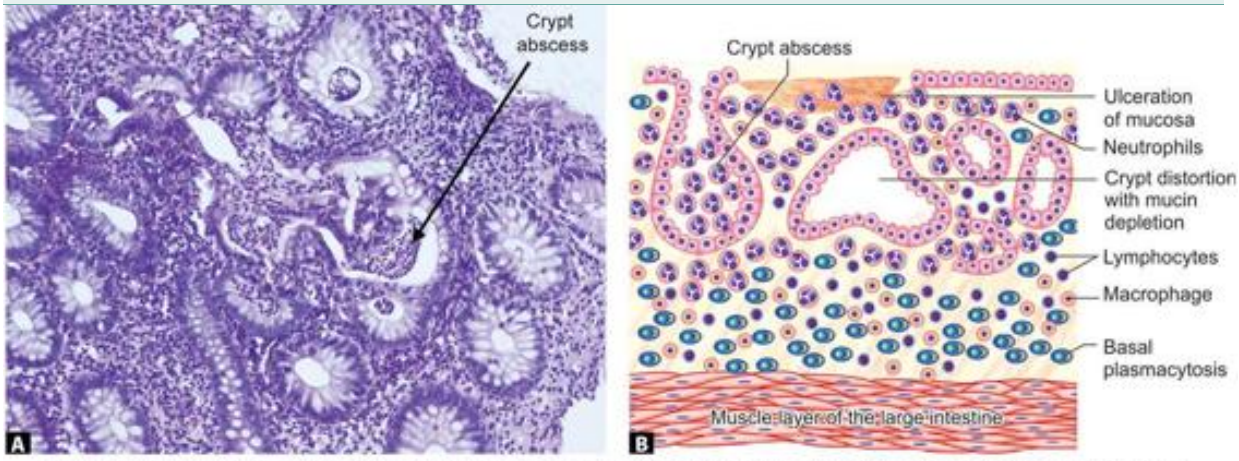
TABLE 18.8: Terminology used for ulcerative colitis depending on the region involved.

Region involved	*Terminology*
Entire colon	Pancolitis
Only the left-side of colon (without extension beyond transverse colon)	Left-sided disease
Limited to the rectum	Ulcerative proctitis
Disease process extends from rectum proximally towards the splenic flexure	Ulcerative proctosigmoiditis
Mild mucosal inflammation of the distal ileum	Backwash ileitis

Backwash ileitis: Ulcerative colitis involving distal ileum.

BOX 18.5: Microscopic features of ulcerative colitis.

- Inflammatory infiltrates of lymphocytes, plasma cells and macrophages
- Cryptitis and crypt abscesses
- Architectural crypt distortion
- Epithelial metaplasia
- Basal plasmacytosis



Figs. 18.36A and B: Ulcerative colitis: (A) Photomicrograph showing crypt abscess (arrow) and dense inflammatory infiltrate surrounding crypts; (B) Diagrammatic shows microscopic features of ulcerative colitis.

- **Skin:** Erythema nodosum and pyoderma gangrenosum.
- **Liver:** Primary sclerosing cholangitis and pericholangitis.
- **Thromboembolic phenomena:** Deep vein thrombosis of the lower extremities.

Complications

Q. Write short note on complications of ulcerative colitis.

- **Toxic megacolon**: In fulminant cases, the inflammation and inflammatory mediators can damage the muscularis propria and disturb neuromuscular function. This may lead to colonic dilation and toxic megacolon → may lead to perforation.
- **Colorectal cancer**: It may develop in long-standing UC.
- **Hemorrhage** from intestinal lesions → blood loss.
- **Electrolyte disturbances** due to diarrhea.

Prognosis: It depends on the severity of active disease and disease duration.

Differences between ulcerative colitis and Crohn disease are listed in **Table 18.9**.

Ulcerative colitis: Severe ulcerating inflammatory bowel disease.

UC: Diffuse disease is limited to the colon and rectum. Inflammation involves only the mucosa and submucosa.

Ulcerative colitis: Early acute ulcerative colitis:
- Mucosal congestion, edema and hemorrhages
- Cryptitis and crypt abscess
- Chronic inflammatory cells (lymphocytes, plasma cells and macrophages) and basal plasmacytosis.

Ulcerative colitis: Granulomas and skip-lesions are not seen.

Ulcerative colitis:

Four Cs:	Four Ms:
1. Congestion	
1. Mucosal atrophy	
2. Crypt abscess	
2. Mucin depletion	
3. Crypt distortion	
3. Metaplasia	
4. Chronic inflammation	4. Mucosal dysplasia

Ulcerative colitis: Exacerbations and remissions.

IBD: Both CD and UC can have extraintestinal manifestations.

Complications of ulcerative colitis: (1) Toxic megacolon; (2) Development of colorectal carcinoma; (3) Intestinal hemorrhage;
(4) Electrolyte imbalances.

INTUSSUSCEPTION

Q. Write short note on intussusception.

Intussusception is an **invagination of one segment of intestine into another** immediately **adjacent distal segment**.

Causes

- **Infants and children**: Usually, there is **no underlying anatomic defect**.
- **Older children and adults**: A **mass or tumor in the wall of the bowel** disturbs normal peristaltic contractions forcing the lesion and a segment of proximal bowel into a distal segment of intestine. The lesion act as the point of traction and causes intussusception. These lesions **include polyps, ingested foreign bodies**, Meckel diverticulum, etc.

MORPHOLOGY

Sites: **In children**, most common site is **ileocolic** and **in adults** it is **colocolic.**

Gross (Figs. 18.37A and B): An intussusception consists of **three parts**:

1. Entering/inner tube (intussusceptum)
2. Returning or middle tube
3. Sheath or outer tube (intussuscipiens)

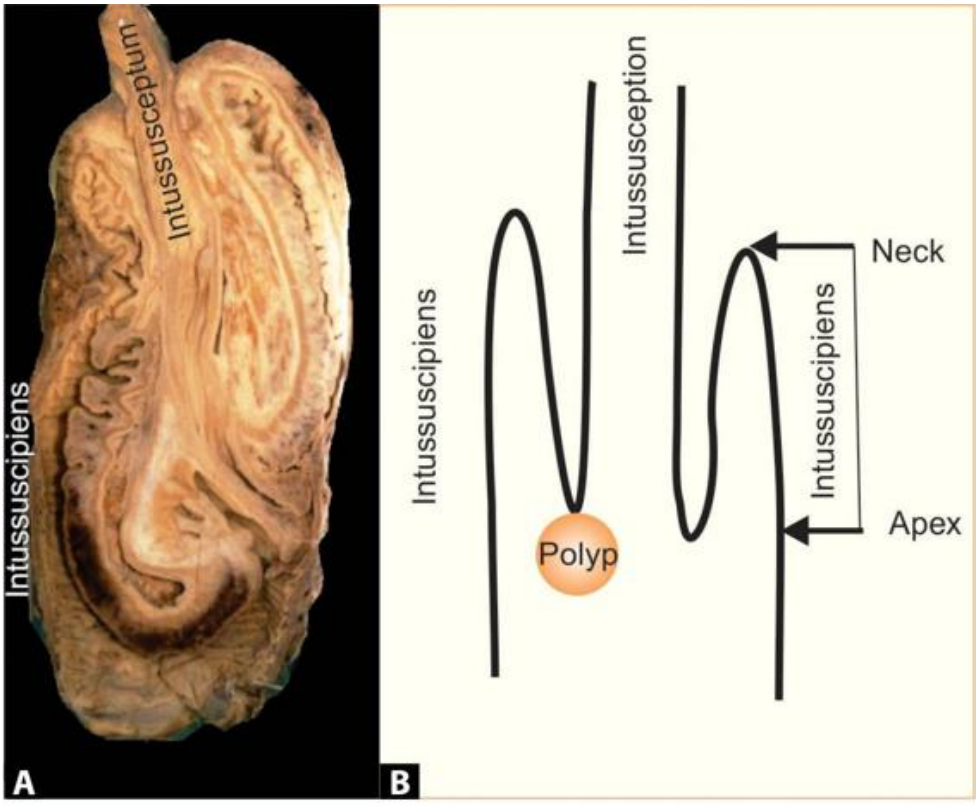
Effect: Untreated intussusception may lead to intestinal obstruction, compression of vessels and infarction of the bowel.

Q. Mention the differences between ulcerative colitis and Crohn disease.

TABLE 18.9: Differences between Crohn disease and ulcerative colitis.

Characteristics	*Crohn disease*	*Ulcerative colitis*
1. Macroscopic		
Bowel region involved	Ileum ± colon	Colon and rectum
Distribution	Skip lesions	Diffuse
Luminal narrowing/stricture	Positive	Rare
Involved bowel wall	Thick	Thin
2. Microscopic		
Inflammation	Transmural	Limited to mucosa and submucosa
Pseudopolyps	Few	Many
Ulcers	Deep, fissures	Superficial, broad-based
Lymphoid reaction	Marked	Moderate
Fibrosis	Marked	Minimal to none
Serosal inflammation	Marked	Absent
Granulomas	Seen in ~35%	Absent
Fistulae/sinuses	Seen	Absent
Crypt abscess	Uncommon	Usual
3. Clinical		
Fissure/fistula	Observed with colonic involvement	Not seen
Fat/vitamin malabsorption	Seen	Absent
Development of malignancy	In colonic involvement	Yes
Recurrence after surgery	Common	Not observed
Toxic megacolon	No	Yes

Note: All features may not be seen in a single case.



Intussusception: Highest incidence between 4 and 10 months of age.

Intussusception: Ileocolic is the most common type.

Figs. 18.37A and B: Intussusception: (A) Ileoileal intussusception; (B) Mechanism and nomenclature of intussusception.

Intussusception: Telescoping/invagination of one segment of intestine into another immediately adjacent distal segment.

Intussusception: Three parts
1. Intussusceptum
2. Returning or middle
3. Intussuscipiens

HIRSCHSPRUNG DISEASE

Q. Write short note on Hirschsprung disease.

Hirschsprung disease is a **congenital abnormality** that is also known as **congenital aganglionic megacolon**. It may occur as isolated or in combination with other developmental abnormalities. About 10% of all

cases are found in children with Down syndrome and serious neurologic abnormalities are present in another 5%. It is **more common in males**. When occurs in females, is usually severe and ends to involve longer aganglionic segments.

Pathogenesis

The neural crest cells migrate into the intestinal wall during embryogenesis. The enteric neuronal plexus develops from these neural crest cells.

- **Premature arrest of migration of neural crest cells**: Hirschsprung disease results **from a congenital defect in colonic innervation.** When **the normal migration of neural crest cells from cecum to rectum is arrested prematurely or when the ganglion cells undergo premature death**. This produces a **distal intestinal segment without** both the **Meissner's submucosal** and the **Auerbach myenteric plexus** ("aganglionosis"). The defect usually begins at the rectum, but extends proximally for variable lengths.
- **Consequences**: The above abnormality lead to **absence of coordinated peristaltic contractions and functional obstruction**. This leads to **dilation proximal to the affected segment**.

Mechanisms: The cause of defective neural crest cell migration is not known. A genetic component may be present. Heterozygous loss of function **mutations in the receptor tyrosine kinase RET is responsible for majority of familial cases** and about 15% of sporadic cases. Mutations in other genes encoding proteins involved in enteric neurodevelopment, including disease-modifying genes, as well as environmental factors are also be involved.

MORPHOLOGY

Segment involved: Hirschsprung disease always affects the rectum and, in most cases, it is limited to the **rectum and sigmoid colon.** But it may additionally involve the variable length of the colon and in severe cases, it can involve the entire colon.

Appearance: The **affected aganglionic region may be grossly normal or contracted**. Normally innervated **proximal colon may undergo progressive dilation** because of functional distal obstruction.

Diagnosis: By demonstrating the absence of ganglion cells in the affected segment.

Clinical Features

Present in neonates as **failure to pass meconium** in the immediate **postnatal period.** This is followed by obstructive constipation often with visible, ineffective peristalsis. It may progress to abdominal distension and bilious vomiting.

Complications: These include enterocolitis, fluid and electrolyte disturbances, perforation, and peritonitis.

Treatment: Surgical resection of the aganglionic segment with anastomosis of the normal proximal colon to the rectum.

POLYPS OF COLON

Definition: A gastrointestinal polyp is a **mass that protrudes into the lumen of the gut**.

Polyps are most common in the colon but may occur in the esophagus, stomach, or small intestine. They are of clinical importance because of their tendency to undergo malignant transformation.

Classification of Polyps (Box 18.6)

Q. Classify colorectal polyps.

BOX 18.6: Classification of gastrointestinal polyps.

According to gross appearance

- Sessile: Polyps do not have a stalk
- Pedunculated: Polyps have a well-defined stalk

According to histopathological appearance

- Non-neoplastic
  - Inflammatory
  - Hamartomatous
  - Hyperplastic
- Neoplastic
  - Benign: Adenoma (Tubular, tubulovillous and villous)
  - Malignant

Non-neoplastic Polyps

Inflammatory Polyps

- These are raised nodules of inflamed, regenerating epithelium. These lesions are not precancerous and are commonly found in **ulcerative colitis (Fig. 18.34B)**.
- **Microscopy:** Composed of a distorted and inflamed mucosal glands and granulation tissue.

Hamartomatous Polyps

- **Rare tumor-like growths**.
- They **consist of mature tissues that are normally present at the site** in which they develop.
- They may be associated with **intestinal and extraintestinal manifestations**.

Juvenile Polyps

These are **focal hamartomatous malformations of the mucosal epithelium and lamina propria**.

- Majority occur in **children** less than 5 years of age.
- Located in the **rectum** and commonly present with rectal bleeding.

Q. Write a short note on juvenile polyposis colon.

Juvenile Polyposis Coli Syndrome (JPS)

- It is characterized by multiple juvenile or inflammatory polyps distributed throughout the colon or GI tract.
- It is associated with an **increased risk of colonic adenocarcinoma**.

MORPHOLOGY

Gross

- Most are **less than 3 cm** in diameter.
- **Pedunculated, spherical, smooth-surfaced** and red in color
- Cut section shows **cystic spaces**.

Microscopy (Fig. 18.38)

- **Cystically dilated glands** filled with mucin and inflammatory debris.
- **Lamina propria** is expanded due to **mixed inflammatory infiltrates**.

Peutz–Jeghers (PJ) Syndrome (Figs. 18.39A to C)

Q. Write short note on Peutz–Jeghers syndrome and its components.

- Rare **autosomal dominant** syndrome

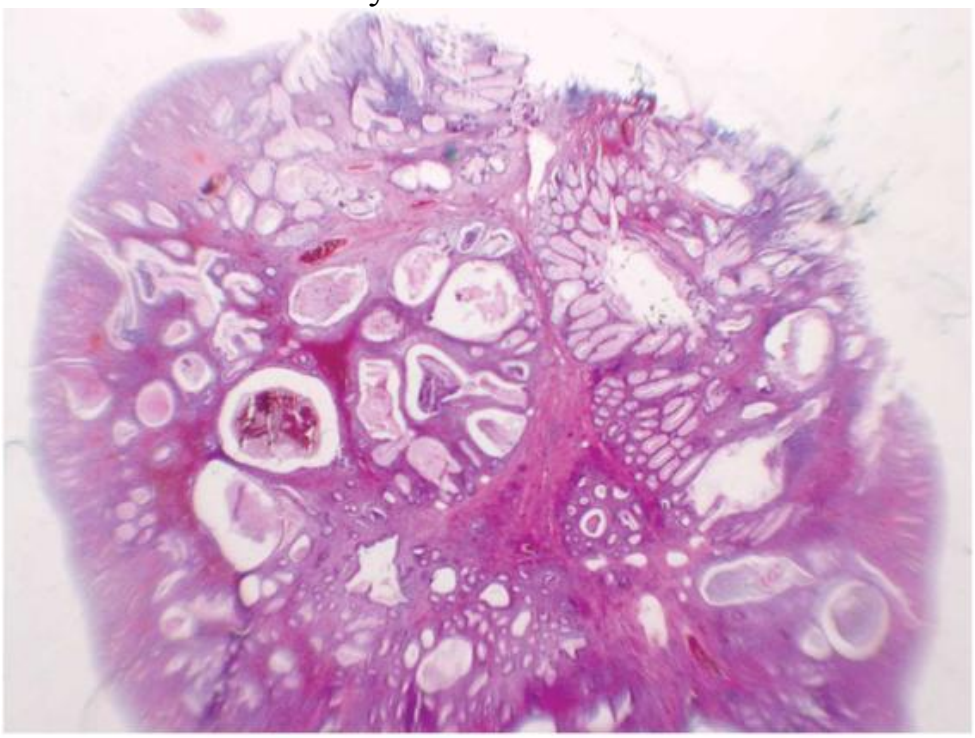


Fig. 18.38: Whole-mount view of a juvenile polyp showing cystically dilated glands.

- **Median age** of presentation is **11 years.**
- The gene *STK11* on chromosome 19 has been found in a proportion of these patients.
- Characterized by **multiple GI hamartomatous polyps (Figs. 18.39A and C)** and **mucocutaneous (**mouth, buccal mucosa and genitalia region) **hyperpigmentation (Fig. 18.39B)**.
- Associated with an **increased risk** of **several malignancies** and include cancers of the colon, pancreas, breast, lung, ovaries, uterus and testicles.

MORPHOLOGY

- **Site**: Most common in the **small intestine**.
- **Number**: Intestinal polyps are usually present in **dozens**.

- **Size: Varies** from a few millimeters to several centimeters in diameter.

Q. Write a short note on microscopic appearance of Peutz-Jeghers (PJ) polyps.

Gross

Large and pedunculated with a lobulated contour.

Microscopy

- Consists of **hyperplastic mature epithelium** appropriate to the anatomic site (where it develops) and **divided by broad bands of mature smooth muscle**.
- Small intestinal PJ polyps consist of crypts and villi of varying lengths divided by **characteristic arborizing bands of smooth muscle fibers (commonly fan out from the center of the polyp and has tree-like appearance) intermixed with lamina propria (Fig. 18.39C)**.

Hyperplastic Polyps

- Due to metaplastic proliferation of differentiated colonic epithelium.
- No malignant potential
- Most common non-neoplastic polyps of the colon and are frequently seen in the rectum.
- Age: Sixth and seventh decades of life.

MORPHOLOGY

Gross

- **Site**: It is most common in the left colon.
- **Size**: Small, smooth, sessile, nodular protrusions of the mucosa, less than 5 mm in diameter.
- **Number**: Single or multiple.

Microscopy

Composed of **elongated colonic crypts** lined by epithelial cells with a pseudopapillary configuration → **"saw-toothed" or serrated appearance**.

Neoplastic Polyps

Q. Write a short note on neoplastic polyps of intestine.

- The most **common and important** neoplastic polyps are **colonic adenomas (**benign neoplasms of intestinal epithelium) with the **potential for transformation** to **colorectal adenocarcinomas**.
- Polyps can occur singly, synchronously in few numbers or as part of a familial polyposis syndrome.

Classification of Neoplastic Polyps

They are classified depending on:

1. **Growth pattern:** (a) Pedunculated, (b) sessile, or (c) flat or depressed. Pedunculated adenomas have thin fibromuscular stalks containing prominent blood vessels derived from the submucosa.
2. **Architecture:** Adenomas can be classified as **tubular**, **tubulovillous**, or **villous**.

MORPHOLOGY

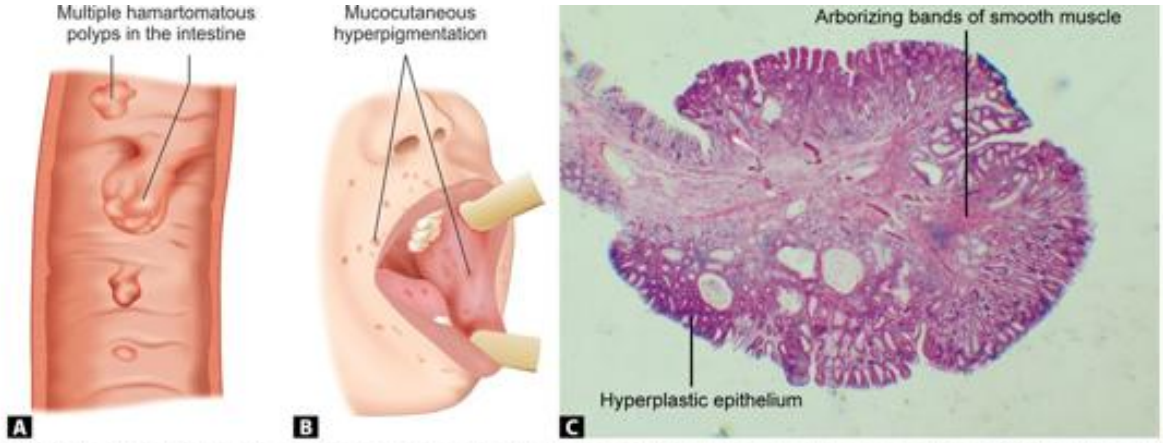
General Features

- **Size**: Range from 0.3 to 10 cm in diameter.
- **Site**: **Almost 50%** all adenomatous polyps of the colon are located **in the rectosigmoid region**. The remaining 50% are evenly distributed throughout the rest of the colon.
- **Microscopy: All colorectal adenomas are low-grade dysplastic lesions** characterized by the presence of epithelial dysplasia. The epithelial dysplasia may be classified as mild, moderate and severe dysplasia.
- **Classification:** Depending on the architecture, adenomas can be classified as **tubular**, **tubulovillous**, or **villous**.

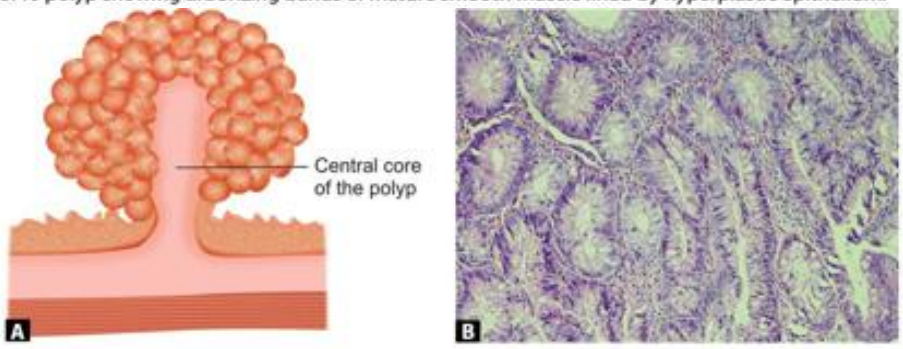
Tubular Adenomas (Adenomatous Polyps)

They constitute two-thirds of the adenomas of large intestine.

- **Gross:** Appear as **small, smooth-surfaced, pedunculated poly-ps** usually **less than 2 cm** in diameter **(Fig. 18.40A)**. Tubular adenoma over 2 cm have higher risk of invasive carcinoma.
- **Microscopy:** Consists of **closely packed** small **rounded or tubular glands (Fig. 18.40B)** embedded in a **stroma** (increase in the number of glands and cells per unit area compared to the normal mucosa). The cells lining the glands are crowded and contain enlarged hyperchromatic nuclei. May **show variable degree of epithelial dysplasia**.



Figs. 18.39A to C: (A) Multiple hamartomatous polyps in the intestine; (B) Mucocutaneous hyperpigmentation; (C) Whole-mount view of PJ polyp showing arborizing bands of mature smooth muscle lined by hyperplastic epithelium.



Figs. 18.40A and B: Tubular adenoma: (A) Shows a pedunculated tubular adenomatous polyp cut section, which shows a central core (diagrammatic); (B) Photomicrograph shows closely packed tubular glands with dysplasia of lining epithelium.

Villous Adenomas (Villous Papilloma)

Q. Write short note on villous adenoma.

They constitute one-tenth of colonic adenomas and are **predominantly** found **in the rectosigmoid region**.

- **Gross: Large, broad-based, sessile**, elevated lesions with **cauliflower-like surface (Fig. 18.41A)**. **Most are over 2 cm**, but may be as large as 10–15 cm in diameter.
- **Microscopy:** Composed of **thin, long, finger-like projections,** (papillary, crown-like growth) which superficially **resemble the villi of the small intestine (Fig. 18.41B)**. The lining epithelium shows dysplasia similar to tubular adenomas. However, **villous adenomas** (larger than 2 cm) likely **contain foci of carcinoma more commonly than tubular adenomas**.

Q. List the differences between villous adenoma and tubulovillous adenoma.

Tubulovillous Adenomas

They show a **mixture of both tubular and villous elements**. **Polyps with more than 25% and less than 75% villous component** are called as tubulovillous.

Sessile Serrated Adenomas

They are more common in the right colon and histologically overlap with hyperplastic polyps. They have malignant potential, but does not show dysplasia. Microscopically, consists of serrated architecture throughout the full length of the glands, including the base of the crypt, dilatation of crypt and lateral growth.

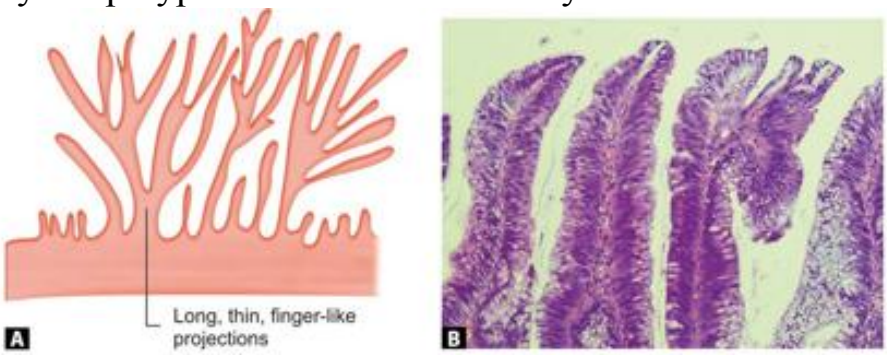
Intramucosal Carcinoma

It is term used when dysplastic epithelial cells break the basement membrane and invade into the lamina propria or muscularis mucosae. Since, there are no functional lymphatic channels in the colonic mucosa, intramucosal carcinomas do not have metastatic potential and complete polypectomy is usually curative.

Polyposis Syndromes

Q. Write short note on familial adenomatous polyposis.

- Polyps in the gastrointestinal system can develop either as sporadic lesions or as part of a polyposis or hereditary cancer syndrome.
- Several syndromes are characterized by the presence of colonic polyps and increased rates of colonic cancer.
- The most common syndromes include familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer syndrome.



Figs. 18.41A and B: Villous adenoma: (A) Diagrammatic; (B) Thin, long, finger-like projections covered by dysplastic epithelium.

Familial Adenomatous Polyposis

Definition: Familial adenomatous polyposis (FAP) is an **autosomal dominant** inherited syndrome characterized by **hundreds to thousands of adenomas** throughout the colorectum **(Fig. 18.42)** as

well as a numerous **extracolonic manifestations**.

- Patients develop few adenomas by the age of 21.
- **Genetic features**: It is caused by **germ-line** inactivation (5q) **mutations** of the ***adenomatous polyposis coli (APC)*** **gene**. *APC* is a tumor suppressor gene and its products modulate a specific signaling cascade (WNT signaling) that regulates cell proliferation **(***refer* **Fig. 18.44)**. Every cell of FAP patient has one inactive APC allele and inactivation of second allele initiate the early phase of neoplastic change.
- Minimum of **100 polyps are necessary for a diagnosis** of FAP.
- **Morphology:** It is similar to sporadic **adenomas**.

All untreated FAP patients **develop colorectal adenocarcinoma**, often before age 30. Prophylactic colectomy prevents colorectal cancer.

- ***Gardner syndrome***: When the **extraintestinal manifestations of FAP are prominent**, the condition is known as Gardner syndrome. The extraintestinal lesions include **osteomas of mandible**, **skull, and long bones, epidermal cysts, desmoid tumors in the abdominal wall, thyroid tumors, etc**.
- ***Turcot syndrome:*** It is rare syndrome characterized by **colonic adenomas** with **tumors of the central nervous system** (particularly medulloblastomas).

Hereditary Nonpolyposis Colorectal Cancer (HNPCC)/Lynch Syndrome

- HNPCC is the **colon cancer family syndrome not classically associated with large numbers of colonic adenomas**.
- **Despite the name** hereditary **nonpolyposis** colorectal cancer, **adenomas do develop** (though not in large numbers) in HNPCC patients.
- Transmitted as an **autosomal dominant** condition.
- HNPCC syndrome is characterized by increased risk of:
  - **Colorectal cancer:** HNPCC patients develop colonic cancer at **younger ages** than sporadic type and are often found in the **right proximal colon**. HNPCC-associated carcinoma are relatively nonaggressive, despite being poorly differentiated and mucinous type.
  - **Extra-colonic cancers:** These include **cancers of the endometrium, ovary, stomach and small intestines, and urinary bladder**.

- **Genetics of HNPCC:** Caused by **germline mutations** in **DNA mismatch repair (MMR) genes**. These genes **encode proteins involved in the detection, excision and repair of errors that occur during replication of DNA.**
  - **One result of defective DNA MMR** gene is a phenomenon referred to as **microsatellite instability (MSI)**. MSI occurs in about 90% cancers in HNPCC.
  - Appearance of abnormally long (due to increase in the number of nucleotide repeats) or short (due to decrease in the number of nucleotide repeats) microsatellites in a DNA (in normal tissue versus tumor) is referred to as **microsatellite instability**.



Fig. 18.42: Familial adenomatous polyposis with numerous polyps in the colon.

  - MSI is divided into three groups depending on the alterations in microsatellite length: MSI-High (MSI-H), MSI-Low (MSI-L), and MS-Stable (MS-S).
  - About six **mismatch repair (MMR) genes** have been identified which are designated as *hMSH2*, *hMSH6*, *hMLH1*, *hMLH3*, *hPMS1* and *hPMS2*, but the majority of HNPCC cases involve two genes namely—***MSH2* and *MLH1***.

- The MMR genes act as tumor suppressors and loss of both copies of the genes result in unrestrained growth and ultimately neoplastic transformation.
- HNPCC patients inherit one defective copy of an MMR gene, and the second copy of MMR gene is lost or inactivated as a somatic event. Loss of the second MMR gene often occurs due to methylation of the gene promoter.

Sigmoid colon: Most common site for polyps and cancer.

Inflammatory polyps develop in: Ulcerative colitis.

Hamartomatous polyps: Occur sporadically or as a part of genetic diseases.

Hamartoma: Jumbled mass of tissue indigenous to the part.

Hamartomas are **NOT** preneoplastic precursor lesions.

Peutz Jeghers polyp associated with:
- Mucocutaneous pigmentation
- Extragastrointestinal cancers.

PJ polyp (PJP): Most common in small intestine.

PJP: Incresaed risk of cancers of colon, breast and gynecologic cancers.

PJ polyps: Hamartomatous polyps likened to trees in which the trunk and branches are smooth muscle fibers.

Hyperplastic polyps: Most common non-neoplastic polyps of colon.

Neoplastic polyp: Most common is adenoma.

Risk factors for malignant change in polyps of colon:
- Large size (over 2 cm)
- Villous architecture
- Dysplasia
- Multiple polyps

Tubular adenoma: Most common neoplastic polyps in the GI tract. Like a raspberry on a stalk.

Villous adenoma: Increased risk of developing carcinoma.

FAP: Caused by APC mutations and patients have more than 100 adenomas and 100% develop colonic cancer if untreated.

Adenomatous polyposis coli gene (*APC*): On the short arm of chromosome 5.

Familial polyposis: Highest malignant potential.

Gardner syndrome: Colonic adenomas, epidermoid cysts, osteomas and desmoids.

Turcot syndrome: Colonic adenomas with brain tumors.

HNPCC: Caused by mutations (inactivation) in DNA mismatch repair genes.

Microsatellites represent short (1–6 nucleotides) repetitive DNA sequences (tandem repeats) scattered throughout the genome.

COLORECTAL CANCER: ADENOCARCINOMA

CC: Describe the etiology, pathogenesis, pathology and distinguishing features of carcinoma of the colon.

Q. Classify tumors of large intestine. Discuss etiopathogenesis (including molecular changes), morphology and staging of carcinoma of the colon (colorectal carcinoma). List the precancerous conditions of carcinoma colon.

Adenocarcinoma of the colon (colorectal carcinoma) is the **most common malignant tumor of the GI tract**.

Etiology and Risk Factors (Box 18.7)

Q. Write short note on etiopathogenesis of carcinoma of colon.

The rate of colorectal cancer has increased significantly, probably as a result of changes in lifestyle and diet.

BOX 18.7: Risk factors for colorectal cancer.

Factors that increases the risk

- Diet
  - Animal fat

  - Insulin resistance
  - Fiber
  - Deficiencies of vitamins A, C and E
- Hereditary syndromes
  - Polyposis coli
  - MYH-associated polyposis
  - Nonpolyposis syndrome (Lynch's syndrome)
- Inflammatory bowel disease: Ulcerative colitis and Crohn disease
- Others
  - *Streptococcus bovis* bacteremia
  - Increasing age
  - Family history of colonic cancer in first degree relative.
  - Prior colorectal cancer

Factors that reduces the risk

- Dietary factors; Diets rich in cruciferous vegetables (e.g., cauliflower, Brussels sprouts and cabbage) and vitamin A
- Protective effect of aspirin or other NSAIDs

Factors that Increases the Risk

Dietary Factors

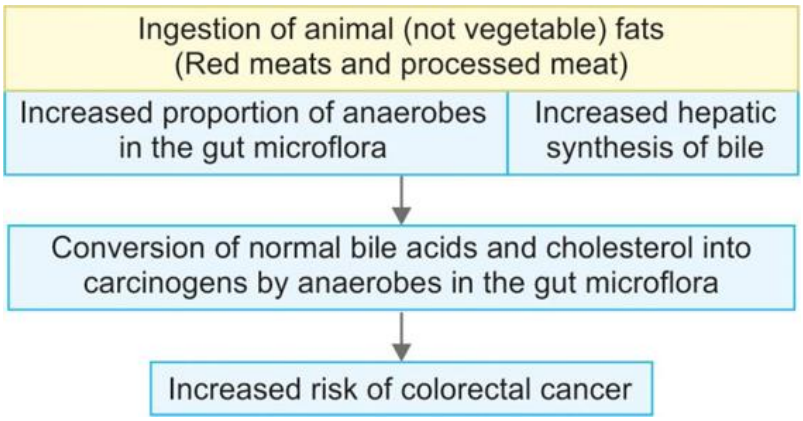
The etiology for most cases of colorectal cancer appears to be related to environmental factors. Dietary factors are closely associated with increased colorectal cancer rates.

- **Low intake of dietary fiber (unabsorbable vegetable fiber):** It is associated with decreased stool bulk → leads to **slower transit of fecal contents** through the colon and altered composition of the intestinal microbiota. These changes may **increase synthesis of toxic oxidative** by-products of bacterial metabolism, which **remains in contact with the colonic mucosa for longer periods**. The dietary fiber may bind to potential mutagens and dilute their concentration by increasing stool bulk. However, studies have *failed* to show any value for dietary fiber in preventing the recurrence of colorectal adenomas or the development of colorectal cancer.
- **High intake of refined carbohydrates and fat**: High level of animal fat (found in red meats and processed meat) in the diet is associated with **increased incidence of colorectal cancer**. High fat intake increases the hepatic synthesis and secretion of bile into the intestine. The contents present in the bile such as cholesterol and bile acids can be converted into

carcinogens by intestinal bacteria. Sequence of events due to ingestion of animal fat involved in the risk of colorectal cancer is presented in **Flowchart 18.3**.

- **Insulin resistance:** Increased calories intake along with physical inactivity is associated with a higher prevalence of obesity. Obese individuals develop insulin resistance and increased circulating levels of insulin. This leads to higher circulating concentrations of insulin-like growth factor type I (IGF-I). IGF-I may stimulate proliferation of the intestinal mucosa.

Flowchart 18.3: Sequence of events due to ingestion of animal fat involved in the risk of colorectal cancer.



Hereditary Factors and Syndromes

Family history of the colorectal cancer is found in up to 25% of patients which suggests the possibility of a hereditary predisposition. Inherited colorectal cancers can be divided into two main groups namely—(i) uncommon polyposis syndromes, and (ii) the more common nonpolyposis syndromes.

Polyposis coli (Familial polyposis of the colon)

Familial adenomatous polyposis (FAP) is a rare **autosomal dominant** inherited syndrome characterized by the appearance of thousands of adenomatous polyps (adenomas) throughout the colorectum (*refer* **Fig 18.42**) as well as a numerous **extracolonic manifestations.** It is caused by **germ-line** inactivation (5q) **mutations** of the ***adenomatous polyposis coli (APC)*** **gene**. Colectomy should be performed as the primary

therapy/prevention. **If the polyposis is not treated surgically, colorectal cancer will develop in almost all patients** aged <40. It is discussed on page 585.

MYH-associated polyposis

MYH-associated polyposis (MAP) is a rare autosomal recessive condition caused due to mutation in the *MUT4H* gene. This condition may be one of the risk factor for colorectal cancer occurring in younger individuals.

Hereditary nonpolyposis colorectal cancer (HNPCC)

HNPCC is also known as *Lynch's syndrome* and is transmitted as an autosomal dominant condition. **Despite the name** hereditary **nonpolyposis** colorectal cancer, **adenomas do develop** (though not in large numbers) in HNPCC patients. Colon cancers in HNPCC patients develop at **younger ages** than sporadic colon cancers and are often found in the **right colon** (*refer* page 585-6).

The colonic adenocarcinoma may evolve from the pre-existing adenomas, referred to as adenoma-carcinoma sequence discussed below (*refer* pages 588-9) under pathogenesis.

Inflammatory Bowel Disease

Patients with long-standing inflammatory bowel disease (IBD) have increased risk of colorectal cancer. Cancers develop more commonly in patients with ulcerative colitis than in those with Crohn disease. In case of ulcerative colitis, the risk is higher in younger patients with pancolitis.

Other Factors

- ***Streptococcus bovis* bacteremia:** Patients who develop endocarditis or septicemia from this fecal bacterium have been found to have high incidence of occult colorectal tumors. The reason is unknown.
- **Increasing age:** It is the most important risk factor. Risk is low before age 40 and it increases steadily to age 50, after which it doubles with each decade.
- **Family history of colonic cancer** in first degree relative.

- **Prior colorectal cancer:** It increases the risk for a subsequent tumor
- **Tobacco use:** Cigarette smoking is linked to the development of colorectal adenomas, particularly after >35 years of tobacco use. No biologic explanation for this association has yet been proposed.

Factors that Reduces the Risk

Dietary Factors

Diets rich in cruciferous vegetables (e.g., cauliflower, Brussels sprouts and cabbage) and **vitamin A** may be associated with a **lower incidence of colorectal cancer**. **Deficiencies of vitamins A, C and E,** which act as antioxidants (free-radical scavengers) may increase the damage caused by oxidants.

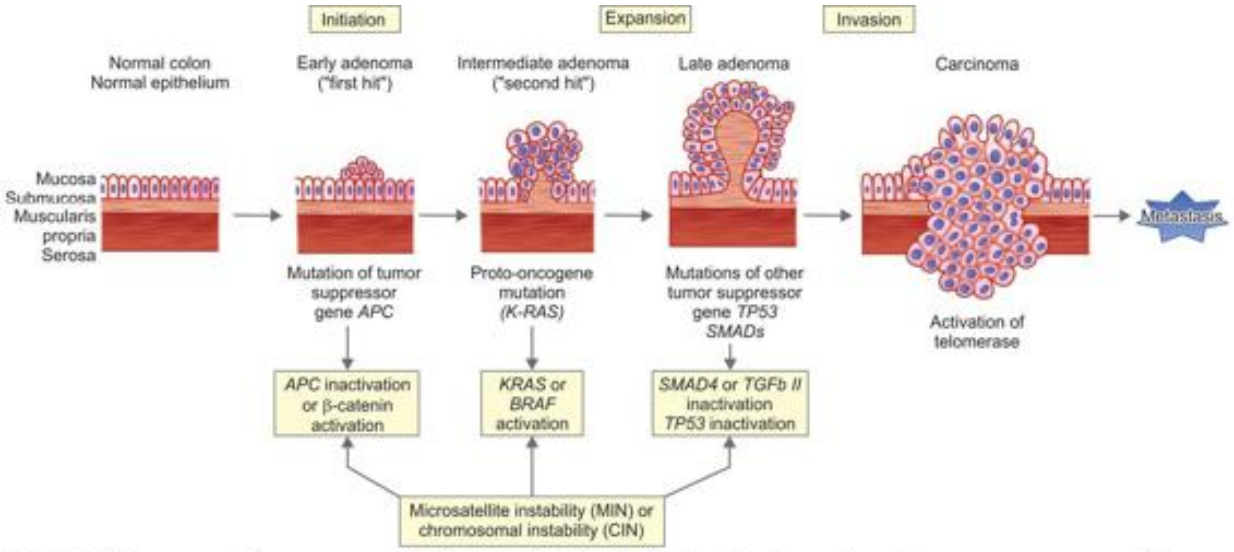


Fig. 18.43: Adenoma-carcinoma sequence showing morphological and molecular changes. Loss of tumor suppressor gene *APC* occurs early followed by mutations of *K-RAS*, *SMADs* and inactivation of the tumor suppressor gene *TP53*, and activation of telomerase.

Protective Effect of Aspirin or other NSAIDs

- Pharmacologic chemoprevention: Ingestion of therapeutic agents, such as aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce the risk of colorectal cancer.
- It may be due to these agents causing **inhibition of the enzyme cyclooxygenase-2 (COX-2),** which is highly expressed in 90% of colorectal carcinomas and 40–90% of adenomas.
- COX-2 is required for production of prostaglandin E_2, which promotes epithelial cell proliferation, particularly after injury.

Pathogenesis

Most colorectal cancers develop from the combination of multiple molecular alterations. They can be mainly divided into (1) **genetic abnormalities** (that **activate oncogenes** or **inactivate tumor suppressor genes**) and (2) **epigenetic abnormalities**.

Genetic Pathways

Two distinct genetic pathways have been described: (A) Classic adenoma-carcinoma sequence, (B) microsatellite instability pathway.

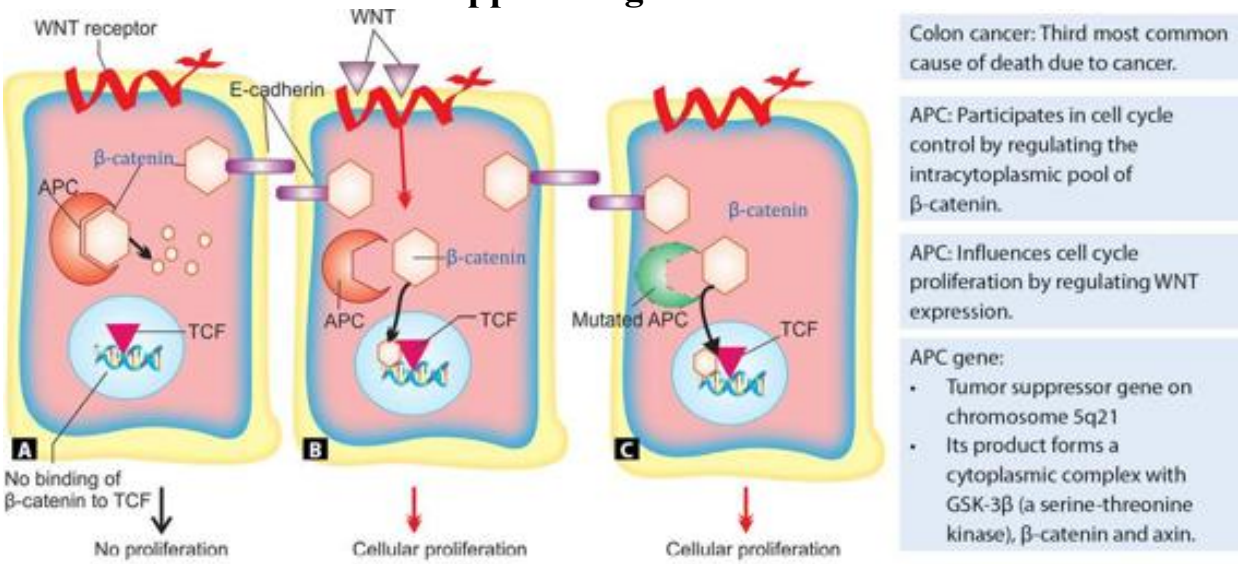
A. Adenoma-carcinoma Sequence(APC/β-catenin Pathway)

Q. Write short note on adenoma-carcinoma sequences in colonic carcinoma.

Most colorectal cancers (regardless of etiology) arise from adenomatous polyps. Adenoma-carcinoma sequence is seen in **about 80% of sporadic colonic cancers**. **According to this pathway (Fig. 18.43)**, **the morphologic progression in a specific step-wise sequence from normal mucosa to adenoma to invasive cancer** is accompanied by a **series of multiple molecular alterations**. Invasive cancers develop more frequently in sessile, serrated (i.e., "flat") polyps and villous adenomas.

1. **Inactivation of *APC* tumor suppressor gene (Figs. 18.44A to C)**
   - **APC** is an important **negative regulator of** β**-catenin,** a component **of the WNT signaling pathway.** Normally, the product of *APC* gene (APC protein) binds to and causes degradation of β-catenin and prevents proliferation of cells.
   - **Both copies of *APC* gene** must be **inactivated** either by mutation or epigenetic (methylation) events. This leads to **accumulation of** β**-catenin**, which forms a complex with TCF → activates transcription of c-*MYC*, *cyclin D1*, and other genes → **promotes cell proliferation**. One of the syndrome associated with APC inactivation is familial adenomatous polyposis (*refer* page 585).

2. ***K-RAS* mutations: Loss of APC function is followed by mutations in proto-oncogene *K-RAS*, which results in oncogene *K-RAS* → promotes cell proliferation and also prevents apoptosis. *K-RAS* mutation is a late event.**
3. ***SMAD2* and *SMAD4*** (tumor suppressor gene) **mutations:** These may develop as the neoplasm progresses. These genes are effectors of TGF-β signaling, which is normally a potent inhibitor of cell proliferation. The loss of these tumor suppressor genes may lead to uncontrolled cell proliferation.
4. **Mutation of *TP53* (tumor-suppressor gene): It is seen in 70–80% of colon cancers,** but is not commonly affected in adenomas. *TP53* mutations also occur at late stages of tumor progression. **Mechanism of inactivation of tumor suppressor gene:**



Figs. 18.44A to C: Role of APC in regulating the β-catenin. APC and β-catenin are components of the WNT signaling pathway: (A) In resting cells, β-catenin forms a complex with APC protein. This complex destroys β-catenin; (B) When cells are stimulated by WNT molecules, the β-catenin is not destroyed and its cytoplasmic levels increase. β-catenin translocates to the nucleus, where it binds to TCF, a transcription factor that activates cell cycle progression; (C) When *APC* is mutated or absent, the β-catenin cannot be destroyed and cell enters into the cell cycle.

- **Chromosomal deletions**
- **Methylation of a CpG-rich zone, or CpG island** (a region of some genes that frequently includes the promoter and transcriptional start site). Cytosines in CpG dinucleotides are normally unmethylated. Methylating the cytosine can turn the gene off **(gene silencing)**.

5. **Activation of telomerase:** Telomere plays a role in stabilizing the chromosome. It shortens with each cell division until cell senescence develops (*refer* pages 46 and 219).

- Telomerase is required to maintain telomere stability and hence cell immortality.
- Most adenomas do not show telomerase activity, but colorectal carcinoma have increased telomerase activity.
- **Expression of telomerase** also increases as lesions progress.

Adenoma-carcinoma sequence showing morpho-logical and molecular changes are shown in **Figure 18.43**.

B. Microsatellite Instability Pathway(Defective DNA Repair) (Fig. 18.45)

Microsatellites are repeated sequences (tandem repeats) of **one to six (1–6) nucleotides in the genome**. They become **unstable during normal cellular replication**, leading to insertion or deletion of bases within these regions. **DNA mismatch repair (MMR) genes rapidly correct these errors** to **maintain microsatellite length**.

- **Mutations in normal DNA repair genes** (**mismatch-repair defects) leads to** accumulation of microsatellites → referred to as **microsatellite instability** (**MSI).** About six **mismatch repair (MMR) genes** have been identified, which are designated as *hMSH2*, *hMSH6*, *hMLH1*, *hMLH3*, *hPMS1* and *hPMS2* (*refer* HNPCC in pages 585-6).
- Microsatellites are typically formed in noncoding regions. However, some microsatellite sequences are located in the coding or promoter region of genes involved in regulation of cell growth. For example, **microsatellite instability** involving:
  - **Type II TGF-β receptor:** Normally, **TGF-β** inhibits colonic epithelial cell proliferation and mutation in type II TGF-β receptor can lead to uncontrolled cell growth.
  - **Pro-apoptotic gene *BAX*:** Its mutation can lead to loss of *BAX*, which may increase the survival of genetically abnormal clones of cells.

C. CpG Island Hypermethylation Phenotype (CIMP)

This is a subset of colon cancers with microsatellite instability in which there are no mutations in DNA mismatch repair enzymes. These tumors have the

CpG island hypermethylation phenotype (CIMP).

- In these tumors, there is **hypermethylation of MLH1 promoter region**, which reduces MLH1 expression and repair function.
- **Activating mutations in the *BRAF* oncogene** are common in these tumors.
- KRAS and TP53 are not usually mutated.

Thus, combination of microsatellite instability, *BRAF* mutation, and methylation of specific targets, such as *MLH1*, are seen in this pathway of carcinogenesis.

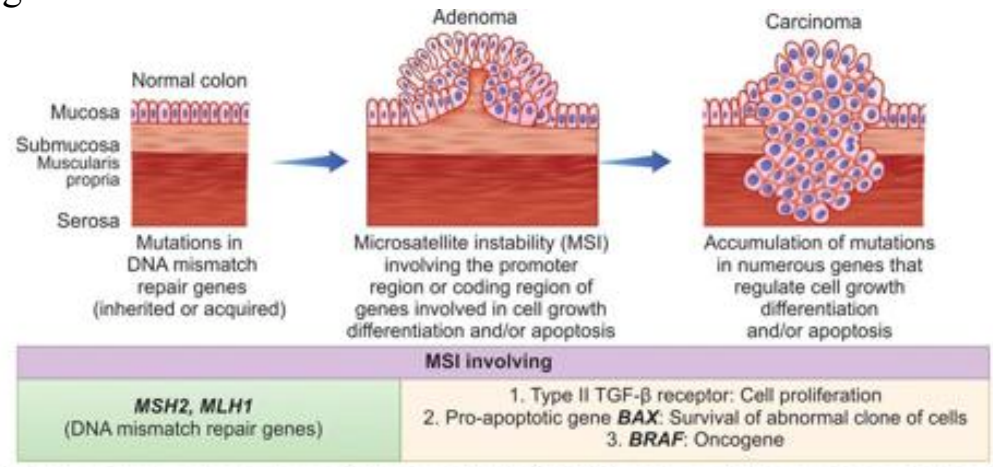


Fig. 18.45: Morphological and molecular changes in the microsatellite instability pathway of colon carcinogenesis. Defects in mismatch repair genes result in microsatellite instability and allows accumulation of mutations in numerous genes involved in cell survival and proliferation.

Epigenetic Abnormalities (refer page 206)

Definition: Epigenetics is a **reversible**, **heritable** alteration in gene expression, which occurs without mutation.

- It is unrelated to gene nucleotide sequence.
- Epigenetic events may enhance progression along both genetic pathways mentioned above.
- Epigenetic changes involve histone modification and DNA methylation, both of which affect gene expression.
- In **carcinoma colon cells, silencing of DNA repair genes** (mismatch-repair gene MLH1) **by hypermethylation** can occur.

MORPHOLOGY

Q. Write short note on morphology of carcinoma colon.

Gross

Location: Colonic adenocarcinomas can be found in **any location** of the colon.
Types: Grossly colonic carcinomas can be categorized into four general types:

- **Exophytic polypoid mass in the right-side of colon (Fig. 18.46A):** Tumors in the proximal colon usually grow into the lumen as bulky, **exophytic (cauliflower-like), polypoid** masses and extend along one wall of the cecum and ascending colon. They rarely cause intestinal obstruction.
- **Annular and constricting tumors in the left-side of colon (Fig. 18.46B):** These tumors are annular lesions that produce the characteristic **"napkin-ring"** or "**apple core**" **constrictions** and luminal narrowing. It **may be associated with intestinal obstruction** and dilatation with attenuation and flattening of the mucosal folds of colon proximal to the tumor. The tumors are firm due to associated desmoplasia.
- **Diffuse/tubular tumors (Fig. 18.46C):** These are similar to linitis plastica of the stomach. They show diffuse flattening **and thickening of the colon**, initially involving the mucosa, but later involve the entire wall of intestine.

- **Infiltrative and ulcerating tumors (Fig. 18.46D):** These cancers are **usually raised, have irregular edges** and a **central, excavated ulcerated area** that often infiltrate the deep layers of the bowel wall.

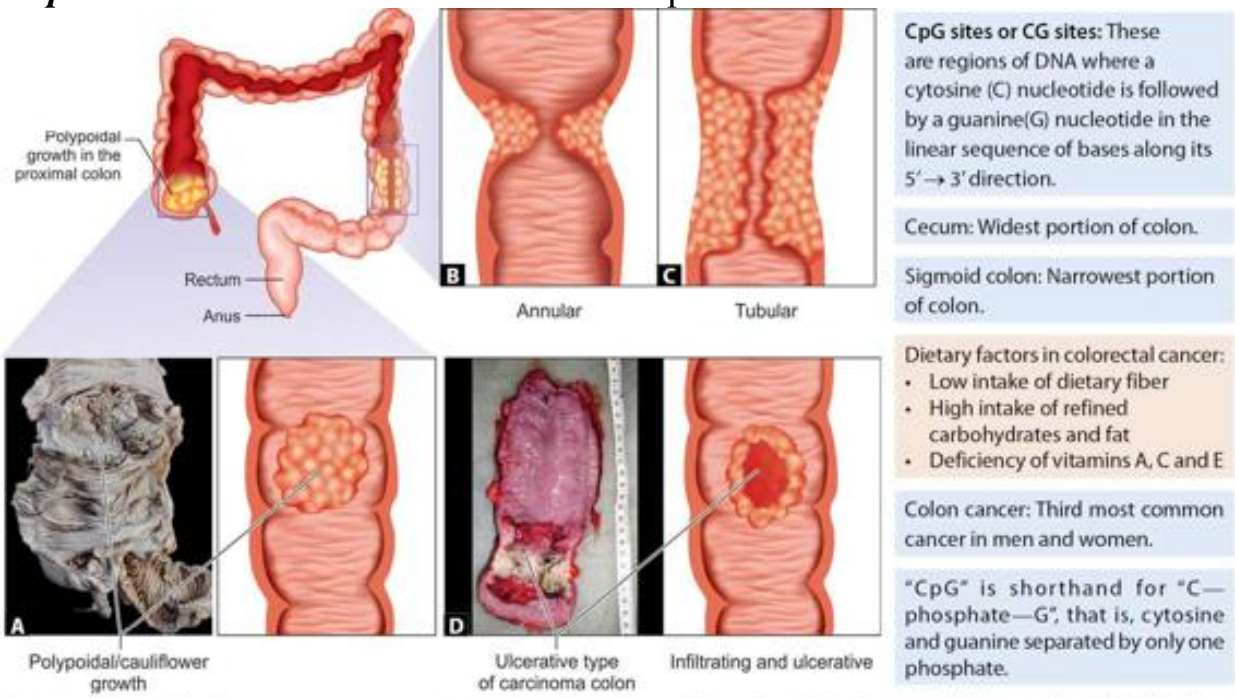
Microscopy (Figs. 18.47A and B)

- Majority of colonic cancers are **adenocarcinomas** and are similar on microscopic examination. Adenocarcinoma may be **well-differentiated, moderately or poorly differentiated**.
- Most of the tumors show **glands of variable size and configuration** separated by moderate amount of stroma. Mitotic figures are usually abundant. The **lumen** of the glands is usually **filled with inspissated eosinophilic mucus** and **nuclear and cellular debris ("dirty" necrosis)**. The invasive component of these tumors may show stromal desmoplasia → causes firm consistency.
- Poorly differentiated carcinoma shows only few glands.
- **Mucinous adenocarcinomas:** They secrete abundant mucin and accumulate within the intestinal wall and are associated with poor prognosis.
- **Signet-ring carcinoma:** It consists of **signet-ring cells** similar to those in gastric cancer and constitute more than 50% of the tumors.

Clinical Features

Q. Differences between the carcinoma of colon on right and left side.

- Tumors in the cecum and other **right-sided colon** cancers usually present with **fatigue and weakness** due to **iron deficiency anemia**. Thus, it is important that **iron deficiency anemia in an older man or postmenopausal woman should be considered due to GI cancer until otherwise proved**.
- **Left-sided cancers** may produce ***occult bleeding*, altered *bowel habits or pain and* discomfort** in the left lower quadrant.



CpG sites or CG sites: These are regions of DNA where a cytosine (C) nucleotide is followed by a guanine(G) nucleotide in the linear sequence of bases along its 5′→3′ direction.

Cecum: Widest portion of colon.

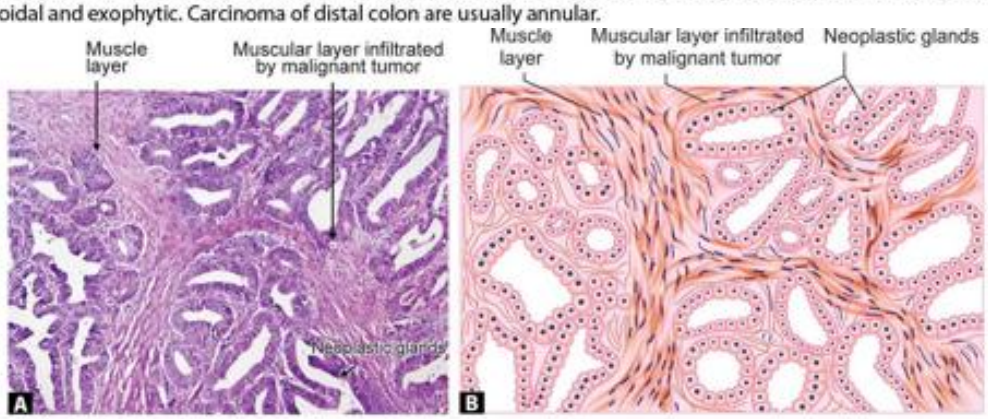
Sigmoid colon: Narrowest portion of colon.

Dietary factors in colorectal cancer:
- Low intake of dietary fiber
- High intake of refined carbohydrates and fat
- Deficiency of vitamins A, C and E

Colon cancer: Third most common cancer in men and women.

"CpG" is shorthand for "C—phosphate—G", that is, cytosine and guanine separated by only one phosphate.

Figs. 18.46A to D: The four common macroscopic varieties of carcinoma of the colon: (A) Exophytic/cauliflower/polypoidal (left side—gross specimen); (B) Annular; (C) Tubular; and (D) Infiltrating and ulcerative (left side—gross specimen). Carcinoma of proximal colon are usually polypoidal and exophytic. Carcinoma of distal colon are usually annular.



Figs. 18.47A and B: Adenocarcinoma of colon composed of tumor cells arranged in glandular pattern: (A) H & E photomicrograph; (B) Diagrammatic.

Methods of Investigation of Colon Cancer

1. **Occult blood** loss in the stool by Guaiac test.
2. **Tumor markers: Elevated levels of carcinoembryonic antigen (CEA) and CA 19-9.**
3. Flexible sigmoidoscopy
4. **Colonoscopy helps in direct visualization of cancer and may be used to take a biopsy:** Investigation of choice.
5. **Radiology:**
   - **Double-contrast barium enema:** It is the radiological investigation of choice, when colonoscopy is contraindicated. It characteristically shows **"apple core" appearance.**
   - **Ultrasonography:** Used as a screening investigation for liver metastases.
   - **Spiral CT:** In elderly patients when contrast enemas or colonoscopy are not diagnostic or are contraindicated.

Staging and Prognosis

Q. Write a short note on staging of carcinoma of colon.

- **Two most important prognostic factors are depth of invasion and the presence or absence of lymph node metastases.**
- Invasion into the muscularis propria reduces the survival rate which is reduced further in the presence of lymph node metastases.
- Poorly differentiated and mucinous carcinomas are associated with poor prognosis.

Dukes and Kirklin, and Astler-Coller staging were being used presently replaced by **TNM (tumor-nodes-metastasis) classification** and staging system from the American Joint Committee on Cancer.

Spread

- **Direct spread:** The tumor can spread in a transverse, longitudinal, or radial direction.
  - **Transverse spread** circumferentially round the intestinal wall causes intestinal obstruction.

- **Longitudinal spread** along both directions.
- **Radial spread** directly into the submucosa into the muscularis propria and then out into the serosa, pericolic fat, lymphatics and veins in the mesentery alongside the bowel wall and sometimes into the peritoneal cavity.

- **Lymphatic spread:** Tumor may spread through lymphatics into the regional lymph nodes. Lymph nodes draining the colon are grouped as:
  - N1: Nodes in the immediate vicinity of the bowel wall.
  - N2: Nodes arranged along the ileocolic, right colic, midcolic, left colic and sigmoid arteries.
  - N3: Apical nodes around the superior and inferior mesenteric vessels.
- **Blood spread: Venous invasion** may give rise to blood-borne metastases in the **liver** (through portal vein). It may also spread to **lungs and bones**.
- **Transcoelomic spread:** Rarely, it can spread by dislodging tumor cells from the serosa of the bowel or via the subperitoneal lymphatics to other structures within the peritoneal cavity.

Q. On a routine health maintenance examination and laboratory evaluation, a 65-year-old male was found to be having microcytic hypochromic anemia. His stool sample was positive for occult blood. A colonoscopy was performed and showed a 4 cm bulky, exophytic (cauliflower-like), polypoid lesion projecting into the cecum. Biopsy was performed and microscopic examination confirmed the clinical suspicion. It was followed by colonic resection.

(a) What is the probable diagnosis? (Ans: Carcinoma of colon /cecum- Exophytic polypoid type).

(b) Describe the etiopathogenesis of the disease (refer pages 586-90).

(c) Describe the morphological features of this lesion (refer page 590).

Q. A 70-year-old man complained of change in bowel habits for the past one year. He also complained that the defecation was more difficult and the caliber of stools has decreased. Physical examination, did not reveal any significant findings. His stool sample was examined for occult blood and was found to be positive. Colonoscopy showed an annular lesion encircling the descending colon. Biopsy examination confirmed the diagnosis and was followed by colonic resection.

(a) What is the probable diagnosis? (Ans: Carcinoma of descending colon-annular type).

(b) Describe the morphological features of this lesion (refer page 590).

(c) Mention the various methods of investigation of this condition (refer page 591).

Microsatellites: Repeated sequences (tandem repeats) of one to six (1–6) nucleotides in the genome. Microsatellites become unstable during normal cellular replication, leading to insertion or deletion of bases within this region.

Microsatellite instability (MSI): Accumulation of microsatellites.

Defects in MMR genes:
- Microsatellite instability
- Mutations in many genes involved in cell proliferation, differentiation and/or apoptosis

Carcinoma colon:
- Exophytic polypoidal mass in the right colon
- Annular constricting mass in the left colon

Most common site of colon cancer is sigmoid colon. Least common site of colonic cancer is:

Hepatic flexure.

Colon cancer: Mucin production worsens the prognosis since mucin aids in tumor extension

Colonic cancer: Most important prognostic factors:
- Depth of invasion
- Lymph node status

Carcinoembryonic antigen (CEA) may be elevated in colonic cancer.

The tumor can spread in a longitudinal, transverse or radial direction; it spreads round the intestinal wall and usually causes intestinal obstruction before it invades adjacent structures.

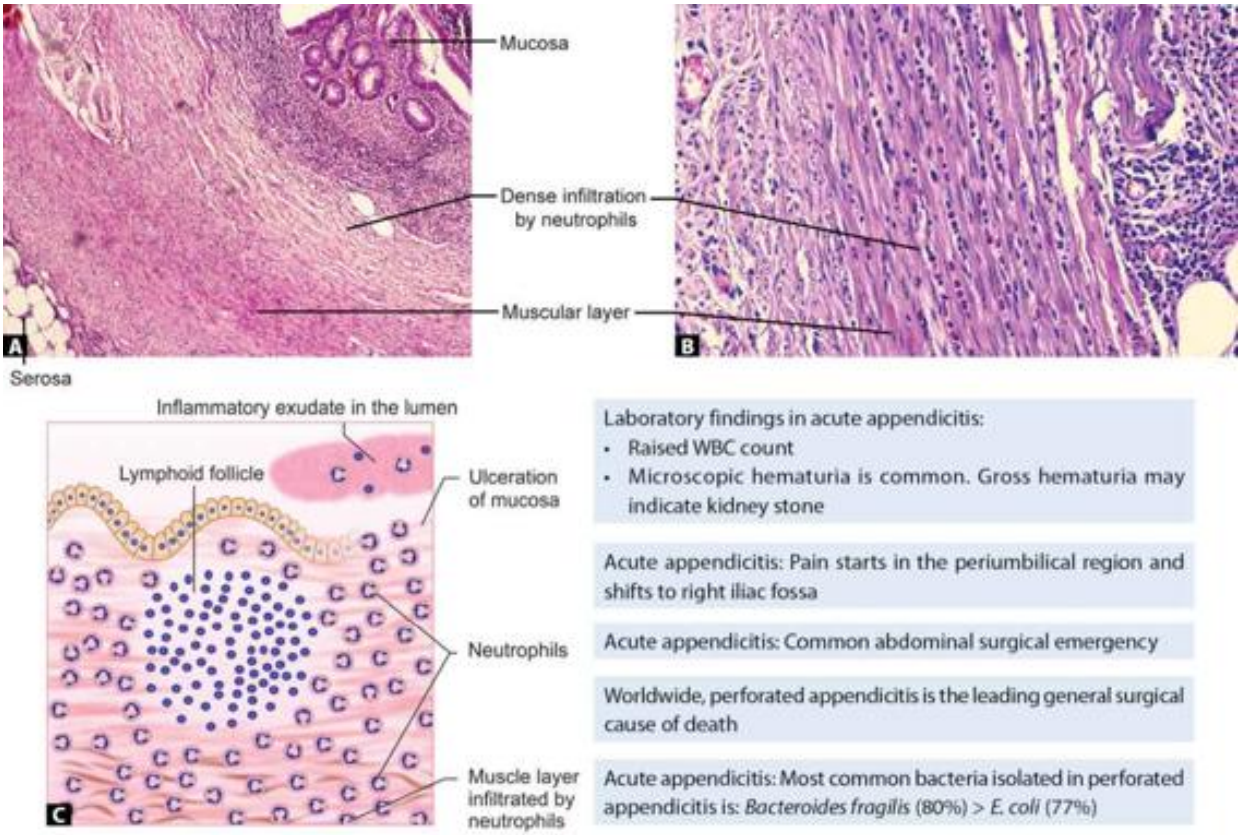
Carcinoma colon: Most **common site of metastasis liver > lung.**

- Hematemesis is the vomiting of blood.
- Hemoptysis is defined as coughing of blood or blood-stained mucus originating from below the vocal cords.
- Epistaxis is defined as bleeding from the nostril, nasal cavity, or nasopharynx.
- Hematochezia is the passage of fresh blood through the anus, usually in or with stools (contrast with melena).
- Melena refers to the dark black, tarry feces that are associated with upper gastrointestinal bleeding. The black color and characteristic strong odor are caused by hemoglobin in the blood being altered by digestive enzymes and intestinal bacteria.

ACUTE APPENDICITIS

Q. Write a short note on acute appendicitis.

- The appendix is prone to acute and chronic inflammation.
- Acute appendicitis is an acute inflammatory process involving the appendix.
- Acute appendicitis can occur in any age group but is most common in adolescents and young adults.



Figs. 18.48A to C: (A) Photomicrograph (low power) of acute appendicitis showing inflammation of mucosa, submucosa and muscular layer; (B) (High power view) shows numerous neutrophils in the muscular layer; (C) Diagrammatic appearance of acute appendicitis.

Pathogenesis

- The etiology of appendicitis is multifactorial and may involve obstruction, ischemia, infections and hereditary factors.
- Acute appendicitis is thought to begin with luminal obstruction due to the factors, which progressively increase the intraluminal pressure.
- The obstruction is usually caused by small stone-like mass of stool, or *fecalith* or less commonly, a gallstone, tumor, or mass of worms *(Oxyuriasis vermicularis)*.
- Ischemic injury and stasis of luminal contents favor bacterial proliferation, trigger inflammatory response.
- Inflammation produces edema and neutrophilic infiltration of the lumen, muscular wall and periappendiceal soft tissues.
- The pressure produced by inflammation and edema predisposes to the development of gangrene, perforation, and peritonitis.

MORPHOLOGY

Gross

- The appendix may be **swollen and erythematous**
- The **serosa** initially appears dull and gray and later may be **covered by a purulent exudate**
- Perforation secondary to gangrene can follow and form abscess.

Microscopy (Figs. 18.48A to C)

- The early lesions show **mucosal erosions**.
- Later, the inflammation extends into the lamina propria, and collections of neutrophils may also be seen in the lumen of the appendix.
- Diagnosis of acute appendicitis should be made when **muscularis propria shows infiltration by neutrophils**.
- In severe cases, neutrophilic exudate produces fibrinopurulent reaction in the serosa. When focal abscesses develop within the wall, it is termed as **acute suppurative appendicitis**.
- When appendix shows large areas of hemorrhagic ulceration and gangrenous necrosis, it is known as **acute gangrenous appendicitis.** This may rupture leading to suppurative peritonitis.

Clinical Features

Acute appendicitis presents with pain in the periumbilical region, which ultimately localizes to the right lower quadrant. This is followed by nausea, vomiting, low-grade fever and mild leukocytosis.

McBurney's sign: A classic physical finding is deep tenderness located two-thirds of the distance from the umbilicus to the right anterior superior iliac spine (McBurney's point).

Complications: Gangrenous appendicitis, perforation, pylephlebitis, portal venous thrombosis, liver abscess, and bacteremia.

CHAPTER 19

Hepatobiliary and Pancreatic Disorders

LIVER

BILIRUBIN METABOLISM AND BILE FORMATION (FIG. 19.1)

CC: Describe bilirubin metabolism, enumerate the etiology and pathogenesis of jaundice, distinguish between direct and indirect hyperbilirubinemia.

Q. Write short note on bilirubin metabolism.

Bilirubin Production

Source of Bilirubin

- **Major** (85%) is derived from the **catabolism of hemoglobin** during the breakdown of senescent red cells.
- **Minor** (15–20%) is derived from the degradation of heme produced from **other sources** (e.g., the P-450 cytochromes) and from premature destruction of hemoglobin in developing red cell precursors in the bone marrow.

Bilirubin Formation

- Heme liberated from above sources is oxidized to **biliverdin** by heme oxygenase.
- **Biliverdin** is immediately **reduced to bilirubin** by the enzyme biliverdin reductase. The bilirubin formed is known as **unconjugated bilirubin**.

Transport of Bilirubin/Bilirubin Binding

- Unconjugated bilirubin formed in the periphery is liberated into the circulation → reversible **binding of bilirubin to serum albumin** (albumin–bilirubin complex). Unconjugated bilirubin is insoluble in aqueous solutions at physiologic pH.
- The unconjugated bilirubin is **transported to the liver** in plasma.

Hepatic Processing of Bilirubin

Excretion of bilirubin from the body is one of the major function of the liver. Metabolism of bilirubin in the liver consists of four separate but interrelated events:

- **Hepatic uptake from the circulation**: On reaching the sinusoidal plasma membrane of the hepatocyte, the unconjugated bilirubin (albumin–bilirubin complex) is dissociated → enters the hepatocytes.
- **Binding:** Within the hepatocyte, bilirubin binds to several proteins in the cytosol known collectively as **glutathione-S-transferases** (also termed ligandin).
- **Conjugation with glucuronic acid**: **Unconjugated bilirubin** (not water-soluble) combines with one or two molecules of glucuronic acid in the presence of uridine diphosphate (UDP)–glucuronyltransferase (UGT1A1) → forms **water-soluble bilirubin diglucuronide (conjugated bilirubin).**
- **Biliary excretion/secretion:** Conjugated bilirubin is excreted into bile → reaches the small intestine.

Intestinal Phase of Bilirubin Metabolism

In the intestine, conjugated bilirubin has two fates:

1. **Most of the conjugated bilirubin deconjugated** (in the distal small intestine and colon) by bacterial β-glucuronidases → **to colorless urobilinogens**. The urobilinogen has two fates:
   i. **Excretion of urobilinogen**: **Most (80%)** of the urobilinogen is **excreted in the feces as** stercobilinogen (is responsible for the normal color of the stool).
   ii. Reabsorption of urobilinogen:

- About 20% of urobilinogens is reabsorbed in the terminal ileum and colon into portal circulation → reaches the liver → re-excreted into the bile.
- A small amount of reabsorbed urobilinogen is excreted in the urine as urobilinogen.

  Note: (1) In obstructive jaundice, urine does not contain urobilinogen. (2) In hepatocellular and hemolytic jaundice, urinary urobilinogen is markedly increased (more than 4 mg/day).

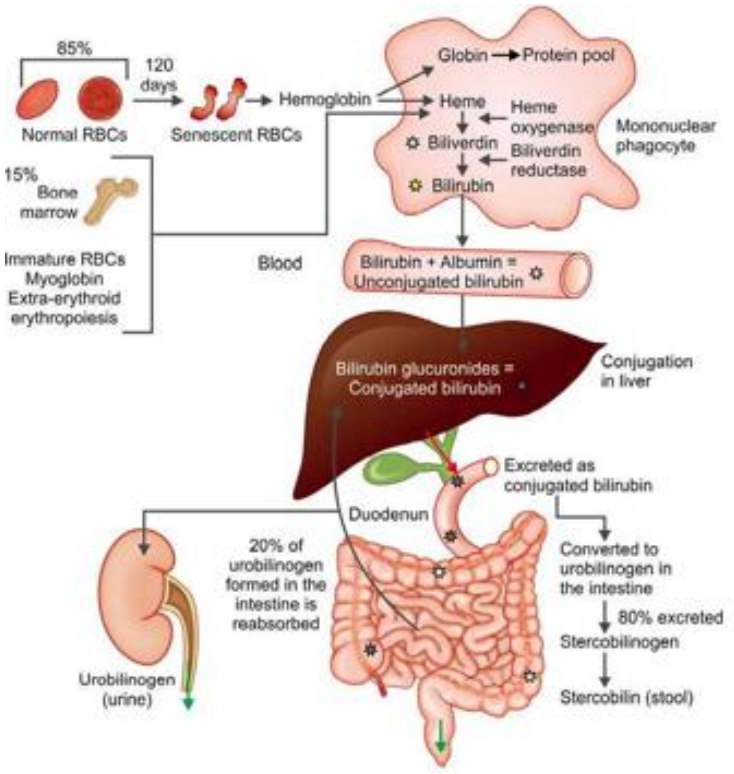
2. Part of the conjugated bilirubin is excreted in the stool as such (bilirubin glucuronide).

Renal Excretion of Bilirubin

- Unconjugated bilirubin is tightly bound to albumin; cannot be filtered by the glomeruli and not excreted in the urine.
- Conjugated bilirubin is filtered by the glomeruli and appears in the urine (bilirubinuria).

Obstructive jaundice: Stercobilinogen is absent in the stool and stool appears pale and clay colored.

Stool: Urobilinogen → stercobilinogen responsible for color of stool.



Kernicterus: Irreversible brain injury (neurological damage) in infants caused due to high concentrations of free unconjugated bilirubin which crossed immature blood-brain barrier

Enterohepatic circulation:
- Process of excretion of bile by liver into intestine and return to the liver.
- Helps to maintain a large endogenous pool of bile acids for digestive and excretory purposes

Kupffer cells are found in liver derived from macrophage–monocyte system

Hepatic stellate cells (Ito cell):
- Found in space of Disse
- High in lipid content
- Store vitamin A
- Synthesize extracellular collagen

Unconjugated bilirubin: End product of heme degradation

Unconjugated bilirubin:
- Lipid soluble and water insoluble
- Toxic to the brain in newborns

Intestinal bacteria: Convert conjugated bilirubin to urobilinogen

Fig. 19.1: Bilirubin metabolism. Major bilirubin is derived from the breakdown of senescent circulating RBCs. The bilirubin binds to serum albumin and delivered to the liver. In the liver, bilirubin forms conjugated water-soluble bilirubin and excreted into bile. In the gut, bacteria deconjugate the bilirubin and degrade it to colorless urobilinogens. About 80% urobilinogens excreted in the stool, and about 20% is reabsorbed. Minor fraction of reabsorbed is excreted into urine as urobilinogen.

Cholestasis: Systemic retention of bilirubin and other solutes eliminated in bile.

Urobilinogen: ~20% recycled to liver and kidney.

JAUNDICE

Q. Write a short note on hyperbilirubinemia.

Definition: Jaundice is defined as **yellowish pigmentation** of **skin, mucous membranes and sclera** due to **increased levels of bilirubin** in the blood. The scleral involvement is because it is rich elastic tissue that has special affinity for bilirubin.

- **Normal serum bilirubin level**: In the normal adult range from 0.3 to 1.2 mg/dL.
- **Jaundice**: Clinically detected when the serum bilirubin level is above 2.0–2.5 mg/dL. With severe disease, the levels may be as high as 30–40 mg/dL.

Mechanism of Jaundice

Various mechanisms can produce jaundice **(Table 19.1)**. Generally, one of these mechanisms predominates. However, more than one mechanism may be responsible for jaundice.

TABLE 19.1: Various mechanisms of jaundice.

Mechanism	***Type of hyperbilirubinemia***
Excessive extrahepatic production of bilirubin	Unconjugated hyperbilirubinemia
Reduced hepatocyte uptake	
Impaired conjugation	
Decreased hepatocellular excretion	Predominantly conjugated hyperbilirubinemia
Impaired bile flow	

Both unconjugated bilirubin and conjugated bilirubin may accumulate in systemic circulation. There are two main pathophysiologic differences between the two forms of bilirubin.

- **Unconjugated bilirubin:**
  - **Insoluble in water** at physiologic pH and is present in the circulation forming tight complexes with serum albumin.
  - **Cannot be excreted in the urine** even when its levels are high in the blood.
  - In **hemolytic disease of the newborn** (erythroblastosis fetalis) → excessive unconjugated bilirubin → crosses the blood brain barrier → reach the brain → causes severe neurologic damage → referred to as **kernicterus**.
- **Conjugated bilirubin:**
  - **Water-soluble, nontoxic**, and only loosely bound to albumin in the plasma.
  - **Excess conjugated bilirubin in plasma** can be **excreted in urine**.

Classification of Jaundice

Q. Write a short note on classification of jaundice.

1. **Based on the underlying cause (Box 19.1)**
   - Predominantly unconjugated
   - Predominantly conjugated

2. **Based on pathological mechanism:**
   - Hemolytic jaundice
   - Hepatocellular jaundice
   - Obstructive jaundice

Normal bilirubin level: Balance between rate of bilirubin production and rate of biliary excretion.

Hemolytic (Prehepatic) Jaundice

Increased destruction of red blood cells (hemolysis) **or their precursors** (ineffective erythropoiesis) causes increased production of bilirubin. **Unconjugated bilirubin** accumulates in the plasma and results in jaundice. **Jaundice is usually mild**, because normal liver can easily handle the increased bilirubin production.

Laboratory Findings

Show features of hemolysis (*refer* pages 306-7).

Serum findings

- **Predominantly unconjugated hyperbilirubinemia.** Serum bilirubin is raised (< 6 mg %).
- **Other liver function tests** (e.g., serum ALP, transferases and albumin) are **normal**, however, **LDH is increased**.

Urine findings

- **No bilirubin in urine**, because the unconjugated bilirubin is not water-soluble and cannot pass into the urine; hence the term **'acholuric jaundice'.**
- **Urinary urobilinogen is increased** (>4 mg/24 hours).

Hepatocellular (Hepatic) Jaundice

- Dysfunction of any of the hepatic steps in bilirubin metabolism may cause jaundice. Hepatocellular jaundice occurs as a **consequence of parenchymal liver disease** which leads to an inability of the liver to transport bilirubin across the hepatocyte into the bile.

- Defect in bilirubin transport across the hepatocyte may occur at any point between the uptake of unconjugated bilirubin into the hepatocyte and transport of conjugated bilirubin into biliary canaliculi.

Laboratory Findings

Serum findings

- **Raised transaminases (AST, ALT):** Acute jaundice with AST >1000 U/L is highly suggestive of an **infectious cause** (e.g., hepatitis A, B), **drugs** (e.g., paracetamol) or **hepatic ischemia.**
- **Both unconjugated and conjugated bilirubin level rises** in the blood.

Urine findings

- Bilirubin present.
- Urobilinogen present.

Cholestatic (Obstructive/Surgical/ Posthepatic) Jaundice

Cholestasis means failure of bile flow. Its cause may be anywhere between hepatocyte and duodenum **(Fig. 19.2)**. Cholestatic jaundice is usually a **'surgical jaundice'** meaning a cause that requires surgical intervention. Cholestasis can be intrahepatic or extrahepatic.

Consequences of cholestasis:

- **Retention of bile acids and bilirubin** in the liver and blood.
- **Deficiency of bile acids in the intestine.**

Laboratory Findings

Serum findings

- **Serum bilirubin markedly raised** and is predominantly conjugated (representing >50% of the total bilirubin) hyperbilirubinemia.

BOX 19.1: Classification of jaundice based on the underlying cause.

A. Predominantly unconjugated hyperbilirubinemia

1. Increased production of bilirubin
   - Hemolytic anemias

- Resorption of blood from internal hemorrhage (e.g., GI bleeding, hematomas)
- Ineffective erythropoiesis (e.g., pernicious anemia, thalassemia)

2. Reduced hepatic uptake
   - Drug that interfere with membrane carrier systems
   - Diffuse liver disease (hepatitis, cirrhosis)
   - Some cases of Gilbert syndrome
3. Impaired bilirubin conjugation
   - Physiologic jaundice of the newborn
   - Crigler–Najjar syndrome types I and II
   - Gilbert syndrome
   - Diffuse liver disease (e.g., hepatitis, cirrhosis)

B. Predominantly conjugated hyperbilirubinemia

1. Decreased hepatocellular excretion
   - Deficiency of canalicular membrane transporters
     - Dubin–Johnson syndrome
     - Rotor syndrome
   - Liver damage or toxicity (e.g., hepatitis)
2. Impaired intra/extrahepatic bile flow
   - Inflammatory destruction of bile ducts (e.g., primary biliary cholangitis)
   - Gallstones
   - Carcinoma of pancreas

- **Serum alkaline phosphatase (ALP) markedly raised** (3–4 times that of normal).
- Minimal biochemical changes of liver parenchymal damage.
- Increase in serum GGT/γ-GT (gamma glutamyl transpeptidase), and 5'nucleotidase
- Antimitochondrial antibody (in primary biliary cholangitis).

Urine findings

- Bilirubin (bilirubinuria ++) present (conjugated bilirubin is soluble in urine). Urine is yellow because of conjugated bilirubin.
- Urobilinogen absent. This is because conjugated bilirubin in bile does not enter the bowel. Therefore, the intestinal bacteria cannot convert conjugated bilirubin into urobilinogen. There is less reabsorption of urobilinogen (enterohepatic circulation), and less is excreted in the urine.

Liver function tests are discussed in detail on pages 409-11.

Distinguishing Obstructive from Non-obstructive Jaundice Based on Liver Function Tests

Non-obstructive jaundice includes prehepatic and hepatic jaundice, whereas obstructive jaundice is usually posthepatic obstruction **(Fig. 19.2)**.

Serum transaminases: A **significant elevation of serum transaminases** out of proportion to the other liver enzymes indicates a **primarily hepatocellular disorder** (i.e. hepatitis). Alcoholic and other toxic hepatitis almost always are associated with marked elevation of the aspartate aminotransferase (AST) level compared to the alanine aminotransferase (ALT), whereas in viral hepatitis the reverse is usually true.

Serum alkaline phosphatase: Predominant elevation of alkaline phosphatase (ALP) level indicates intrahepatic cholestasis and is usually due to an infiltrative process (e.g., metastatic carcinoma) or drug reaction. **Serum ALP markedly raised in obstructive (posthepatic) jaundice.**

Serum bilirubin: Raised bilirubin level can result from either direct (conjugated) or indirect (unconjugated) causes. Although many liver disorders can produce elevations of both, as a practical matter unconjugated hyperbilirubinemia is due to significant hemolysis.A predominant elevation in conjugated bilirubin favors extrahepatic cholestasis from biliary obstruction.

Laboratory Investigations in Jaundice (Table 19.2)

CC (Skill and Perform): Interpret liver function and viral hepatitis serology panel. Distinguish obstructive from non-obstructive jaundice based on clinical features and liver function tests. Skill assessment: Procedure require certification.

Q. Write short note on laboratory investigations in jaundice.

Distinguishing features between direct and indirect hyperbilirubinemia are presented in **Table 19.3.**

Van den Bergh reaction: It was a colorimetric method of estimation of serum bilirubin level and done to know the nature/type of jaundice. Presently, this method is not used.

Principle: It was a chemical reaction in which serum of patient is mixed with diazo reagent (contains diazotised sulfanilic acid). Bilirubin reacts with

diazotised sulfanilic acid in the diazo reagent to produce purple colored azobilirubin.

Interpretation:

- **Direct positive**: If the serum contains conjugated bilirubin, red color develops immediately and it is called direct positive.

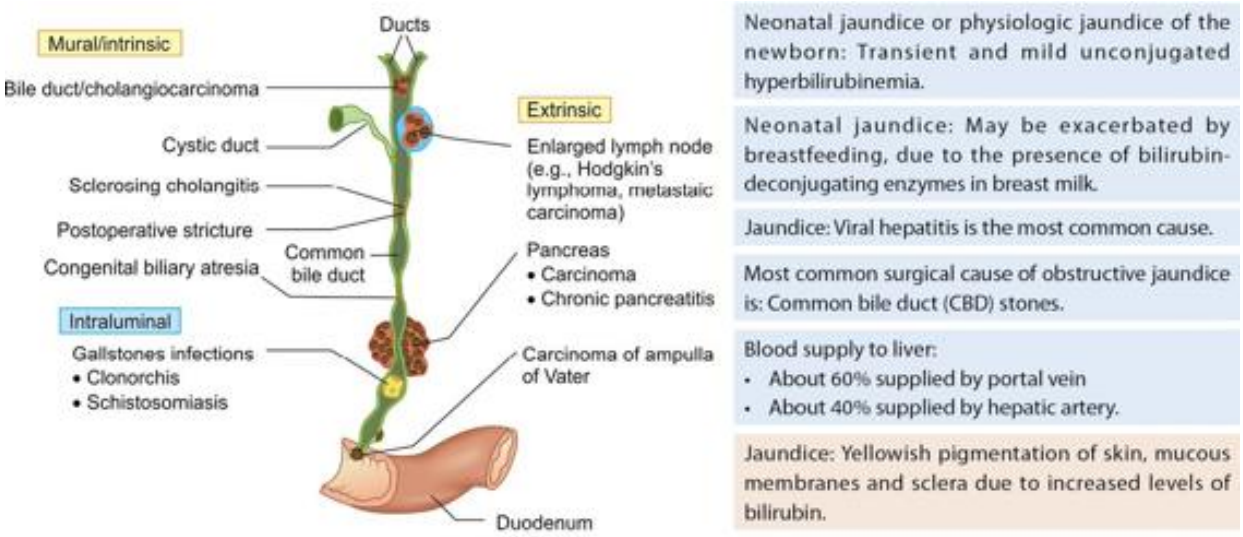


Fig. 19.2: Causes of obstructive jaundice.

TABLE 19.2: Laboratory investigations in jaundice.

Hemolytic Jaundice:

- Show features of hemolysis
- Predominantly unconjugated hyperbilirubinemia. Serum bilirubin is raised (>6 mg %).
- No bilirubin in urine, because the unconjugated bilirubin is not water-soluble and cannot pass into the urine; hence, the term 'acholuric jaundice'.
- Urinary urobilinogen is increased (more than 4 mg/24 hours).
- Other liver function tests (e.g., serum ALP, transferases and albumin) are normal. However, LDH is increased

Hepatocellular Jaundice

- Raised transaminases (AST, ALT): Acute jaundice with AST >1000 U/L is highly suggestive of an infectious cause (e.g., hepatitis A, B), drugs (e.g., paracetamol) or hepatic ischemia
- Imaging
- Liver biopsy

TABLE 19.2: Laboratory investigations in jaundice.

Cholestatic (Obstructive/Surgical) Jaundice

- *Serum findings*
  - Serum bilirubin markedly raised and is predominantly conjugated hyperbilirubinemia
  - Serum ALP markedly raised (3–4 times that of normal)
  - Minimal biochemical changes of liver parenchymal damage
  - Antimitochondrial antibody (in primary biliary cholangitis)
- *Urine findings*
  - Bilirubin present
  - Urobilinogen absent

- In **indirect positive** test, patient's serum is first treated with alcohol and later mixed with diazo reagent. The development of red color, indicates the presence of unconjugated bilirubin in the serum.
- **Biphasic reaction**: If both conjugated and unconjugated bilirubin are present the reaction is termed biphasic reaction.

Jaundice: Occurs when the equilibrium between bilirubin production and excretion is disturbed. Serum bilirubin level is above 2.0–2.5 mg/dL.

During first 2 weeks of life the process of conjugation and excretion of bilirubin is not fully mature.

Unconjugated bilirubin: Insoluble in water and cannot be excreted in urine.

Other classifications of jaundice: (1) Preheaptic, hepatic and post-hepatic and (2) Medical and surgical.

Clinical features and laboratory findings useful in differentiating different types of jaundice are presented in **Table 19.4**.

HEREDITARY HYPERBILIRUBINEMIAS (TABLE 19.5)

Crigler–Najjar Syndrome

Q. Write short note on Criggler–Najar syndrome.

Crigler–Najjar Syndrome Type I

- Rare, **autosomal recessive** disorder due to **complete absence of hepatic UGT1A1**.
- Characterized by **chronic, severe**, **unconjugated hyperbilirubinemia** → produce severe jaundice, icterus and **death** secondary to kernicterus **within 18 months** of birth.
- **Bile** does contain conjugated bilirubin → hence, it is **colorless**.

TABLE 19.3: Distinguishing features between direct and indirect hyperbilirubinemia.

Feature	***Unconjugated bilirubin***	***Conjugated bilirubin***
Definition	It is the bilirubin that is not conjugated in the liver	It is bilirubin diglucuronide formed by conjugation of unconjugated bilirubin with glucuronic acid in the liver
Normal serum level	More	Less (less than 0.25 mg/dL)
Water solubility	Not soluble in water	Soluble in water
Solubility in lipids and alcohol	Soluble in fat and alcohol	Insoluble in fat and alcohol.
Serum albumin binding	High	Low
Presence in bile	Not present in bile	Present in bile
Presence in urine (renal excretion)	Not present in urine (absent)	Not present in urine, but appears when there in high plasma level
van den Bergh reaction	Indirect (total minus direct)	Direct positive
Toxicity	Toxic to the tissues. It can cause brain damage leading to kernicterus (neurologic damage) in infants	Not toxic to the tissues

TABLE 19.4: Clinical features useful in differentiating different types of jaundice.

Feature	***Hemolytic (prehepatic)***	***Hepatocellular (intrahepatic)***	***Obstructive (posthepatic)***
Clinical features			
Jaundice: Color (depth)	Lemon yellow (mild)	Orange yellow (variable)	Greenish yellow (deep)
Pruritus	Absent	Variable	Present
Bleeding tendency	Absent	Present	Present (late)

Anemia	Present	Absent	Absent
Splenomegaly	Present	Variable	Absent (may develop later when cirrhosis develops)
Palpable gallbladder	—	—	May be present
Features of hepatocellular failure	Absent	Present (early)	Present (late)
Laboratory findings			
Conjugated bilirubin	Absent	Raised	Raised
AST or ALT	Normal	Increased	Normal
ALP	Normal	Normal	Raised
Urine bilirubin	Absent	Present	Present
Urine urobilinogen	Present	Present	Absent

(AST: asparate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase)

- Treatment with **phenobarbital** (which induces microsomal enzymes including UGT), has **no effect**.
- **Liver** is **morphologically normal** by light and electron microscopy. Invariably fatal.

Crigler–Najjar Syndrome Type II

- Less severe, nonfatal disorder.
- Partial deficiency of UGT1A1 enzyme.
- Mode of inheritance—autosomal dominant with variable penetrance.
- **Phenobarbital treatment can improve** bilirubin glucuronidation by inducing hypertrophy of the hepatocellular endoplasmic reticulum.

Gilbert Syndrome

- Relatively **common, harmless,** inherited disorder with no clinical consequences.
- Caused **mutations in the UGT1 gene** → leads to inadequate synthesis of the UGT1A1 enzyme (activity is about 30% of normal), a less severe reduction than in Crigler–Najjar syndromes.
- **Autosomal recessive** mode of inheritance.
- Usually asymptomatic.

- **Mild, chronic unconjugated fluctuating hyperbilirubinemia** not associated with functional derangements.

Dubin–Johnson Syndrome

Q. Write short note on Dubin–Johnson syndrome.

Etiology

- Benign autosomal recessive disorder.
- Due to the **complete absence of multidrug resistance protein 2 (MRP2)** → defect in hepatocellular excretion of bilirubin glucuronides across the biliary canalicular membrane.

MORPHOLOGY

- Liver is **darkly pigmented** because of **coarse melanin-like pigmented granules** within the enlarged lysosomes present in the cytoplasm of hepatocyte.
- **Pigment composed of polymers of epinephrine metabolites.**

Clinical Features

- Chronic or recurrent conjugated hyperbilirubinemia.
- Most patients are asymptomatic and have a normal life expectancy.

Rotor Syndrome

- Rare form of asymptomatic **conjugated hyperbilirubinemia**.
- Due to many defects, such as hepatocellular uptake, intracellular binding and excretion of bilirubin pigments.
- Inherited as an **autosomal recessive** trait.
- **Liver is morphologically normal**.

Crigler–Najjar syndrome: Basic abnormality is impaired conjugation of bilirubin.

Dubin–Johnson syndrome: Liver is darkly pigmented due to melanin-like granules of epinephrine metabolites.

TABLE 19.5: Hereditary hyperbilirubinemias.

Hereditary disorder	*Mode of inheritance*	*Defects in bilirubin metabolism*	*Characteristics*
Unconjugated hyperbilirubinemia			
Crigler–Najjar syndrome type I	Autosomal recessive	Absent UGT1A1 activity	Fatal
Crigler–Najjar syndrome type II	Autosomal dominant with variable penetrance	Decreased UGT1A1 activity	Usually mild
Gilbert syndrome	Autosomal recessive	Decreased UGT1A1 activity	Harmless
Conjugated hyperbilirubinemia			
Dubin–Johnson syndrome	Autosomal recessive	Impaired biliary excretion of bilirubin glucuronides	Liver with pigmented granules in cytoplasm, harmless
Rotor syndrome	Autosomal recessive	? Decreased hepatic uptake ? Decreased biliary excretion	Harmless

(UGT: uridine diphosphate–glucuronyl transferase; UGT1A1: UDP Glucuronosyltransferase Family 1 Member A1).

Presence of urobilinogen in urine rules out obstructive jaundice.

HEPATIC FAILURE

CC: Describe the pathophysiology and pathologic changes seen in hepatic failure and their clinical manifestations, complications and consequences.

Hepatic failure is clinical syndrome that is the most severe clinical consequence of liver disease. It occurs when the liver cannot sustain its vital activities and about 80% to 90% of hepatic functional capacity must be lost to produce hepatic failure.

It may develop as acute, chronic, and acute-on-chronic liver failure.

Acute Liver Failure

Definition: Acute liver failure is defined as an acute liver disease that produces encephalopathy (and coagulopathy) within 6 months of the initial liver injury in the absence of pre-existing liver disease. It is the result of sudden and massive hepatic destruction. Acute liver failure is termed as fulminant **liver failure** when the encephalopathy develops within 2 weeks of the onset of jaundice, and as **subfulminant liver failure** when the encephalopathy develops within 3 months.

Causes (Box 19.2): Massive hepatic necrosis in acute liver failure is most commonly produced by:

- **Acute viral hepatitis:** Hepatitis A and B infections.
- **Toxic exposure drugs:** Accidental or deliberate ingestion of acetaminophen (paracetamol).
- Autoimmune hepatitis

BOX 19.2: Mnemonic for causes of acute liver failure.

A: **A**cetaminophen, **a**utoimmune hepatitis, hepatitis **A**
B: Hepatitis **B**
C: **C**ryptogenic, hepatitis **C**
D: **D**rugs/toxins, hepatitis **D**
E: Hepatitis **E**
F: **F**atty liver of pregnancy

Mechanism of hepatocellular necrosis: It may be due to direct toxic damage (e.g., acetaminophen) to hepatocytes, but more often due to combination of toxicity and immune-mediated hepatocyte destruction (e.g., hepatitis virus infection).

MORPHOLOGY

Acute liver failure shows **massive hepatic necrosis**.

Gross

Initially, the liver is enlarged due to swelling and edema produced by inflammation. This is followed by dramatic decrease in the size (becomes small and shrunken) due to destruction/loss of parenchyma.

Microscopy

It shows broad/large regions/zones of parenchymal loss/destruction surrounded by occasional islands of regenerating hepatocytes. Since, it is an acute process scar is not seen.

Clinical Features

It presents first with nausea, vomiting, worsening jaundice, and fatigue. They are followed by the onset of life- threatening encephalopathy, coagulation defects, and portal hypertension associated with ascites. The end result is development of multiorgan failure and death.

Other manifestations of acute liver failure are:

- **Jaundice** (yellow discoloration of the skin) and **icterus** (yellow discoloration of the sclera) due to retention of bilirubin. Jaundice (hyperbilirubinemia) in hepatic failure is mostly conjugated, although unconjugated bilirubin levels may also be increased. Cholestasis increases the risk of life-threatening bacterial infection.

- **Hepatic encephalopathy**: It is due to disorder of neurotransmission in the central nervous system and neuromuscular system. It consists of spectrum of disturbances in consciousness. They range from mild behavioral abnormalities, to marked confusion and stupor, to deep coma and death. The progression of encephalopathy following acute injury may occur over days, weeks, or months. Impaired neuronal function, brain edema are correlated to raised levels of ammonia in blood. Associated fluctuating, neurologic signs include rigidity, hyperreflexia and asterixis.
- **Asterixis**: Asterixis is characteristic sign characterized by the nonrhythmic rapid extension-flexion movement of the head and extremities. This is best seen as "flapping" of the hands when the arms are held in extension with dorsiflexed wrists.
- **Coagulopathy**: Liver produces vitamin K-dependent and vitamin K-independent clotting factors. The production of these coagulation factors is massively decreased in acute liver failure because of impaired synthesis by damaged hepatocytes. This is responsible for coagulopathy. It presents as easy bruising and can lead to life-threatening or fatal intracranial bleeding. Liver is also helping in removing activated coagulation factors from the circulation.The damaged liver is not able to remove the activated coagulation factors and this may sometimes paradoxically produce disseminated intravascular coagulation, thereby further exacerbating the bleeding tendency.
- **Portal hypertension**: It occurs when there is reduced blood flow through the portal venous system. This may be due to obstruction at the prehepatic, intrahepatic, or posthepatic level. Though it can manifest in acute live failure, portal hypertension is more common in chronic liver failure (*refer* page 601). In chronic liver disease, portal hypertension develops over months to years. If portal hypertension develops in acute liver failure within days to weeks, the site of obstruction is usually intrahepatic. Its major clinical consequences are ascites and hepatic encephalopathy.
- **Hepatorenal syndrome**: It is a type of renal failure developing in patients with liver failure. There will be neither intrinsic morphologic nor functional cause for this renal failure. The main renal functional abnormalities include sodium retention, impaired free-water excretion, and decreased renal perfusion and glomerular filtration rate. It presents

with a decrease in urine output and rising blood urea nitrogen and creatinine levels.

Laboratory findings: Initially, the serum transaminase levels are markedly raised into the thousands. As hepatocytes are lost, serum transaminase values level decrease rapidly because of the disappearance of their source, namely —hepatocytes.

Chronic Liver Failure and Cirrhosis

Chronic liver failure follows years or decades of insidious, progressive liver injury.

Causes: These include **chronic hepatitis B, chronic hepatitis C, nonalcoholic fatty liver disease** (NAFLD)**, and alcoholic liver disease. Liver failure in chronic liver disease is usually associated with cirrhosis.**

Cirrhosis is discussed in detail on pages 620 and 623-4. It has to be noted that not all cirrhosis leads to chronic liver failure and not all end-stage chronic liver disease is associated with cirrhosis of liver.

Child–Pugh classification (Child–Pugh score) is a system for assessing the prognosis chronic liver disease primarily cirrhosis. Child–Pugh classification of cirrhosis distinguishes between class A (well compensated), B (partially decompensated), and C (decompensated). It correlates with different histological features histologically.

Clinical Features

In about 40% of patient with cirrhosis, there may not be symptoms until the most advanced stages of the disease. Even at late stages when symptoms occur, the initial symptoms may be nonspecific as anorexia, weight loss and weakness. Later patient may develop signs and symptoms of acute liver failure namely jaundice, encephalopathy, and coagulopathy (discussed under acute liver failure above on page 600). Apart from these symptoms, chronic liver failure may show following additional features. Portal hypertension is more frequent and more complex in chronic liver failure than in acute liver failure. Portal hypertension and its consequences are discussed on pages 625-7. Chronic liver diseases are one of the risk factors for the development of hepatocellular carcinoma (HCC) (*refer* page 634-6).

MORPHOLOGY

Liver failure in chronic liver disease is most commonly associated with cirrhosis (*refer* pages 622 and 623), the cause of which varies.

- **Fibrosis**: In cirrhosis with thin fibrous septa separated by large islands of intact hepatic parenchyma are less likely to develop portal hypertension. In cirrhosis with broad bands of dense scar, dilated lymphatic spaces and with less intervening parenchyma are likely to develop portal hypertension and to end-stage disease.
- **Ductular reactions**: In chronic liver disease, as the disease progresses, there is increase in ductular reactions which is usually most prominent in those with cirrhosis. These ductular reactions are derived from activation and differentiation of stem cells. Ductular reactions may stimulates some amount of the scarring in chronic liver disease and may cause further progression of the liver disease.
- **Regression of fibrosis**: Although uncommon, regression of fibrosis may rarely occur even in a fully established cirrhosis. This is characterized by thinning, compactness and eventual fragmentation of scars. As fibrous septa break apart, adjacent nodules of regenerating parenchyma may coalesce to form larger islands of parenchyma. All livers with cirrhosis show evidence of both progression and regression and their balance depends on the severity and persistence of the underlying disease.

Acute-on-Chronic Liver Failure

It is the **sudden development of signs of acute liver failure in patients suffering from years of stable, well-compensated and advanced chronic liver disease.**

Cause: These patients, usually suffer from well-established cirrhosis with extensive vascular shunting. There are large volumes of functioning liver parenchyma with a borderline vascular supply. Such liver is vulnerable to superimposed, potentially lethal insults. The short-term mortality in these patients is about 50%. The cause sudden decompensation in patient with chronic liver disease is:

- Hepatitis D superinfection in patient with chronic hepatitis B
- Resistance to medical therapy in patients with medically suppressed viral hepatitis B in whom viral mutants arise.
- Ascending bacterial cholangitis in patients with primary sclerosing cholangitis
- Replacement of liver parenchyma by primary or metastatic carcinoma.
- Systemic disorder: e.g., sepsis and its attendant hypotension, acute cardiac failure or a superimposed toxic injury in a patient with well-compensated cirrhosis.

VIRAL HEPATITIS

CC: Describe the etiology and pathogenesis of viral and toxic hepatitis: distinguish the causes of hepatitis based on the clinical and laboratory features. Describe the pathology, complications and consequences of hepatitis.

Q. Classify viral hepatitis. Describe the structure, course of disease and serological markers for hepatitis B virus.

Definition: Viral hepatitis may be defined as viral **infection of hepatocytes** that produces necrosis and inflammation of the liver.

Causes

Q. List the different causative viruses of hepatitis.

Most cases of hepatitis are caused by a group of **five separate, unrelated** viruses that have a particular affinity for the liver known as **hepatotropic virus** (hepatitis viruses A, B, C, D, and E). **All except HBV are RNA viruses**.

- Hepatitis **A and E** cause **infectious** hepatitis and transmitted mainly by the fecal-oral route or ingestion of contaminated water.
- Hepatitis **B, C, and D** cause **serum hepatitis**. They are transmitted mainly by parenteral routes and less commonly by intimate or sexual exposure. They can produce chronic hepatitis, which may progress to cirrhosis and hepatocellular carcinoma.
- **In few cases** of an acute viral hepatitis–like syndrome, the **known hepatitis virus cannot be identified** as etiological agent. They are termed acute non-A, non-B, non-C, non-D, non-E (non-A–E) hepatitis or acute hepatitis of unknown cause.

Q. Write a short note on laboratory diagnosis of acute viral hepatitis.

Hepatitis A Virus

- **Hepatitis A virus** (HAV): It is a **nonenveloped, 27 nm, RNA virus**. The virus replicates mainly in the liver. It has outer capsid protein (HAVAg).

- **Source of infection**: Only source of infection is **acutely infected person**.
  - Virus is excreted in bile → excreted in stool/feces of infected persons for about 2 weeks before the onset of symptoms and then for a further 2 weeks or so.
  - Disease is **maximally infectious just before the onset of jaundice**.

Mode of Transmission

Fecal-oral route by ingestion of contaminated water and foods. HAV viremia is transient. Hence, blood-borne transmission does not occur.

Incubation period: **3–6 weeks** (mean ~ 4 weeks).

Outcome of HAV Infection

HAV cause a mild, **benign, self-limited acute hepatitis**. HAV **does not produce chronic hepatitis or a carrier state** and fulminant hepatitis develops only rarely.

Laboratory and Serological Findings for HAV Hepatitis

Q. Serological markers of hepatitis a with graphic form.

Prodromal Stage

- Serum bilirubin is usually normal.
- **Bilirubinuria** and increased urinary excretion of urobilinogen.
- **Raised serum AST or ALT** precedes the jaundice.

Icteric Stage

- Serum **bilirubin is raised**.
- Serum **AST raised** and maximum levels are reached within 1–2 days after the appearance of jaundice, and may rise above 500 IU/L.
- **Serum ALP** is usually **less than 300 IU/L**.

Diagnosis (Fig. 19.3)

1. **Demonstration of virus in feces:** The virus can be demonstrated during late incubation period and the preicteric phase by electron microscopy.
2. **Detection of antibody:**
   - **IgM anti-HAV (IgM antibody against HAV)**: It appears in blood at the onset of symptoms and is a **reliable marker of acute infection**. It reaches peak levels in 2–3 weeks and disappears after 3–4 months.
   - **IgG anti-HAV:** IgM anti-HAV is followed by the appearance of IgG anti-HAV. It persists for years and **provides a lifelong immunity** against reinfection by HAV.

Prophylaxis

Improved sanitary practices and **prevention of fecal contamination of food and water. HAV vaccine** is effective in preventing infection.

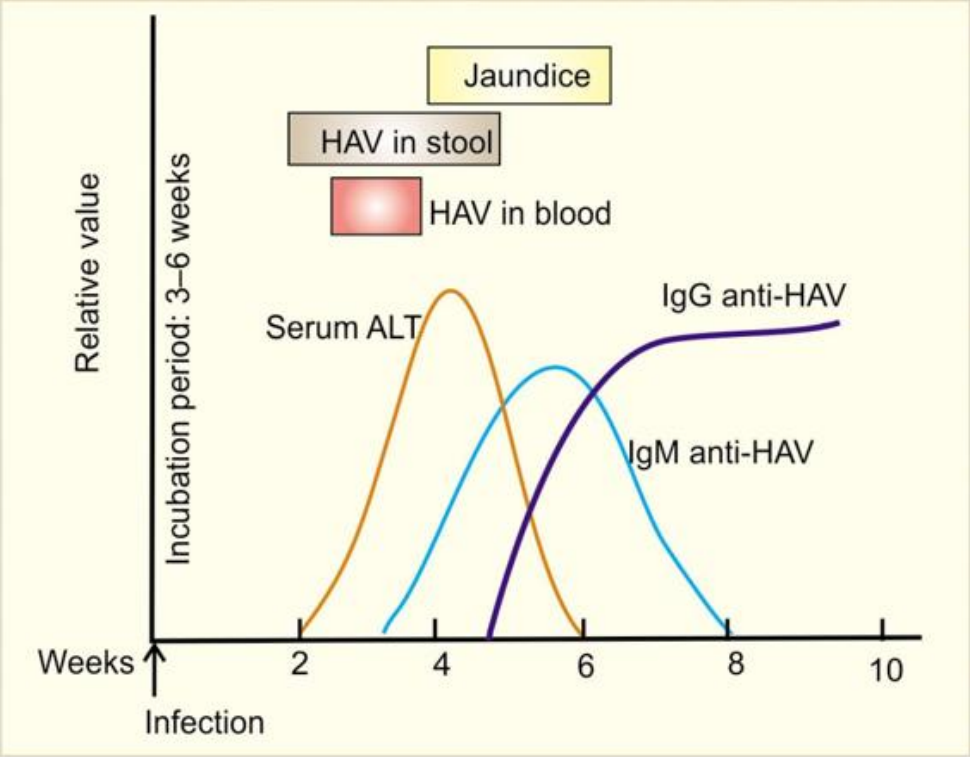


Fig. 19.3: Laboratory and serologic course markers in acute hepatitis A. (ALT: alanine aminotransferase; HAV: hepatitis A virus).

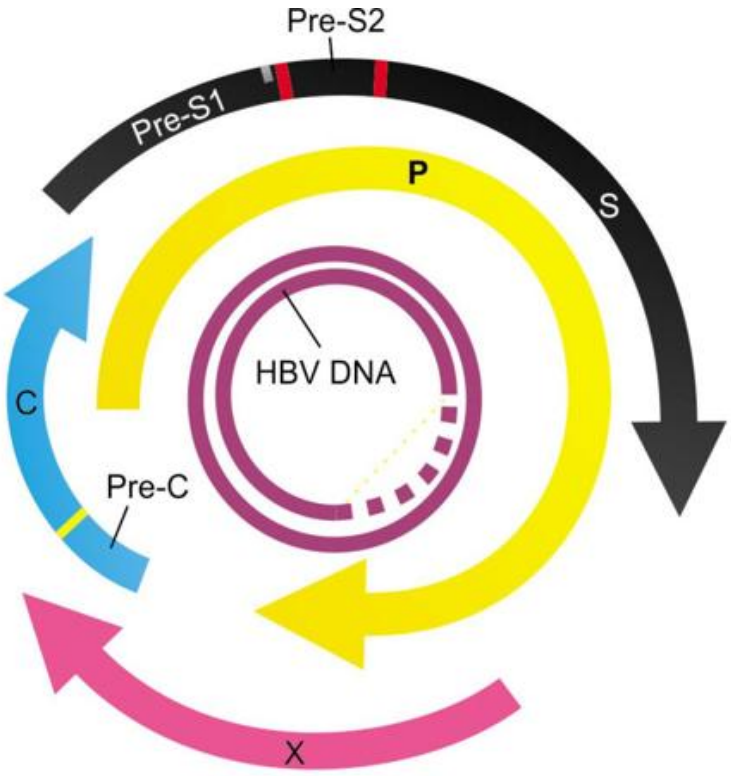


Fig. 19.4: Diagrammatic representation of various genes and respective encoded proteins in HBV. HBV-DNA encodes four proteins, namely—(1) DNA polymerase required for viral replication (P), (2) Surface protein (S), (3) Core protein (C), and (4) X protein.

Hepatitis B Virus

Q. Describe the structure of hepatitis B virus.

Structure of HBV

- Hepatitis B virus (HBV): It is a **hepatotropic DNA virus** belonging to the family Hepadnaviridae.
- HBV **virion**: It is spherical and **double-layered**.
- **Dane particle**: It is the complete viral particle/virion.

Genome of HBV

It consists of partially double-stranded circular DNA and has four genes **(Fig. 19.4)**.

- **HBsAg (S gene): HBsAg, hepatitis B surface antigen** is a product of S gene, which is **secreted into the blood in large amounts**. HBsAg is **immunogenic**.
- **HBcAg (C gene):** The **C** gene produces two antigenically different products:
  - Hepatitis B core antigen (HBcAg): It remains intracellular within the hepatocytes and do not circulate in the serum. Hence, not detectable in the serum of patients.
  - Hepatitis Be antigen (HBeAg): It is secreted into serum and is a surrogate (substitute) marker for high levels of viral replication. It is essential for the establishment of persistent infection.
- **HBV polymerase (P gene): A polymerase (Pol)** is a product of P gene and DNA polymerase enzyme is **needed for virus replication**.
- **HBxAg (X gene): HBx protein** is **necessary for virus infectivity** and has been **implicated in the pathogenesis of liver cancer in HBV infection**.

Source of infection: Human suffering from hepatitis or carrier is the only source of infection.

Mode of Transmission

- **Vertical/congenital transmission**: From mother [who is carrier for HBV (90% HbeAg+, 30% HbeAg–)] to child may occur in utero, **during parturition or soon after birth**.
- **Horizontal transmission**: It is the **dominant mode** of transmission.
  - **Parenteral**:
    - **By percutaneous and mucous membrane:** Exposure to infectious body fluids, through minor cuts/abrasions in the skin or mucous membranes. HBV can survive for long periods on household articles, e.g., toys, toothbrushes and may transmit the infection.
    - **Intravenous route:** Through transfusion of unscreened infected blood or blood products. This mode of spread is rare now, because of routine screening of all blood donors for HBV and HBC. Other modes include intravenous drug abuse with sharing of needles and syringes, tattooists or acupuncturists.
  - **Close personal contact:** Unprotected heterosexual or homosexual intercourse. The virus can be found in semen and saliva.

Incubation period: It ranges from **4–26 weeks**.

Sequelae/Outcome of HBV Infection (Fig. 19.5)

Q. Write short note on sequelae course/outcome of hepatitis B virus infection.

1. **Acute hepatitis** with recovery and clearance of the virus.
2. **Chronic hepatitis**.
3. Progressive chronic disease ending in **cirrhosis**.
4. **Fulminant hepatitis** with massive liver necrosis.
5. Asymptomatic **carrier** state.
6. Hepatocellular carcinoma.

Sequence of Serological Markers for HBV Hepatitis

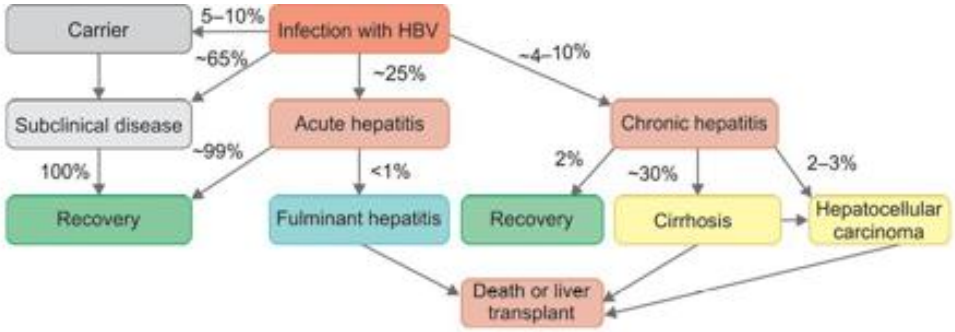


Fig. 19.5: Potential outcomes of hepatitis B infection.

Q. Discuss the laboratory diagnosis (serological markers) of hepatitis B virus infection.

The natural course of the disease can be followed by serum markers **(Figs. 19.6A and B).**

HBsAg

- It is the **first virologic marker**, which appears in serum before the onset of symptoms. It peaks during the disease and becomes undetectable within 3–6 months.
- **Significance**: **Present in the serum in both acute and chronic hepatitis B; indicates an infectious state**. **Loss of HBsAg** plus the development of anti-HBs **denotes recovery**.

Anti-HBs

Q. Mention the antibodies of hepatitis B infection.

- It is antibody to HBsAg and **detectable in serum after the disappearance of HBsAg**.
- **Significance**: Anti-HBs is a **protective antibody** and present in the serum in the recovery phase and in immunity (i.e., vaccination) and **may persist for life providing protection.** This is the basis for current vaccination using noninfectious HBsAg.

HBeAg, HBV-DNA, and DNA Polymerase

- They appear in serum soon after HBsAg.
- **Significance:** Usually not helpful in the diagnosis of hepatitis B, but may be **valuable in assessing prognosis**. They **indicate active viral replication**.
- **HBeAg or HBV- DNA**
  - Their persistence 6 weeks after the onset of symptoms indicates infectivity and probably develop chronic hepatitis B.
  - Their absence is a favorable serologic finding and if associated with appearance of anti-HBe antibodies indicates low infectivity.
- **Anti-HBe** is present in the serum in the recovery phase.

Anti-HBc

- **HBcAg is not found in the serum**. But its antibody, IgM anti-HBc appears in serum a week or two after the appearance of HBsAg. After about 6 months, the IgM anti-HBc antibody is replaced by IgG anti-HBc.
- **Significance**:
  - IgM anti-HBc is the **earliest antibody marker** seen in the serum, long before anti-HBe or anti-HBs.
  - **IgM anti-HBc indicates recent infection** (first 6 months).
  - **IgG anti-HBc indicates remote infection** (beyond 6 months). IgG anti-HBc remains lifelong in the serum and its presence **indicates previous infection with HBV** even when all the other viral markers are not detectable.

HBV DNA by PCR is helpful in confirming the diagnosis.
Serological findings in HBV are summarized in **Table 19.6**.

Prevention

Hepatitis B can be prevented by **vaccination** and by the **screening of donor blood, organs, and tissues**. The **vaccine is purified HbsAg** and induces a protective anti-HBs antibody response.

Hepatitis C Virus

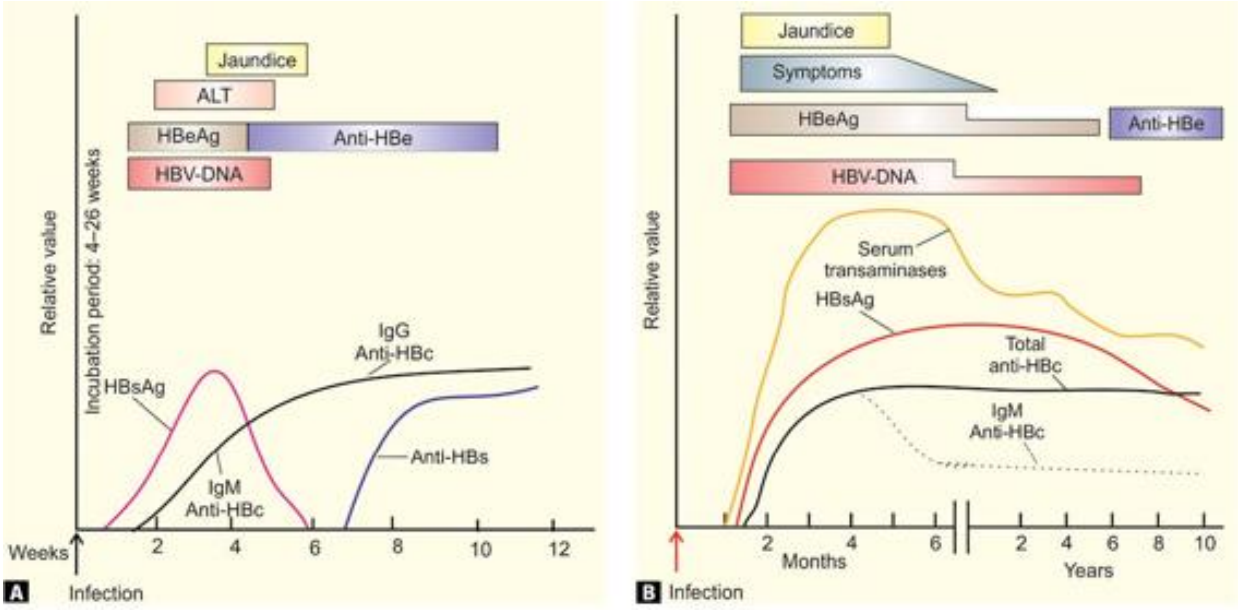
HCV is a **small, enveloped (contains specific proteins)**, **single-stranded RNA virus**. It is a member of the Flaviviridae family. It is the major cause of liver disease.

Mode of spread (Box 19.3): It mainly spreads by the **parenteral route** as a blood-borne infection**.** It may also spread by **sexual contact**. The major route of infection in children is vertical perinatal transmission from the mother.

Features

Incubation period: 2–26 weeks (mean 6–12 weeks).

HCV has several proteases of which one polymerase, namely—viral RNA polymerase has a low fidelity (accuracy/exactness). This makes the viral genome inherently unstable and gives rise to new genetic variants at a high pace. The **emergence of an endogenous, newly mutated strain, its genomic instability and antigenic variability, makes it difficult to produce an effective HCV vaccine.** About six major HCV genotypes have been identified and each with one or more "subspecies." Usually, infections in an individual are caused by a virus of a single genotype, but new genetic variants are generated in the host as long as viral replication persists. As a result, each patient usually comes to be infected with a population of divergent but closely related HCV variants known as *quasispecies*.



Figs. 19.6A and B: Sequence of serologic markers: (A) Acute hepatitis with resolution; (B) Chronic hepatitis caused by HBV.

TABLE 19.6: Summary of serological findings in HBV.

Antigens		***Antibodies***			***Interpretation***
HBsAg	***HBeAg***	***Anti-HBc***	***Anti-HBs***	***Anti-HBe***	
+	+	IgM	-	-	**Acute hepatitis B**, highly infectious
+	+	IgG	-	-	**Chronic infection** or carrier state, **high infectivity**
+	-	IgG	-	+/-	**Chronic infection** or carrier state, **low infectivity**
-	-	-	+	-	**Immunity** following HBV vaccine

Testing for HBeAg, anti-HBe, HBV DNA, and anti-HBs does not help in the diagnosis of hepatitis B but may be valuable in assessing prognosis.

Life cycle of HCV (Fig. 19.7): The proteins in the viral envelop of HCV attaches to the receptor on surface of liver cell. The virus enters into the liver cell through receptor-mediated endocytosis. They release its RNA genome into the cytoplasm of liver cells (hepatocytes). The genome of virus encodes a single polyprotein that is processed by several proteases into 10 functional proteins. The most important of these viral proteins are (i) viral protease (needed for complete processing of the polyprotein); (ii) NS5A (a protein that is required for assembly of HCV into mature virions); and (iii) a viral

RNA polymerase (required for replication of the viral genome). Similar to HIV, knowledge about the life cycle of HCV (viral replication and assembly) has facilitated the development of highly effective anti-HCV drugs for the treatment of HCV infection. **These drugs specifically target the viral protease, RNA polymerase, and NS5A protein, all of which are required for production of virus(Fig. 19.7).** Combination therapy with these drugs (a strategy similar to triple drug therapy for HIV) has proven to be very effective. The goal of current therapy is to eradicate HCV RNA, which is defined by the absence of detectable HCV RNA in the blood 6 months after treatment is stopped. This is associated with a high probability of cure. **Presently, more than 95% of HCV infections are curable**, and this can be expected to improve further as new anti-viral drugs become available. The major disadvantage is that these drugs are very expensive.

BOX 19.3: Most common risk factors for HCV infection [as per the Centers for Disease Control and Prevention (CDC)].

- Intravenous drug abuse
- Multiple sex partners
- Needle stick injury
- Having had surgery within the last 6 months
- Multiple contacts with an HCV-infected individual
- Employment in the medical or dental field

Sequelae/Outcome of HCV Infection

Q. Sequelae of hepatitis C virus infection.

1. **Acute hepatitis**.
2. **Chronic hepatitis:** It **occurs in the majority of individuals infected by HCV.** It can be prevented by screening procedures.
3. **Cirrhosis:** It develops over 5–20 years in 20–30% of patients.
4. Fulminant hepatic failure is rare.

Serum markers HCV hepatitis (Figs. 19.8A and B): The elevated titers of **IgG anti-HCV** after an active infection **do not confer effective immunity**. Diagnosis is confirmed by PCR for HCV DNA.

Hepatitis D Virus

Hepatitis D virus (HDV) is a **defective RNA virus,** which requires depends on HBV for its replication and expression. HDV is found only in HBV carriers. The duration of HDV infection is determined by the duration of HBV infection.

Patterns of HDV Hepatitis

HDV causes **Delta hepatitis** with two clinical patterns.

- **Acute coinfection**: It develops when an individual is **exposed simultaneously** to serum containing **both HDV and HBV**. The HBV infection first becomes established and the HBsAg is necessary for development of complete HDV virions.
- **Superinfection**: It occurs when an **individual already infected with HBV** is exposed to a **new dose HDV**.

Mode of spread: Parenteral route and sexual contact.

Outcome of HDV Infection

- **Coinfection of HBV and HDV**
  - **Acute hepatitis B + D**: It is usually transient and self-limited and clinically similar to acute hepatitis B.
  - **Chronic hepatitis**: It is similar to chronic hepatitis B.
- **Superinfection with HDV in a chronic HBsAg carrier**
  - **Acute hepatitis**: It may be severe in a HBV carrier, or chronic hepatitis B.
  - **Chronic hepatitis**.
  - **Cirrhosis and hepatocellular cancer** (HCC).

Serological Markers of HDV

Q. Write a short note on serological markers of hepatitis D viral hepatitis.

- **HDV RNA:** It is detectable in the blood and liver **before and in the early days of acute disease**.
- **Anti-HDV**: **IgM anti-HDV-most reliable indicator of recent HDV exposure**.

Hepatitis E Virus

- Hepatitis E Virus (HEV) is an unenveloped, RNA virus in the **Hepevirus** genus. Viral particles are 32–34 nm in diameter.
- HEV infection is responsible for more than 30–60% of cases of sporadic acute hepatitis in India.
- Hepatitis E occurs primarily in young to middle-aged adults.

Source of infection: HEV is a **zoonotic disease with animal reservoirs**, such as monkeys, cats, pigs, and dogs. Virions are shed in stool during the acute illness.

Mode of transmission: It is an **enterically transmitted, water-borne** infection.

Incubation period:~6 weeks.

Outcome of HEV Infection

It causes self-limiting acute hepatitis. It does not cause chronic liver disease. But it has a **high mortality rate (about 20%) among pregnant women.**

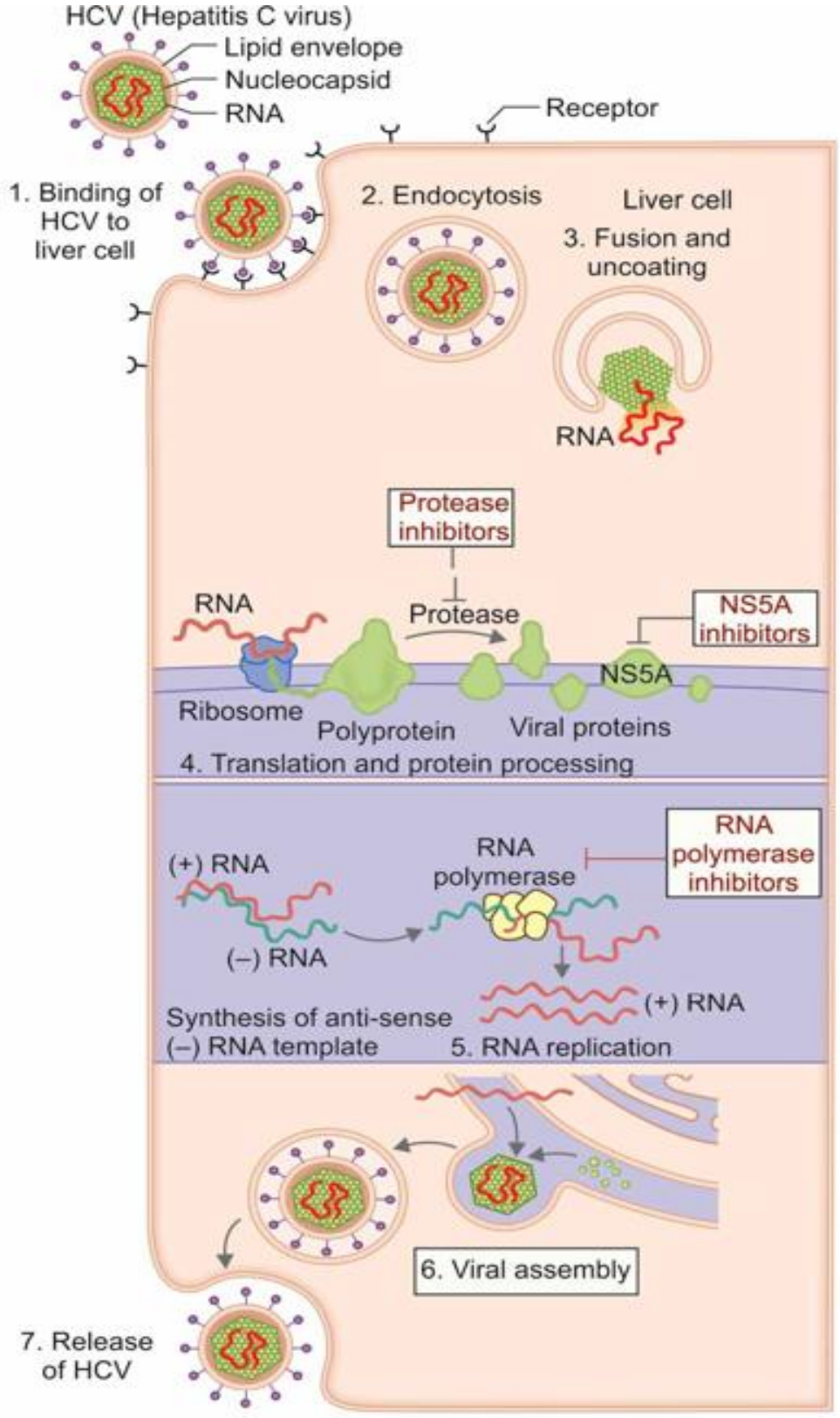
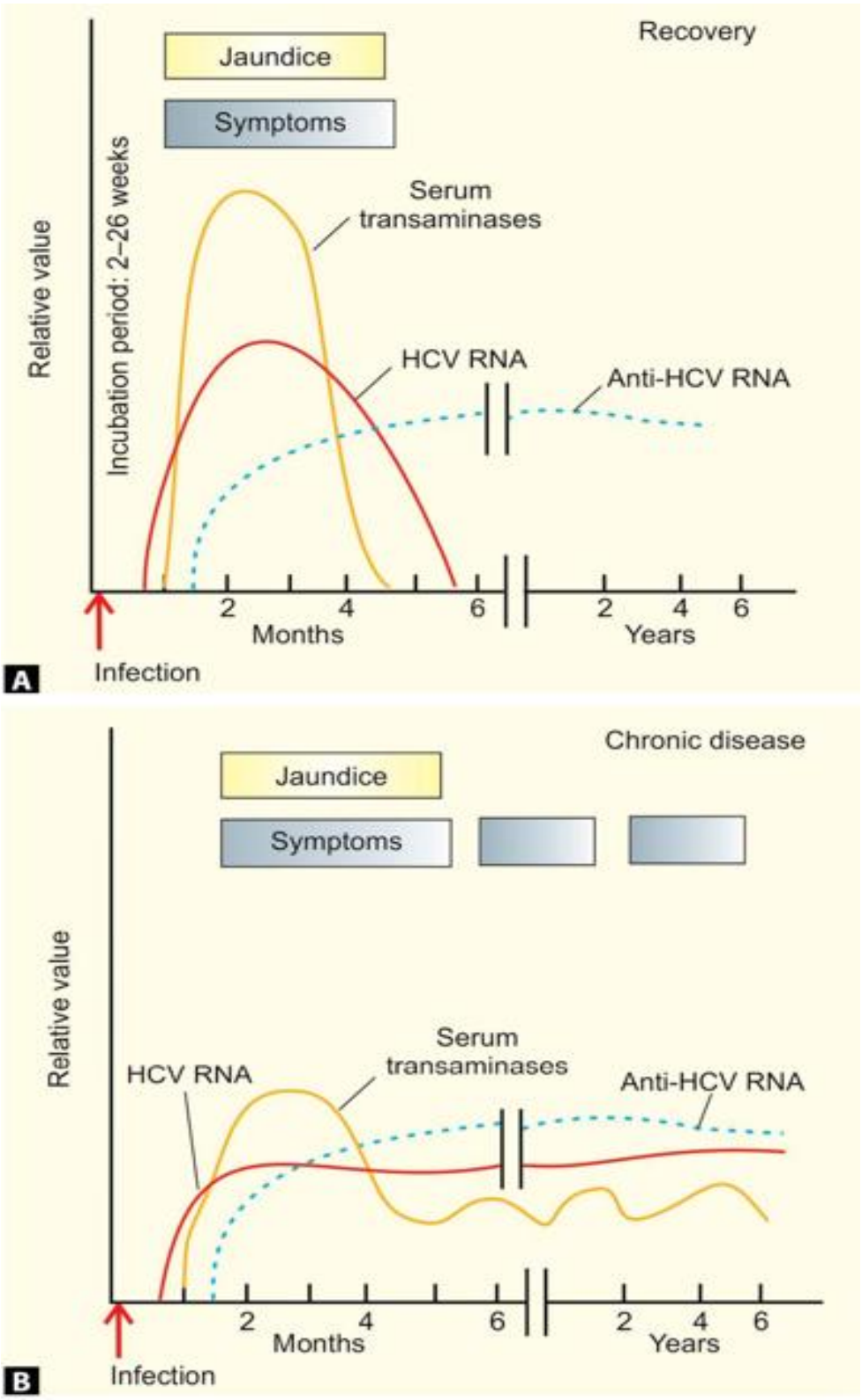


Fig. 19.7: Life cycle of hepatitis C. Various steps are shown along with steps that can be effectively targeted with anti-viral drugs.



Figs. 19.8A and B: (A) Sequence of serologic markers for acute hepatitis caused by HCV; (B) Sequence of serologic markers for chronic hepatitis caused by HCV.

Diagnosis of HEV

- Before the onset of clinical illness, HEV RNA and HEV virions can be detected in stool and serum.
- After the onset of clinical illness, serum aminotransferases rise and elevated IgM anti-HEV titers also occur simultaneous. After recovery, the IgM is replaced with a persistent IgG anti-HEV titer PCR for HEV RNA.

Salient features of hepatitis viruses are presented in**Table 19.7**.

Clinical Features of Viral Hepatitis

- Asymptomatic acute infection with recovery (serologic evidence only).
- Acute hepatitis (anicteric or icteric)
- Fulminant hepatitis with massive to submassive hepatic necrosis.
- Chronic hepatitis (without or with progression to cirrhosis).
- Chronic carrier state.

Acute Asymptomatic Acute Infection with Recovery

It is usually incidentally identified because of mild elevation of serum transaminases or presence of antiviral antibodies.

Acute Hepatitis

It can be caused by any one of four hepatotropic viruses. This can be divided into four phases:

1. **Incubation period** (varies depending on the type of virus).
2. **Symptomatic preicteric phase:** It presents with nonspecific symptoms, such as malaise, nausea, poor appetite, and vague abdominal pain.
3. **Symptomatic icteric phase:** Jaundice and yellow sclera, dark-colored urine (conjugated hyperbilirubinemia), and pruritis (bile salt retention).
4. **Convalescence.**

Investigations

- **Biochemical**: Raised serum bilirubin and aminotransferase levels.
- **Serology:** Hepatitis viral genome in the liver and serum followed by antibodies to viral antigens.

- **Microscopy:** Varying degrees of necrosis of hepatocyte and inflammation (*refer* page 609).
  - Chronic hepatitis: It may be without or with progression to cirrhosis (*refer* page 611)
  - Chronic carrier state (discussed below, pages 608-9).

Fulminant Hepatitis

Fulminant hepatitis with massive to submassive hepatic necrosis (*refer* page 612).

Laboratory Findings in Acute Viral Hepatitis

Liver Function Test

- **Serum aminotransferases (AST, ALT): Raised** and maximum levels are observed during the prodromal phase (400–4000 IU/L). They progressively decline during icteric and recovery phase. Serum ALT is more specific for necrosis of liver cell than serum AST. Serum ALT is the last liver enzyme to return to normal.
- **Serum bilirubin:** When jaundice appears, the serum bilirubin usually rises to levels ranging from 5–20 mg/dL. It may continue to rise despite falling serum aminotransferase levels. Both conjugated and unconjugated bilirubin levels are equally **raised.** Bilirubin levels >20 mg/dL extending and persisting late into the course of viral hepatitis are likely to be associated with severe disease.
- **Serum alkaline phosphatase:** It may be normal or only mildly elevated (less than two times the normal).
- **Serum protein:** Normal

TABLE 19.7: Summary of features of hepatitis viruses.

Type	*Type of virus*	*Route of transmission*	*Mean incubation period*	*Frequency of chronic liver disease*	*Diagnosis*
A	ssRNA	Fecal-oral	2–4 weeks	Never	IgM anti-HAV
B	Partially dsDNA	Parenteral, sexual contact, perinatal	1–4 months	5–10%	HBsAg or antibody to HBcAg
C	ssRNA	Parenteral	7–8 weeks	~80%	Anti-HCV, HCV RNA
D	Circular defective ssRNA	Parenteral	Same as HBV	10% (coinfection); 90–100% (superinfection)	Anti-HDV, HDV RNA; Coinfection—IgM anti-HBc and anti-HDV; Superinfection—IgG anti-HBc and anti-HDV
E	ssRNA	Fecal-oral	4–5 weeks	In immunocompromised hosts only	IgM/IgG anti-HEV

(ss: single stranded; ds: double-stranded DNA; HBcAg: hepatitis B core antigen; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HCV: hepatitis C virus; HDAg: hepatitis D antigen; HDV: hepatitis D virus; HEV: hepatitis E virus)

Less common causes of viral hepatitis:
1. Cytomegalovirus
2. Epstein–Barr virus

Rare causes of viral hepatitis:
1. Herpes simplex
2. Yellow fever

Urine: Bilirubinuria (in early stages), and **increased urinary urobilinogen**.

Hematological Tests

- **Neutropenia and lymphopenia** are transient. These are followed by a **relative lymphocytosis.**
- **Prothrombin time** (PT) measurement is important in acute viral hepatitis. This is because it is prolonged in severe cases with signifies extensive hepatocellular necrosis damage and indicate a worse prognosis.
- **Erythrocyte sedimentation rate (ESR) is raised**.

Serological Findings

Hepatitis A (*refer* page 603 and **Fig. 19.3**), hepatitis B (*refer* page 604 and **Fig. 19.6A**), hepatitis C (*refer* page 606 and **Fig. 19.8**), hepatitis D (*refer* page 607) and Hepatitis E (*refer* page 607).

Liver biopsy: It is not necessary, except when the diagnosis is questionable or when clinical evidence suggests chronic hepatitis.

Carrier State in HBV

Definition: A "carrier" is an individual **who harbors** and **can transmit an organism (HBV)**, but **does not manifest symptoms**.

Factors Determining Carrier State

- **Age at infection:** If infection occurs **in children perinatally** → high rate of developing carrier state. The carrier rate is lowest when adults are infected.
- **Impaired immunity** → carrier state.

Types of Carriers

Carrier state constitutes reservoirs for infection.

- **Inactive carriers (healthy carriers)**: They carry one of the viruses but have no liver disease. They have **normal or** only **mildly raised serum aminotransferase** values **without HBeAg,** but **show anti-HBe**. Majority do not progress to liver disease.
- **Active carriers**: They harbor the viruses and have non-progressive liver damage. They are usually asymptomatic, but show intermittent or persistent **elevation of serum aminotransferase levels**.

MORPHOLOGY

The **liver cells** of HBV carriers show **ground glass appearance** due to HBsAg in the cytoplasm **(Fig. 19.9)**. These cells **stain orange with Orcein stain**.

Morphology of Acute and Chronic Hepatitis

Q. Write a short note on microscopy of acute viral hepatitis.

Q. Morphology of liver in viral hepatitis.

- **Acute versus chronic hepatitis:** Differentiation is by duration and microscopic pattern of cell injury. Hepatitis may be caused by viruses as well as other agents (e.g., drugs, toxins, autoimmune).
- **General morphological features**: Majority of microscopic changes caused by hepatotropic viruses (A, B, C, D and E) are generally similar.

Acute Hepatitis

MORPHOLOGY

Gross

- Normal or may be swollen in acute hepatitis
- **Involvement** may be **diffuse or patchy**
- Cut section muddy-red, mushy with yellow or green discoloration due to jaundice.

Microscopy (Fig. 19.10)

1. **Hepatocyte injury: Ballooning degeneration:** It is seen with mild injury. It is characterized by **swelling of hepatocytes**, empty and pale-stained cytoplasm, with clumping of cytoplasm around the nucleus.

Q. Write a short answer on Councilman body.

Hepatocyte necrosis:

- **Dropout necrosis**: Rupture of the cell membrane of ballooned hepatocytes → leads to cell death and focal loss of hepatocytes → necrotic cell **dropout** → collapse of sinusoidal collagen reticulin framework → **aggregates of macrophage** around necrotic hepatocyte.
- **Acidophilic or apoptotic or Councilman body:** It is caused by anti-viral cytotoxic (effector) T cells. Apoptotic hepatocytes shrink → become **intensely eosinophilic** and have a densely staining **pyknotic or fragmented nuclei**. They may be surrounded by effector T cells. The remnants of apoptotic hepatocytes may be extruded into the sinusoids → appear as **acidophilic or Councilman bodies**.
- **Bridging necrosis (Fig. 19.11):** It is a confluent (uniting band/zone) necrosis of hepatocytes observed in severe acute hepatitis**.** This **band/zone of necrosis** may extend from **portal tract to portal tract**, **central vein to central vein, or portal-to-central regions of adjacent lobules**.

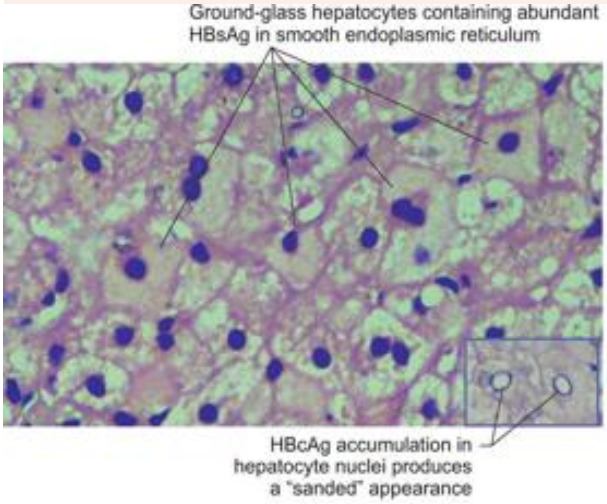
2. **Inflammation:** It involves **all areas** of the lobule and is a **characteristic and prominent feature** of acute hepatitis.
   - **Mononuclear inflammatory cells:** The inflammatory cells in acute hepatitis mainly consists of **lymphocytes and macrophages.**
   - **Lobular hepatitis**: It is **inflammation** involving **liver parenchyma away from portal tract**. Unlike chronic hepatitis, the inflammatory infiltrate is usually not concentrated in portal tracts but is seen throughout the lobule.
   - **Interface hepatitis:** It can **occur in acute and chronic hepatitis** (*refer* page 611).
3. **Kupffer cells:** They show **hypertrophy and hyperplasia** and contain lipofuscin pigment as a result of phagocytosis of hepatocellular debris.
4. **Lobular disarray**: It is due to combination of necrosis of hepatocytes, accompanying regeneration and mononuclear inflammatory infiltrate → disruption of the normal orderly architecture of the liver cell plates. Rarely **cholestasis** may be found, characterized by the bile plugs in canaliculi and brown pigmentation of hepatocytes.

HBV is 100 times as infectious as human immunodeficiency virus (HIV) and 10 times as infectious as hepatitis C virus (HCV).

Four Bs of mode of transmission of HBV:
- **B**lood
- **B**irth
- **B**lood bank
- **B**ody fluids

HBV is not transmitted by breastfeeding.

HBsAg: Persistence after 6 months indicates chronic HBV.



HBsAg: Responsible for ground-glass hepatocytes

Ground-glass hepatocytes: HBV-infected liver cells having finely granular cytoplasm packed with spheres and tubules of HBsAg

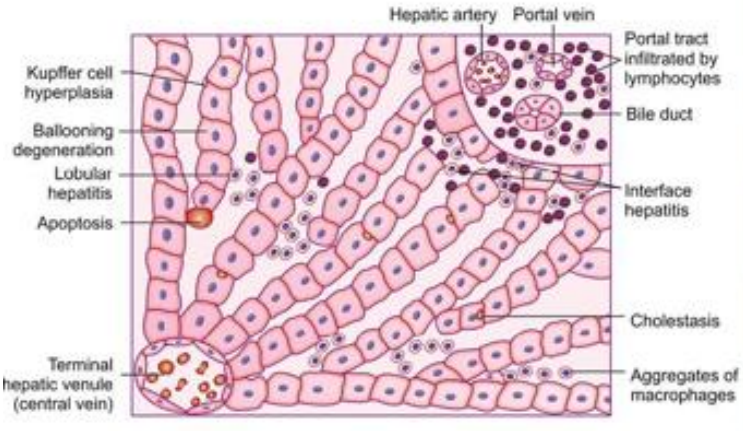
HBcAg gives sanded nuclei appearance

Healthy/inactive carrier of HBV:
- HBsAg+
- IgG anti- HBc+

Viral hepatitis: Infection of hepatocytes by viruses

Hepatotropic viruses: A, B, C, D, and E. All except HBV are RNA viruses

Fig. 19.9: Chronic HBV carrier showing few liver cells with diffuse granular cytoplasm giving rise to ground-glass hepatocytes due to abundant HBsAg in the smooth endoplasmic reticulum. Inset shows hepatocyte nuclei with sanded appearance due to accumulation of HBcAg.



HAV and HEV: Causes acute hepatitis and never causes chronic hepatitis

HBV, HCV and HDV: Can cause chronic hepatitis

HAV: Most common viral cause of jaundice

HAV:
- IgM anti-HAV indicates infection
- IgG anti-HAV indicates recovery/vaccination

HBsAg: Also named Australia antigen, because of its first detection in Australian aborigine

HBsAg: First viral antigen to appear and last one to disappear with recovery

Fig. 19.10: Microscopic features of acute hepatitis.

Carrier state: Presence of HBsAg in the serum for 6 months or more after the initial detection.

Anti-HBs: Protective antibody; develops after recovery/immunization.

HBeAg and HBV-DNA: Infective particles of HBV.

HBcAg: Never appears in the blood.

Marker during window period of HBV: IgM anti-HBc.

Most useful indicator of prior infection with HBV is anti-HBc Ag.

IgG anti-HBc: Present after 6 months of infection.

Two Cs of HCV:
- **C**hronic hepatitis—more often
- **C**irrhosis.

HBV and HCV infections: Increased risk of HCC even in the absence of cirrhosis.

Three Ds of HDV:
- **D**elta agent
- **D**efective virus
- **D**epends on HBV

HCV and HDV: Does not produce protective antibody.

HDV: Defective virus; causes hepatitis with HBV co-infection or superinfection.

Chronic viral hepatitis: Produced by HBV, HCV and HDV.

HEV: High mortality rate (about 20%) among pregnant women.

Three Es of HEV
- **E**ndemic in equatorial regions
- **E**pidemic frequent
- **E**xpectant mother have high mortality

HAV and HEV: Cause only acute hepatitis and never chronic hepatitis.

Phases of acute hepatitis: Preicteric, icteric and convalescence.

Acute hepatitis: Aminotransferase levels peak before the appearance of jaundice.

Acute hepatitis: Serum ALT is the last enzyme to return to normal.

Types of carriers:
- Healthy carriers
- Active carriers

Active/infective carrier of HBV:
- HBsAg+
- HBeAg+
- HBV-DNA+
- IgG anti-HBc+

Inflammation is scattered throughout the hepatic lobule and is termed as "spotty necrosis" or lobular hepatitis. Inflammatory cells: Both in acute and chronic viral hepatitis are mainly T cells.

Councilman bodies: Acute viral hepatitis.

Microscopy of acute hepatitis:
- Hepatocyte injury
- Inflammation
- Sinusoidal cell (Kupffer cells) reactive changes
- Lobular disarray

Chronic hepatitis C: Also shows

- Steatosis (fatty change)
- Lymphoid aggregates in the portal tract
- Bile duct damage.

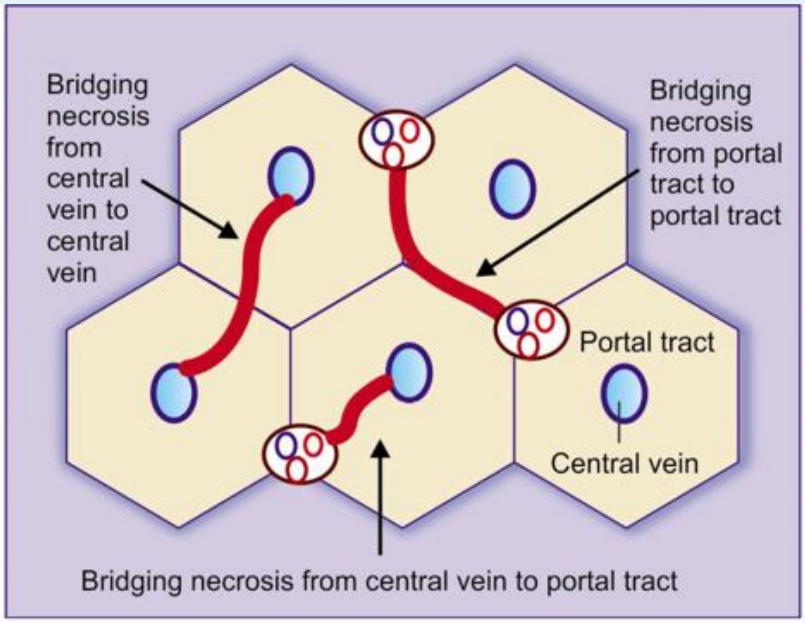


Fig. 19.11: Patterns of bridging necrosis in severe hepatitis.

Bridging necrosis : Band of necrosis from
- Portal tract to portal tract
- Central vein to portal tract
- Portal tract to central vein of adjacent lobule.

CHRONIC HEPATITIS

Definition: Chronic hepatitis is defined as **symptomatic**, **biochemical, or serologic evidence of hepatic disease** for **more than 6 months**. Microscopically, there should be inflammation and necrosis in the liver.

Causes

Hepatitis may be caused by viruses as well as other etiological agents **(Table 19.8)**. The viruses include:

- **HCV**: It is the most common cause of chronic viral hepatitis and mostly asymptomatic.
- **HBV:** Chances of chronic hepatitis is high if the **infection occurs at a younger age**. **Maternal-to-infant transmission** is a major risk factor.
- **HDV+HBV:** Either superinfection or co-infection → chronic hepatitis.

MORPHOLOGY

Q. Write short note on chronic hepatitis.

Gross

- **Early stage**: Liver may appear **normal.**
- **Later stage**: Liver feels **firm** because of increased fibrosis → may progress to macronodular cirrhosis.

Microscopy (Figs. 19.12A and B)

Range from mild to severe hepatitis. Regardless of etiology, it is characterized by a combination of (1) portal inflammation, (2) interface hepatitis, (3) parenchymal inflammation and necrosis, and (4) fibrosis.

1. **Portal inflammation:** In **mild hepatitis,** inflammation is **limited to portal tracts** and predominantly consists of **lymphocytes, macrophages, and occasional plasma cells.**
2. **Interface hepatitis (piecemeal necrosis/periportal necrosis):** It is an **important feature** characterized by **spillover of inflammatory cells** (lymphocytes and plasma cells) **from portal tract into the adjacent parenchyma** at the limiting plate → associated with degenerating and apoptosis of periportal hepatocytes.
3. **Parenchymal inflammation and necrosis:** It is variable in severity but usually spotty. Mononuclear inflammatory cells surround the damaged hepatocytes. **Bridging necrosis** between portal tracts and portal tracts-to-terminal hepatic veins **may be seen**.
4. **Fibrosis**: It is the **hallmark of chronic liver damage.** Continued inflammation and associated necrosis → leads to progressive fibrosis at the limiting plate and enlargement of the portal tract**.**
   a. **Initially:** Fibrosis is seen only in the portal tracts.
   b. **Later**: Periportal septal fibrosis occurs. This is followed by bridging /linking of fibrous septa (bridging fibrosis) between adjacent fibrotic portal tracts (i.e., portal to portal) or portal-central.

Clinical Features

Highly variable.

- **Fatigue:** It is the most common symptom.
- **Other symptoms:** Malaise, loss of appetite, and bouts of mild jaundice.

Physical Findings

These are few, such as spider angiomas, palmar erythema, mild hepatomegaly, hepatic tenderness, and mild splenomegaly.

Laboratory Findings

Laboratory features of chronic hepatitis B cannot adequately distinguish mild form severe hepatitis.

- ***Biochemical investigations:***
  - **Serum aminotransferase: Mild** to moderate **elevation** and may fluctuate in the range of 100−1000 units/dL. ALT (SGPT) tends to be more raised than compared to AST (SGOT). Once cirrhosis develops AST levels exceeds ALT.
  - **Serum alkaline phosphatase:** Normal or only marginally elevated
  - **Serum bilirubin:** May be normal or raised in the range of 3−10 mg/dL.
  - **Serum proteins: Hypoalbuminemia** in severe cases and hyperglobulinemia in severe or end-stage cases
  - **Prothrombin time:** Prolonged in severe or end-stage cases
- ***Serological markers*** *(**Box 19.4** and refer* pages 604-5 and **Fig. 19.6A**).
- ***Urine*** shows increased bilirubin and urobilinogen.

BOX 19.4: Serological markers of chronic hepatitis B.

- Positive HBsAg
- Positive IgG anti-HBc, negative IgM anti-HBc
- Positive HBe antigen or rarely, positive anti-HBe
- Positive HBV-DNA

Consequence

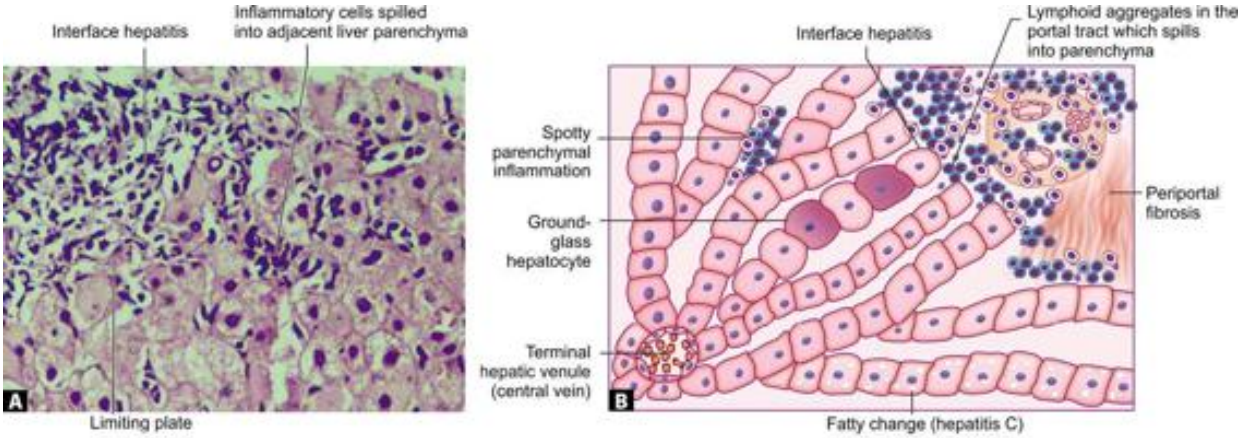
Continued loss of hepatocytes and fibrosis → results in **cirrhosis**. Cirrhosis is characterized by irregularly sized nodules separated by broad fibrous scars and is referred to as **post-necrotic cirrhosis**. It is usually **macronodular or mixed micro- and macronodular** type. The term post-necrotic is not specific and is applied to any cirrhosis in which the liver shows large, irregular-sized nodules with broad scars.

FULMINANT HEPATIC FAILURE

TABLE 19.8: Major causes of chronic hepatitis.

Virus	*Other causes*

TABLE 19.8: Major causes of chronic hepatitis.	
Virus	***Other causes***
• Chronic hepatitis BChronic hepatitis C • Chronic hepatitis D	• Autoimmune hepatitis • Drug-induced chronic hepatitis • Wilson's disease • Cryptogenic hepatitis (non-A–E hepatitis)



Figs. 19.12A and B: Microscopic features of chronic hepatitis: (A) Photomicrograph; (B) Diagrammatic.

Definition: Hepatic insufficiency progresses **within 2–3 weeks** from **onset of symptoms** to hepatic encephalopathy, in patients who do not have chronic liver disease.

Causes

- **Viral hepatitis:** HBV and HAV. Occasionally, HCV and others.
- **Noninfectious causes:** Acetaminophen toxicity.
- Unknown.

Pathogenesis

- It varies depending on etiology. HBV-induced fulminant hepatitis shows massive apoptosis and the factors which predispose to fulminant hepatitis include advanced age and female sex.
- When the destruction is massive, regeneration is disorderly and result in nodular masses of liver cells. Fibrous scarring may lead to cirrhosis.

MORPHOLOGY

Morphological features are almost similar irrespective of the causative agent. It can be subdivided into submassive and massive necrosis.

Gross

- Distribution of liver destruction varies.
- May involve **the entire liver or only random areas**.
- **Massive necrosis: Liver may shrink → 500–700 g.** It appears **shrunken, red covered by a wrinkled, too-large capsule**. **Cut section**, the **necrotic areas appear muddy red, mushy** with areas of hemorrhage.

Microscopy

- **Massive necrosis** of **hepatocytes** in contiguous lobules and the reticulin framework is collapsed in these regions.
- **Minimal inflammatory reaction**.
- If patient survives for several days, there may be inflammatory cells that phagocytose the necrotic cells.
- If the parenchymal framework is preserved, regeneration can completely restore the liver architecture.

Prognosis: The mortality is ~80% without liver transplantation, and ~35% with transplantation.

Chronic hepatitis: Symptomatic, biochemical, or serologic evidence of hepatic disease for more than 6 months.

Basis of present classification of chronic hepatitis:
- Etiology: Cause of hepatitis
- Grade: Histologic activity
- Stage: Degree of progression.

Interface hepatitis: Spillover of inflammatory cells from portal tract into the adjacent parenchyma at the limiting plate.

Scoring systems: Used to assess the severity and progression of liver damage due to HBV and HCV infection. Key elements in these systems are as follows: Inflammation and hepatocyte destruction (grade), and the severity of fibrosis (stage).

Serological markers of chronic hepatitis B:
- Positive HBsAg
- Positive IgG anti-HBc, negative IgM anti-HBc
- Positive HBe antigen or rarely, positive anti-HBe
- Positive HBV-DNA

Chronic infection by HBV and HCV: Increased risk for hepatocellular carcinoma.

DRUG- AND TOXIN-INDUCED LIVER INJURY

Liver is the main organ involved in metabolizing major drugs as well as detoxification, Liver can undergo injury due to many therapeutic and environmental chemical/agents.

Mechanism of liver injury: Liver injury may be due produced by 3 main mechanisms. These include **(i) due to direct cell (hepatocytic) toxicity, (ii) through hepatic conversion of a xenobiotic** (is a chemical substance not naturally produced or expected to be present in humans**)** compound **to an active toxin, or (iii) immune mechanisms** (e.g., drug or a metabolite may act as a hapten that may convert a cellular protein into an immunogen).

Classification of Drug Toxic Reactions

Drug toxic reactions may be classified as **predictable** (intrinsic) or **unpredictable** (idiosyncratic) reactions. Both type of injury may manifest immediately or take weeks to months.

Predictable (Intrinsic) Reactions/Hepatotoxins

The defining characteristics of predictable drug-induced hepatoxicity are as follows:

- The extent of liver injury is dose-dependent manner (e.g., carbon tetrachloride, the mushroom poison phalloidin, the analgesic acetaminophen). It always produces liver cell damage in sufficiently high doses. It affects almost all or most individuals who consume the drug.
- Liver necrosis is zonal and usually, but not always, centrilobular.
- The time duration between the exposure and development of liver cell necrosis is short.

Example: One of the examples for predictable hepatotoxin is **acetaminophen.** Acetaminophen is not a toxic by itself but its toxic metabolite is produced by the cytochrome P-450 system in acinus zone 3 hepatocytes. As these hepatocytes in zone 3 undergoes necrosis, the hepatocytes in the zone 2 take over this metabolic function, and becomes injured. In severe overdoses, the zone of damage extends to the periportal hepatocytes, causing in acute hepatic failure. Acetaminophen may be used as suicidal or may be produce accidental overdoses. Since, the cytotoxicity

depends on the cytochrome P-450 system, it may be upregulated by other agents taken in combination with acetaminophen (e.g., alcohol or codeine).

Unpredictable (Idiosyncratic)Reactions/Hepatotoxins

The liver injury caused by this group of drugs can occur with low frequency (i.e., in rare individuals), irrespective/independent of dose and without obvious predisposition **(idiosyncratic reaction)**. It depends on idiosyncrasies of the host, especially the propensity to mount an immune response to the antigenic stimulus or the rate at which the agent can be metabolized. **Most drug reactions are unpredictable and appear to be due to idiosyncratic reactions.** It develops in individuals with metabolic or genetic predispositions. Some drugs or their metabolites may trigger immunologic reactions in the liver (autoimmune hepatitis).

Examples: ***Chlorpromazine*** causes cholestasis in patients who are slow to metabolize it to an innocuous by-product. **Halothane** (anesthetic agent) and its derivatives can cause a fatal immune mediated hepatitis in some patients exposed to this on multiple occasions.

Diagnosis of Drug- or Toxin-induced Liver Injury

No specific test is available to predict or diagnose drug induced hepatotoxicity. It may be made on the basis of the following, namely:

- Temporal association of liver damage with drug or toxin exposure
- Recovery (usually) upon removal of the inciting drug/agent
- Exclusion of other potential causes.
- **Drug-induced liver injury can mimic any type of liver disease** and its severity may vary from asymptomatic elevations of transaminases to a very serious acute liver failure or chronic liver disease. It is necessary to obtain any history of medications, drugs, potential toxins such as herbal products, dietary supplements, topical applications (e.g., ointments, perfumes, shampoo), and environmental exposures (e.g., cleaning solvents, pesticides, fertilizers) from patients with raised liver enzymes or jaundice.
- **Rule out other liver diseases** (e.g., viral hepatitis, genetic disease, autoimmune hepatitis, alcoholic hepatitis).

- **Liver biopsy is usually of limited value** in the diagnosis of drug injury. This is because, microscopic patterns of acute or chronic drug-induced liver disease overlap with non–drug-related diseases.

Patterns of Drug-induced Liver Disease

Drug toxicities can produce wide variety of microscopic changes similar to those in non-drug-induced liver diseases. However, few drugs show characteristic patterns of liver toxicity. Many drugs and chemicals can produce liver injury. List of the more common drugs and toxins that cause liver injury according to the type of morphologic changes are presented in **Table 19.9.** It has to be noted that a single agent can produce more than one pattern of injury.

ALCOHOLIC LIVER DISEASE

CC: Describe the pathophysiology, pathology and progression of alcoholic liver disease including cirrhosis.

- Chronic and excessive alcohol (ethanol) consumption is **one of the major causes** of liver disease.
- Alcoholic liver disease (ALD) constitutes a **spectrum of disorders** directly related to the excessive alcohol use.
- ALD consists of **three major, distinctive, but overlapping lesions (Fig. 19.13):** (1) Hepatic steatosis (fatty liver), (2) alcoholic hepatitis, and (3) alcoholic cirrhosis.

Metabolism of Ethanol (Fig. 19.14)

The liver is the main organ involved in the ethanol metabolism.

Formation of Acetaldehyde

Ethanol is metabolized to acetaldehyde by three enzyme systems present in the liver, namely—(1) Alcohol dehydrogenases (ADHs), (2) cytochrome P-450 2E1 (CYP2E1), and (3) catalase (least important).

1. **Alcohol dehydrogenase (ADH):** It is present in the cytoplasm of the liver and is the **main enzyme system involved in alcohol metabolism**

at low concentrations.

2. **Cytochrome P-450 2E1 (CYP2E1):** When blood alcohol levels are high, the **microsomal** (present in microsomes) **ethanol-oxidizing system** (MEOS) participates in its metabolism. The enzyme involved is cytochrome P-450 2E1 (CYP2E1 located in the smooth ER). It **can also generate reactive oxygen species**.
3. **Catalase**: It is present in peroxisomes and is of **minor importance**.

Formation of Acetic Acid

Acetaldehyde, **is converted to acetic acid** by acetaldehyde dehydrogenase (ALDH) in the mitochondria. The acetic acid is then **utilized in the mitochondrial respiratory chain.**

Etiology of Alcoholic Liver Disease

TABLE 19.9: Patterns of injury in drug and toxin-induced hepatic injury.

Pattern of injury and morphologic changes	***Examples of drug/toxic agents***
Cholestasis (bile accumulates in hepatocytes and canaliculi)	
• Bland/pure cholestasis, without inflammation	Contraceptive pills, anabolic steroids, antibiotics (e.g., sulfamethoxazole), HAART (Highly active anti-retroviral therapy)
• Cholestatic hepatitis (lobular necrosis and inflammation)	Antibiotics, phenothiazines, statins
Hepatocellular necrosis	
• Spotty necrosis	Methyldopa, phenytoin
• Centrilobular necrosis	Acetaminophen, carbon tetrachloride, toxin of the mushroom *Amanita phalloides*
• Massive necrosis	Acetaminophen, halothane
Chronic hepatitis	Isoniazid
Fatty liver (hepatic steatosis)	
• Large and small fat droplets	Ethanol, corticosteroids, methotrexate, total parenteral nutrition, accidental exposure to carbon tetrachloride
• Microvesicular steatosis (small fat droplet)	Tetracycline, aspirin (Reye syndrome*), HAART, valproate

• Steatohepatitis with Mallory–Denk bodies	Ethanol, amiodarone (antiarrhythmic agent)
Fibrosis (periportal and pericellular) and cirrhosis	Alcohol, methotrexate, enalapril, vitamin A
Noncaseating epithelioid **granulomas**	Sulfonamides, isoniazid, amiodarone
Vascular lesions	
• Sinusoidal obstruction syndrome (veno-occlusive disease): obliteration of central veins	High-dose chemotherapy, bush teas
• Budd–Chiari syndrome (occlusion of hepatic veins)	Oral contraceptives by inducing hypercoagulability
• Peliosis hepatis (blood-filled cavities without endothelial lining)	Anabolic sex steroids, contraceptive steroids, antiestrogen tamoxifen
Mass lesions and altered hepatic morphology	
Nodular regenerative hyperplasia (nodular both grossly and microscopically without fibrosis).	Antimetabolites (e.g., 6-thioguanine), azathioprine
Neoplasms	
• Hepatocellular adenoma	Oral contraceptives, anabolic steroids
• Hepatocellular carcinoma	Alcohol, thorotrast
• Cholangiocarcinoma	Thorotrast
• Angiosarcoma	Inhalation of vinyl chloride, intravenous administration of the radioactive contrast agent thorium dioxide (thorotrast)

*Reye syndrome is a rare (uncommon) acute disease of children characterized by microvesicular steatosis, hepatic failure and encephalopathy. Symptoms usually begin after a febrile illness (e.g., influenza or varicella infection) and may correlate with aspirin intake.

Q. Discuss the pathogenesis of alcoholic cirrhosis.

Risk Factors

They influence the development and severity of alcoholic liver disease.

- **Gender: Females are more susceptible** to ALD than males. Females develop advanced liver disease with substantially less alcohol intake. Estrogen increases gut permeability to endotoxins (gut-derived) and increased production of pro-inflammatory cytokines and chemokines (from macrophages and Kupffer cells) resulting in injury to liver cells.

- **Genetic factors**: Genetic predisposition is likely and **polymorphisms in detoxifying enzymes** (aldehyde-dehydrogenase-ALDH) have been identified.
- **Associated conditions: Iron overload** and **chronic infections with HCV and HBV** can **increase the severity** of ALD and **hasten the progression** of alcoholic liver disease to cirrhosis in chronic alcoholics. Even **moderate alcohol intake increases the risk of cirrhosis and hepatocellular cancer in HCV-infected patients**.

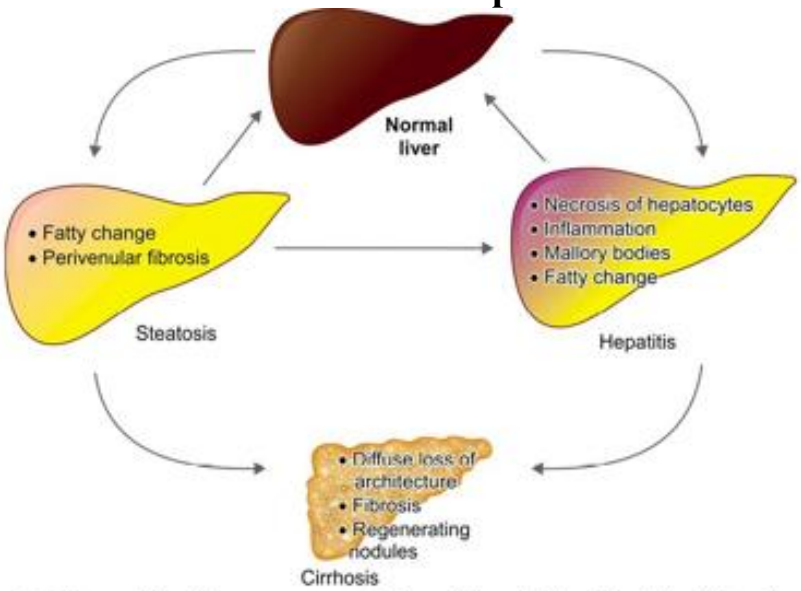
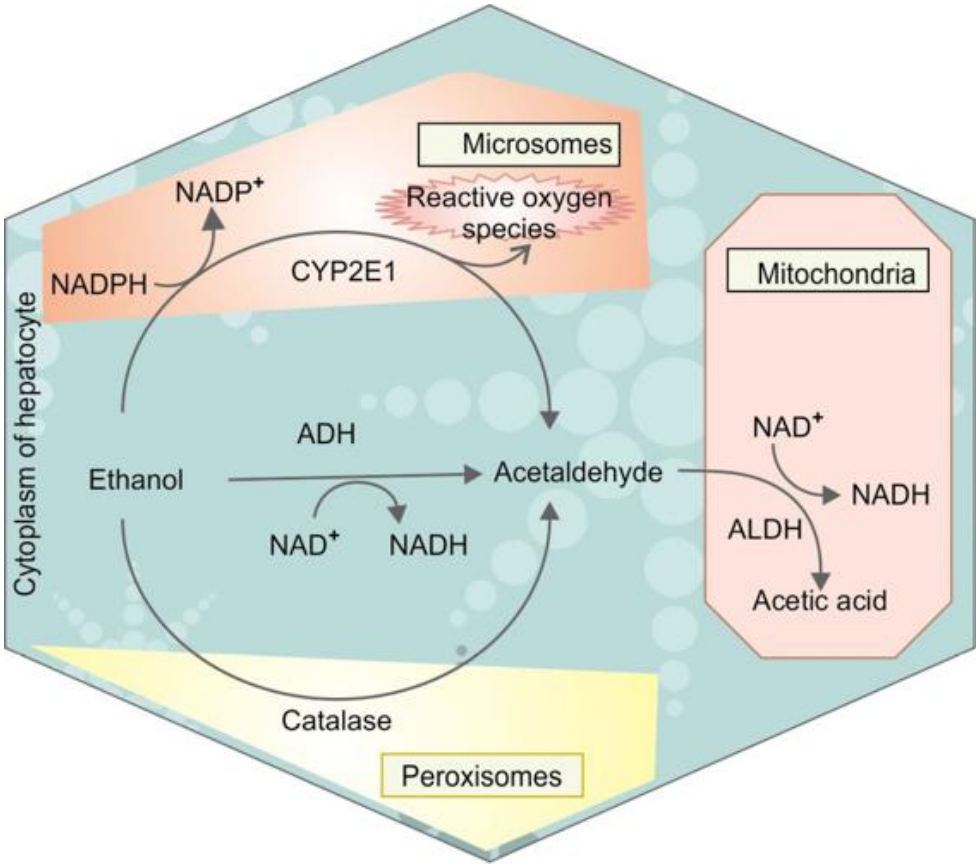


Fig. 19.13: Interrelationships among steatosis, hepatitis, and cirrhosis in alcoholic liver disease.



Causes of post-necrotic cirrhosis: Viral hepatitis, autoimmune hepatitis, hepatotoxins (carbon tetrachloride, mushroom poisoning), drugs (acetaminophen, α-methyldopa), and rarely alcohol.

Cryptogenic cirrhosis: Cause not known.

Fulminant hepatic failure: Hepatotropic virus is the most common cause.

Fulminant hepatic failure:
- Liver shrinks to 500–700 g
- Wrinkled capsule
- Massive necrosis of hepatocytes
- Minimal inflammatory reaction

Fig. 19.14: Ethanol metabolism in hepatocyte. Ethanol is converted to acetaldehyde by three different routes: (1) Most important route is in the cytosol by alcohol dehydrogenase (ADH) (2) in the microsomes (by CYP2E1), and (3) in the peroxisomes (by catalase). Acetaldehyde is oxidized by aldehyde dehydrogenase (ALDH) to acetic acid in mitochondria. Oxidation through CYPs in the microsomes may also generate reactive oxygen species.

($NADP^+$: Nicotinamide-adenine dinucleotide phosphate; NADPH: reduced form of nicotinamide-adenine dinucleotide phosphate;NAD^+: nicotinamide adenine dinucleotide; NADH: reduced form of nicotinamide adenine dinucleotide)

- **Amount and duration of alcohol intake** (drinking patterns): These are the **most important risk** factors. The time required to develop **ALD is directly related to the amount of alcohol consumed**. Consumption of moderate amounts of alcohol is usually not injurious, but excessive amounts causes damage.
  - Short-term ingestion of about 80 g of alcohol (six beers or 8 ounces of 80-proof liquor) over one to several days → produces mild, reversible hepatic steatosis.
  - Daily intake of 80 g or more of ethanol → increases the risk for severe hepatic injury.
  - Daily consumption of 160 g or more for 10–20 years → severe injury.

Pathogenesis of Alcoholic Liver Disease

Q. Write a short note on etiopathogenesis and morphology of alcoholic liver disease.

Pathogenesis of alcoholic liver injury is not completely known. Alcohol is a **direct hepatotoxic** and **its metabolism in the liver initiates several pathogenic process**. About 10–15% of alcoholics develop cirrhosis **(Fig. 19.14)**.

Mechanisms of Liver Injury by Ethanol (Fig. 19.15)

Ethanol is metabolized to a highly reactive and potentially toxic compound namely **acetaldehyde** in the liver**(Fig. 19.14)**. Acetaldehyde **plays an etiologic role in alcoholic liver disease**. The oxidation of ethanol produces several toxic agents and damages the metabolic pathways. The most important mechanisms are discussed below:

1. Oxidative Stress/Reactive Oxygen Species

Metabolism of alcohol in the liver by CYP2E1 in the microsomes produces **reactive oxygen species** (such as hydrogen peroxide and superoxide ions), which **cause lipid peroxidation** of cell membranes leading to injury (*refer* page 19) to hepatocyte.

2. Immune and Inflammatory Mechanisms by Forming Chemical Adducts

Acetaldehyde forms chemical adducts with cellular proteins in hepatocytes and **form neoantigens** → which initiate immune response → cause **cell injury similar to autoimmune-like diseases**.

3. Increased Redox (NADH:NAD^+) Ratio

- This is due to decreased NAD and may occur in both cytoplasm as well as the mitochondria of hepatocytes.
- **Decreased NAD^+ in cytoplasm**: Oxidation of ethanol in the cytoplasm of hepatocytes by ADH decreases the amount of nicotinamide adenine dinucleotide (NAD^+) and increase in NADH (reduced form NAD). Decreased NAD^+ **inhibits oxidation of fatty acid** and leads to **accumulation of fat** in the liver. It may also cause lactic acidosis.
- **Mitochondrial dysfunction:** The **acetaldehyde** formed from ethanol **is converted to acetic acid in mitochondria**. This reaction also increases, causes NADH/NAD^+ ratio in the mitochondria and generates reactive oxygen species (superoxide ions). Normally, antioxidant glutathione is transported from the cytoplasm into the mitochondria and can neutralize oxidants. This transport of glutathione is impaired in alcoholic liver disease. Due to **depletion of glutathione,** the generated reactive oxygen species produce **mitochondrial dysfunction.**

4. Increased Production of Proinflammatory Cytokines

- **Lipopolysaccharide (endotoxin) from gram-negative bacteria**: In the intestinal flora, alcohol causes the release of endotoxin (lipopolysaccharide-LPS) from gram-negative bacteria. **LPS enter** the **portal circulation → stimulates production** of **TNF-α** (tumor necrosis factor α) and other **cytokines** (IL-6, and TGF-α) from macrophages and Kupffer cells → produces **injury to liver cells**.
- **Impaired proteasome function:** Normal function of the ubiquitin-proteasome pathway is to removeirregular and damaged proteins. In

alcoholic cirrhosis, the **function of proteasome is impaired → inefficient degradation of ubiquitin → accumulation** of **large amounts of ubiquitin** in the hepatocytes **in the form of Mallory bodies**. Impaired proteasome function also causes → death of hepatocytes and release cytokines, such as interleukin (IL)-8 and IL-18. IL-8 attracts neutrophils and IL-18 sustains inflammation and causes damage to liver cells.

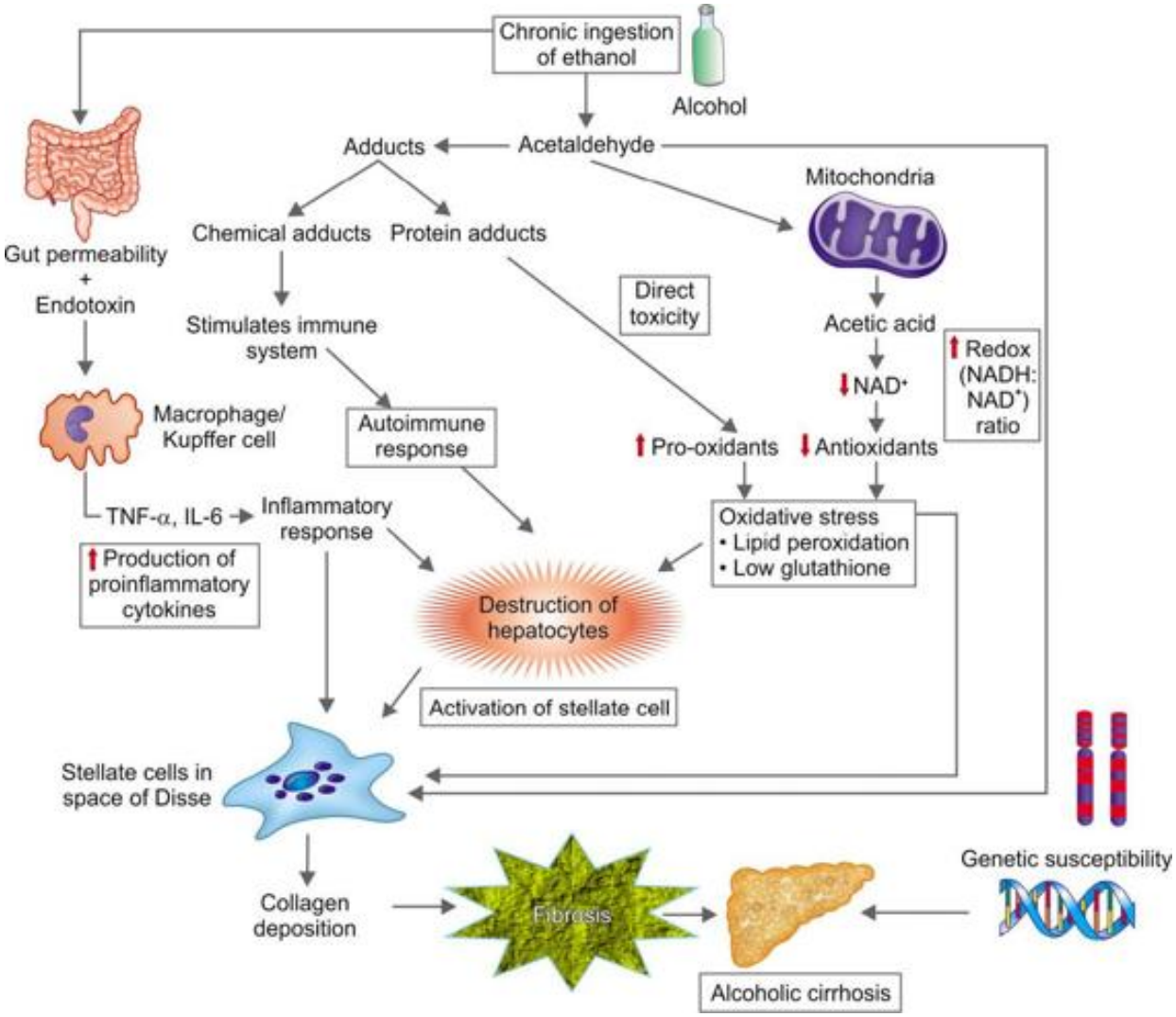
5. Direct Toxicity by Forming Protein Adducts

Acetaldehyde can form adducts with reactive residues on proteins or small molecules (e.g., cysteines) and form toxic molecules which can directly damage the hepatocytes. This is in addition to damage produced by immunological mechanisms mentioned above.

6. Hypoxic Damage

The centrilobular area of the hepatic lobule has the lowest oxygen tension and high susceptibility to hypoxia induced damage. **Chronic alcohol consumption** increases oxygen demand by the liver resulting in a hypoxia of the centrilobular region.

7. Reduced Levels of ADH and ALDH Isozymes



Alcoholic liver disease includes:
1. Hepatic steatosis
2. Alcoholic hepatitis
3. Alcoholic cirrhosis

Risk of ALD:
- Variable and not everyone who drinks heavily will develop alcoholic liver disease
- Cirrhosis develops after 10–15 years

Drinking pattern and liver disease:
- Type of beverage does not affect risk
- Damage is more in continuous rather than binge drinkers

Acetaldehyde: Highly reactive and potentially toxic compound

Fig. 19.15: Pathogenesis of alcoholic liver disease.

The **efficiency of alcohol metabolism** in the **liver depends on** the expression **levels of ADH and ALDH isozymes.** Persons having genetic variants with low ALDH activity cannot oxidize acetaldehyde and cannot tolerate alcohol.

8. Abnormal Metabolism of Methionine

Alcohol also causes impaired hepatic metabolism of methionine, S-adenosylmethionine, and folate. This causes **decreased levels of glutathione and sensitizes the liver to oxidative injury**.

9. Malnutrition and Deficiencies of Vitamins

When alcohol becomes a major source of calories in the diet of an alcoholic, the individual may develop malnutrition and vitamin deficiencies (such as thiamine). Additional factors, such as by impaired digestive function, (due to chronic gastric and intestinal mucosal damage and pancreatitis) may further contribute these defects.

10. Induction of Enzymes

CYP2E1 and other cytochrome P-450 enzymes in the liver are induced by alcohol and increases alcohol catabolism in the endoplasmic reticulum. When alcohol concentration in the blood is high, it competes with other compounds metabolized by the same enzyme system. This increases the **conversion of other compounds like drugs (e.g., acetaminophen) to toxic metabolites.**

Mechanisms of Fibrosis/Cirrhosis (Fig. 19.16)

Stellate cells (Ito cells or perisinusoidal cells) are present in the space of Disse between hepatocytes and sinusoidal endothelial cells. Normally, the stellate cells are quiescent and store vitamin A.

Activation of Stellate Cells

One of the characteristic features of cirrhosis is fibrosis. Alcohol activates hepatic stellate cell → **transformed into highly fibrogenic cells with myofibroblast-like contractile property** → produce collagen (fibrosis) and lose their stored vitamin A. Myofibroblasts are also capable of constricting sinusoidal vascular channels thereby increasing vascular resistance within the liver.

Causes of **stellate cell activation**:

- **Cytokine and chemokine:** It is produced by Kupffer cells, endothelial cells, hepatocytes, and bile duct epithelial cells. For example, transforming growth factor β (TGF-β).
- **Inflammatory cytokines:** These are produced by chronic inflammation and include: Tumor necrosis factor (TNF), lymphotoxin, interleukin 1β (IL-1β), and lipid peroxidation products.
- **Oxidative stress.**

Mechanisms of Steatosis in Chronic Alcoholism (refer Fig. 2.9)

Alcohol is a hepatotoxin and **steatosis is** the **reversible** manifestation of chronic alcoholism. Mechanisms by which alcohol causes steatosis of liver are (*refer* page 23):

1. Increases the catabolism of fat in the peripheral tissues (lipolysis) and increases delivery of free fatty acids to the liver. Most of the fat deposited in the liver is derived from the diet.
2. Increases the synthesis of fatty acid in the liver.
3. Decreases the oxidation of fatty acids by mitochondria.
4. Increases the production of triglycerides.
5. Impairs the assembly and secretion/release of lipoproteins.

MORPHOLOGY

Hepatic Steatosis (Fatty Liver)

Gross: The **liver is enlarged** (can cause massive enlargement of the liver and may weigh about 4–6 kg), **soft, yellow and greasy** (***refer*** **Fig. 2.10**).

Microscopy (Figs. 19.17 and *refer* Fig. 2.11)

1. **Microvesicular steatosis (Fig. 19.17A):** It is the **accumulation of small, clear vacuoles of lipid within the cytoplasm of hepatocytes**. It is the initial and most common histologic response. It affects **acinar zone 3 (perivenular/centrilobular region of lobule)** where alcohol dehydrogenase (the major enzyme responsible for alcohol metabolism) is located and hence, affected first. The nuclei of affected hepatocytes are centrally located and cytoplasm looks foamy.
2. **Macrovesicular steatosis (Fig. 19.17B):** It **develops with continuing alcohol ingestion**. This creates **clear large, lipid vacuole/s** (single or multiple), which **compress and displace the hepatocyte nucleus to the periphery** of the cell. However, with the cessation of alcohol drinking fatty change are **completely reversible** and liver returns to normal.
3. **Absence of inflammation or fibrosis**: There is usually neither inflammation nor fibrosis, but continued alcohol intake leads to **fibrosis around the terminal hepatic veins (perivenular)** and adjacent to sinusoids.

Alcoholic Hepatitis (Alcoholic Steatohepatitis)

Q. Write short note on morphology of alcoholic hepatitis.

Alcoholic hepatitis **may be a precursor** to the development of **cirrhosis**.

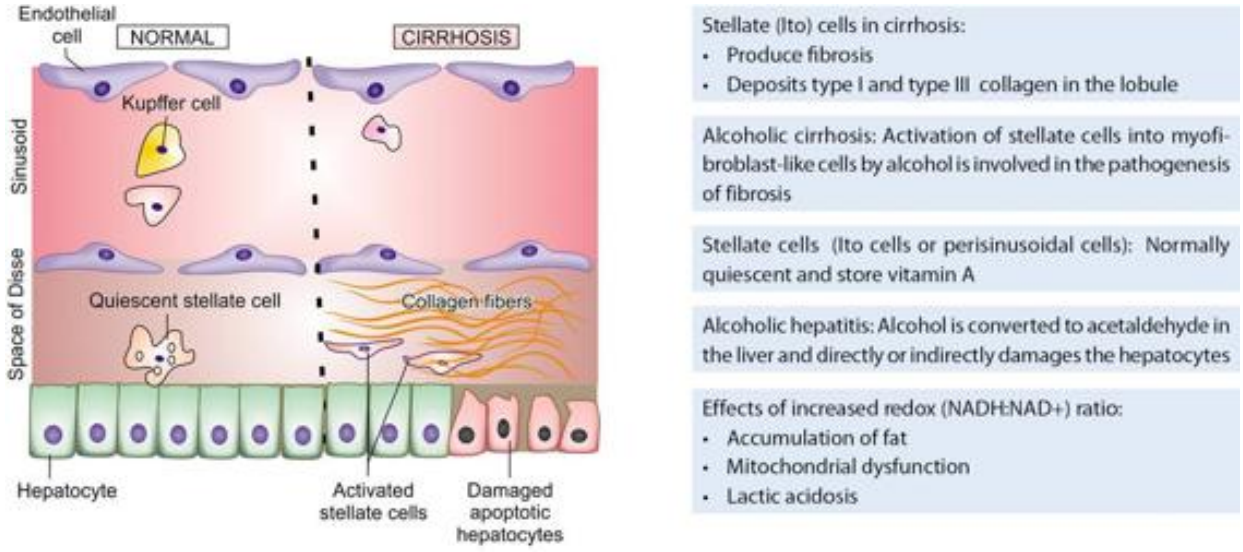
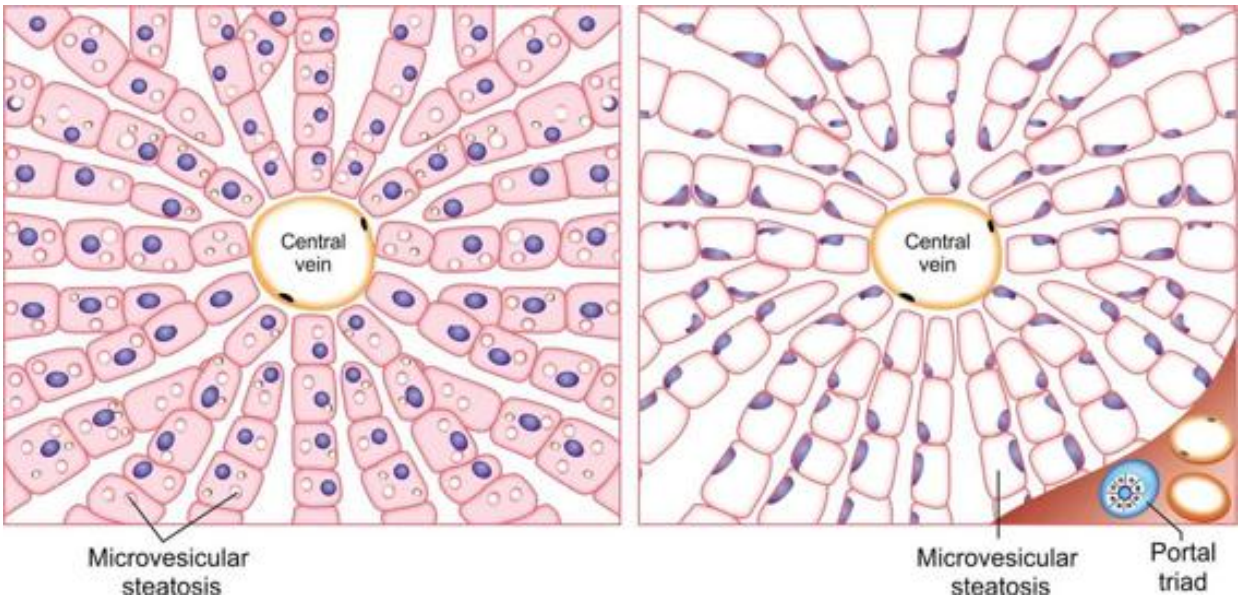


Fig. 19.16: Pathogenesis of fibrosis in cirrhosis of liver. Activation of the stellate cell is followed by proliferation of fibroblasts and the deposition of collagen in the space of Disse.



Figs. 19.17A and B: Hepatic steatosis: (A) Diagrammatic appearance of microvesicular steatosis; (B) Macrovesicular steatosis.

MORPHOLOGY

Gross

The liver may be **enlarged**; **yellow** due to steatosis and **firm** due to increased fibrosis.

Q. Write a short note on Mallory bodies, its microscopic appearance and conditions associated with Mallory body.

Microscopy (Figs. 19.18A and B)

Alcoholic hepatitis has four characteristic features and the lesions are predominantly centrilobular.

1. **Ballooning degeneration of hepatocyte:** It is characterized by **swollen liver cells** (hepatocytes) having pale-stained, finely granular or clumped cytoplasm. This is **due to accumulation** of **fat, water and proteins.** It is predominant in centrilobular region (zone 3 of acinus). Severe ballooning degeneration may lead to → hepatocyte necrosis.
2. **Mallory bodies (Mallory–Denk bodies/Mallory hyaline):** They consist of **tangled skeins of cytokeratin intermediate filaments** (**such as cytokeratin 8 and 18).** They appear as **dense, eosinophilic ropey cytoplasmic inclusions/clumps**, usually situated in a **perinuclear** location, in the degenerating hepatocytes **(Figs. 19.18** and *refer* **Fig.**

2.24A). Mallory bodies are a **characteristic but not specific** feature of alcoholic liver disease.

3. **Neutrophilic infiltration**: They are commonly seen **around ballooned hepatocytes**, particularly those containing Mallory bodies. The **portal tracts** may be infiltrated by **lymphocytes and macrophages** but is not a prominent feature.
4. **Alcoholic steatofibrosis:** Alcoholic hepatitis may activate sinusoidal stellate cells and portal fibroblasts. This produces fibrosis which initially starts as sclerosis of central veins followed by perisinusoidal fibrosis in the space of Disse of the centrilobular region. The fibrosis spreads further outward to the periphery of lobule, enclosing single or small groups of hepatocytes in a chicken wire fence pattern. In late stages, fibrosis may link fibrous tissue from central vein to portal tracts. Eventually, nodularity may develop leading to cirrhosis.
5. **Variable degree of steatosis**: Described earlier under steatosis.

Features of alcoholic cirrhosis are discussed below:

Alcoholic Cirrhosis

Q. Define and classify cirrhosis. Describe the morphological features (pathology) and complications of alcoholic cirrhosis.

Q, Write short note on morphology of alcoholic cirrhosis.

Cirrhosis is **chronic**, **irreversible** and **end-stage** of alcoholic liver disease. It is characterized by diffuse loss of architecture with fibrosis and parenchymal nodular regeneration. Usually evolves slowly and insidiously. It is also known as **Laennec cirrhosis, portal cirrhosis and nutritional cirrhosis**.

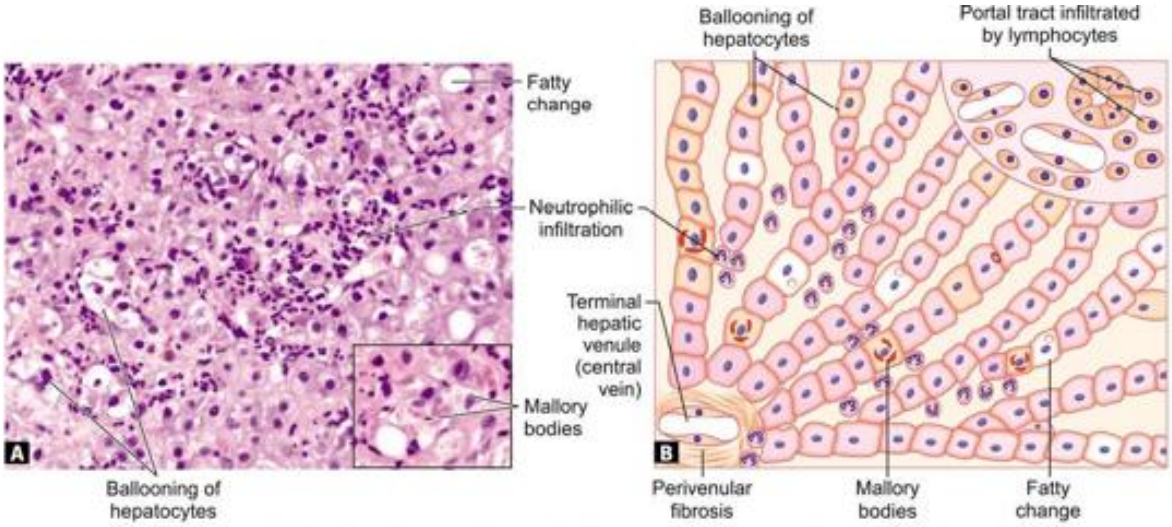
MORPHOLOGY

Gross

Early stage: Liver appears **yellow tan, fatty, and enlarged**, usually weighs above 2 kg.
Late stage (Figs. 19.19A and B): Liver appears brown, nonfatty (loses fat), **diffusely nodular** and **firm.** The **size** of liver progressi-vely becomes **smaller** than normal **(shrunken).** The weight may be sometimes less than 1 kg.

- **Capsular surface** of liver is **nodular** or may have a pig-skin texture.
- As it advances, the nodularity becomes more prominent and nodules are less than 3 mm (**micronodular**) → **"hobnail"** appearance on the surface of the liver.

- With time, the nodules may coalesce to form larger nodules and forms a **mixed micronodular and macronodular pattern**.
- End-stage alcoholic cirrhosis resemble cirrhosis due to other causes and the underlying etiology cannot be determined by both gross and microscopic examination.



Figs. 19.18A and B: Alcoholic hepatitis showing ballooning degeneration of hepatocytes, fatty change, Mallory bodies and neutrophilic infiltration: (A) Photomicrograph and inset shows Mallory bodies; (B) Diagrammatic.

Microscopy (Figs. 19.20A to C)

1. **Loss of architecture:** The hepatocyte injury and consequent fibrosis involves the entire liver → diffuse loss of the architecture.
2. **Regenerating nodules**:
   - The liver cell damage and fibrosis → stimulate the surviving hepatocytes to regenerate and proliferate → form regenerating nodules surrounded by fibrous septa. The regeneration nodules lack the normal structure of the liver lobules or acini.
   - Initially, the regenerating nodules are uniform with diameters less than 0.3 cm and are called as **micronodules.**
   - Later wider bands of fibrosis create nodules larger than0.3 cm and are called **macronodules.**
   - Finally, the liver shows **mixed micronodular and macronodular pattern. Mallory bodies** are usually **not seen** at this stage.
3. **Fibrosis:** It is initially delicate and extend through sinusoids from **central-to-portal regions as well as from portal tract to portal tract.** Later, there are **broad bands of fibrosis**.
4. **Vascular reorganization:** The parenchymal damage and fibrosis disrupt the vascular architecture of the liver. New vascular channels will be formed in the fibrotic septa, which connect the vessels in the portal region (hepatic arteries and portal veins) to terminal hepatic veins, shunting blood from the parenchyma.

Clinical Features

Hepatic Steatosis (Fatty Liver)

It may produce hepatomegaly and there may be mild elevation of serum bilirubin, alkaline phosphatase and g-glutamyl transpeptidase (GGTP). Alcohol withdrawal and with the provision of an adequate diet, the liver can return to normal.

Alcoholic Hepatitis

It appears acutely, usually following a bout of heavy drinking. Symptoms may be nonspecific, such as malaise, anorexia, weight loss, upper abdominal discomfort and tender hepatomegaly. Repeated bouts of alcoholic hepatitis may lead to cirrhosis in about one-third of patients within a few years. Alcoholic hepatitis may be superimposed on cirrhosis.

Alcoholic Cirrhosis

Symptoms are similar to other forms of cirrhosis (*refer* pages 625-7).

Laboratory Findings

Alcoholic Steatosis

Biochemical findings:

- Moderately **raised ALT and aspartate aminotransferase (AST) with AST: ALT >1.**
- γ-GT (gamma glutamyl transpeptidase) level is a sensitive test to determine whether the individual is taking alcohol. There is increased synthesis of this due to induction of the smooth endoplasmic reticulum by alcohol.

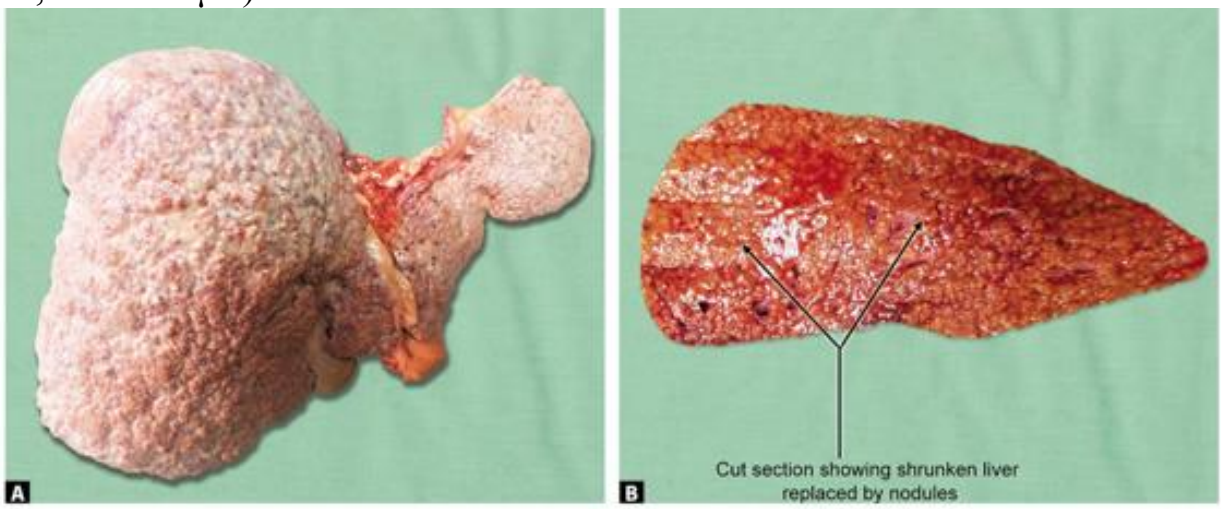
Liver biopsy: It shows accumulation of fat in perivenular hepatocytes and later in entire hepatic lobule.

Alcoholic Hepatitis

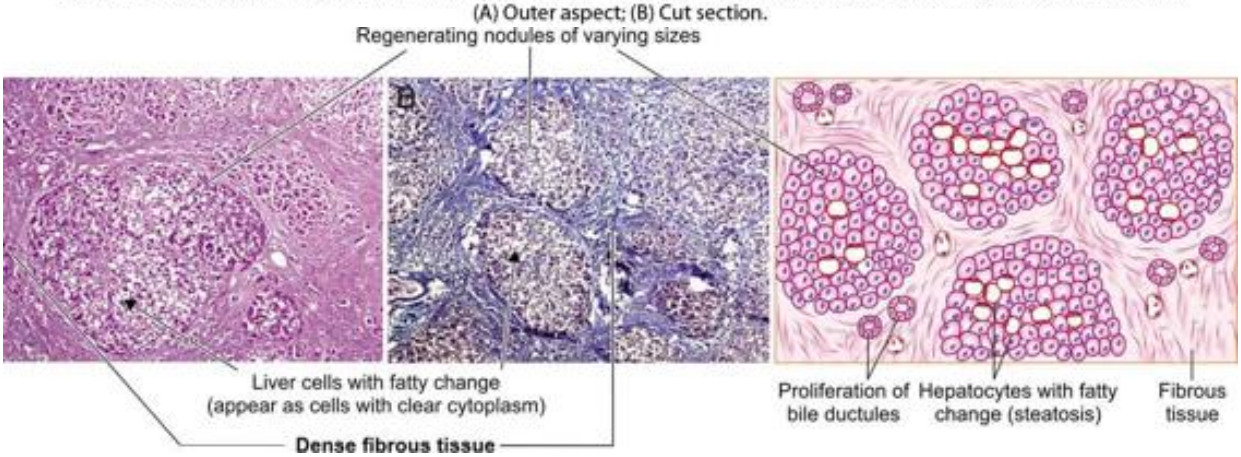
Biochemical findings:

- **Serum aminotransferase (AST and ALT) raised** to 2–7 times of normal (usually <400 IU). **AST: ALT ratio is >2** (two times greater increase in serum AST than ALT). This is because AST is located in the mitochondria, and alcohol damages the mitochondria in hepatocytes.
- **Serum bilirubin**: Raised
- **Serum alkaline phosphatase**: Mildly elevated
- **Serum albumin**: Decreased
- **Prothrombin time**: Prolonged

Hematological findings: Absolute neutrophilic leukocytosis (12,000 to 14,000 cells/μL).



Figs. 19.19A and B: Gross appearance of alcoholic cirrhosis showing diffuse replacement of parenchyma by micronodules: (A) Outer aspect; (B) Cut section.



A
B
C

Figs. 19.20A to C: Microscopic appearance of alcoholic cirrhosis: (A) Photomicrograph; (B) Masson trichrome; (C) Diagrammatic showing abnormal regenerating nodules of varying sizes separated by

fibrous tissue. In B, fibrous tissue appears blue (Masson trichrome).

Urine: Bilirubin (**bilirubinuria)** and urobilinogen are moderately elevated.

Alcoholic Cirrhosis

Investigations are helpful for assessing the severity and type of liver disease.

Liver function tests

- **Hyperbilirubinemia**: Due to raise in both conjugated and unconjugated bilirubin. Not very common with cirrhosis. Could suggest superadded hepatitis, hepatocellular carcinoma, congestive or obstructive etiology.
- **Serum proteins:** Show **reversal of A:G ratio.**
  - **Serum albumin is decreased** (hypoalbuminemia) and is due to reduced synthesis by liver.
  - **Serum globulin is increased** (hyperglobulinemia) due to stimulation of reticuloendothelial system.
- **Serum transaminases:**
  - **AST (SGOT) is raised**
  - **ALT (SGPT) is raised** and usually less than 300 units/dL.
  - **AST:ALT ratio: More than 2 in alcoholic cirrhosis** and **less than 2 in cirrhosis complicating viral hepatitis**.
- **Alkaline phosphatase:** It may be slightly elevated.
- **Prothrombin time: Prolonged** due to reduced synthesis of clotting (especially vitamin K-dependent) factors.

Hematological tests:

Peripheral blood picture: **Anemia and acanthocytosis** (spur-like projections on RBC), **leukopenia and thrombocytopenia** (due to hypersplenism and bone marrow suppression by alcohol).

Serological markers: For hepatitis B and C.

Other biochemical markers:

- **Blood ammonia** estimation: It is a reliable investigation when hepatic encephalopathy is suspected. Raised blood ammonia is due to:
  - Decreased clearance by liver
  - Shunting of portal venous blood to systemic circulation.
- **Serum electrolytes**: Low sodium (hyponatremia) indicates severe liver disease and is due to either defect in free water clearance or to excess diuretic therapy. Others findings include hypokalemia, hypomagnesemia, hypophosphatemia.

Hepatic steatosis:
- Microvesicular
- Macrovesicular
- No inflammation
- No fibrosis

Panlobular micro- and macrovesicular steatosis indicates alcohol as etiology.

Alcoholic hepatitis:
- Ballooning degeneration
- Mallory bodies
- Neutrophilic infiltration
- Steatosis
- Perivenular fibrosis

Mallory bodies: Seen in
- Alcoholic hepatitis
- Nonalcoholic fatty liver disease (NAFLD)
- Primary biliary cirrhosis (PBC)
- Wilson disease
- Chronic cholestatic syndromes
- Hepatocellular tumors

Mallory bodies: Tangled skeins of cytokeratin intermediate filaments (such as cytokeratin 8 and 18).

Alcoholic cirrhosis:
- Loss of architecture
- Fibrosis
- Regenerating nodules

AST/ALT >2 is highly suggestive of alcohol as the cause of liver disease.

Causes of death in alcoholic liver disease
- Hepatic coma
- Massive gastrointestinal hemorrhage
- Intercurrent infection
- Hepatorenal syndrome
- Hepatocellular carcinoma

NONALCOHOLIC FATTY LIVER DISEASE

- **Nonalcoholic fatty liver disease (NAFLD) is a common disease in which fatty liver develops in individuals who do not drink alcohol.** The liver changes may be any of the three types, namely—(i) steatosis, (ii) steatohepatitis (usually inflammation is less prominent than in alcoholic liver disease), and (iii) cirrhosis.
- **Nonalcoholic steatohepatitis (NASH):** The term is used when there is clinical features of liver injury (e.g., elevated transaminases), and the histological features of hepatitis.

Etiopathogenesis (Fig. 19.21)

Q. List four abnormalities associated with nonalcoholic fatty liver disease (NAFLD).

Increased prevalence of NAFLD is seem in **association with insulin resistance** and the **metabolic syndrome**. Other commonly associated abnormalities with NAFLD are:

1. Type 2 diabetes (or family history of diabetes).
2. Obesity, primarily central obesity.
3. Dyslipidemia (hypertriglyceridemia, low high-density lipoprotein cholesterol, high low-density lipoprotein cholesterol).
4. Hypertension.

Initiating events in NAFLD are (i) the development of obesity and (ii) insulin resistance (within both adipose tissue and the liver). Both these factors increase the mobilization of free fatty acids from adipose tissue and are taken up by hepatocytes. This stimulates the synthesis of fatty acids within hepatocytes. More than 50% of the lipid found in hepatocytes in NAFLD is derived from adipose tissue, and the remaining are due to de novo synthesis in liver cells. Precise mechanism by which accumulation of lipid in hepatocytes predisposes to the development of liver injury and NASH is not known. Liver injury causes activation of stellate cells in space of Disse resulting in deposition of collagen, and hepatic fibrosis. These changes along with hepatocyte damage lead to full-blown NASH.

Clinical Features

- NAFLD is the most common cause of **incidental elevation of serum transaminases**. Most of the patients with hepatic steatosis are asymptomatic and those with active steatohepatitis or fibrosis may also be asymptomatic. Some patient may complain of fatigue, malaise, right upper-quadrant discomfort, or symptoms of chronic liver disease.
- Liver biopsy is needed to detect NASH and distinguish it from uncomplicated NAFLD. NAFLD is a significant contributor to the pathogenesis of "cryptogenic" cirrhosis.
- Current therapy: Obesity reduction and reversal of insulin resistance. Lifestyle modifications to reduce weight (diet and exercise) is the most effective form of treatment.

Pediatric NAFLD: It is becoming more common due to increased problem of obesity and metabolic syndrome.

CIRRHOSIS

Definition: Cirrhosis is an **end stage** of any chronic liver disease. It is a **diffuse process** (entire liver is involved) characterized by **fibrosis** and conversion of normal architecture to structurally **abnormal regenerating nodules** of liver cells.

Morphological Characteristics

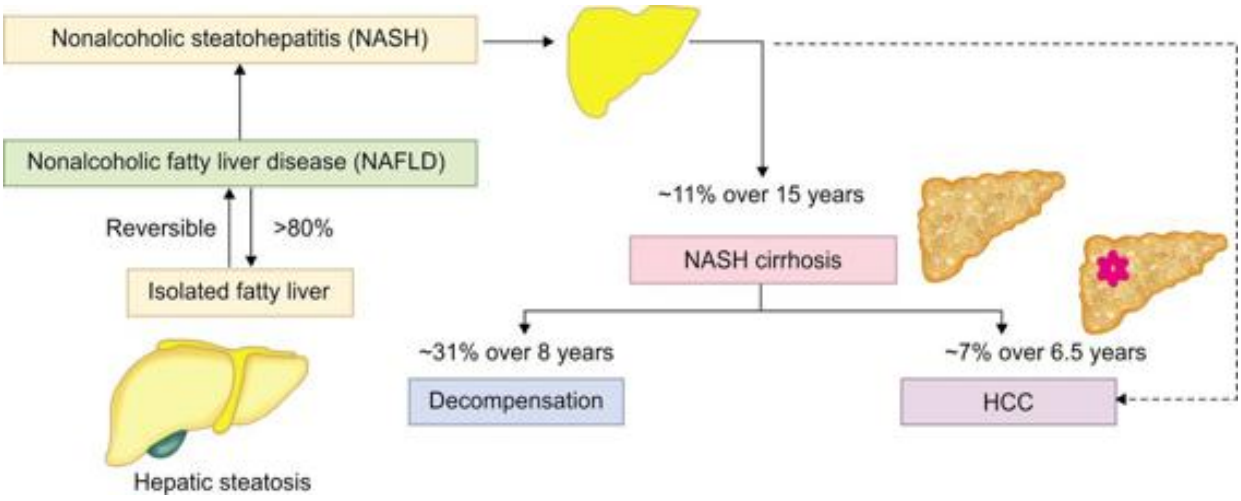


Fig. 19.21: Natural history of nonalcoholic fatty liver disease (NAFLD).

The three main morphologic characteristics of cirrhosis are as follows:

1. Fibrosis

It is the characteristic feature of progressive liver damage. The fibrous tissue form delicate bands or broad scars and link portal tracts with one another and portal tracts with terminal hepatic veins.

2. Regenerating Nodules

Liver cell damage is compensated by regeneration of hepatocytes. These regenerating hepatocytes forms nodules and are surrounded by fibrosis. Nodularity results from cycles of hepatocyte regeneration and scarring. The regenerating liver cells does not maintain the normal architecture. The size of nodules vary from very small (<0.3 cm, micronodules) to large (several centimeters, macronodules). The pattern of scarring may be scars either linking portal tracts to each other or linking of portal tracts to central veins.

3. Loss of Architecture

The hepatocyte injury and consequent fibrosis are diffuse processes, which occur in the entire liver. This disrupts the architecture of the **entire liver**.

Classification

Morphological Classification (Fig. 19.22)

Depending on the size of the regeneration nodules cirrhosis is classified as (i) micronodular, (ii) macronodular, and (iii)mixed.

Micronodular Cirrhosis

It is characterized by **regular** and **small nodules** measuring **less than 3 mm in diameter**. The **fibrous tissue septa** are **usually thin** and fibrous septa bridge portal tracts and central veins (portal-portal and/portal-central) resulting in a small nodule without central structures (e.g., alcoholic cirrhosis).

Macronodular Cirrhosis

It is characterized by the presence of nodules of variable size, more **irregular** than in the micronodular cirrhosis and usually **larger than 3 mm in diameter**. The **fibrous tissue septa are broad** and the nodules are more variable in composition and are often composed of multiple acini. Micronodular cirrhosis can be converted into a macronodular form by continued regeneration and expansion of existing nodules. For example, cirrhosis associated with chronic hepatitis. The macronodular cirrhosis has an **increased risk** of developing **carcinoma** of liver.

Mixed Cirrhosis

It consists of both micronodules and some macronodules.

Depending on the activity, each of these forms may sub-classified as an active and inactive form.

- **Active form:** It is characterized by evidence of continuing liver cell necrosis and inflammatory reaction.
- **Inactive form:** It shows neither liver cell necrosis nor inflammation.

Etiological Classification

This classification takes into consideration clinical, biochemical, immunological or biopsy features. Main causes of cirrhosis are shown in **Box 19.5**.

Pathogenesis

Four important processes are involved:

1. **Death of liver cells with loss of architecture**: The pathogenesis of hepatocyte injury varies depending on the etiological agent. The mechanism by which alcohol causes hepatocyte injury is discussed on pages 616-18.
2. **Fibrosis (Fig. 19.16):** It is mainly due to the **activation of hepatic stellate cells,** which are transformed into highly fibrogenic cells called myofibroblasts**.** It is discussed in detail in pages 618.
3. **Regenerating nodules:** The liver cell damage and fibrosis stimulate the surviving hepatocytes to regenerate and proliferate to form regenerating

nodules (*refer* page 620).

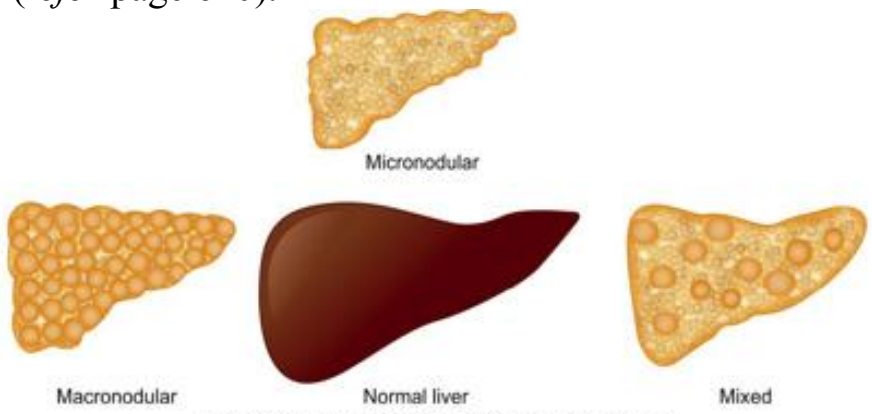


Fig. 19.22: Morphological classification of cirrhosis.

4. **Vascular reorganization:** The parenchymal damage and fibrosis disrupt the vascular architecture of the liver (*refer* page 626).

The net outcome is formation of a fibrotic and nodular liver. The blood supply to hepatocytes is severely compromised, as well as ability of liver cells to secrete substances into blood.

Clinical Features

The clinical features of cirrhosis range widely:

- **Initial phase:** It is termed as "**compensated**" cirrhosis, the patient may be asymptomatic.
- **Later phase:** It is termed as "**decompensated**" cirrhosis, presents with complications of portal hypertension or liver dysfunction (or both).

Nonspecific clinical manifestations: Anorexia, weight loss, weakness, and in advanced disease, symptoms and signs of hepatic failure may develop.

Hepatic failure is usually precipitated by systemic infection or gastrointestinal hemorrhage.

Cirrhosis: End-stage process with
- Bridging fibrous septa
- Most or entire liver involvement
- Regenerating nodules consisting of senescent and replicating cells.

Cirrhosis: Difficult to identify the cause on morphological features alone, because all advanced cases of cirrhosis tends to have a similar appearance.

Classification of cirrhosis:
- Morphological classification

- Etiological classification

Morphological classification:
- Micronodular
- Macronodular
- Mixed

Cause of death in cirrhosis:
- Progressive liver failure
- Complication related to portal hypertension
- Development of hepatocellular carcinoma

PORTAL HYPERTENSION

CC: Describe the etiology, pathogenesis and complications of portal hypertension.

Q. Write a short note on portal hypertension.

Definition: Portal hypertension is defined as the **elevation of the hepatic venous pressure** above 7 mm Hg.

Causes of Portal Hypertension

The causes of portal hypertension can be divided into three categories:

- **Prehepatic causes:** Obstructive thrombosis of the portal vein before it ramifies within the liver or massive splenomegaly with increased splenic vein blood flow.
- **Posthepatic causes:** Severe right-sided heart failure, constrictive pericarditis, and hepatic vein outflow obstruction.

BOX 19.5: Main causes (etiology) of cirrhosis.

- Alcohol is one of the most common causes
- Viral hepatitis (HBV and HCV)
- Nonalcoholic steatohepatitis (NASH)
- Hemochromatosis
- Autoimmune liver disease (autoimmune hepatitis and primary biliary cirrhosis)
- Intrahepatic or extrahepatic biliary obstruction: Recurrent biliary obstruction (e.g., gallstones)
- Metabolic disorders: Wilson's disease
- 'Cryptogenic' (hidden cause) or idiopathic
- Drugs and toxins

- Indian childhood cirrhosis

- **Intrahepatic causes:** Cirrhosis (main cause), schistosomiasis, massive fatty change and diffuse fibrosing granulomatous disease (e.g., sarcoidosis).

Pathogenesis of Portal Hypertension in Cirrhosis

It is produced by a combination of two simultaneously occurring processes:

1. **Increased intrahepatic resistance to blood flow through the liver:**
   - **Deposition of fibrous tissue** (scarring): Fibrosis and compression by regenerative nodules (fixed component) **increases the sinusoidal vascular resistance**. **In the fibrous septa**, **anastomosis develop between the arterial and portal system**. These impose arterial pressures on the low pressure portal venous system and contribute to portal hypertension.
   - **Active vasoconstriction: Contraction of vascular smooth muscle cells and myofibroblasts** (functional component) also **increases the sinusoidal vascular** resistance. Intrahepatic vasoconstriction is jointly due to deficiency in intrahepatic nitric oxide (NO) by sinusoidal endothelial cells as well as increased production of the vasoconstrictor ET-1.
2. **Increase in portal venous inflow (flow): It results from the hyperdynamic circulation/consequences of portal hypertension (Fig. 19.23)**.

Consequences of Portal Hypertension

Q. What are the complications of cirrhosis?

Q. Write a short note on complications of portal hypertension.

i. Ascites.
ii. Formation of portosystemic venous shunts.
iii. Congestive splenomegaly.
iv. Hepatic encephalopathy.

Ascites

Q. Write a short note on pathogenesis of ascites due to alcoholic cirrhosis.

Ascites is defined as the **accumulation of excess fluid** in the **peritoneal cavity**.

The **most common** (85% of cases) **cause** of ascites is **portal hypertension caused by cirrhosis**.

Pathogenesis of Ascites in Cirrhosis (Fig. 19.24)

It is complex process and involves the following mechanisms:

- **Portal hypertension:** It increases the hydrostatic pressure in portal vein.
- **Hypoalbuminemia:** It is due to decreased synthetic function in a cirrhotic liver → reduces the plasma oncotic pressure.
- **Splanchnic vasodilation and hyperdynamic circulation**. It reduces systemic arterial blood pressure → activates **renin-angiotensin-aldosterone system** with the development of hyperaldosteronism → leads to **sodium retention** → fluid accumulation and expansion of the extracellular fluid volume.

  The combination of portal hypertension, splanchnic arterial vasodilation, and sodium and water retention increases the hydrostatic pressure as well as permeability of interstitial capillaries. It causes extravasation of fluid into the peritoneal cavity.
- **Percolation of hepatic lymph into the peritoneal cavity:** In cirrhosis, hepatic lymphatic flow exceeds thoracic duct capacity. The excess lymph may percolate (pass through liver) into the peritoneal cavity and cause ascites.

Portosystemic Shunts/Varices and Variceal Hemorrhage

It is one of the complications of portal hypertension due to cirrhosis. In portal hypertension, increased portal vascular resistance leads to increase in the portal system pressure → the blood flow is reversed from portal to systemic circulation by dilation of collateral vessels and development of new vessels. Portosystemic shunting/bypasses occurs at the sites where the systemic and portal circulation shares common capillary beds.

Main Sites of Portosystemic Shunting/Bypasses

Main sites are **(Fig. 19.23)**:

- **Esophagogastric junction:** These collaterals produce gastroesophageal varices (in about 40% of patients). They can cause massive, frequently fatal hematemesis (especially when there is associated coagulopathy).
- **Veins around and within the rectum:** It results in rectal varices (manifest as hemorrhoids).
- **Retroperitoneum:** Collaterals may form in the retroperitoneum, especially in females and communicate between the ovarian vessels and iliac veins.
- **Umbilicus:** Produce prominent collaterals around the umbilicus. They appear as dilated subcutaneous veins extending from the umbilicus toward the rib margins (**caput medusae**).

Splenomegaly and Hypersplenism

Portal hypertension is associated with long-standing congestion and may cause **congestive splenomegaly**. The spleen is enlarged and varies in size. Massive splenomegaly may give rise to the syndrome of **hypersplenism**. Hypersplenism is characterized by a decrease in the lifespan of all of the formed elements of the blood (pancytopenia). **Pancytopenia** of hypersplenism is due to the prolonged transit time of blood through the hyperplastic spleen.

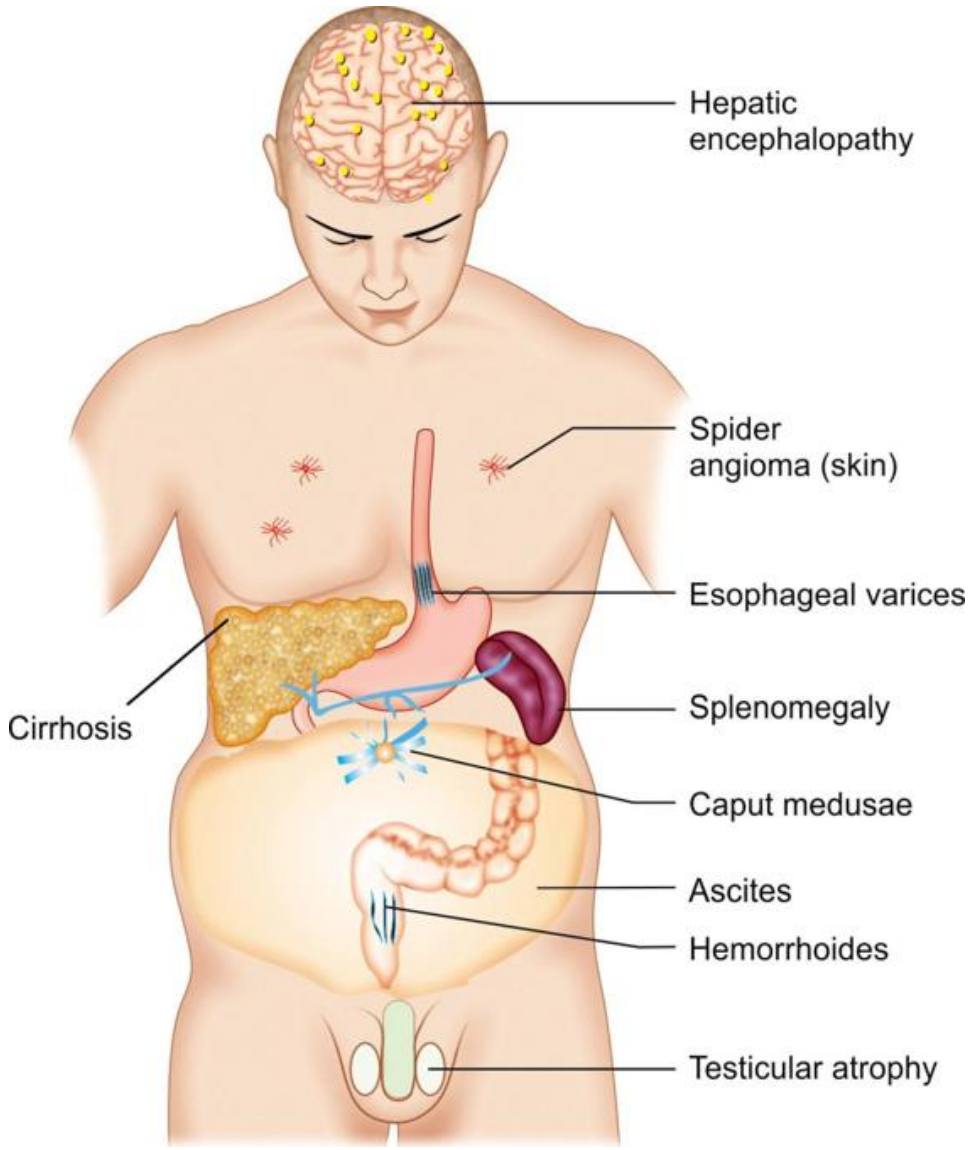
MORPHOLOGY

Gross

The **spleen is firm and enlarged**; up to 1000 g. Cut surface is uniformly deep red.

Microscopy

The spleen shows dilated sinusoids with thickening of wall due to fibrous tissue. Focal areas of hemorrhages may lead to the formation of **fibrotic, iron-laden nodules**. These are known as **Gamna–Gandy bodies** (*refer* Figs. 5.3B and C).



Endocrine Complications are Associated with Cirrhosis

It may be either due to the direct effects of alcohol abuse or hepatic dysfunction.

In Men

Hyperestrogenism

- Chronic liver failure → reduced hepatic catabolism of estrogens + weak androgens are converted to estrogenic compounds in peripheral tissues →

hyperestrogenism → leads to **feminization**.

- The portosystemic shunts secondary to portal hypertension in cirrhosis allow these hormones to bypass the liver.
- Feminization is characterized by **gynecomastia, a female body habitus, and a female distribution of pubic hair**. Hyperestrogenism also causes vascular manifestations, which include **spider angiomas** (upper trunk and face) and **palmar erythema** (due to local vasodilatation).

Hypogonadism

Chronic alcoholics also develop hypogonadism, which is manifested as **testicular atrophy, impotence, and loss of libido**. These are due to direct toxic action of alcohol.

In Women

They may show features of **gonadal failure**, presenting as oligomenorrhea, amenorrhea, infertility, ovarian atrophy, and loss of secondary sex characteristics. These effects are **due to direct toxic action of alcohol on gonads**.

Others

- **Pruritis:** Chronic severe jaundice can cause pruritus (itching). Its intensity may very severe and the skin scratch may be so deep to produce raw areas in the skin that is prone to repeated potentially life-threatening infection. In some patients, severe pruritus can be relieved by and is a primary indication for liver transplantation. Pruritus may also develop in other disorders associated with cholestasis and may be to the increased bile salts in the body.
- **Cause of death:** Hepatic encephalopathy, bleeding from esophageal varices and bacterial infections (resulting from damage to mucosa in the GIT and Kupffer cell dysfunction) and development of hepatocellular carcinoma.

Q. A 62-year-old male with a history of alcohol abuse, complained of gradual weakness, anorexia,

weight loss. He was chronic alcoholic for the last 22 years. On examination, there was jaundice, spider angiomas on the skin and gynecomastia. Abdominal palpation reveled ascites and splenomegaly.

(a) What is your probable diagnosis? (Ans: Alcoholic cirrhosis with portal hypertension).

(b) Discuss the etiopathogenesis of this condition (refer pages 615-9).

(c) List the different forms of liver diseases associated with alcohol intake (refer page 615).

(d) Describe the morphological changes in the target organ involved (refer page 620).

(e) What are the clinical manifestations of portal hypertension? (refer pages 625-7).

Q. A 65-year-old male with a history of alcohol abuse was brought to casualty with history hematemesis 2 hours back. Physical examination revealed mild jaundice, spider angiomas, mild pedal edema and gynecomastia. There was distension of abdomen due to ascites. The liver was firm nodular liver and spleen was just palpable. Upper GI endoscopy revealed dilated bleeding blood vessels in esophagus. Sclerotherapy was done and bleeding was controlled.

(a) What is your probable diagnosis? (Ans: Alcoholic cirrhosis with bleeding from esophageal varices).

(b) Describe the morphological changes in the liver (refer page 620).

(c) What is the cause of hematemesis? (refer page 626).

(d) List the complications (refer pages 625-7).

Portal hypertension in cirrhosis: Due to increased intrahepatic resistance to blood flow through the liver and increase in portal venous inflow.

Most common cause of portal hypertension in adults: Cirrhosis followed by non-cirrhotic portal fibrosis.

Most common cause of portal hypertension in children: Extrahepatic portal vein obstruction (EHPVO).

Portal vein hypertension in cirrhosis: Due to intrasinusoidal hypertension produced by compression caused by regenerating nodules.

Fig. 19.23: Major clinical consequences of portal hypertension in cirrhosis.

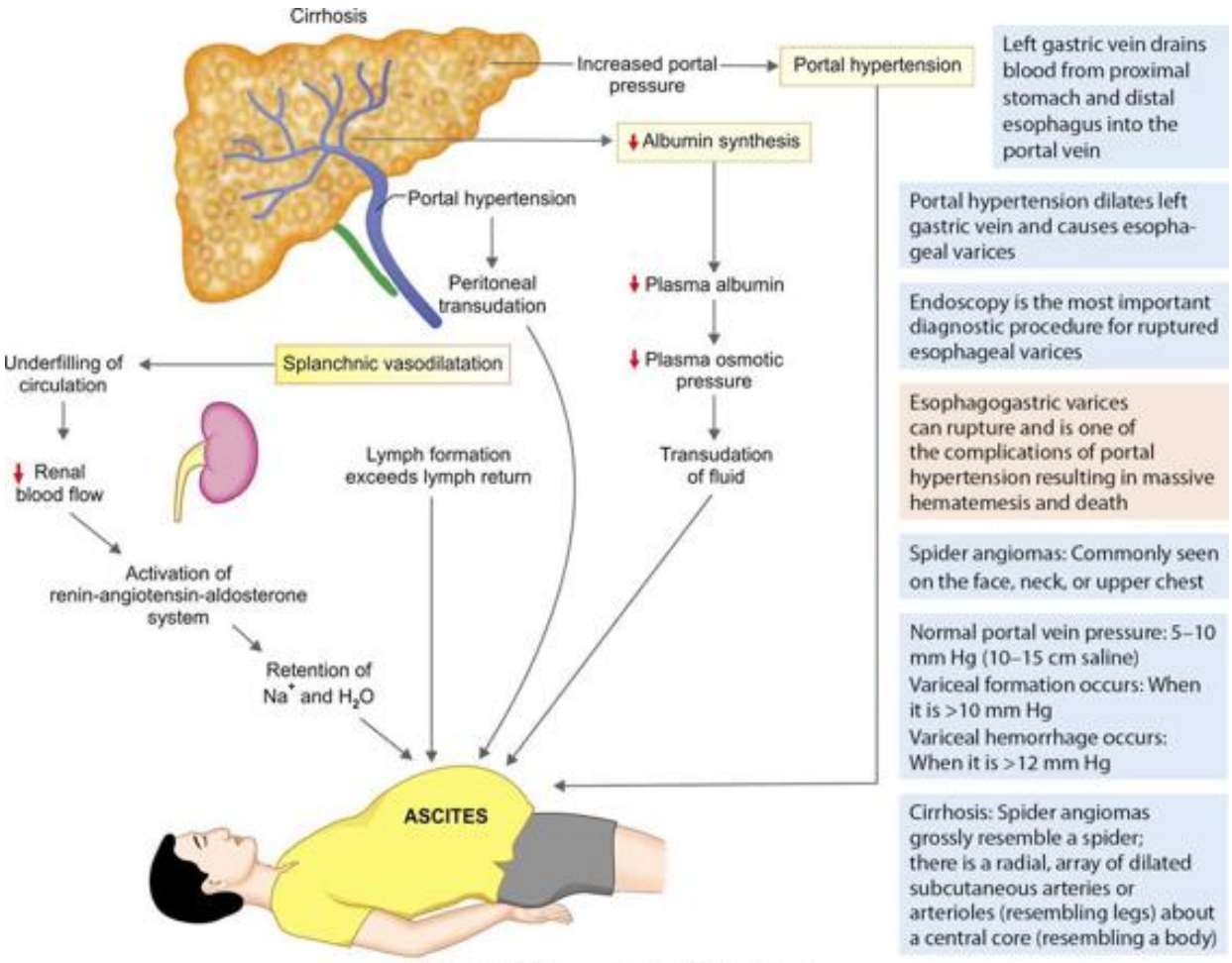


Fig. 19.24: Pathogenesis of ascites in cirrhosis.

Pathogenesis of ascites in cirrhosis:
- Portal hypertension
- Hypoalbuminemia
- Secondary hyperaldosteronism
- Percolation of hepatic lymph into the peritoneal cavity

Ascites in cirrhosis: Transudate.

Cirrhosis: In males produces hyperestrinism → gynecomastia, spider angioma, and palmar erythema.

HEMOCHROMATOSIS

Q. Write short essay/note on hemochromatosis.

Definition: Hemochromatosis is defined as **excessive accumulation of body iron**.

Classification of Iron Overload

Hemochromatosis may be a primary (hereditary hemochromatosis) or secondary to other acquired or genetic disorders **(Box 19.6)**.

- **Primary (hereditary) hemochromatosis/hemochromatosis:** It is a homozygous-recessive inherited disorder due to excessive absorption of iron.
- **Secondary (**acquired) **hemochromatosis/hemosiderosis**: Accumulation of iron in tissues may occur secondary to other disorders.

Pathogenesis

In hemochromatosis, there may be **mutations in HJV, TfR2 and HFE,** which leads to absence of hepcidin → leads to absorption of iron even when there is substantial **elevation of body iron stores** → leads to accumulation of iron mainly in the liver. Symptoms develop usually when the stored iron exceeds 20 g.

Mechanism of Tissue Damage

Excessive iron causes tissue damage by the following mechanisms:

1. **Free radical formation**: It damages the tissue by lipid peroxidation.
2. **Activation of hepatic stellate cells**: It stimulates collagen formation.
3. **Interaction of reactive oxygen species and of iron with DNA:** It results in lethal cell injury or predisposition to hepatocellular carcinoma.

Organ/Tissues Involved

Hemosiderin gets deposited in the following organs (in decreasing order of severity): **Liver, pancreas, myocardium,** pituitary gland, adrenal gland, thyroid and parathyroid glands, joints, and skin. Mainly it presents with **cirrhosis** and **pancreatic fibrosis**.

Morphology of Liver

MORPHOLOGY

Gross

In early stages, liver may appear grossly normal or slightly darker in color. With progressive accumulation of iron, the liver (other organs, such as the pancreas) become **chocolate-brown color**. **Cirrhosis** due to hemochromatosis is **initially micronodular**, and **later** become **macronodular** cirrhosis.

Microscopy

Iron deposits first appear as **finely granular golden-yellow pigment in the cytoplasm** of periportal hepatocytes. Hemosiderin is easily recognized with **Prussian blue stain** (*refer* **Fig. 2.12**), which stains them blue. As iron continues to accumulate, iron accumulates in hepatocytes throughout the lobule, within the bile duct epithelium and Kupffer cells. Fibrosis develops slowly. Initially, fibrosis develops at the periportal region, later forms portal-portal bridging fibrosis and leads to micronodular cirrhosis.

Clinical Features

- Excessive iron accumulation is a **slow and progressive process** and symptoms usually develop during the fifth to sixth decades of life. Males are more affected than females (5 to 7:1).
- Classic triad is characterized by:
  a. **Micronodular cirrhosis** in all patients
  b. **Diabetes mellitus** (75–80%) → bronze diabetes
  c. **Skin pigmentation** (75–80%).

Death may result from cirrhosis, cardiac disease or hepatocellular carcinoma.

Hemosiderosis: Acquired iron overload.

Hemochromatosis: Mutations in *HJV*, *TfR2* and *HFE* gene which leads to absence of hepcidin. Iron damages by synthesis of hydroxyl-free radicals.

Hemochromatosis: The actions of iron on cells are reversible and if damage is not severe, removal of excess iron with therapy promotes recovery of tissue function.

Laboratory findings in hemochromatosis:
- Raised serum iron
- Raised % saturation
- Raised serum ferritin
- Reduced total iron binding capacity (TIBC)

Hemochromatosis: Micronodular cirrhosis, diabetes mellitus and brown skin pigmentation—bronze diabetes.

WILSON'S DISEASE

Q. Write a short note on Wilson's disease.

Wilson's disease is an **autosomal recessive disorder of copper metabolism,** leads to progressive accumulation of toxic levels of copper in the liver and various other tissues. **Copper causes tissue toxicity** and end-organ damage principally the **liver, brain, and eye**.

Etiology

- Major method of elimination of copper from the body is by excretion in the bile and is regulated by Wilson's disease gene, **ATP7B**.
- **In Wilson's disease → mutation of the ATP7B gene →** causes deficiency in the ATP7B protein → **failure to excrete copper in bile →** copper **accumulates within the liver →** cause toxic **liver injury through the ROS** produced by the **Fenton reaction**.

Organs Involved

Liver and extrahepatic sites include: Central nervous system, kidneys, endocrine organs, heart, and musculoskeletal system.

BOX 19.6: Classification of iron overload.

1. **Hereditary hemochromatosis**

- Mutations of genes encoding HFE, transferrin receptor 2 (TfR2), or hepcidin
- Mutations of genes encoding HJV (hemojuvelin: Juvenile hemochromatosis)

2. **Hemosiderosis (secondary hemochromatosis)**
   - Parenteral iron overload: Transfusions, long-term hemodialysis, aplastic anemia, sickle cell disease
   - Ineffective erythropoiesis with increased erythroid activity: β-thalassemia, sideroblastic anemia
   - Increased oral intake of iron
   - Chronic liver disease: Chronic alcoholic liver disease

MORPHOLOGY

Liver

It is the main organ involved.

Gross

In early stages, the liver may be grossly normal or show mild degree of steatosis. In later stages, progressive fibrosis leads to **macronodular cirrhosis**.

Microscopy

In early Wilson's disease, the changes may be mild. The changes may be as follows:

- **Fatty change (steatosis)**: Mild to moderate steatosis is common and may be microvesicular or macrovesicular.
- A**cute hepatitis: It is similar to viral hepatitis**, except accompanying **fatty change**.
- **Chronic hepatitis**: It is common.
- **Other features include:** Hepatocyte necrosis, macrovesicular steatosis, vacuolated hepatocellular nuclei, and **Mallory bodies**. As the **disease progresses**, **periportal fibrosis** may develop and progresses to bridging fibrosis and **cirrhosis.**
- **Massive liver necrosis:** It is rare and is similar to that caused by viruses or drugs.

Brain

Affects the **basal ganglia**, particularly the **putamen** and shows **atrophy**.

Eye Lesions

Q. Write a short note on Kayser–Fleischer ring.

Kayser–Fleischer rings (Fig. 19.25) are formed **due to green-to-brown deposits of copper in Desçemet's membrane** (in the limbus) of the cornea.

Clinical Features

- **Age at onset:** It ranges between 6 and 40 years of age.
- **Liver involvement:** It can produce **acute or chronic liver disease**.
- **Neuropsychiatric manifestations**: These include: Mild behavioral changes, frank psychosis, or a **Parkinson disease–like syndrome** (such as tremor).

Biochemical Findings

- **Decreased serum ceruloplasmin**.
- **Increased copper content in liver** (the most sensitive and accurate test).
- **Increased urinary excretion of copper** (the most specific screening test).
- **Serum copper** levels: It is **not useful for diagnosis**, since they may be low, normal, or elevated, depending on the stage of the disease.

Wilson disease: Reduced incorporation of copper into ceruloplasmin and failure to excrete copper into the bile.

Ceruloplasmin: Enzyme synthesized in the liver that contains copper.

Wilson disease: Accumulation of copper due to mutation of the metal ion transporter ATP7B gene.

Wilson's disease: Copper is not visible on routine H and E stains. May be demonstrated by special stains, such as rhodanine or rubeanic acid.

Kayser–Fleischer ring: Green-to-brown deposits of copper in Desçemet's membrane of the cornea.

POSTNECROTIC CIRRHOSIS

Postnecrotic cirrhosis is also known as posthepatitic cirrhosis, macronodular cirrhosis and coarsely nodular cirrhosis.

Liver shows large and irregular nodules of with broad bands of fibrous connective tissue. It is **most commonly develops after previous viral hepatitis**.

Etiology

- **Viral hepatitis:** Most common viral hepatitis associated with postnecrotic cirrhosis is hepatitis B and C.
- **Drugs and chemical hepatotoxins:** Phosphorus, carbon tetrachloride, mushroom poisoning, acetaminophen and α-methyldopa.
- **Others:** Include certain infections (e.g., brucellosis), parasitic infestations (e.g., clonorchiasis), metabolic diseases (e.g., Wilson's disease) and advanced alcoholic liver disease.
- **Idiopathic:** Where the etiology is unknown.

MORPHOLOGY

Typically, postnecrotic cirrhosis is **macronodular** type.

Gross

Liver is usually small, distorted with numerous with **irregular macronodules of varying sizes** ranging from 3 mm to a few centimeters in diameter.

Microscopy

- Loss of architecture.
- **Macronodules:** Liver is replaced by macronodules larger than 3 mm.
- **Fibrosis:** The fibrous septa separate the macronodules.
- **Inflammation:** Fibrous septa may show mononuclear inflammatory cell infiltrate especially in cases following HCV chronic hepatitis.

Clinical Features

- More common in females than in males.
- Features of cirrhosis similar to alcoholic cirrhosis.
- Splenomegaly and hypersplenism may be observed.
- Postnecrotic cirrhosis following hepatitis B and C virus infection in early life, is more frequently may develop hepatocellular carcinoma later.

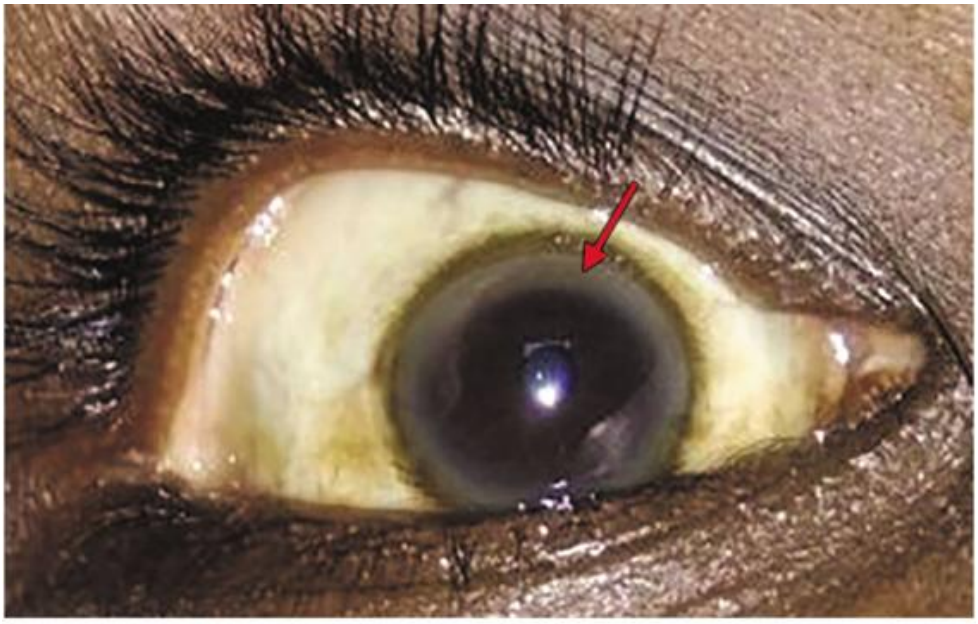


Fig. 19.25: Kayser–Fleischer ring (arrow).

SECONDARY BILIARY CIRRHOSIS

Q. Write a short note on secondary biliary cirrhosis.

Definition: Cirrhosis developing secondary to prolonged obstruction of the extrahepatic biliary tree.

Etiology

Q. Write a short note on causes of secondary biliary cirrhosis.

- **In adults: Extrahepatic cholelithiasis** (gallstones), **malignant tumors of the biliary tree or head of the pancreas**, and **strictures** due to previous surgical procedures.
- **In children**: **Biliary atresia, cystic fibrosis, choledochal cysts** (a cystic anomaly of the extrahepatic biliary tree), and paucity of bile duct syndromes (insufficient intrahepatic bile ducts).

MORPHOLOGY

Gross

Liver is nodular and shows **yellow-green pigmentation**. **Hard to cut** and appear **finely granular**.

Microscopy

- **Loss of** normal **architecture** of the liver.
- Coarse **fibrous septa** subdivide the liver. Under **low power**, it produces a characteristic of irregular **"jigsaw puzzle piece" nodules**.
- Fibrous septa shows **distended small and large bile ducts**, which **contain inspissated pigmented material**.
- **Extensive proliferation of smaller bile ductules** is seen mainly at the interface between septa in former portal tracts and the parenchyma.
- **Liver cells** may show extensive **feathery degeneration** and formation of **bile lakes**.
- Obstruction favors ascending bacterial infection and associated with neutrophilic infiltration of bile ducts; severe **pylephlebitis** and may lead to abscesses.

Secondary biliary cirrhosis:

- Jigsaw puzzle piece nodules
- Small and large bile ducts distended with bile
- Proliferation of bile ductules
- Bile lakes

AUTOIMMUNE CHOLANGIOPATHIES

Autoimmune cholangiopathies consists of two distinct immunologically-mediated disorders which involve intrahepatic bile ducts. These are (i) primary biliary cholangitis and (ii) primary sclerosing cholangitis.

Primary Biliary Cholangitis

Definition: Primary biliary cholangitis (PBC) is an autoimmune liver disease characterized by **nonsuppurative, inflammatory destruction of small- and medium-sized intrahepatic bile ducts** (cholangitis). There is no involvement of large intrahepatic ducts and the extrahepatic biliary tree. Previously, this was known as primary biliary cirrhosis, but most patients do not progress to cirrhosis. Hence, the term primary biliary cholangitis is preferred.

- **Gender**: It usually affects **middle-aged women**, with a female to male ratio of more than 6:1.
- **Age**: It may occur between 20 and 80 years of age, with peak incidence between **40 and 50 years of age**.

Etiology and Pathogenesis

- The exact cause and pathogenesis of PBC is not known. It is thought to be an autoimmune disorder. **Genetic and environmental factors play role** in the pathogenesis of the PBC. Family members of PBC patients have an increased risk.
- **Mechanism of intrahepatic bile destruction:** Many mechanisms have been proposed and these include:
  - **Aberrant expression of MHC class II** molecules on bile duct epithelial cells
  - **Accumulation of autoreactive T cells** around bile ducts
  - **Antimitochondrial antibodies** to hepatocytes or other antibodies against cellular components (nuclear pore proteins, and centromeric proteins, etc.)
  - The **characteristic autoantibody** detected in PBC is **antimitochondrial antibodies.** They target the E2 component of the pyruvate dehydrogenase complex (PDC-E2). PDC-E2–specific T cells are also detected in these patients, supporting immune-mediated pathogenesis.
- **Consequences of bile duct destruction:** Destruction of bile ducts leads to **impaired secretion of bile, cholestasis, and inflammatory reaction in the portal tract**. This results in **hepatic damage**, **fibrosis** and ends up in **cirrhosis and liver failure**. Cirrhosis develops several years after the onset of disease and most patients are diagnosed at a pre-cirrhotic stage. Hence, the older term primary biliary cirrhosis is somewhat misleading.

MORPHOLOGY

Gross

- **Early-stage,** PBC may have **few gross findings**, weight may be normal or mildly increased (because of inflammation).
- In **late-stage** PBC, the liver shows uniform and bile stasis stains the **liver green**.

Microscopy

Three distinct stages:

- **Stage I—florid duct lesion**: It is characterized by **inflammation and injury to interlobular bile duct epithelial cells**. The bile ducts are **actively destroyed** and surrounded by a dense collection of **lymphocytes, macrophages and plasma cells.**

Lymphocytes may form **lymphoid follicles and few with germinal centers**. **Noncaseating epithelioid granulomas** may be seen in the portal tracts. The lesion is quite patchy in distribution.

- **Stage II—scarring: Inflammatory process destroys small bile ducts**. This is accompanied by **proliferation of bile ductules** and **fibrosis** at the periphery of portal triad → **obstruction to intrahepatic bile flow** → leads to **inflammation, and necrosis** of the adjacent periportal hepatic parenchyma.
- **Stage III—of cirrhosis:** Fibrosis in the portal tract and portal-portal septal fibrosis. If not treated, it follows one of two paths to end-stage disease: (1) most classic pathway in which there is increasingly widespread loss of bile ducts, slowly leading to cirrhosis and eventually to profound cholestasis or (2) development of prominent portal hypertension rather than severe cholestasis. Fortunately, both of these outcomes are rare.

Laboratory Findings

- **Serum alkaline phosphatase** (markers of cholestasis), **γ-glutamyltransferase and cholesterol (hypercholesterolemia) are raised.**
- Hyperbilirubinemia occurs in late stages.
- **Antimitochondrial antibodies** are **characteristic and are essential for the diagnosis** of PBC. They are found in 90–95% of patients.
- **Liver biopsy:** PBC is confirmed by liver biopsy and lesion is considered diagnostic if a florid duct lesion is present.

Clinical Features

- Insidious in onset.
- Commonly present with **pruritus, fatigue**, and **abdominal discomfort**.
- **Other features:** These include: Skin pigmentation (due to melanin deposition), eyelid xanthelasmas (cholesterol-rich macrophages), steatorrhea, osteomalacia and/or osteoporosis (due to malabsorption of vitamin D).
- **Cirrhotic stage:** Jaundice, hepatic decompensation, portal hypertension and variceal bleeding develop.

Prognosis: Increased risk of hepatocellular carcinomas.

Cause of death: Liver failure, massive hemorrhage from esophageal varices and intercurrent infection.

Treatment: Early treatment with oral ursodeoxycholic acid results in dramatic improved outcomes by slowing progression of disease. Liver

transplantation is the best treatment for advanced liver disease.

Primary biliary cholangitis (PBC): Destruction of bile ducts in the portal triad by autoimmune mechanism.

PBC: Three stages
1. Florid ductal lesion
2. Scarring
3. Cirrhosis

Primary biliary cholangitis: Jaundice develops late. Pruritis develops before jaundice.

Anti-mitochondrial antibodies are present in about 95% of primary biliary cholangitis and are essential for diagnosis the diagnosis of PBC.

PBC: Increased risk of HCC.

Primary Sclerosing Cholangitis

Definition: Primary sclerosing cholangitis (PSC) is characterized by inflammation and obliterative fibrosis of intrahepatic and extrahepatic bile ducts.

- Irregular biliary strictures **lead to dilation of preserved segments which produces** characteristic **"beading" of the intrahepatic and extrahepatic biliary tree in MRI**.
- Inflammatory bowel disease most commonly ulcerative colitis, coexists in about 70% of patients with PSC. PSC usually occur in the third to fifth decades of life and has a 2:1 male predominance.

Pathogenesis: PSC is probably due to an immunologically mediated injury to bile ducts.

Atypical perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) are found in up to 80% of patients.

MORPHOLOGY

Morphologic changes seen in large ducts (intrahepatic and extrahepatic) is different from that seen in smaller intrahepatic ducts.

- **Large duct inflammation is similar to** that of ulcerative colitis and consists of neutrophils infiltrating into the epithelium superimposed on a chronic inflammatory

background. Inflamed areas progress to strictures as scarring narrows the lumen.

- **Smaller ducts:** They usually show a striking **circumferential, "onion skin" fibrosis** around an atrophic duct lumen which may eventual obliterate the lumen, leaving a "tombstone" scar. Inflammation is minimal. Because the likelihood of sampling small-duct.

Progression: As the disease progresses, the liver becomes markedly cholestatic, leading to cirrhosis. Biliary intraepithelial neoplasia and cholangiocarcinoma may develops in up to 7% of patients.

Clinical Features

- Patients may be asymptomatic and may show persistent elevation of serum alkaline phosphatase. Some may present with progressive fatigue, pruritus.
- Liver transplantation is the only definitive treatment.

LIVER ABSCESSES

Q. Write a short note on liver abscesses.

Definition: Localized collection of pus inside the liver parenchyma is known as liver abscess. Liver abscesses are common in developing countries.

General Features

Etiology

- *Entamoeba histolytica:* Amebic liver abscess is caused by ***Entamoeba histolytica***.
- **Liver abscess by other protozoa and helminthic organisms:** Secondary infection of hydatid cyst caused by echinococci and less commonly other organisms.
- **Pyogenic abscess:** Bacterial infections in the liver may manifest as pyogenic abscess. Such abscesses usually represent a complication of bacterial infection elsewhere.
- **Fungal abscess:** *Candida*.

Mode of Infection

The causative agent may reach the liver by any one of the following routes.

- **Portal vein:** The majority of hepatic abscesses used to result from portal spread of intra-abdominal infections (e.g., appendicitis, diverticulitis, colitis).
- **Artery:** Through hepatic artery, e.g., during septicemia.
- **Ascending infection** in the biliary tract (ascending cholangitis).
- **Direct invasion of the liver** from a nearby source or a penetrating injury.

MORPHOLOGY

Gross and microscopic appearances are similar to abscesses in other sites and are usually filled with purulent debris. Bacteria may be demonstrated with special stains.

Gross (Fig. 19.26)

- Liver abscesses may be **multiple or solitary**. Bacteremic spread through the arterial or portal system produces multiple small abscesses, whereas direct extension and trauma usually cause solitary large abscesses.
- **More common in the right lobe.**
- Size is variable, ranging up to 10 cm.

Clinical Features

They present with **fever, right upper quadrant pain** and **tender hepatomegaly**. Jaundice may develop when there is extrahepatic biliary obstruction.

Amebic Liver Abscess

Q. Write short note on amebic liver abscess.

Most common extraintestinal complication of amebic dysentery (*refer* pages 569-70 and **Fig. 18.26**).

Q. Write a short note on morphology (gross and microscopy) of amebic liver abscess.

Gross

Amebic abscess ranges from 8–12 cm in diameter and appears well circumscribed. **Amebic abscess are usually located in the subdiaphragmatic region.** The **abscess cavity contains** thick, dark material that has been likened to **anchovy paste (sauce) (Fig. 19.27) or chocolate.**

Microscopy

The trophozoites can be demonstrated in the periphery of the necrotic debris.

Clinical features: The symptoms are similar to pyogenic abscesses. Surgical drainage of large abscesses is important.

Complications:

- If an amebic abscess continues to grow, it may rupture into the **(1) thoracic cavity to produce empyema or a lung abscess, (2)** may rupture into the **peritoneal cavity**, where it produces **peritonitis**, a complication associated with a mortality rate as high as 40%.
- The amebae may also invade the blood, in which case **abscesses of the brain and lung** may ensue.

Liver abscess—route of infection:
1. Portal vein
2. Arterial blood
3. Ascending infection in the biliary tract
4. Direct invasion

Amebic liver abscess:
- Solitary
- More common in right lobe

Amebic liver abscess: Anchovy paste (sauce) or chocolate colored.

MALIGNANT TUMORS OF LIVER

Malignant tumors of liver can be primary or metastatic. Most primary cancers of liver arise from hepatocytes and are termed **hepatocellular carcinoma** (HCC). Less common are cancers that arise from bile duct known as cholangiocarcinomas.

Two rare primary liver cancers are: Hepatoblastomas and angiosarcomas.

Hepatoblastoma

Q. Write a short note on hepatoblastoma.

- Most common liver tumor arising in young childhood.
- **Malignant tumor** and usually fatal.

MORPHOLOGY

Gross

Appears as a **solitary, large mass** in the **right lobe**.

Microscopy

Two variants

1. **Epithelial type:** It consists of small **polygonal fetal cells or smaller embryonal cells**. These tumor cells form **acini, tubules, or papillary** structures.
2. **Mixed epithelial and mesenchymal type:** It is characterized by areas of **both epithelial and mesenchymal differentiation**. The mesenchymal component may be **primitive**, **mesenchyme** (with spindle or stellate cells with little cytoplasm), or show differentiation towards osteoid, cartilage, or striated muscle.

Hepatocellular Carcinoma

- Hepatocellular carcinoma (HCC) is a **malignant tumor derived from hepatocytes or their precursors**.
- **Predominantly in males** with a M:F ratio of 2.4:1.

Etiopathogenesis

Q, Write short note on etiopathogenesis of HCC.

It is multifactorial disease and complex in pathogenesis. It is **probably a multistep process** that involves various risk factors. **Three major** and **several minor risk factors** are associated with HCC **(Table 19.10)**.

Major Risk Factors

1. **Chronic hepatitis:** The risk of liver cancer in individuals infected with **both HCV and HBV is three times higher than with either alone.**
   - **HBV infection:** Chronic HBV infection is strongly associated with HCC, especially **vertical transmission** from infected mothers to her child is a **major risk factor**.
     - **Repeated cycles of liver cell necrosis and regeneration** in chronic hepatitis B is the soil for HCC. HBV associated cirrhosis is another major risk factor.

- The **genome of HBV is integrated** into the **host DNA of liver cells** → may **activate proto-oncogenes into oncogene**, which causes tumor.
- **X gene of HBV encodes HBV X-protein**, which is a transcriptional activator of many genes. **It can inactivate tumor suppressor genes** and **cause cell transformation**.

- **HCV infection**: Most patients with HCV who develop HCC have underlying cirrhosis. **HCV core protein may interact with many cellular proteins** to cause HCC.

2. **Cirrhosis: Strong association between HCC and cirrhosis** and both frequently coexists.
   - **Alcoholic cirrhosis** predisposes to HCC.
   - Male gender, age, and duration of cirrhosis are the major risk factors for HCC in patients with cirrhosis.
3. **Aflatoxin B_1 (chemical carcinogen)**: It is a toxin produced by the fungus ***Aspergillus flavus***. This fungus **contaminates improperly stored peanuts and grains**. Dietary exposure to aflatoxin B_1 is an important risk factor for hepatocellular carcinoma.
   - **Aflatoxin** can **bind covalently with cellular DNA of hepatocytes** and cause a specific **mutation of *TP53* tumor suppressor gene.**
   - Aflatoxin B_1 and HBV interact synergistically in the pathogenesis of hepatocellular carcinoma.
4. **Nonalcoholic steatohepatitis (NASH)**

Minor Risk Factors

These include: Genetic factors, age, gender, chemicals, hormones, and nutrition.

- **Hemochromatosis:** Excessive free iron may be carcinogenic and generates mutagenic reactive oxygen species.
- **Wilson's disease**: It is characterized by accumulation of copper in the liver.
- **Metabolic disorders**:
  - With cirrhosis: Inherited metabolic disorders complicated by cirrhosis. Examples, α_1-antitrypsin deficiency and type 1 hereditary tyrosinemia.

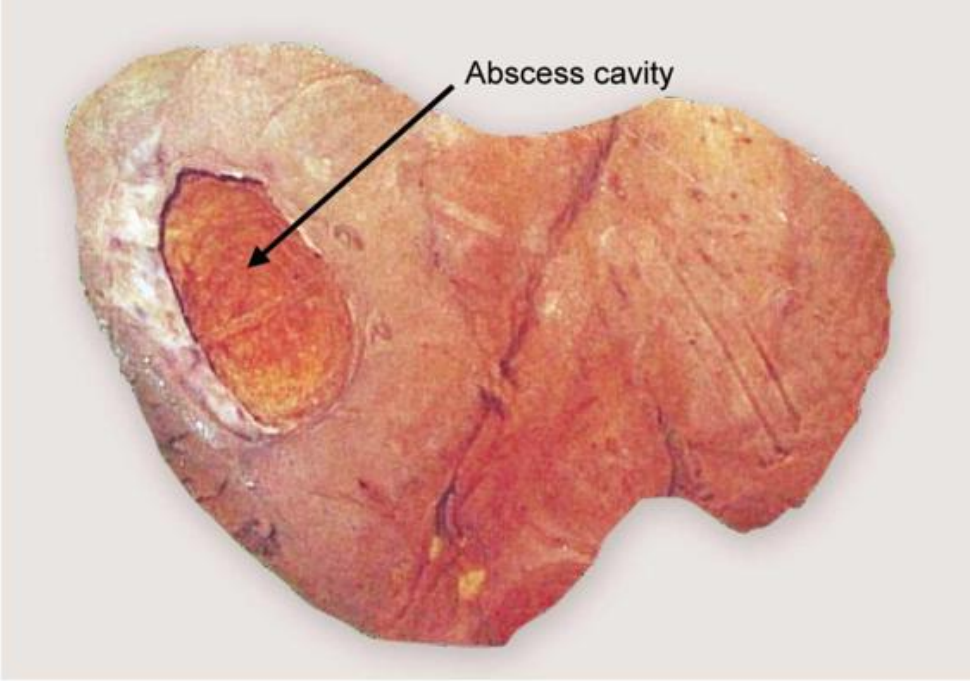
- Without cirrhosis: Certain inherited diseases in the absence of cirrhosis. Example, type 1 glycogen storage disease.

- **Oral contraceptive steroids**.
- **Cigarette smoking**.
- Alcohol consumption.

Molecular Basis of HCC

Both genetic and epigenetic alterations have been detected in hepatocellular carcinoma.

Genetic Alterations



Liver is the most common site of abdominal visceral abscess.

Pyogenic liver abscess: Accounts for majority of liver abscess.

Most common cause of pyogenic liver abscess:
1. *E. coli*—Western countries
2. *Klebsiella pneumoniae*—Asian countries
3. *Staphylococcus* in children

Fig. 19.26: Liver with an abscess cavity in the right lobe.



Fig. 19.27: Anchovy sauce appearance of amebic liver abscess.

These include aneuploidy, point mutations, and both loss and gain of chromosomal components.

- **Mutation in tumor DNA repair genes: Chronic hepatitis** of any cause (viruses, alcohol, and metabolic or autoimmune) **leads to repeated cycles of liver cell death, regeneration, and repair** → may lead to many **mutations in DNA repair genes**.
- **Activation of oncogene**: Point mutations in cellular proto-oncogenes, such as ***KRAS*** may result in oncogene. Others include: **Overexpression of growth factor TGF-α, point mutation or overexpression of WNT signal transduction proto-oncogenes** β-catenin. About 50% of HCC cases are associated with activation of WNT or AKT pathways. X protein of the hepatitis B virus have been shown to have oncogenic effects.
- **Inactivation of tumor suppressor gene: Integration of HBV genome** into host hepatocyte genomic DNA may cause inactivation of tumor suppressor genes (e.g., *TP53*).

Epigenetic Alterations

- **c-MYC amplification** by epigenetic alterations.

- **Telomerase:** Cells may become immortal, through activation of the cellular telomerase enzyme.

Precursor lesions of HCC

Q. Write a short note on precursor lesions of HCC.

1. **Hepatocellular adenoma** particularly those with β-catenin activating mutations.
2. **Cellular dysplasias in chronic liver disease:** May be seen in chronic liver disease, before or after development of cirrhosis. These include:
   - **Small-cell change:** It is probably premalignant. These liver cells have high nuclear–cytoplasmic ratio and mild nuclear hyperchromasia and/or pleomorphism.
   - **Large-cell change:** It is a marker of increased risk of HCC in the liver and in hepatitis B. They may also be directly premalignant. These cells larger than normal liver cells having large, multiple, moderately pleomorphic nuclei with normal nuclear–cytoplasmic ratio.
3. **Dysplastic nodules:** These are nodules having different appearance than cirrhotic nodules, that are usually detected radiologically or in resected specimens of cirrhosis.
   - **Low-grade dysplastic nodules:** They may or may not transform to higher grade lesions, but they are indicator of higher risk for HCC. They do not have cytological or architectural atypia.
   - **High-grade dysplastic nodules:** Important precursors of HCC in viral hepatitis and alcoholic liver disease. The cells of these nodules have cytological (e.g., small- cell change) or architectural features suggestive of, but not sufficient for diagnosis of frank HCC.

MORPHOLOGY

Gross (Figs. 19.28A to C)

Q. Write short note on morphology of HCC.

Three patterns. All patterns may **cause enlargement of liver**, particularly the large unifocal and multinodular patterns. **Areas of necrosis and hemorrhage** are common in all patterns.

1. **Unifocal** (usually large): Tumor appears as **large** circumscribed **single mass** in a portion of the liver **(Figs. 19.29A and B).**

2. **Multifocal:** This pattern shows **multiple nodules** of variable size which are widely distributed.
3. **Diffusely infiltrative:** This type is characterized by **large part of the liver or sometimes entire liver infiltrated by homogeneous indistinct tumor nodules.** The tumor may blend into a cirrhotic liver background and may be difficult to differentiate from the regenerating nodules of cirrhosis.

Color: HCCs are usually **light brown, yellowish-white or gray** in color. **Production of bile** by tumor cells may cause **greenish-brown discoloration** of the tumor.

Microscopy (Figs. 19.30A and B)

HCC graded as well-differentiated, moderately differentiated, and undifferentiated (pleomorphic) forms.

1. **Well-differentiated HCC**: **Tumor cells can be recognizable as hepatocytic in origin**. **Bile production by tumor cells** is the **hallmark** of hepatocellular carcinoma. Tumor cells are arranged in trabecular and acinar (pseudoglandular) pattern.
   - **Trabecular pattern**: It shows malignant hepatocytes arranged in trabeculae **(several layers of malignant cells)** or **irregular anastomosing plates**. The tumor **cells are polygonal** and have **abundant, slightly granular cytoplasm**. The **nuclei** are **large** and **hyperchromatic** and show **prominent nucleoli**.
   - **Acinar, pseudoglandular pattern** (adenoid): In this pattern, malignant hepatocytes are arranged around **a lumen and resemble glands**. The lumen may contain bile. The acini formed by the tumor cells are not true glands, hence the name pseudoglandular.

TABLE 19.10: Risk factors for hepatocellular carcinoma.

Major risk factors	*Minor risk factors*
• Chronic hepatitis: HBV/HCV • Alcoholic cirrhosis • Aflatoxin B_1 • Non-alcoholic steatohepatitis (NASH) • Metabolic syndrome and its attendant obesity, diabetes mellitus, and NAFLD	• Hereditary hemochromatosis • Wilson's disease • Tyrosinemia • α_1-Antitrypsin deficiency • Glycogen storage disease • Oral contraceptives • Cigarette smoking

2. **Moderately differentiated HCC:** This grade may show **s**olid, scirrhous, and clear-cell pattern.
   - **Solid variety:** The tumor cells usually are small and may show considerable variation in shape. Bile production is rare.
   - **Scirrhous variety:** Malignant cells are arranged in narrow bundles separated by **abundant fibrous stroma**.
   - **Clear cell variety:** It consists of predominantly or exclusively clear cells. The clear cytoplasm is due to glycogen or, in some cases due to fat.

3. **Poorly or undifferentiated** HCC: It consists of **pleomorphic cells with great variation in size and shape**. The nuclei also are extremely variable in size and shape. Many bizarre-looking anaplastic giant cells can be seen.

Globular hyaline structures may be seen in the cytoplasm of all types of hepatocellular carcinoma. They represent **alpha-fetoprotein,** α_1**-antitrypsin**, or other proteins. **Mallory's hyaline may be occasionally** seen.

Spread

- **Local spread:** HCC may first spread within the liver itself and develop satellite nodules. **Intrahepatic metastases (by vascular invasion/direct extension)** more likely to occur when the size of tumors reach 3 cm. Local invasion of the diaphragm is common.
- **Lymphatic spread: HCC may spread** to portal lymph nodes, perihilar, peripancreatic, and para-aortic nodes.
- **Blood spread**:
  - **All patterns of HCCs have a strong tendency for invasion of vessels**.
  - This may result in extensive intrahepatic metastases.
  - The portal vein and its branches are infiltrated by tumor.
  - Occasionally, long, snake-like tumor masses may **invade the portal vein** and occlude portal circulation.
  - Rarely, tumor may invade inferior vena cava and extend into the right side of the heart through the hepatic veins.
  - It may metastasize to the **lungs**.

Fibrolamellar HCC

Q. Write short note on fibrolamellar HCC.

- It is a **distinctive uncommon variant** of **HCC** and constitutes about 5% of HCCs.
- **Age and sex**: It occurs in **young patients without cirrhosis**.
- E**tiology:** Unknown.

MORPHOLOGY

Gross

Single large, hard "scirrhous" well-circumscribed tumor with central stellate fibrous scar.

Microscopy

It consists of **large, polygonal cells with** abundant **deeply eosinophilic** (oncocytic) **cytoplasm** and **prominent nucleoli**. The tumor cells are arranged **in nests or cords,** and separated by **parallel bands of abundant dense collagen bundles**.

- P**rognosis:** It is **better** than the conventional HCC.

Clinical Features of HCC

- Most patients present with **ill-defined upper abdominal pain, malaise, fatigue** and **weight loss**.
- On examination, liver appears enlarged, irregular or nodular.

Laboratory Findings—Serum Marker

- **Alpha-fetoprotein: About 50%** hepatocellular carcinoma is associated with **high serum levels of alpha-fetoprotein**. However, α-fetoprotein levels are often raised in other neoplastic and non-neoplastic liver diseases and in some extrahepatic disorders.
- α-L-**fucosidase**: It is raised in HCC and also in cirrhosis.
- **Serum des-α-carboxy prothrombin**: It is **raised in a majority of hepatocellular carcinoma.**

Causes of Death

1. Cachexia.
2. Gastrointestinal or esophageal variceal bleeding.
3. Liver failure with hepatic coma.
4. Rupture of the tumor with fatal hemorrhage (rare).

Most common benign tumor of liver: Hemangioma.

Angiosarcoma of the liver: Highly aggressive neoplasms, associated with exposure to vinyl chloride (plastic pipes), arsenic, or Thorotrast.

Hepatoblastoma: Most common primary hepatic tumor of childhood.

Remember risk factors for HCC as:
Major ABCC (A = Aflatoxin, B = HBV, C = HCV, C = Cirrhosis).
Minor HCC (H = Hemochromatosis, C = Cigarette, C = Contraceptive).

HCC: Chronic infection by HBV and HCV, pre-existing cirrhosis and aflatoxin B_1 are major risk facors.

HCC: Most common primary malignant tumor of liver.

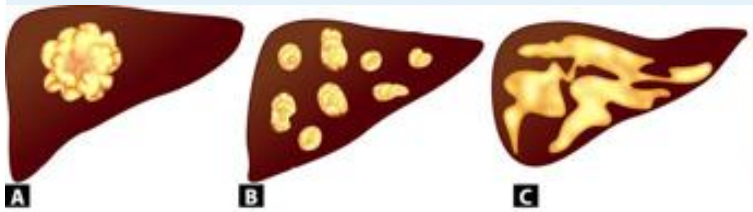
One of the concepts is that some HCCs may arise from epithelial stem cells of liver.

HCC: Tumor cells recapitulate varying degrees of normal liver architecture. Bile production by neoplastic cells is hallmark. Strong tendency for invasion of vessels.

Tumor marker for fibrolamellar HCC: Neurotensin.

Fibrolamellar HCC:

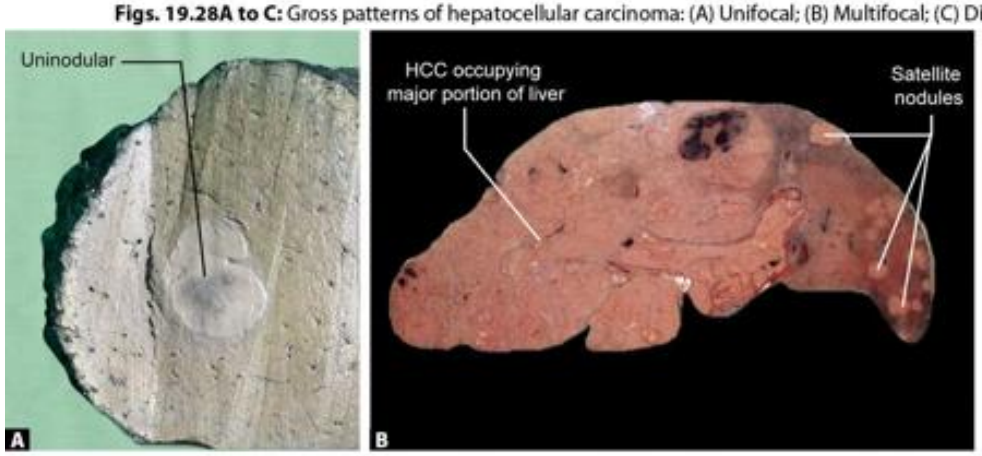
- Young adults without cirrhosis
- Well circumscribed with central stellate fibrous scar
- Grows slowly
- Better prognosis.



Lectin fraction -3 of AFP (AFP-l3):

- Highly specific to HCC
- Indicator of poorly-differentiated HCC
- Unfavorable prognosis

Figs. 19.28A to C: Gross patterns of hepatocellular carcinoma: (A) Unifocal; (B) Multifocal; (C) Diffuse infiltrative.



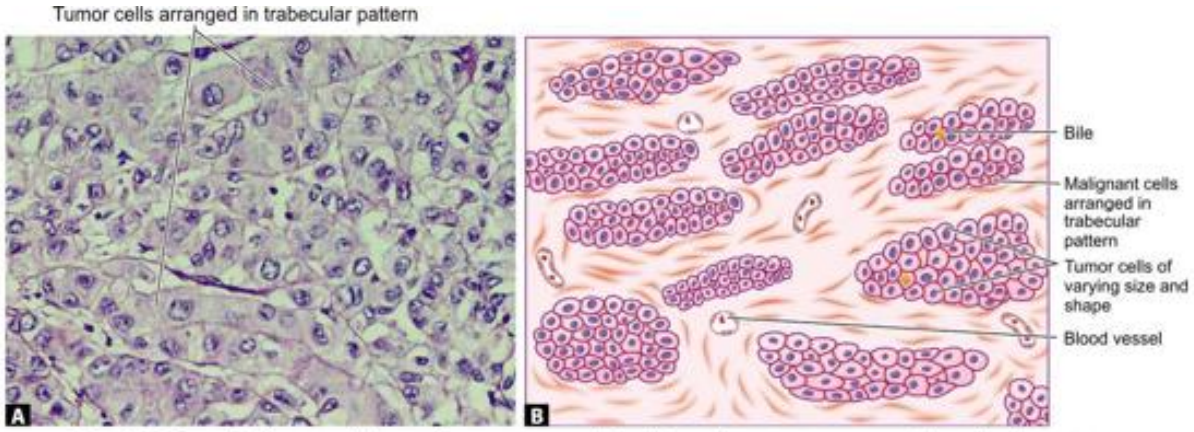
Hep-par 1: Used in diagnosing hepatocellular carcinoma

HCC gross: Three patterns

1. Unifocal
2. Multifocal
3. Diffusely infiltrative

Hep-par 1 (hepatocyte paraffin 1): Specific for hepatocyte mitochondria and is considered the most specific and sensitive marker of normal and neoplastic hepatocytes

Figs. 19.29A and B: Hepatocellular carcinoma (A) Uninodular; (B) Liver with a large gray-white tumor occupying the major portion of liver along with satellite nodules.



Figs. 19.30A and B: Hepatocellular carcinoma composed of malignant hepatocytes arranged in trabecular pattern: (A) Photomicrograph; (B) Diagrammatic.

HCC: Associated paraneoplastic syndromes include polycythemia (PTH-related protein), hypoglycemia (insulin-like factor), hypercalcemia and erythrocytosis (EPO-erythropoietin).

Hepatocellular carcinoma: Most common symptom—abdominal pain > weight loss.

HCC: Raised serum AFP. Staining for Glypican-3 is used to distinguish early HCC from dysplastic nodules.

Neoplasm with alpha-fetoprotein:
- Hepatocellular carcinoma
- Nonseminomatous germ cell tumors (e.g., endodermal sinus tumor/yolk-sac tumor) of testis.

Non-neoplastic conditions false positive with alpha-fetoprotein:
- Cirrhosis
- Massive liver necrosis
- Chronic hepatitis (especially HCV)
- Normal pregnancy, fetal distress or fetal death, fetal neural tube defects (e.g., anencephaly and spina bifida)

CHOLANGIOCARCINOMA (CCA)

- It is the **second most common hepatic malignant tumor of liver**.
- **Site:** It may **arise anywhere in the biliary tree**, from the large intrahepatic bile ducts at the porta hepatis to the smallest bile ductules (within liver).

Risk Factors

- **Primary sclerosing cholangitis** (PSC).

- **Congenital fibropolycystic diseases of the biliary system,** such as Caroli disease and choledochal cysts.
- **HCV infection.**
- Previous **exposure to thorotrast** (formerly used in radiography of the biliary tract).
- **Chronic infection** of the biliary tract by the liver fluke **opisthorchis sinensis**.
- **Premalignant lesions:** Biliary intraepithelial neoplasias (low to high grade, BilIN-1, -2, or -3).

Classification

Cholangiocarcinoma is classified according to their location.

- **Intrahepatic (**about 10%)
- **Extrahepatic forms** (about 80–90%)
  - **Perihilar tumors** (50–60% of all CCAs): These are known as **Klatskin tumors** and are located at the **junction of the right and left hepatic ducts** forming the common hepatic duct.
  - **Distal bile duct (bile duct carcinomas) tumors** (20–30%): They arise **near the ampulla of Vater**. They **also include periampullary carcinomas,** which consists of adenocarcinoma of the duodenal mucosa and pancreatic carcinoma.

MORPHOLOGY

Gross

- **Extrahepatic CCAs: These** are **usually small lesions** and appear as **firm, gray nodules within the bile duct wall**.
- **Intrahepatic CCAs**: They develop in the intrahepatic portal tract **(Fig. 19.31)**.

Microscopy

- **Adenocarcinomas**: Well-differentiated adenocarcinomas consist of well-defined **glandular and tubular structures lined by cuboidal to low columnar epithelial cells**.
- **Marked desmoplasia:** It is characterized by dense collagenous stroma separating the glandular structures.

Workers exposed to polyvinyl chloride may develop: Angiosarcoma of liver.

Cholangiocarcinoma of liver—one of the risk factor is: Opisthorchis/Clonorchis sinensis infection.

Klatskin tumors are located at the junction of right and left hepatic ducts. They are the most common subtype of cholangiocarcinoma.

The marker of choice for differentiating HCC and its fibrolamellar variant is AFP.

METASTATIC TUMORS

Q. List four common primary sites of metastatic tumors.

- Metastasis to liver is **more common than primary tumors of liver**. Apart from liver, lungs are also most often involved in the metastatic spread of cancers.
- **Most common site of primary** tumor producing hepatic metastases are the (i) **gastrointestinal tract (colon), (ii) breast, (iii) lung, and (iv) pancreas.** However, any cancer in any site of the body may spread to the liver, including leukemias, melanomas, and lymphomas.

MORPHOLOGY

Gross (Fig. 19.32)

- The liver is **enlarged** and may weigh several kilograms.
- The liver may show only **one nodule or** may be completely replaced by **multiple nodules of metastatic deposits**.
- On the surface of the liver, the metastatic **nodules appear as umbilicated masses.**
- **Central umbilication is due to** necrosis (outgrow blood supply) and hemorrhage in the central area of the nodule.

Microscopic

The metastatic deposits usually appear **similar to the primary tumor.** Occasionally, they may be so undifferentiated that the primary site cannot be determined.

Clinical Features

- **Weight loss:** It is a common early finding.
- **Portal hypertension with splenomegaly, ascites**, and **gastrointestinal bleeding** may occur.

- **Jaundice**: It may be either due to obstruction of the bile ducts or replacement of most of the liver parenchyma.
- Hepatic failure may develop.

Laboratory findings: May show **increase** in the **serum alkaline phosphatase** level.

Prognosis: Most patients die within a year.

Metastasis to liver: Most common malignant tumors of liver. Common primary includes GI tract, breast, lung and pancreas.

GALLBLADDER

ACUTE CHOLECYSTITIS

Definition: Acute cholecystitis is a acute **diffuse inflammation** of the gallbladder.

Types

Q. Write short note on cholecystitis.

- **Acute calculous cholecystitis:** It is associated **with gallstone** and is the most common serious complication of gallstone.
- **Acalculous cholecystitis:** It is not associated with gallstones and may occur in severely-ill patients.

Pathogenesis

Acute Calculous Cholecystitis

Acute inflammation of the gallbladder develops in **about 90% of cases as a complication due to obstruction of the neck or cystic duct by gallstones.** The inflammation of gallbladder may be brought out by **three factors**,

namely— (1) chemical inflammation, (2) mechanical inflammation, and (3) bacterial inflammation.

1. **Chemical inflammation**: Inflammation of the obstructed gallbladder may be due to chemical irritation.
   - **Production of lysolecithin**: The phospholipase from the epithelium of the gallbladder may act on lecithin present in bile and release **toxic** lysolecithin → can cause chemical inflammation.
   - **Decreased glycoprotein**: **Normally, glycoprotein** produced by gallbladder mucosa has **protective role.** Its **decreased production** leads to the direct mucosal cell damage by the detergent action of concentrated bile salts.
   - **Supersaturation of bile with cholesterol:** It may cause toxic damage to the epithelium.
   - **Prostaglandins:** These are **released within the wall of the distended gallbladder** and contribute to **inflammation of mucosa and wall of the gallbladder**.
2. **Mechanical inflammation: Obstruction of the cystic duct** increases intraluminal pressure and distention of the gallbladder → leads to disturbance of motility (dysmotility) of the gallbladder → results in **obstruction of venous flow and ischemia** of the gallbladder mucosa and wall.
3. **Bacterial inflammation:** It may play a role in acute cholecystitis, but **usually bacterial invasion is a secondary event**. The most frequent organisms include *Escherichia coli*, *Klebsiella* species, *Streptococcus* species, and *Clostridium* species.

Acute Acalculous Cholecystitis

- Gallstones are not seen in 5–10% of patients with acute cholecystitis.
- **Risk factors** include:
  - Major trauma and burns
  - Sepsis with hypotension and multisystem organ failure
  - Immunosuppression
  - Diabetes mellitus
  - Nonbiliary major surgical operations

- Systemic infections (tuberculosis, syphilis, actinomycosis, etc.).

- **Ischemic injury**: It is the **main pathogenic event** in acute acalculous cholecystitis.
- **Contributing factors**: These include inflammation and edema of the wall-compromising blood flow, dehydration, gallbladder stasis, and accumulation of microcrystals of cholesterol (biliary sludge).

MORPHOLOGY

Both **acute acalculous** and **calculous cholecystitis** are **morphologically similar**, except that stones are not seen in the acalculous form.

Gross (Fig. 19.33A)

- **Size:** Gallbladder is usually **enlarged, tense** and **edematous**.
- **Serosa:** It is covered by **fibrinous exudate** and the serosa may appear bright red due to subserosal hemorrhage. In severe cases, serosa may be covered by suppurative exudate.
- **Wall:** It is **thickened** (up to 2 cm) due to edema and hemorrhage.
- **Mucosa:** It is **red or purple** and may show **ulcerations**.
- **Lumen:** In **calculous cholecystitis**, the lumen may contain one or more **stones**. The lumen is filled with turbid fluid.

Q. Write a short note on microscopy of acute cholecystitis.

Microscopy (Figs. 19.33B and C)

- Mucosa shows focal **ulcerations**.
- **Wall of the gallbladder** shows **edema, hemorrhage** and **acute inflammatory cells**. Secondary bacterial infection may lead to suppuration in the gallbladder wall.
- **Widespread necrosis** is found in **gangrenous cholecystitis**.

Complications

Q. Write a short note on complications of acute cholecystitis.

- **Empyema of the gallbladder:** Gallbladder with obstructed cystic duct may be distended with pus (purulent exudate).
- **Gangrenous cholecystitis:** Gallbladder transformed into a green-black necrotic organ **(Fig. 19.33C)**.

- **Acute gaseous or emphysematous cholecystitis**: It is due to invasion of gas-forming organisms (such as clostridia and coliforms).
- **Perforation of the gallbladder:** It is due to secondary bacterial infection.
- **Bile peritonitis:** It may result from the discharge of bile from the distended gallbladder into the peritoneal cavity.
- **Pericholecystic abscess, abscesses in the liver or abdominal cavity**.
- **Fistula into the intestine or duodenum**: Perforations may lead to fistula formation with neighboring organs, such as small or large intestine creating a **cholecystenteric fistula.**

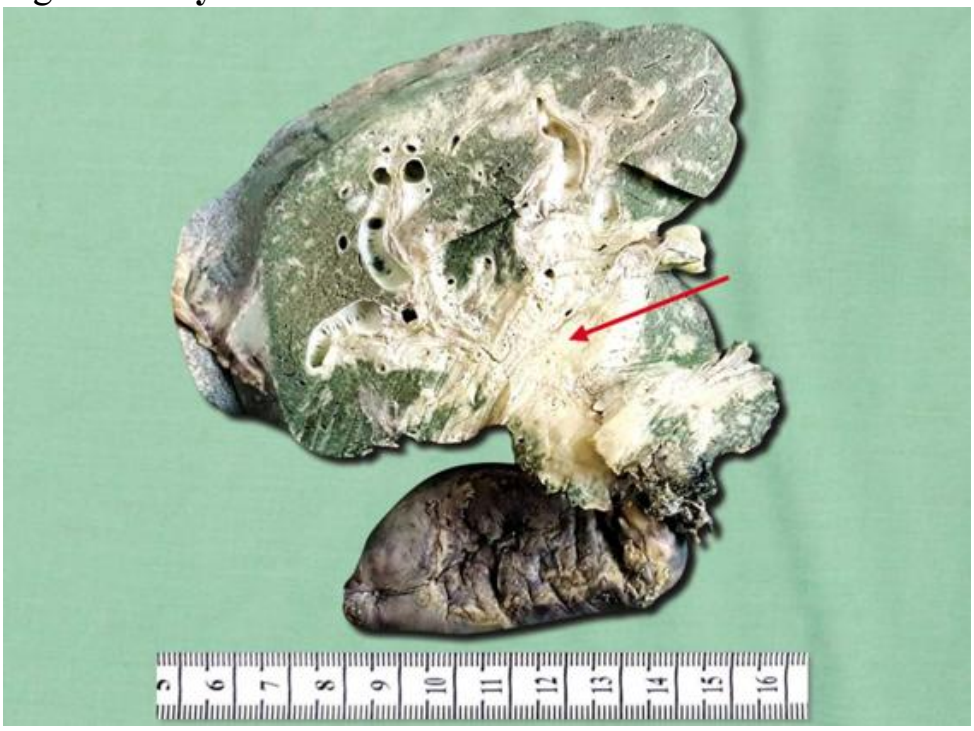


Fig. 19.31: Gross appearance of intrahepatic cholangiocarcinoma.



Fig. 19.32: Metastasis to liver showing multiple nodules (arrows) with umbilication.

- **Gallstone ileus**: **Through the fistula**, large gallstone can **pass into the bowel** → result in gallstone ileus or intestinal obstruction.

Clinical Features

- Acute cholecystitis presents with **progressive right upper quadrant** or epigastric pain.
- It is frequently associated with **mild fever, anorexia, nausea**, and **vomiting**. Acute calculous cholecystitis may also **appear suddenly** as an **acute surgical emergency**.
- **Acute acalculous cholecystitis** tends to be **more insidious in onset**.

Laboratory findings: Mild to moderate **leukocytosis** and mild elevations in serum alkaline phosphatase values may be seen.

Functions of gallbladder:

- Reservoir of bile
- Concentration of bile
- Secretion of mucus
- Acidification of bile

Gallbladder: Lacks
- Muscularis mucosa
- Submucosa

Cholecystitis: Inflammation of the gallbladder is known as cholecystitis. It may be acute, chronic, or acute superimposed on chronic.

Common diseases of the gallbladder: Gallstones, acute and chronic cholecystitis.

Cholecystitis: Most commonly occurs in association with gall- stones.

Pathogenesis of acute calculous cholecystitis:
- Chemical inflammation
- Mechanical inflammation
- Bacterial inflammation

Acute cholecystitis:
- Right upper quadrant pain
- Fever
- Leukocytosis

CHRONIC CHOLECYSTITIS

- **Most common disease of the gallbladder**.
- Associated with **gallstones in ~90%** of the cases.
- May also develop following recurrent attacks of acute cholecystitis.

MORPHOLOGY

Extremely variable and depends on the severity and the duration of the disease.

Gross (Fig. 19.34)

- **Size:** Gallbladder may be **normal** or may be **shrunken** in severe cases.
- **Serosa:** It is usually **smooth and glistening**, but may be dull due to fibrosis.
- **Cut section:** The **wall is thickened, opaque and gray-white**.
- **Lumen**: It usually shows **stones**.

Microscopy (Figs. 19.35A and B)

Q. Write a short note on microscopy of chronic cholecystitis.

- **Mild cases**: The inflammation is **scanty** and show **lymphocytes, plasma cells, and macrophages** in the **mucosa and in the subserosal fibrous tissue**.
- **Advanced cases:** It shows **marked subepithelial and subserosal fibrosis**, associated with **mononuclear cell infiltration**.
- **Mucosal epithelium**: It may be **normal or atrophic** or show focal ulceration.
- **Rokitansky–Aschoff sinuses:** These are **irregularly shaped, tubular structures** formed due to **invagination of mucosal epithelium deep into the wall** of the gallbladder**.** They represent herniations or diverticula resulting from increased intraluminal pressure.

Morphological Variants

- **Cholecystitis glandularis:** In this varaint **mucosal folds fuse together** and form crypts of epithelium buried in the gallbladder wall.
- **Porcelain gallbladder**: It is characterized by **extensive dystrophic calcification** within the gallbladder wall (predominantly in the muscle and lamina propria).
- **Acute on chronic cholecystitis**: Superimposition of acute inflammation on chronic inflammation indicates acute on chronic cholecystitis.
- **Xanthogranulomatous cholecystitis:** It is characterized by **marked thickening of wall** and **diffuse or nodular collections of macrophages containing neutral fat and lipofuscin** (ceroid) pigment.

Clinical Features

- Usually present as **recurrent attacks** of either **steady or colicky epigastric or right upper quadrant pain**.
- Other features **include nausea**, vomiting, and **intolerance for fatty foods**.

Complications

- **Bacterial superinfection** with cholangitis or sepsis.
- **Gallbladder perforation and local abscess** formation.
- **Gallbladder rupture with diffuse peritonitis.**
- **Biliary enteric (cholecystenteric) fistula**, with passage of gallstones into adjacent organs, such as intestine. It may result in **gallstone-induced intestinal obstruction (ileus).**
- **Porcelain gallbladder has increased risk of cancer**.

Rokitansky–Aschoff sinuses: Irregular, tubular structures formed due to invagination of gallbladder mucosal epithelium deep into the wall.

CHOLELITHIASIS (GALLSTONES)

Q. Discuss the etiopathogenesis of cholelithiasis. Describe the morphology of different kinds/types of gallstones. List the complications.

- Cholelithiasis is a **common disorder** and found in ~10 to 20% of adult populations in developed countries.
- **Majority (>80%) are "silent"**.

Classification of Gallstones

Q. Classify gallstones/cholelithiasis.

Depending on the Chemical Composition

Most widely used classification is based on the relative amount of cholesterol within stones. There are two main types of gallstones, namely—(i) cholesterol stones and (ii) pigment stones.

Cholesterol Gallstones

They contain **more than 90% of crystalline cholesterol monohydrate** + calcium bilirubinate + calcium carbonate. **Cholesterol stones composed of mainly cholesterol** are **radiolucent**.

- **Mixed cholesterol gallstones:** They contain a **mixture of cholesterol (60–89%)** + calcium bilirubinate + calcium carbonate + mucin glycoprotein.

Pigment Gallstones

They develop when there is **increased unconjugated bilirubin**. They are composed of **calcium bilirubinate (calcium salts of unconjugated bilirubin) + calcium carbonate** + less **than 20% of cholesterol**. Pigment stones are subclassified as **black and brown pigment** stones.

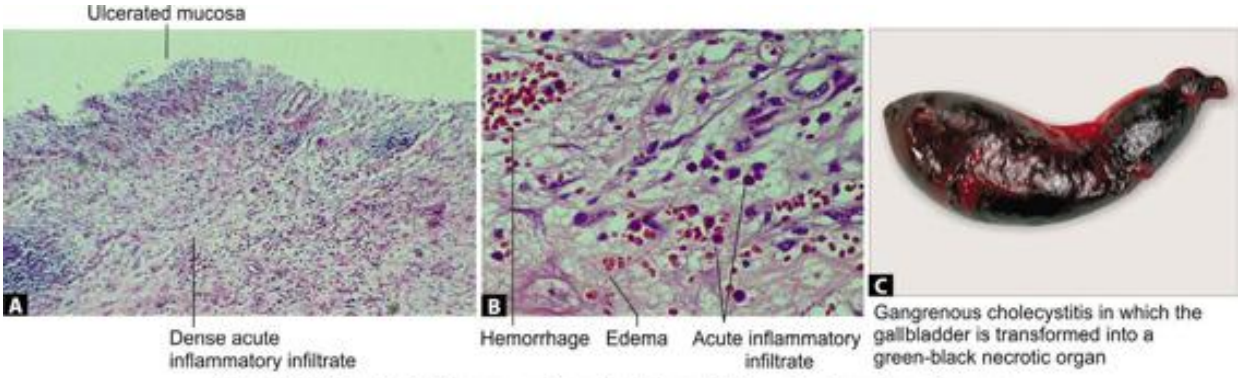
- **Black pigment stones:** They are common in India (40%).
  - Generally occur in patients with older age, cirrhosis and chronic hemolytic states.

- Contains 10–90% calcium bilirubinate + other calcium salts (50–75% —such as phosphate and carbonate), mucin glycoprotein. They have very low cholesterol concentration.
- About 50–75% are radiopaque because of calcium salts.

- **Brown pigment stones:** These are more common in Asia.
  - They form in the bile duct and are associated with bile stasis and infections in the gallbladder and biliary tree. They are rare in the gallbladder.
  - Composed of calcium bilirubinate + calcium palmitate (calcium salts of free fatty acids) + mucin glycoprotein + fatty acids and cholesterol less than 20%. Calcium phosphate and calcium carbonate are usually not present (in contrast they are present in black pigment stones).
  - Usually radiolucent.

Old classification is presented in **Box 19.7**.

Depending on the Location



Figs. 19.33A to C: (A) Microscopy of acute cholecystitis showing ulceration of mucosa; (B) High-power view showing edema, hemorrhage and acute inflammatory infiltrate; (C) Gangrenous cholecystitis.

- **Intrahepatic stones:** They are predominantly **brown pigment stones**.
- **Gallbladder stones:** These are **mainly cholesterol stones** and **few are black pigment stones**.
- **Choledocholithiasis** (bile duct stones): Composed mostly of **mixed cholesterol stones**.

Risk Factors for Cholesterol Stones (Box 19.8)

Q. Write short note on risk factors for cholesterol stones.

Cholesterol gallstones are uncommon in developing countries. They are is formed probably due to combination of genetic susceptibility and environmental factors.

1. **Advancing age:** It is one of the **major risk factor**. Prevalence of cholesterol gallstones increases with age until, at 60 years. As age advances increase in gallstone is associated with the metabolic syndrome and obesity.
2. **Female sex hormones:**
   - **Female gender:** Gallstone is **two times more** common in females than in males and higher prevalence is observed in premenopausal women and both may be due to effect of estrogen.**Estrogen** increases the secretion of cholesterol and decrease the secretion of bile acids by the liver → favors → lithogenic bile secretion by the liver.
   - **Pregnancy:** In the last trimester of pregnancy, the **gallbladder empties more slowly** and causes **stasis** and increases the **precipitation of cholesterol crystals**. Progesterone, which is the predominant hormone during pregnancy inhibits the discharge of bile from the gallbladder.
   - **Oral contraceptives:** They **increases** both **uptake and synthesis of cholesterol**.
3. **Environmental factors:**
   - **Drugs**: **Clofibrate**, used to lower blood cholesterol results in **excessive secretion of cholesterol in the bile**. Drugs, such as octreotide and ceftriaxone also predispose to gallstones.
   - **Obesity and rapid weight loss:** These are strongly associated with **increased biliary cholesterol secretion**. Obesity enhances cholesterol absorption, synthesis, and secretion. There is probably a linear relation between body weight and the risk of gallstones.
   - **Diet high in calories and cholesterol:** They are associated with increased risk.
4. **Acquired disorders: Gallbladder stasis** (neurogenic or hormonal) → promote cholesterol and pigment gallstones.
5. **Genetic predisposition:** Prevalence of cholesterol stones is highest in North American Indians, Chilean Indians.
6. **Familial predisposition**: Gallstones are twice as common in first-degree relatives of patients with gallstones. ***ABCG5*** **and** ***ABG2*** **genes**

participate in biliary cholesterol secretion and its **variants increases the risk** for the development of cholesterol gallstones.

7. **Metabolic abnormalities:** These associated with high blood cholesterol levels (e.g., diabetes, some genetic hyperlipoproteinemias, and PBC).

Pathogenesis of Cholesterol Stones (Fig. 19.36)

Q. Write short note on pathogenesis of cholesterol stones.

The cholesterol stone formation is **complex multifactorial process**. Cholesterol gallstone formation involves four simultaneous processes namely, (1) cholesterol supersaturation, (2) gallbladder hypomotility, (3) acceleratednucleation and crystallization and (4) growth to stone-sized aggregates.

Cholesterol Supersaturation

- **Cholesterol is secreted by the hepatocytes as vesicles** (unilamellar or multilamellar) **or micelles**.
- **Vesicles** are spherical structures consisting of **cholesterol surrounded by one or more layers of phospholipid bilayers** but **does not contain bile salts**.
  - **Unilamellar vesicle:** It **consists of cholesterol surrounded by one phospholipid bilayer**.
  - **Multilamellar vesicle:** It consists of **cholesterol surrounded by more than one phospholipid bilayer**.
- **Micellation**: It is the **process of conversion of vesicle into complex aggregates of water-soluble molecules** known as mixed **micelles** by **the detergent-like action of bile acids**.
  - Mixed micelles = bile acids + phospholipids + cholesterol, in which the lipophilic (fat soluble) portions of the three molecules aggregate in the center of the structure while the hydrophilic (water-soluble) portions are oriented at the periphery, thus allowing solubility in bile.
- **Supersaturation:** It is a condition in which **cholesterol concentrations of bile exceed** the **solubilizing** (achieved by phospholipids and bile

acids) **capacity of bile**. It is one of the important mechanisms in the formation of lithogenic (stone-forming) bile.

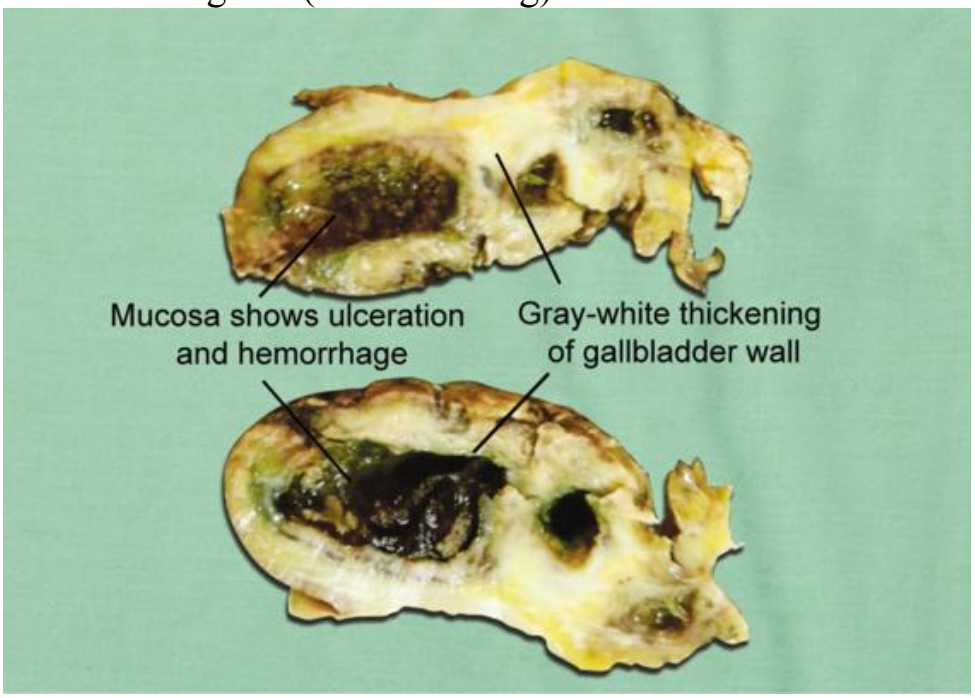
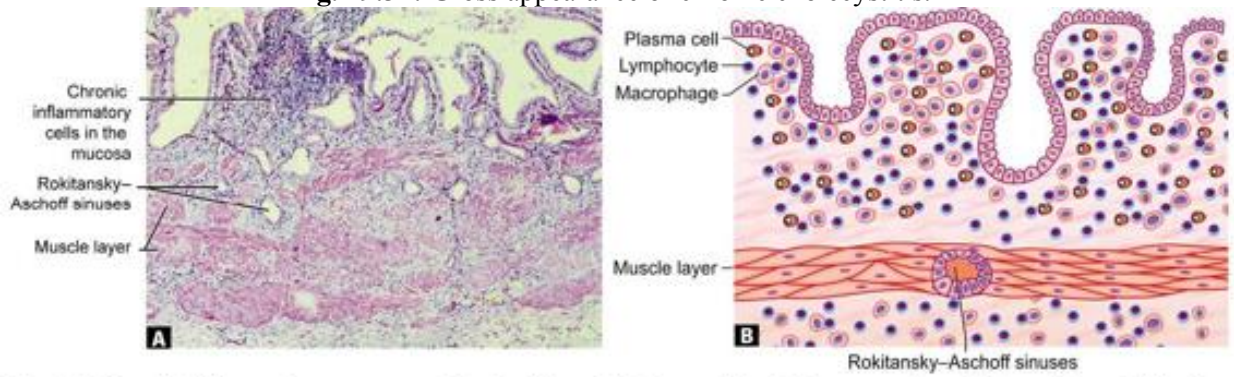


Fig. 19.34: Gross appearance of chronic cholecystitis.



Figs. 19.35A and B: Microscopic appearance of chronic cholecystitis. Outpouching of the mucosa through the wall forms Rokitansky–Aschoff sinus. Mucosa is infiltrated by inflammatory cells: (A) Photomicrograph; (B) Diagrammatic.

- **Causes of cholesterol supersaturation**: It may be due to **increased biliary cholesterol secretion** (most important cause), **decreased bile acid synthesis, or both**.
  - **Increased biliary cholesterol secretion**: It is observed in obesity, high-caloric diet and cholesterol-rich diets, certain drugs (e.g., clofibrate), pregnancy, oral contraceptives, rapid weight loss, and increased activity of hMG-CoA reductase (the rate limiting enzyme of hepatic cholesterol synthesis).
  - **Decreased bile salts**: Crohn disease.

Gallbladder Hypomotility

- Normal motility of the gallbladder empties and completely excretes its bile along with all supersaturated bile or crystals. Thus, motility does not allow stones to grow.
- Abnormalities in gallbladder emptying (decreased motility/hypomotility) → **causes gallbladder stasis → promote the aggregation of cholesterol crystals → formation of biliary sludge** (also known as **microlithiasis**).

Nucleation and Precipitation of Cholesterol Monohydrate Crystals

Nucleation

With supersaturation, cholesterol can no longer remain soluble.

Excess of cholesterol is carried as unilamellar cholesterol-rich vesicles → which fuse into large multilamellar vesicles → they nucleate to form solid microscopic cholesterol monohydrate crystals. The **nucleation** is the first and crucial step in gallstone formation and is **accelerated in lithogenic bile**.

Accelerated Nucleation

It may be due to either an excess of pronucleating factors or a deficiency of antinucleating factors.

- **Pronucleating factors: Mucin** and **certain non-mucin glycoproteins** and **biliary calcium** are important pronucleating factors.
  - **Hypersecretion of mucus:** Mucin glycoproteins are normally secreted continuously from the gallbladder. Hypersecretion of mucin **accelerates growth and precipitation of cholesterol monohydrate crystals.** Mucus traps the nucleated crystals and favors their aggregation into stones.
  - **Calcium salts:** Calcium carbonate, calcium bilirubinate and calcium phosphate in the bile can **serve as a nidus for cholesterol crystallization**.
- **Anti-nucleating factors**: These include apolipoproteins A-1 and A-2, other glycoproteins and lecithins.

Growth to Stone-sized Aggregates

- **Biliary sludge:** It is defined as **suspension of precipitates of cholesterol monohydrate crystals or calcium bilirubinate granules in bile.** Before the appearance of stones, there is always the formation of a 'biliary sludge'. It implies supersaturation of bile with either cholesterol or calcium bilirubinate. Biliary sludge can resolve, persist, or progress. When it progresses to stones its crystals grow to form plates, in part because of impairment in gallbladder contractility.
- **Microlithiasis: After nucleation, crystallization** occurs, and **cholesterol monohydrate crystals grow into microstones** (microlithiasis/crystals in bile). Microlithiasis is identified by the presence of sludge by ultrasonography (US) or the presence of birefringent cholesterol crystals in bile.
- **Gallstone:** The **microstones increase** in size by gradual addition of more cholesterol and other constituents → **form macroscopic gallstones**.

BOX 19.7: Older classification of gallstones.

- **Pure gallstone** (10%)
  - Cholesterol
  - Bile pigment (bilirubin)
  - Calcium carbonate
- **Mixed stone** (80%): Mixture of cholesterol, bile pigment and calcium carbonate in varying proportion
- **Combined stone** (10%): Pure gallstone nucleus with mixed stone shell or mixed gallstone nucleus with pure gallstone shell

BOX 19.8: Risk factors for gallstones.

Cholesterol Stones

1. **Advancing age**
2. **Female sex hormones**: Female, pregnancy, parity and oral contraceptives
3. **Environmental factors**: Drugs (octreotide, ceftriaxone, clofibrate therapy), obesity and metabolic syndrome, rapid weight reduction, diet high in calories and cholesterol
4. **Acquired disorders:** Gallbladder stasis
5. **Genetic predisposition**
6. **Hereditary factors**: Familial predisposition, inborn disorders of bile acid metabolism
7. **Metabolic abnormalities:** Diabetes, genetic hyperlipoproteinemias and PBC

Pigment Stones

1. **Demography:** More common in Asians and in rural region more than urban
2. **Chronic hemolysis**
3. **Chronic biliary tract infections**
4. **Gastrointestinal diseases**: Ileal disease (e.g., Crohn disease), ileal resection or bypass, cystic fibrosis with pancreatic insufficiency
5. **Alcoholic cirrhosis**

6. **Pernicious anemia**
7. **Advancing age**

Pathogenesis of Pigment Stones

Q. Write short note on risk factors/pathogenesis of pigment stones.

Risk factors for pigment stones are listed in **Box 19.6**. Pigment stones are subclassified as **black and brown pigment** stones.

Black Pigment Stones

- **Unconjugated bilirubin** is **insoluble in bile**. Under **normal physiologic conditions, it is not secreted into bile** and constitutes less than 1% of total bile pigment.
- **Increased production of unconjugated bilirubin** may **precipitate as calcium bilirubinate** probably **around a nidus of mucinous glycoproteins to form black pigment stones**.
- **Causes of increased unconjugated bilirubin in bile:** These include **chronic hemolysis** (e.g., β-thalassemia, hereditary spherocytosis, sickle cell disease), cirrhosis, severe ileal dysfunction (or bypass) and pancreatitis. In most patients, the predisposing cause is not known.

Brown Pigment Stones (Fig. 19.37)

It can be **found in the gallbladder or within the biliary tree**.

Bile Stasis and Infection

Brown pigment stones **develop secondary to stasis and infection**.

- **Stasis favors the bacterial infection** as well as accumulation of mucus.
- **Biliary infections**
  - **Bacterial infections of biliary tract by enteric bacteria** (predominantly *E. coli*) and with ascending cholangitis favor brown stone formation.
  - **Parasitic infestations:** Brown stones may be found in association with **infestation by *Ascaris lumbricoides* or *Clonorchis sinensis***,

and other helminthes, which may invade the biliary tract.

- Dead bacteria and parasites may act as nuclei and accelerate the precipitation of calcium bilirubinate.

Actions of Bacterial Enzymes on Bile Constituents

In the bile, the bacterial enzymes have following actions:

- **Bacterial enzyme β-glucuronidase → hydrolyzes conjugated bilirubin** in the bile → to **unconjugated**, insoluble bilirubin.
- Bacterial **phospholipases hydrolyze phospholipids** and **liberate free fatty acids** (such as palmitic acid and stearic acid).
- Bacterial enzymes **hydrolyze bile salts** (glycine or taurine-conjugated) into **free (unconjugated) bile acids**.

Formation of Brown Stone

The **products** formed from above bacterial actions: (1) unconjugated insoluble bilirubin, (2) free fatty acids and (3) unconjugated bile acids. They **combine with calcium** to produce → **water-insoluble calcium bilirubinate** → forms brown stone.

MORPHOLOGY

Cholesterol Stones (Fig. 19.38A)

- Site: They occurs only in the gallbladder.
- Appearance: It **varies depending on the cholesterol content;** either purely cholesterol (100%—rare) or have cholesterol as the major chemical component.

Pure Cholesterol Stones

They account for 10% of gallstones.

Appearance (Fig. 19.38A): Single, **pale yellow and round to ovoid**.

- **Finely granular** and hard external surface.

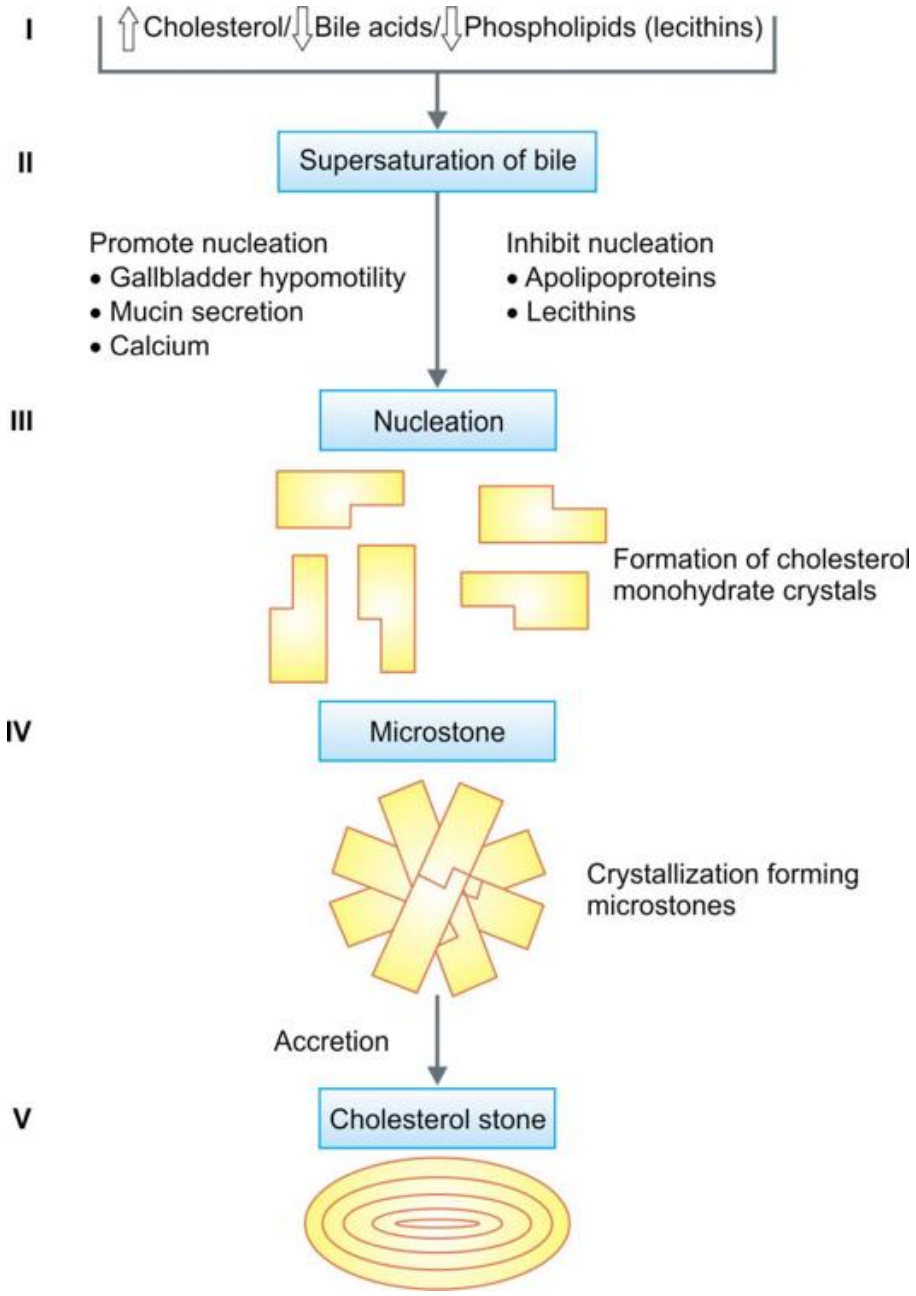
- **Large** and measure **2–4 cm** in diameter.
- **Cut surface:** It is **glistening** and shows **long, thin radiating cholesterol monohydrate crystals**.
- **Radiolucent**.

Mixed Gallstones

Stones with **lower cholesterol content than the pure cholesterol stones** are designated mixed. They are composed of **60–89% cholesterol and proportions of calcium carbonate, phosphates, and bilirubin**. They are **slightly more common than pure cholesterol stones**.

Appearance (Fig. 19.38B): Often **multiple and gray-white to black**.

- **Surfaces may be rounded or faceted**, because of tight apposition.
- **Smaller than pure cholesterol stones** and that range from **0.2 to 3.0** cm in diameter.
- **Cut surface: Laminated** with a dark core.
- **Stones composed largely of cholesterol are radiolucent;** but **those with sufficient calcium carbonate are radiopaque** (10–20%).



Bile salts = Bile acids (cholic acid and chenodeoxycholic acid)+ taurine or glycine.

Bile acids: Water-soluble sterols are the major catabolic products of cholesterol.

Bile acids: Act as detergents and solubilize water-insoluble lipids (secreted by hepatocytes) in the bile, and dietary lipids in the lumen of the gut.

Increased biliary cholesterol:
- Obesity
- Cholesterol-rich diet

- Clofibrate therapy.

Decreased bile acids:
- Primary biliary cirrhosis
- Mutation of *CYP7A1* gene
- Oral contraceptive pills
- Impaired enterohepatic circulation (e.g., Crohn disease, ileal resection).

Decreased biliary lecithin: *MDR-3* gene mutation.

Solubility of cholesterol in bile depends on the concentration of phospholipids and bile acids.

Fig. 19.36: Pathogenesis of cholesterol stones.

Pigment Gallstones

Black pigment stones are **formed in sterile gallbladder bile**, and **brown stones are formed in infected intrahepatic or extrahepatic ducts**.

Black Pigment Stones (Fig. 19.38C)

Composed of either **pure calcium bilirubinate** or in **combination with a variety of other calcium** salts (hydroxyapatite and carbonate).

Appearance: Multiple with an **inverse relationship between size and number**.

- **Shiny**, **black or deep brown**.
- **Usually irregular, spiculated (**"jacklike"**) molded** and may crumble on touch.
- **Relatively small** and range from **0.2–1.5** cm **in diameter**.
- **Arise in sterile gallbladder bile**.
- Because of calcium carbonates and phosphates, **approximately 50–75% are radiopaque**.

Brown Pigment Stones (Fig. 19.38D)

Composed of **calcium salts of unconjugated bilirubin**, with **varying amounts of cholesterol and protein**.

- **Appearance: Multiple** and **dull brown**.
- **Laminated and soft** and may have a **soap-like or greasy consistency.** These stones are **easy to crush endoscopically**.
- Range from **0.2–1.5** cm **in diameter**.
- **Usually radiolucent**.
- Usually **associated with infection of biliary tract and intrahepatic or extrahepatic ducts**.

Clinical Features

- Majority of patients with gallstones remain **asymptomatic** throughout their lives.
- Gallstones usually produce symptoms by causing inflammation or obstruction when they pass into the cystic duct or CBD.
- The characteristic symptom is **biliary colic** that is a constant and often long-lasting pain. Large gallstones usually cannot enter the cystic or common ducts to produce obstruction.

Complications

Q. Write short note on complications of gallstones.

In the Gallbladder

- **Cholecystitis**: It can cause both **acute and chronic cholecystitis.** Rarely it may cause gangrenous or emphysematous cholecystitis.
- **Empyema of the gallbladder**: It is characterized by distention of gallbladder with pus.
- **Hydrops of the gallbladder**: Gallbladder distended with clear watery fluid.
- **Mucocele:** Gallbladder distended by cloudy, mucoid material.
- **Perforation of gallbladder**.
- **Carcinoma:** Gallstones may **increase the risk of carcinoma** of the gallbladder.

In the Bile Ducts

- **Biliary obstruction** (obstructive cholestasis).
- **Acute cholangitis** (inflammation of the biliary tree).
- **Acute pancreatitis**.

In the Intestine

- **Biliary fistulas:** Fistula may develop **between biliary system and bowel or gallbladder and skin**.
- **Gallstone ileus** or **Bouveret's syndrome**: Rarely, a large gallstone may erode directly into an adjacent loop of small intestine and produce intestinal obstruction ("**gallstone ileus" or Bouveret's syndrome**).

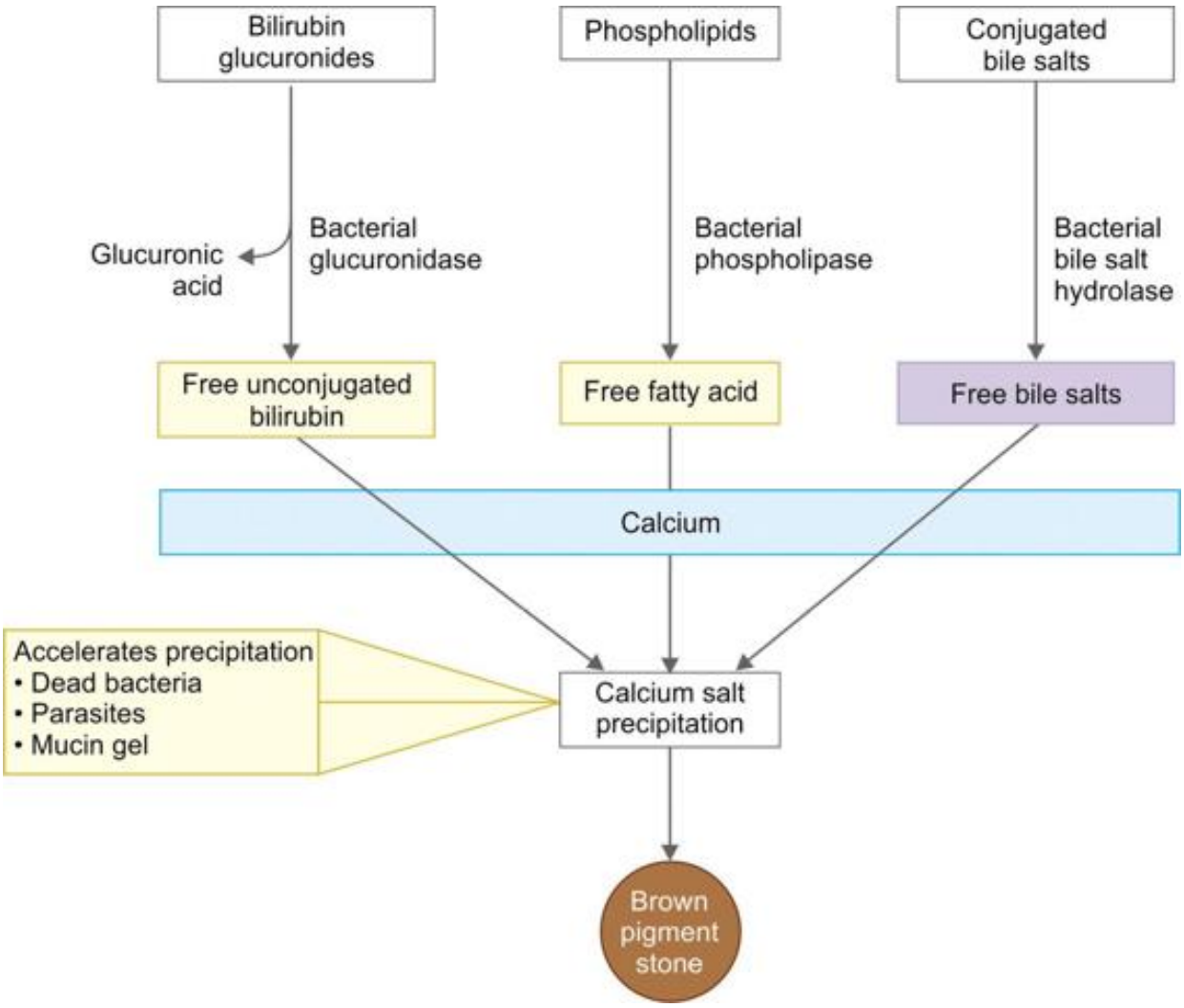
Major solutes of bile:
- Bile acids (80%)
- Phospholipids (includes lecithin and other phospholipids— 16%)
- Unesterified cholesterol (4.0%)

Primary bile acids: Includes cholic acid and chenodeoxycholic acid and are synthesized from cholesterol in the liver.

Gallbladder hypomotility/statsis:
- Total parental nutrition (TPN)
- Prolonged fasting
- Pregnancy
- Massive burns
- Oral contraceptive pills
- Octreotide

Pronucleating factors:
- Mucin
- Non-mucin glycoproteins
- Calcium
- Infection

Anti-nucleating factors:
- Apolipoproteins A-1 and A-2
- Other glycoproteins
- Lecithins



Brown stones:
- Formed in bile duct
- Rare in gallbladder.

Brown pigment stones develop secondary to:
- Bile stasis
- Biliary infection.

Brown pigment stones: Sign of biliary tract infection

Chemical composition of gallstones: Cholesterol, calcium bilirubinate and other calcium salts (carbonate, phosphate and palmitate) are basic constituents.

5 Fs of cholesterol stone:
1. **F**at
2. **F**ertile

3. **F**orty
4. **F**emale
5. **F**amilial

Fig. 19.37: Pathogenesis of brown pigment stones. The enteric bacteria produce β-glucuronidase, phospholipase A, and conjugated bile acid hydrolases. β-glucuronidase hydrolyze unconjugated bilirubin; phospholipase A liberates free fatty acids from phospholipids; and conjugated bile acid hydrolases produce unconjugated (free) bile acids. They form complex with calcium to produce insoluble calcium salts, thereby resulting in stone formation. Dead bacteria and parasites accelerate the precipitation of calcium bilirubinate. The mucin gel in the gallbladder can trap these complex precipitates and facilitate their growth into macroscopic stones.

Black pigment stones associated with:
- Chronic extravascular hemolysis (e.g., hereditary spherocytosis, sickle cell anemia)
- Cirrhosis
- Mechanical prosthetic valves
- Gilbert syndrome
- Cystic fibrosis
- Ileal disease or resection

Cholesterol stones: Most common gallstone. May be pure or mixed.

Black pigment stones: Seen with chronic extravascular hemolysis and consists mainly calcium bilirubinate.

Common complications of gallstones:
- Cholecystitis
- Biliary obstruction
- Carcinoma
- Acute pancreatitis

Hydrops of gallbladder: Due to obstruction of cystic duct.

CARCINOMA OF THE GALLBLADDER

- Carcinoma of the gallbladder **rare**; however, it is the most common malignancy of the extrahepatic biliary tract.
- It is slightly **more common in females** and occurs most frequently during seventh decade of life.
- It is common in parts of northern India.

Etiology

Risk Factor

- **Gallstones**: They predispose to **carcinoma by causing chronic irritation, chronic inflammation and epithelial damage**.
- **Genetic factors**: Prevalence of the gallbladder cancer in American Indians, Hispanic Americans and parts of north India probably reflect the role of genetic factors.
- **Carcinogen**: **Present in the bile acids** are believed to play a role.
- **Calcification of the gallbladder**.
- **Gastrointestinal disorders:** Ulcerative colitis and polyposis syndromes also have an association with gallbladder adenocarcinoma.

Molecular Genetics

- Carcinoma of the gallbladder develops through accumulation of multiple genetic alterations, involving oncogenes *(KRAS* mutation), tumor suppressor genes (*p 53* mutations), and DNA repair genes.
- Microsatellite instability is present in about 10% of cases.

MORPHOLOGY

Gross

- Sites: **Most arise in** the **fundus and neck** (60%), followed by the body (30%).
- **Usually show calculi** and marked fibrosis of the wall.
- **Two patterns of growth.**
- **Diffusely infiltrating (70%):** It usually appear as a **poorly defined area of diffuse thickening of the gallbladder wall** (due to **prominent desmoplasia**) and similar to linitis plastica of stomach. These tumors are **scirrhous, gray-white** and have a very firm consistency. Deep ulceration can cause fistula formation to adjacent viscera.
- **Exophytic/polypoid (30%):** It grows into the lumen as an **irregular, cauliflower mass** and also invade the underlying wall of the gallbladder. The luminal portion of the tumor mass may show areas of necrosis, hemorrhage and ulceration.

Microscopy

- Most gallbladder cancers are **adenocarcinomas** showing varying degrees of differentiation.
- The well-differentiated carcinomas consist of well-formed glands lined by a layer of cuboidal cells and often embedded in a desmoplastic stroma.
- Others tumors may be infiltrative and poorly differentiated to undifferentiated.

Spread

- **Direct spread**: Tumor may spread to liver, peritoneal surfaces, cystic duct and adjacent bile ducts. Biliary-enteric fistulas may develop when tumor penetrates into intestine.
- **Lymphatic spread**: It may spread to perihilar lymphatics and portal-hepatic lymph nodes and in later stages, mediastinal and supraclavicular lymph nodes.
- **Blood spread**: **Liver** (both local spread and hematogenous spread) and **lungs**.

Clinical Features

- **Right upper quadrant abdominal pain, anorexia and nausea and vomiting**.
- Laboratory investigation show **elevated alkaline phosphatase level**.

Risk factors for carcinoma of gallbladder:

- Gallstones
- Porcelain gallbladder.

Carcinoma of gallbladder: Adenocarcinoma.

Carcinoma of gallbladder: Most common symptom is biliary colic.

PANCREAS



A

B



Figs. 19.38A to D: Gallbladder with stones: (A) Showing cholesterol stones; (B) Showing mixed cholesterol stones; (C) Showing multiple black pigment stones; (D) Showing brown stones removed from gallbladder.

ACUTE PANCREATITIS

Definition: Acute pancreatitis is a reversible, **acute inflammation of the exocrine pancreas** due to injury to the parenchyma of the pancreas.

- Inflammatory disease of the exocrine pancreas may be classified as (1) acute pancreatitis or (2) chronic pancreatitis.
- Acute pancreatitis is relatively common. Its **severity varies from focal edema and fat necrosis to widespread hemorrhagic necrosis**.

Etiology

Q. Discuss the etiology and pathogenesis of acute pancreatitis.

Various causes of acute pancreatitis are presented in **Box 19.9. Alcoholism and gallstones account for** about **80% of the cases**.

1. **Alcohol:** It causes acute pancreatitis usually in individuals who consume large quantities of alcohol. **A polymorphism in the detoxifying enzyme uridine 5-diphosphate (UDP) glucuronyl transferase increases the risk for alcoholic induced pancreatitis**. The male-to-female ratio is 6:1.
2. **Gallstones:** It is responsible for acute pancreatitis **in about 30–60% of cases**. The frequency of acute pancreatitis is inversely proportional to the size of gallstones. The male-to-female ratio is 1: 3.
3. **Non-gallstone-related obstruction of the pancreatic ducts:** Due to pancreatic cancer or other periampullary neoplasms, biliary sludge, parasites (e.g., *Ascaris lumbricoides* and *Clonorchis sinensis*).
4. **Genetic factors:** No cause is identified in about 10–20% of patients and are termed idiopathic. Some of these may have a genetic basis. Three susceptibility genes have been identified:

- **Mutation in cationic trypsinogen** (*PRSS1*) **gene:** It is the **most common genetic defect,** which results in resistance to trypsin hydrolysis.
- **Mutation in the pancreatic trypsin inhibitor gene** (serine protease inhibitor, Kazal type 1; *SPINK1)*: *SPINK1* gene is essential for the inactivation of trypsin and for the prevention of the autodigestion of the pancreas by activated trypsin. Mutation of this gene **causes inappropriate activation of trypsin**, which in turn can **activate other digestive proenzymes** resulting in pancreatitis.
- **Mutations in the cystic fibrosis transmembrane regulator** (***CFTR***) gene.

5. **Drugs:** They cause pancreatitis **either by a hypersensitivity reaction** or by the generation of **toxic metabolite**.

Pathogenesis (Fig. 19.41)

Q. Write short essay on pathogenesis of acute pancreatitis.

The exact mechanisms by which the above causes trigger inflammation of the pancreas are not completely known.

Autodigestion of the Pancreatic Substance

According to this theory, pancreatitis **develops due to premature** (inappropriate) **activation of proteolytic pancreatic enzymes** → leads to a **process of autodigestion**.

Activation of Pancreatic Enzymes

Pancreatic enzymes are synthesized in an inactive proenzyme form (e.g., trypsinogen, chymotrypsinogen, proelastase, and lipolytic enzymes, such as phospholipase A_2) and become active when converted into enzyme form. **Premature activation** (before their secretion from the acinar cells) **of pancreatic enzyme**s is responsible for acute pancreatitis.

- **Activation of trypsin:** One of the pancreatic **proenzyme trypsinogen is prematurely activated to trypsin** and it is an **important triggering**

event in the pathogenesis of **acute pancreatitis**.

- **Factors activating trypsin,** e.g., endotoxins, exotoxins, viral infections, ischemia, anoxia. Trypsin also can be spontaneous activated.
- **Actions of trypsin:** Trypsin can **convert many proenzymes into active forms** [e.g., proelastase to → elastase (damages the elastic fibers of blood vessels) and prophospholipase to → phospholipase (degrades fat cells).
- **Activation of kinin, clotting and complement system:** Trypsin acts on kinin system and converts prekallikrein to → kallikrein → activate Hageman factor (factor XII), the clotting and complement systems.

Mechanism of Activation of Pancreatic Enzymes

The exact mechanisms of activation of pancreatic enzymes are not well-understood. However, the fundamental mechanism that transforms the initial injury into pancreatitis appears to be **intracellular activation of digestive proenzymes into active enzymes in the pancreatic acinar cells**. This can involve **three possible pathways**:

1. ***Pancreatic duct obstruction:*** According to this theory, partial or total obstruction of the pancreatic duct alone can cause pancreatitis.
   - Any **pancreatic duct obstruction** (e.g., gallstones, biliary sludge, tumor) can **raise intraductal pressure** → exacerbate back-diffusion across the ducts → lead to the **rupture of ductules and acini** → **liberates enzyme-rich fluid** in the interstitium and **inappropriate activation of digestive proenzymes** → causes injury → initiate local inflammation and interstitial edema. Edema may decrease the local blood flow and cause further ischemic injury to acinar cells.
2. ***Primary acinar cell injury:*** Any injury to the acinar cell can trigger acute pancreatitis.
   - Direct acinar cell injury may be caused by certain **viruses (e.g., mumps), drugs, alcohol and direct trauma** to the pancreas. Injury may also follow ischemia or shock.
   - Cellular injury → **initiates the inflammatory process** → leads to pancreatic edema, hemorrhage → necrosis.
3. ***Defective intracellular transport of proenzymes within acinar cells:*** It is responsible for pancreatitis **due to metabolic injury, alcohol or duct**

obstruction.

- In pancreatic acinar cells, normally digestive enzymes and lysosomal hydrolases are transported in separate pathways.
- **Fusion of lysosome and digestive enzymes within large vacuoles** → may activate the pancreatic proenzymes → cause acute intracellular injury.

Mechanism of Alcohol-induced Pancreatitis

1. **Obstruction of small ductules by proteinaceous plugs:** Chronic alcohol ingestion results in the **secretion of protein-rich pancreatic fluid** → form **inspissated protein plugs** → obstruct small pancreatic ducts. They lead to pancreatitis similar to duct obstruction described above.
2. **Abnormal spasm of sphincter of Oddi:** Alcohol increases pancreatic exocrine secretion and causes **contraction of the sphincter of Oddi** (the muscle at the ampulla of Vater) → pancreatitis.
3. D**irect toxic effects on acinar cells:** This may be caused by **alcohol and its metabolic byproducts**.
4. **Increased amounts of proteases in pancreatic secretions:** Found in alcoholic patients.

Clinical Features

- **Abdominal pain:** It is constant and intense. It is **referred to the upper back** and its severity varies from mild to severe.
- **Other symptoms:** These include anorexia, nausea, and vomiting.

Laboratory Findings

- **Leukocytosis:** It is usually found in moderate to severe acute pancreatitis.
- **Serum amylase:** It is markedly elevated during the **first 24 hours**.
- **Serum lipase:** It is raised **within 72–96 hours** and follows elevated amylase.
- **Glycosuria:** It occurs in 10% of cases.
- **Hypocalcemia:** It is **due to precipitation** of calcium soaps in necrotic fat.
- **Direct visualization of the pancreas:** By radiography.

Complications of Acute Pancreatitis (Table 19.11)

Q. Write short note on complications of acute pancreatitis.

BOX 19.9: Causes of acute pancreatitis.

Metabolic
- **Alcoholism**
- Hyperlipoproteinemia
- Hyperparathyroidism
- Hypercalcemia

Mechanical
- **Gallstones**
- Trauma blunt abdominal trauma
- Iatrogenic injury: During surgery (e.g., endoscopic procedures with dye injection)
- Periampullary neoplasms (such as pancreatic cancer)
- Parasites (*Ascaris lumbricoides* and *Clonorchis sinensis* organisms)

Drugs and toxins
- Drugs: Furosemide, azathioprine
- Toxins: Insecticides, methanol, organophosphates

Genetic
- Mutation in cationic trypsinogen (*PRSS1*) and trypsin inhibitor (*SPINK1*) gene

Vascular
- Shock
- Atheroembolism
- Thrombosis and embolism
- Vasculitis

Infectious
- Viral infections: Mumps, coxsackie virus

Idiopathic (~10–20%)

Acute Interstitial or Edematous Pancreatitis

- It is a **mild and reversible** process.
- **Microscopy:** It shows **interstitial edema** and **mild infiltration** by p**olymorphonuclear leukocytes. Necrosis or hemorrhage is not seen**.

Acute Necrotizing Pancreatitis

Gross

Pancreas

- It is **enlarged** and **swollen**, and shows red-black **areas of hemorrhage** with foci of yellow-white, chalky **fat necrosis** (*refer* page 31 and **Fig. 2.18**). Fat necrosis is due to the action of lipase on triglycerides, which release fatty acids from the fat cells.
- The **fatty acids combine with calcium to form insoluble salts** (calcium soaps). This process of soap formation is known as **saponification**. The calcification **may reduce the level of blood calcium**, sometimes to such an extent of causing neuromuscular irritability.

Extrapancreatic lesions

- **Foci of fat necrosis (**refer **Fig. 2.18):** It may also be seen in **extrapancreatic fat** (omentum and the mesentery of the bowel) and outside the abdominal cavity (subcutaneous fat).
- **Fluid:** The **peripancreatic tissue or peritoneal cavity** may contain a **serous, slightly turbid, brown-tinged fluid**. This fluid may show necrotic fat globules, which are produced by the action of enzymes on adipose tissue. The necrotic tissue appears "**putty-like**," and is similar to "**canned dog food**".

Microscopy (Fig. 19.39)

- **Edema of interstitial tissue: It due to leakage of fluid from the microvasculature.**
- **Acute inflammation:** Seen in the interstitial tissue.
- **Enzymatic fat necrosis (***refer* **Fig. 2.18):** It appears as **granular blue with ghost outlines of the necrotic cells**.
- **Destruction of parenchyma of the pancreas:** Acinar and ductal tissues as well as the islets of Langerhans are necrotic **due to proteolytic digestion.**
- **Destruction of blood vessels:** The **activated elastase** destroys blood vessel and **leads to interstitial hemorrhage**.

Acute Hemorrhagic Pancreatitis

Q. Write a short note on acute hemorrhagic pancreatitis.

- It usually develops in **middle age** (peak incidence at 60 years) and associated with **high morbidity and mortality**.
- **Gross (Fig. 19.40): It shows extensive necrosis, hemorrhage** and fat necrosis within the **pancreatic parenchyma.** In severe cases, marked hemorrhage may convert the pancreas into a large retroperitoneal hematoma.

TABLE 19.11: Complications of acute pancreatitis.

Local complications	***Systemic complications***

TABLE 19.11: Complications of acute pancreatitis.

Local complications	*Systemic complications*
• Sterile pancreatic abscess • Pancreatic pseudocyst • Infection by gram-negative organisms from the alimentary tract	• Shock with acute renal tubular necrosis during the first week • Acute respiratory distress syndrome • Acute renal failure • Hemolysis • Disseminated intravascular coagulation (DIC) • Diffuse fat necrosis

Genetic factors in acute pancreatitis: Mutations in genes: *PRSS 1, SPINK 1, CFTR.*

Safety mechanism in pancreas to prevent autoactivation of trypsin: Pancreatic secretory trypsin inhibitor (PSTI) binds with the active trypsin and inhibits its activity.

MORPHOLOGY

Q. Write short note on morphology of acute pancreatitis.

The changes depend on the duration and severity of the process. Morphologically, acute pancreatitis classified into 3 types, namely—(i) interstitial/edematous, (ii) necrotizing and (iii) hemorrhagic.

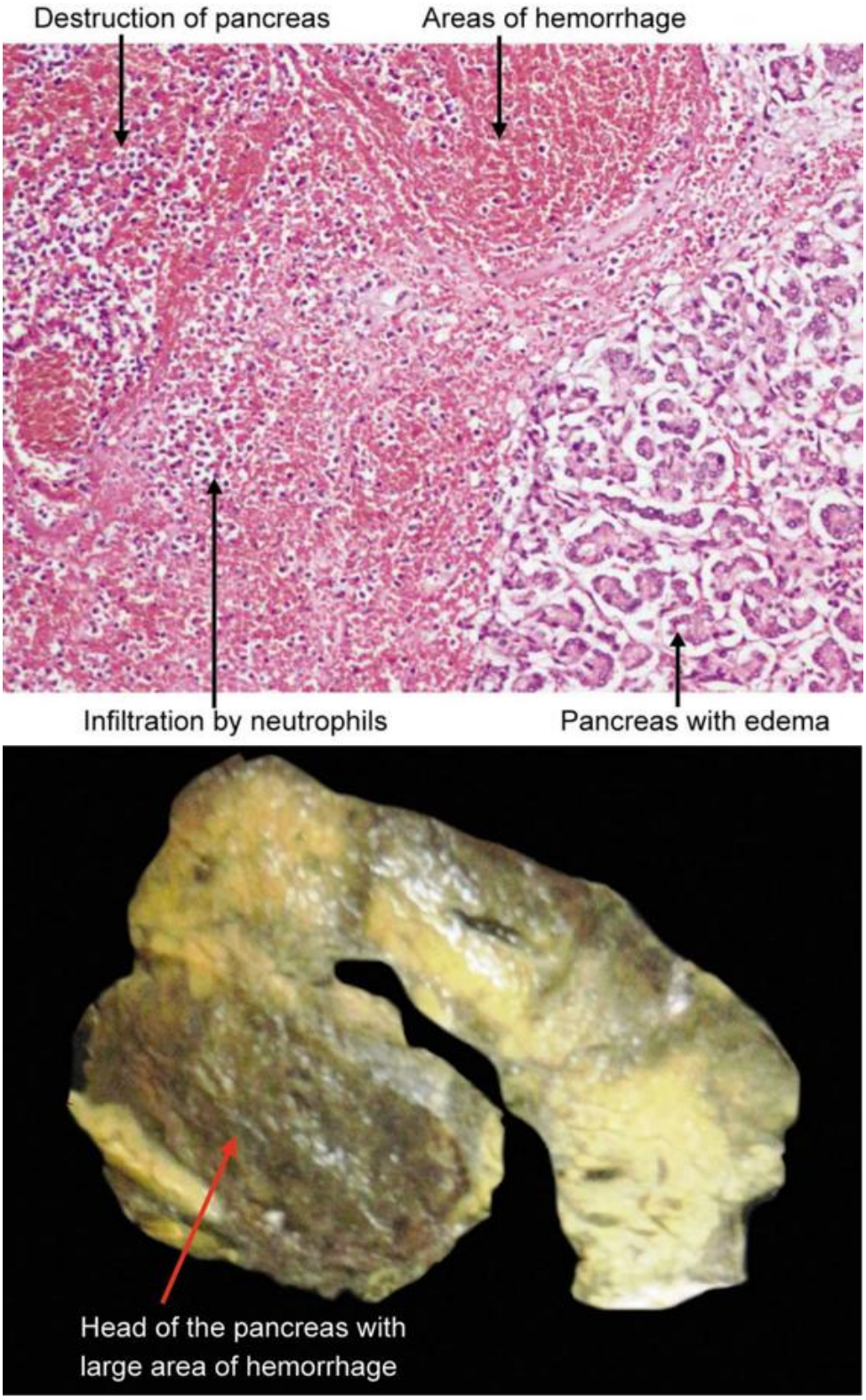


Fig. 19.39: Microscopic appearance of acute hemorrhagic pancreatitis. Histology shows numerous neutrophils, hemorrhage and destruction of parenchyma (left side) and pancreas with edema (right side).

Fig. 19.40: Acute hemorrhagic pancreatitis. Longitudinal section of the pancreas shows dark areas of hemorrhage in the head of the pancreas (left).

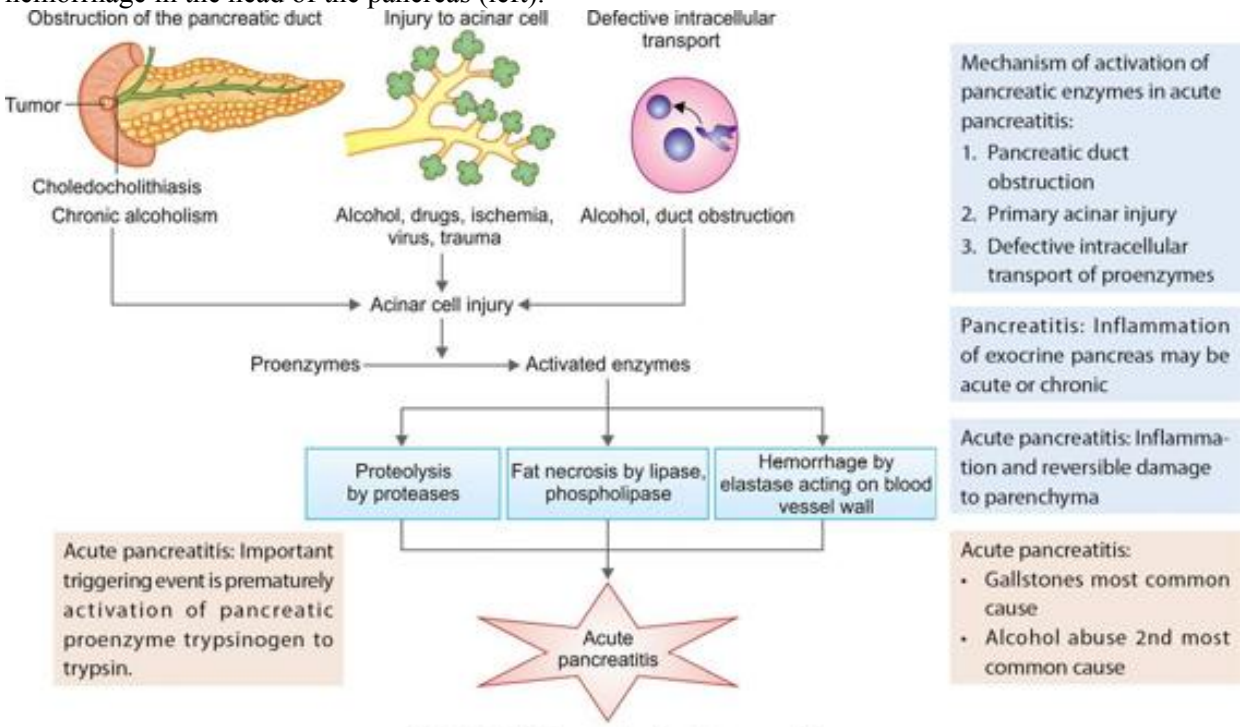


Fig. 19.41: Pathogenesis of acute pancreatitis.

Mechanism of alcohol-induced acute pancreatitis:
1. Small ductules obstruction
2. Abnormal spasm of sphincter of Oddi
3. Direct toxic effect
4. Increased proteases.

Acute pancreatitis: May present as acute abdominal medical emergency.

Acute pancreatitis: C-reactive protein level >130 mg/mL indicates severe pancreatitis. A three-fold or higher elevation of amylase and lipase levels confirm the diagnosis. Lipase is a more specific/diagnostic marker and lasts longer. Amylase can be elevated in other conditions (e.g., peptic ulcer disease, mesenteric ischemia, salphingitis). Persistent of hypocalcemia is a poor prognostic sign.

CHRONIC PANCREATITIS

Definition: Chronic pancreatitis is defined as **chronic inflammation** of the pancreas characterized by the presence of **permanent and progressive morphologic or functional damage to the pancreas**.

Pancreas shows **irreversible damage of exocrine parenchyma, and fibrosis**. In the **late stages**, there may be **destruction of endocrine parenchyma**.

Etiology

- **Alcohol abuse: Chronic alcohol consumption** is the **most common (about 70%) cause** and usually develops in middle-aged males.
- **Less common causes:**
  - **Obstruction of the pancreatic duct:** By pseudocysts, calculi, trauma, or carcinoma.
  - **Hyperparathyroidism:** May develop as a result of the hypercalcemia.
  - ***CFTR* gene mutations: Cystic fibrosis** is caused by inherited mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. This **decreases bicarbonate secretion by pancreatic ductal cells** and **promote protein plugging** and the development of chronic pancreatitis.
  - **Tropical pancreatitis:** It is seen in Africa and Asia and few have a genetic basis.
  - **Hereditary pancreatitis**
  - **Autoimmune pancreatitis**
  - **Idiopathic:** It constitutes about 20% of cases.

Pathogenesis (Fig. 19.42)

Q. Discuss the pathogenesis of chronic pancreatitis.

The pathogenesis of chronic pancreatitis is not clear. **Repeated attacks of acute pancreatitis may lead to chronic pancreatitis**. **Four theories** have been suggested and it is unlikely that all cases of chronic pancreatitis can be explained by a single theory.

Obstruction Theory

Some etiological agents cause **increase protein concentrations** → precipitation of protein → form **ductal plugs** → calcification of ductal plugs to form calculi → further **obstruction of the pancreatic ducts**. They are **found in alcoholic induced chronic pancreatitis**.

Necrosis-fibrosis Theory

According to this hypothesis, the inflammation and scarring resulting from **bouts of acute pancreatitis** → cause **obstruction and stasis** within the ducts → form stones in the duct.

Toxic-metabolic Theory

This theory proposes that toxins such as alcohol and its metabolites can cause **direct toxic damage to acinar cells** → produces necrosis of cells → eventually fibrosis.

Oxidative Stress Theory

- The pancreas is exposed to "oxidative stress" either through the systemic circulation or through reflux of bile into the pancreatic duct.
- **Oxidative stress generates free radicals** in acinar cells with following consequences:
  - Leads to membrane lipid oxidation and acinar cell necrosis.
  - Recurrent inflammation, tissue damage and fibrosis, and the fusion of lysosomes and proenzymes.
  - Alcohol abuse is one of the etiological factors, which induces oxidative stress.

Profibrogenic Chemokines

- Two profibrogenic chemokines, namely—**transforming growth factor** β (**TGF-**β) and **platelet-derived growth factor (PDGF)** have been found in chronic pancreatitis.
- They cause **activation and proliferation of periacinar myofibroblasts** (pancreatic stellate cells), which **deposit collagen** and ultimately lead to **fibrosis**.

MORPHOLOGY

Gross

Chronic pancreatitis can be subdivided into two major forms:

Non-obstructive Chronic Pancreatitis

It is by far the **most common form of chronic pancreatitis** (~95%) and about 80% of the patients are alcoholics.

- Pancreas is **nodular, hard** and may be either enlarged or atrophic.
- **Cut section** may show **dilated ducts** and **calcified concretions**.
- **When the calcification is extensive**, it is termed as **chronic calcifying pancreatitis**.

Obstructive Chronic Pancreatitis

It is the result of narrowing or occlusion of the pancreatic ducts. It is most **commonly due to carcinoma and stones** in the ductal system.

Microscopy

Chronic pancreatitis is characterized by:

- **Parenchymal fibrosis:** The pancreas shows large irregular areas of fibrosis (periductal, intralobular, and interlobular). It is a major feature.
- **Atrophy of acini:** There is reduction in the number and size of the acini. The islets of Langerhans are relatively resistant to chronic pancreatitis in comparison with the pancreatic acini. However, as the disease progresses they may be reduced or disappear.
- **Dilation of the pancreatic ducts:** The **interlobular and intralobular pancreatic ducts are frequently dilated** and their **lumen show protein plugs**.
- **Alterations in the duct epithelium:** It shows **atrophy/hyperplasia/squamous metaplasia.**
- **Chronic inflammatory infiltrate:** Fibrotic areas show infiltration by **lymphocytes, plasma cells, and macrophages**.

Clinical Features

- **Repeated attacks of abdominal pain** or persistent abdominal and back pain.
- **Silent until severe functional damage** develops:
  - **Pancreatic exocrine insufficiency:** Characterized by **weight loss** and **hypoalbuminemic edema** due to chronic malabsorption.
  - **Pancreatic endocrine insufficiency: Diabetes mellitus** may develop.

Prognosis: Long-term outlook is poor.

Chronic pancreatitis: (1) Non-obstructive or (2) Obstructive

Steatorrhea: Seen in chronic pancreatitis and not in acute pancreatitis.

Chronic pancreatitis:
1. Parenchymal fibrosis

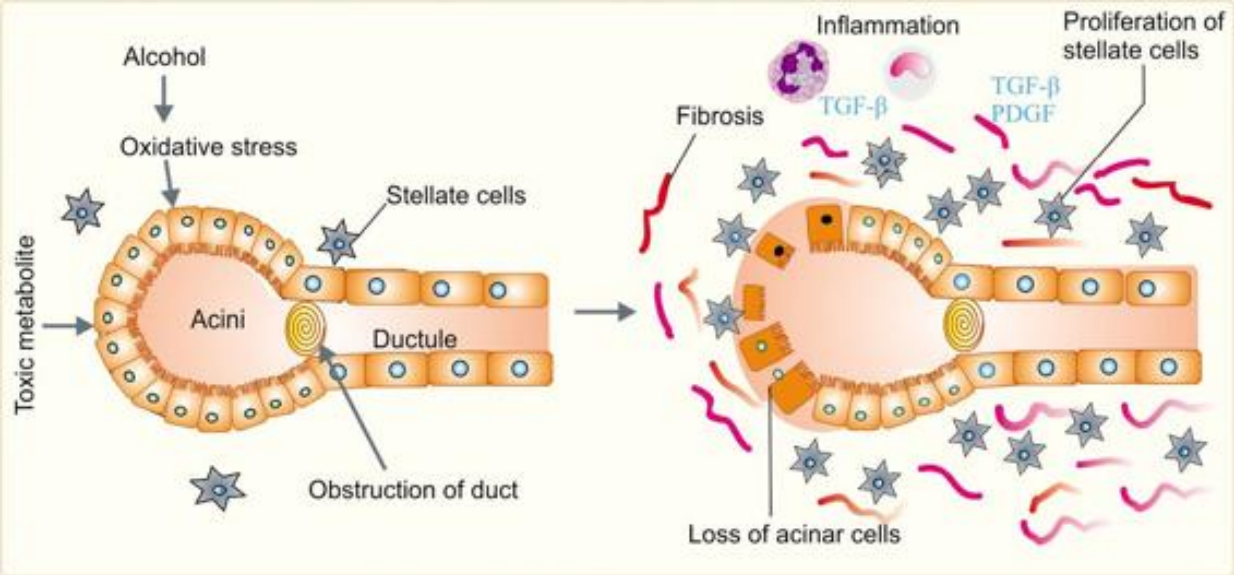
2. Atrophy of acini
3. Dilatation of pancreatic ducts
4. Atrophy/hyperplasia/squamous metaplasia of duct epithelium
5. Chronic inflammation

Complications of chronic pancreatitis:
1. Pancreatic pseudocysts
2. Malabsorption, steatorrhea
3. Secondary diabetes
4. Risk of developing pancreatic cancer

PSEUDOCYST OF PANCREAS (FIG. 19.43)

Q. Write short note on pancreatic pseudocyst.

- Pseudocysts constitute about 75% of cysts in the pancreas.
- Pseudocyst is **not true cyst** and **does not have any epithelial lining**. It is intimately associated with the pancreatic tissues.
- **Causes:** Complication of **acute and chronic pancreatitis**, and **traumatic injury to the pancreas**.



Chronic pancreatitis: Irreversible damage to the parenchyma and scar formation

Pathogenesis of chronic pancreatitis
Four theories include:
1. Obstruction

2. Necrosis-fibrosis
3. Toxic-metabolic
4. Oxidative stress

Chronic pancreatitis: Alcohol abuse is the most common cause

Fig. 19.42: Pathogenesis of chronic pancreatitis. Repeated episodes of acinar cell injury produces profibrogenic cytokines such as transforming growth factor β (TGF-β) and platelet-derived growth factor (PDGF), resulting in the proliferation of stellate cells (myofibroblasts) and produces fibrosis.

MORPHOLOGY

Gross

- Number: It is usually **single**.
- Size: It ranges from 2 to 30 cm in diameter.
- Sites **(Fig. 19.5):** May be seen **within the substance of the pancreas**, or **more commonly in the lesser omental sac or in the retroperitoneum** between the stomach and transverse colon or between the stomach and liver.
- Lumen: It contains **necrotic-hemorrhagic material rich in pancreatic enzymes** (amylase).

Microscopy

The wall of the pseudocysts shows non-epithelial-lined fibrous connective tissue and necrotic pancreatic debris.

Complications

Majority of pseudocysts resolve spontaneously.

- Pseudocyst may **enlarge and compress** or obstruct the duodenum or even perforate into **adjacent structures**.
- May be **secondarily infected** → form an **abscess**.
- Rarely **may rupture** → **chemical or septic peritonitis** or both.

PANCREATIC CARCINOMA

NC: Describe the etiology, pathogenesis, manifestations, laboratory, morphologic features, complications and metastases of pancreatic cancer

Infiltrating ductal adenocarcinoma of the exocrine pancreas (commonly known as "pancreatic cancer") is the most frequent neoplasm of the pancreas (about 85% of all neoplasms).

Etiology and Pathogenesis

Age: It is usually found in the **elderly patients** and about 80% of cases occur between the ages of **60 and 80 years**.

Risk Factors

- **Cigarette smoking** and **alcohol use**
- **Consumption of a diet rich in fats**
- **Chronic pancreatitis**
- **Diabetes mellitus**
- **Inherited genetic defects:**
  - ***BRCA2*** mutations
  - Mutations in ***CDKN2A (p16)***
  - Mutation in the ***PALLD*** gene.

Precursors to Pancreatic Cancer(Pancreatic Intraepithelial Neoplasias)

- Similar to colorectal cancer, there is a progression in the pancreas from non-neoplastic epithelium to well-defined noninvasive lesions in small ducts and ductules to invasive carcinoma.
- The precursor lesions are called "pancreatic intraepithelial neoplasias" (PanINs).

Molecular Carcinogenesis (Fig. 19.44)

1. Telomere Shortening

The epithelial cells in PanINs show critical shortening of telomere length, which predisposes these lesions to accumulate progressive chromosomal abnormalities and to develop invasive carcinoma.

2. Oncogene

Mutation of *KRAS:* It is an early genetic alteration, which in turn activates several intracellular signal transduction pathways and the transcription factors Fos and Jun.

3. Tumor Suppressor Gene

- **Inactivating mutations of *p16/CDKN2A:*** It occurs in 90% of the cases. The p16 protein (product of *p16*) plays an important role in the control of the cell cycle, and its inactivation results in loss of cell cycle checkpoint.
- **Mutational inactivation of *SMAD4***: It occurs in about 55% of pancreatic cancers. ***SMAD4* is only rarely inactivated in other cancers**.
- **Mutations of *TP53*:** It is found in about 75% of pancreatic cancers.

MORPHOLOGY

- **Location:** About **60% in the head, 15% in the body**, and 5% in the tail; in about 20% of cancers diffusely involve the entire pancreas.
- **Gross:** Pancreatic carcinomas are usually appear as **hard, stellate, gray-white, poorly delineated and firm masses**.
- **Microscopy:** Pancreatic ductal adenocarcinoma are graded microscopically into well-differentiated, moderately differentiated, and poorly differentiated. **The tumor cells forms abortive tubular structures (Fig. 19.45) or cell clusters and show infiltration**. The malignant glands are poorly formed and are usually lined by pleomorphic cuboidal-to-columnar epithelial cells.

Spread

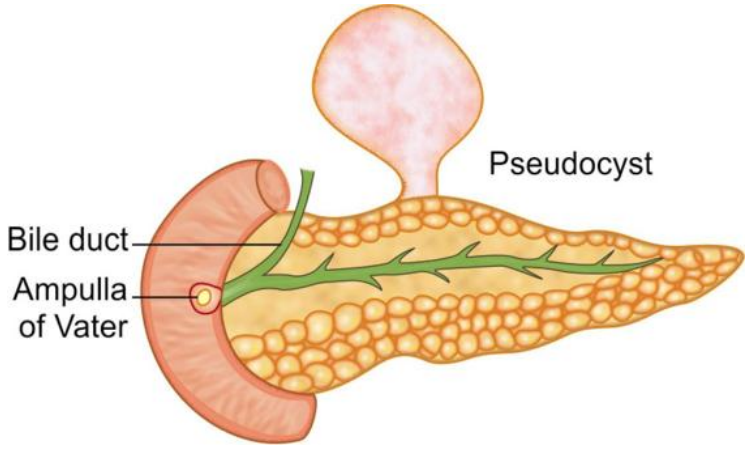
- **Local spread:** Pancreatic cancers usually show **perineural invasion**. They grow along nerves and invade into the retroperitoneum. They can directly spread into peripancreatic soft tissues, spleen, adrenals, vertebral column, transverse colon, and stomach.
- **Lymphatic spread:** Through lymphatics it spreads to peripancreatic, gastric, mesenteric, omental, and portahepatic lymph nodes.
- **Blood spread:** It may spread to the liver, lungs, adrenal, and bones.

Clinical Features

Pancreatic cancers usually remain silent until they infiltrate into adjacent structures.

- **Pain:** It is usually the first and the **outstanding symptom**.
- **Obstructive jaundice:** It is associated **with carcinoma of the head** of the pancreas.

- **Advanced stage:** Symptoms include: Weight loss, anorexia, and generalized malaise and weakness.



- **Carcinomas of the body and tail of the pancreas: It remains silent in the initial period**.
- **Migratory thrombophlebitis (Trousseau sign):** It is **characterized by spontaneously appearing and disappearing (migratory) thrombosis**. It is found in about 10% of patients. It is attributable to the production of platelet-aggregating factors and procoagulants from the carcinoma or its necrotic products.

Laboratory Findings

Raised serum levels of many enzymes and antigens (e.g., **carcinoembryonic antigen and CA19–9 antigen**) are often found in pancreatic cancer. These markers are nonspecific and lack the sensitivity. However, they are useful in follow-up of a patient's response to treatment.

Imaging techniques: Many imaging techniques, such as endoscopic ultrasonography and computed tomography, are useful for the diagnosis, but are not useful as screening tests.

Prognosis: The **course is brief and progressive**.

Pancreatic cancer: Probably arises from precursor lesions known as PanINs.
Carcinoma of pancreas: More than 85% are ductal adenocarcinomas.
Adenocarcinoma of pancreas: Most common gene mutation (oncogene activated by point mutation) is *KRAS*. *KRAS* gene is located on chromosome 12p.
Pancreatic cancer: (1) Highly invasive, (2) Desmoplastic response.

Perineural invasion:
1. Pancreatic cancer
2. Prostatic cancer
3. Adenoid cystic carcinoma
4. Carcinoma of gallbladder
5. Cholangiocarcinoma

Carcinoma head of pancreas: Present with obstructive jaundice.

Trousseau's syndrome/migrating thrombophlebitis: Spontaneously appearing and disappearing (migratory) thrombosis.
Associated malignancies are:
1. Most common is carcinoma of pancreas
2. Carcinoma of lung
3. GI tract malignancies
4. Prostatic cancer
5. Ovarian cancer
6. Lymphoma

Carcinoma of pancreas: May be associated with palpable gallbladder.

Trousseau's sign: Carpopedal spasm in hypocalcemia.

Troisier's sign: Palpable left supraclavicular lymph node (Virchow's node).

Carcinoma of head of the pancreas: Preferred test is contrast-enhanced CT scan.
SPINK1 is only associated with hereditary pancreatitis but not in pancreatic cancer.
Ductal adenocarcinoma of pancreas: Most common in the head of the pancreas.

Pancreatic carcinoma:
1. CA19-9 is the gold standard tumor marker.
2. CT scan-best test

CC: Describe and interpret the abnormalities in liver function tests (Shows how: A skill attribute: is able to interpret/demonstrate a complex procedure requiring thought, knowledge and behavior)

Case 1

History: A 28-year-old homosexual male presented with malaise and anorexia of 1-week duration. He had mild fever. He is not an intravenous drug addict. He was tested for HIV 6 months earlier and test was negative.

Examination: He has mild temperature (38.6°C) and with mild jaundice.

Investigations:

Liver function tests		
Test	***Results***	***Normal range***
Serum aspartate aminotransferase	250 IU/L	12–38 U/L
Serum alanine aminotransferase	300 IU/L	7–41 U/L
Total bilirubin	6 mg/dL	0.3–1.3 mg/dL
Direct (conjugated)	4.5 mg/dL	0.1–0.4 mg/dL
Indirect (unconjugated)	1.5 mg/dL	0.2–0.9 mg/dL
Alkaline phosphatase	106 IU/L	33–96 U/L
Proteins—total	7.0 g/dL	6.7–8.6 g/dL
Albumin	5.0 /g/dL	3.5–5.5 g/dL
Globulins	2.0 g/dL	2.0–3.5 g/dL
Albumin/globulin ratio	2.5:1	1.5–3: 1
Prothrombin time (PT) (Quick's one stage method)	17 seconds	11–16 second

Fig. 19.43: Pseudocyst of pancreas.

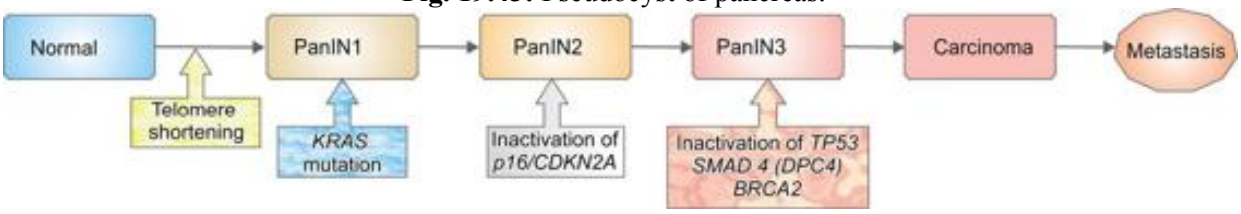
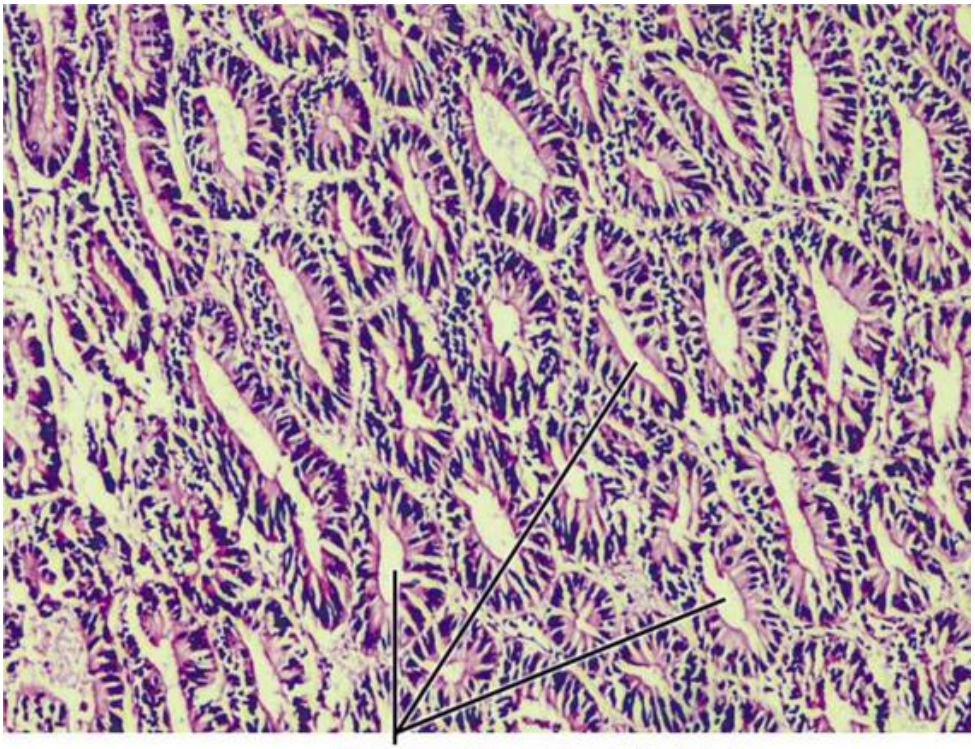


Fig. 19.44: Genetic progression of pancreatic carcinogenesis. The progression from normal epithelium to low-grade pancreatic intraepithelial neoplasia (PanIN) (PanIN1 and PanIN2), to high-grade PanIN3, to invasive carcinoma (left to right) is associated with the accumulation of specific genetic alterations. Telomere shortening and mutations of the oncogene KRAS occur at early stages. It is followed by inactivation of the p16 (tumor suppressor gene) at intermediate stages, and the inactivation of the *TP53, SMAD4 (DPC4)*, and *BRCA2* tumor suppressor genes occur at late stages.



Other tests	
Urine	
Bile pigments	++
Bile salts	+
Urobilinogen	+ (traces)
Feces	Normal colored

HIV test: Negative
Total WBC count: 11,500/µL (4,000–11,000/µL)

Questions

1. What is the probable diagnosis?
2. Interpret the liver function test.
3. What further investigations will help in arriving at the diagnosis?

Answers

1. Probable diagnosis is **hepatocellular jaundice** due to **acute viral hepatitis**.

2. The liver function test is abnormal with a marked rise in the alanine aminotransaminases (high AST/ALT indicates hepatocyte damage) and moderately elevated bilirubin. Normal or mild increase of serum alkaline phosphatase indicate that there is no biliary obstruction. This indicates a hepatocellular jaundice rather than an obstructive jaundice. The raised total WBC count suggests acute viral hepatitis. Acute viral hepatitis may be caused by A, B or C. Since, this patient is a homosexual (a risk factor) probably it may be either hepatitis B or C.
3. Serological tests can be used to know whether there are immunoglobulin M (IgM) antibodies indicating acute infection with hepatitis B or hepatitis C viruses, to confirm the diagnosis. This patient has acute hepatitis B infection. Other serological markers to be performed as mentioned on page 607.

Fig. 19.45: Pancreatic carcinoma shows tumor cells forming abortive tubular structures.

Case 2

History: A 55-year-old woman complained of intermittent dull central epigastric pain of 2-month duration. The pain was not radiating and there was no relationship of pain to eating. Her appetite was normal and there was no history of nausea, vomiting or loss of weight. Her bowel habit was normal. Investigations showed normal blood count, urea, creatinine and electrolytes, and liver function tests. An H2 antagonist was prescribed and follow-up advised.
She was slightly relieved of pain at first, but after 1 month, the pain became more frequent and severe. This time, the patient noticed that pain was relieved by sitting forward and begun to radiate through to the back. The patient's husband noticed that her eyes had become slightly yellow. Few days later, she noticed that her urine had become dark and her stools pale. She also complains of itching.

Examination: Her sclerae was yellow with a slight yellow tinge to the skin. There were scratch marks on skin and there were no other significant findings.

Investigations

Routine hematological investigations were normal.

Liver function tests		
Test	***Results***	***Normal range***
Serum aspartate aminotransferase	80 IU/L	12–38 U/L
Serum alanine aminotransferase	90 IU/L	7–41 U/L
Total bilirubin	12 mg/dL	0.3–1.3 mg/dL

Direct (conjugated)	10.0 mg/dL	0.1–0.4 mg/dL
Indirect (unconjugated)	2.0 mg/dL	0.2–0.9 mg/dL
Alkaline phosphatase	162 IU/L	33–96 U/L
Proteins—total	6.6 g/dL	6.7–8.6 g/dL
• Albumin	4.4 /g/dL	3.5–5.5 g/dL
• Globulins	2.2 g/dL	2.0–3.5 g/dL
Albumin/globulin ratio	2:1	1.5–3: 1
Prothrombin time (PT) (Quick's one stage method)	25 seconds	11–16 second
• After parenteral Vitamin K administration	18 seconds	

Other tests

Urine	
• Bile pigments	Present
• Bile salts	++
Feces	Clay colored

Questions

1. What is the probable diagnosis?
2. What are other causes that can produce this type of changes in liver function tests?
3. What further investigations should be performed?

Answers

1. Points favors a probable diagnosis of **obstructive jaundice**. Features that point to obstructive jaundice are markedly raised serum alkaline phosphatase (suggestive of biliary obstruction) and serum bilirubin, presence of dark urine (indicates a conjugated hyperbilirubinemia arising from biliary obstruction) and pale stools (because conjugated bilirubin is not entering the bowel because of obstruction). The conjugated bilirubin is water soluble and excreted in the urine. Prolonged prothrombin time is reduced after parenteral Vitamin K indicates failure of absorption of Vitamin K due to bile salt deficiency (bile salts are needed for the absorption of the fat-soluble vitamins A, D, E and K) due to biliary

obstruction. Retention of bile salts is responsible for itching. The objective is to define the site of obstruction and its cause. Carcinoma of the pancreas in its early stages can present with non-specific symptoms. The pain has two characteristic features of carcinoma of the pancreas: relief by sitting forward and radiation to the back. The **pancreatic carcinoma** is an important cause of obstructive jaundice. An alternative diagnosis could be gallstones, but the pain is not typical.

2. Other causes of obstructive jaundice (*refer* pages 597-8).
3. Further investigations: These include an abdominal ultrasound, computed tomography (CT) scan, magnetic resonance cholangiopancreatography (MRCP).

Case 3

History: A 62-year-old male is brought to the emergency department at 2 am for vomiting fresh red blood. He is continuing to vomit large amounts of blood. He has no associated abdominal pain. His stools have been dark black for last 2 days. **On enquiry, he was chronic alcoholic for the last 20 years**.

Examination: He is anemic and mildly jaundiced. There are spider nevi on his upper trunk and **gynecomastia**. **Abdominal palpation shows ascites** and spleen is palpable at 4 cm below the costal margin (**splenomegaly)**. His pulse rate is 72/min and the blood pressure 90/60 mm Hg lying down, dropping to 76/48 on standing.

Liver function tests		
Test	***Results***	***Normal range***
Serum aspartate aminotransferase	40 IU/L	12–38 U/L
Serum alanine aminotransferase	80 IU/L	7–41 U/L
Total bilirubin	2.1 mg/dL	0.3–1.3 mg/dL
Direct (conjugated)	0.9 mg/dL	0.1–0.4 mg/dL
Indirect (unconjugated)	1.2 mg/dL	0.2–0.9 mg/dL
Alkaline phosphatase	344 IU/L	33–96 U/L
Proteins—total	5.6 g/dL	6.7–8.6 g/dL
Albumin	1.6 /g/dL	3.5–5.5 g/dL
Globulins	4.0 g/dL	2.0–3.5 g/dL
Albumin/globulin ratio	1:2.5	1.5–3: 1

Prothrombin time (PT) (Quick's one stage method)	22 seconds	11–16 second
Gamma-glutamyl transpeptidase	467 IU/L	9–58 IU/L

Questions

1. What is the likely diagnosis?
2. Give reasons.
3. What are the complications of this condition?

Answers

1. Most likely diagnosis is a variceal bleed due to underlying chronic liver disease secondary to alcohol.
2. Patient has signs of chronic liver disease and his liver function tests are consistent with this. The synthetic function of liver is markedly reduced. This reflected in the reduction of serum proteins, reversal of albumin globulin ratio (1:2.5) and prolonged prothrombin time. Raised gamma-glutamyl transpeptidase is a good guide to alcohol intake of more than 60 g alcohol/day.
3. Complications of cirrhosis (*refer* pages 625-7).

CHAPTER 20

Kidney and Urinary Tract Disorders

HISTOLOGY OF THE KIDNEY

CC: Describe the normal histology of the kidney.

Unit of kidney: The **nephron (Fig. 20.1)** is the **structural unit** of the kidney. Nephron consists of five major parts namely the: (i) glomerulus, (ii) proximal convoluted tubule (PCT), (iii) loop of Henle, (iv) distal convoluted tubule (DCT), and (v) collecting ducts.

Glomerulus (Figs. 20.2 to 20.4)

- It is the **basic filtering unit** of the kidney that consists of a tuft of **anastomozing network of capillaries** formed by branching of the afferent arteriole.
- Capillaries are lined by **fenestrated (window-like openings) endothelium** lying on a **glomerular basement membrane** (GBM). The outer portions of the fenestrated endothelial cell are in contact with the inner surface of the GBM, whereas the central part is in contact with the mesangial cell and mesangial matrix.
- The entire **outer surface of GBM is covered** by **visceral epithelial cell** (podocyte) foot processes. The visceral epithelial cells line the glomerular side of Bowman's space, whereas the **parietal epithelial cells line Bowman's capsule** on the opposite side.
- **Mesangium:** It consists of **mesangial cells** and basement membrane-like **mesangial matrix** and supports the entire glomerular tuft.

Tubules

The tubules constitute the greatest amount of the renal parenchyma. The structure of renal tubular epithelium varies in different parts of the nephron and is correlated with the functional capacity of that part of the tubule.

- **Proximal convoluted tubule (PCT):** It is the first part arising from the glomerulus. It is lined by cuboidal cells with a brush border composed of microvilli.
  - **Functions: Active reabsorption** of filtered sodium, potassium, glucose, amino acids, proteins, vitamins, bicarbonate, phosphate, calcium and uric acid. **Passive reabsorption** of 80% of filtered water.
- **Loop of Henle:** The PCT drains into the straight part of loop of Henle. It consists of thin descending, and thin and thick ascending limbs. The descending limb is continuation of PCT and the ascending limb continues further into distal convoluted tubule (DCT). The descending segment of loop of Henle is lined by simple epithelium whereas the ascending limb is lined by columnar cells.
  - **Functions: Active reabsorption** of sodium, potassium and chloride, and **passive diffusion** of water resulting in concentrated filtrate of urine.
- **Distal convoluted tubule (DCT):** It represents a transition from thick ascending limb from the point where the ascending limb meets the vascular pole of the glomerulus of its origin, to the early collecting ducts. It is lined by cuboidal cells.

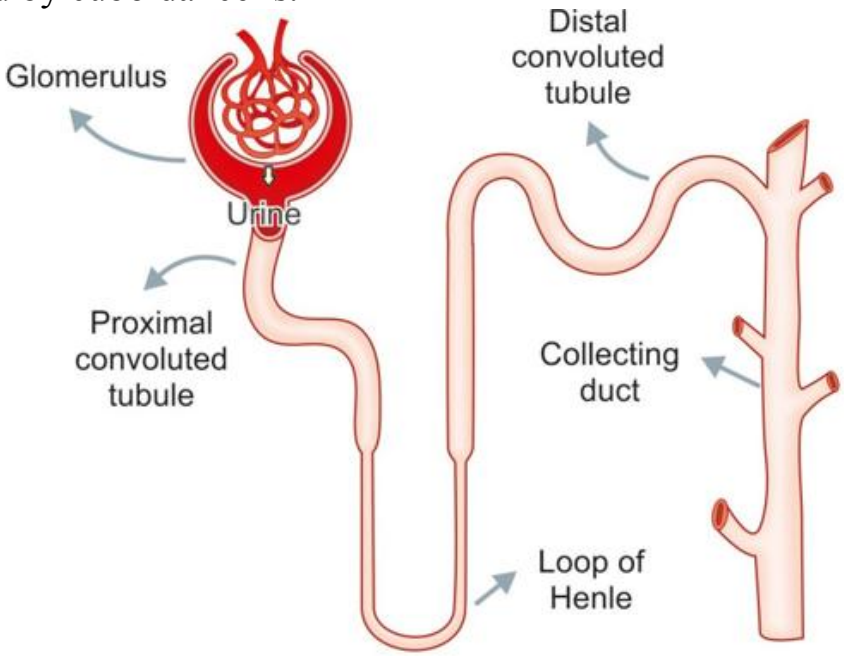


Fig. 20.1: Different components of nephron.



Fig. 20.2: Photomicrograph of light microscopic appearance of glomerulus.

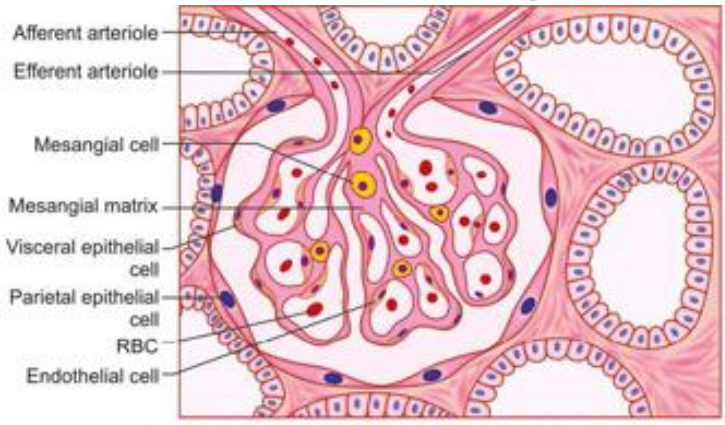


Fig. 20.3: Light microscopic appearance of glomerulus (diagrammatic).

Glomerulus: Basic filtering unit and consists of a tuft of anatomising network of capillaries.

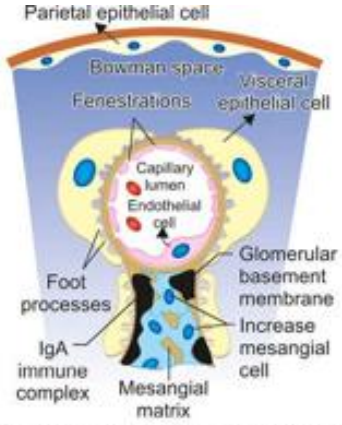


Fig. 20.4: Diagrammatic representation of electron microscopic view of normal renal glomerulus. The relationship of the different cell types to the glomerular basement membrane and mesangial matrix is illustrated using a single glomerular loop.

- **Functions:** Contributes to urinary concentration and acidification.

- **Collecting ducts:** Through these ducts urine reaches the tip of renal papilla. It is lined by cuboidal epithelium without brush border.
  - **Functions:** Reabsorption of water under control of ADH, and secrete H^+ and K^+ ions.

Interstitium

The interstitium in the renal cortex is scanty and consists of a small number of fibroblast-like cells whereas in the medulla, it is more and contains stellate interstitial cells.

Blood Supply

Blood is supplied to each kidney by a main **renal artery** which arises from the aorta at the level of the 2nd lumbar vertebra. It usually divides into anterior and posterior divisions at the hilum. The anterior and posterior divisions divide into segmental branches from which interlobar arteries arise which course between the lobes.

Diseases of the kidney can be divided into four major groups depending on the basic morphologic components affected namely: glomerular diseases, tubules diseases, interstitial diseases, and diseases of blood vessels.

Clinical Manifestations of Renal Diseases (Table 20.1)

Q. Write a short note on azotemia and uremia.

GLOMERULAR DISEASES

CC: Define and classify glomerular diseases. Enumerate and describe the etiology, pathogenesis, mechanisms of glomerular injury, pathology, distinguishing features and clinical manifestations of glomerulonephritis.

Q. Classify glomerular diseases.

Glomerular diseases may be broadly divided into primary and secondary:

- **Primary glomerulonephritis or glomerulopathy (those without inflammatory cells):** Kidney is the only or predominant organ involved.
- **Secondary glomerular diseases:** In these disorders, glomeruli are involved secondary to a systemic disease.

Most common forms of glomerulonephritis are listed in **Box 20.1.**

Clinical Manifestations

Clinical manifestations are grouped into the **five major glomerular syndromes (Table 20.1)**.

Histological Alterations

Glomerulonephritis/glomerulopathies may show one or more of **three basic histological changes** namely: (1) increased cellularity, (2) basement membrane thickening, and (3) hyalinization or sclerosis.

Increased Cellularity (Hypercellularity)

It is characterized by an **increase in the number of cells in the glomerular tufts**. Such **lesion is called proliferative glomerulonephritis**. Hypercellularity may be due to:

- **Cellular proliferation:** It may involve any of the cells of glomeruli (mesangial or endothelial or epithelial cells).
- **Leukocytic infiltration:** These include neutrophils, monocytes, and lymphocytes.

TABLE 20.1: Clinical manifestation of renal diseases.

Disease	*Manifestations*
Glomerular syndromes	
Nephritic syndrome (glomerular disease)	Acute onset of either grossly visible **hematuria** (red blood cells in urine) or microscopic hematuria with dysmorphic red cells and red cell casts on urinalysis, diminished GFR, **mild to moderate proteinuria (with or without edema), azotemia**, and **hypertension**
Rapidly progressive glomerulonephritis	Nephritic syndrome with rapid decline in GFR (within hours to days)
Nephrotic syndrome (glomerular disease)	Heavy/massive proteinuria (more than 3.5 g/day), hypoalbuminemia, severe edema, hyperlipidemia, and lipiduria (lipid in the urine)
Chronic kidney disease (previously termed chronic renal failure)	Persistently diminished GFR less than 60 mL/minute/1.73 m^2 for at least 3 months, from any cause, and/or persistent albuminuria. It is the end result of all chronic renal parenchymal diseases

TABLE 20.1: Clinical manifestation of renal diseases.

Disease	*Manifestations*
Asymptomatic hematuria or proteinuria, or both	Usually due to mild glomerular abnormalities
Others	
Azotemia is biochemical manifestation of kidney injury or extrarenal causes Uremia = azotemia + clinical signs and symptoms + biochemical abnormalities	Raised blood urea nitrogen (BUN) or an elevated serum creatinine due to reduced glomerular filtration rate (GFR) **Renal causes:** Acute or chronic kidney injury **Extrarenal causes:** Prerenal azotemia (e.g., due to hypotension or excessive fluid loss from any cause) or postrenal azotemia (due to obstruction to urine flow distal to the kidney)
Acute kidney injury	**Rapid decline in GFR** (within hours to days), with deranged fluid and electrolyte balance, and **retention** of metabolic waste products normally excreted by the kidney (**urea and creatinine**), **oliguria or anuria** (reduced or no urine flow) in most severe cases. It may be due to glomerular, interstitial, vascular or acute tubular injury
End-stage renal disease (ESRD)	GFR is less than 5% of normal and is the terminal stage of uremia
Renal tubular defects due to diseases that either directly affect tubular structures or cause defects in specific tubular functions	Polyuria (excessive urine formation), nocturia, and electrolyte disorders
Urinary tract obstruction and renal tumors	Features depends on anatomic location and nature of the lesion
Urinary tract infection. It may affect kidney (pyelonephritis) or the bladder (cystitis)	Bacteriuria and pyuria (bacteria and leukocytes in the urine) Infection may be symptomatic or asymptomatic
Nephrolithiasis (renal stones)	Spasms of severe pain (renal colic) and hematuria

Uremia: Azotemia + clinical signs and symptoms + biochemical abnormalities.

- **Formation of crescents:** These are formed due to **proliferation of parietal epithelial cells and infiltration by leukocytes** (*refer* **Fig. 20.10**). This develops **as a response to fibrin** that **leaks into the urinary space through ruptured basement membranes**.

Basement Membrane Thickening

It may be due to:

- **Deposition of amorphous electron-dense** (immune complexes) **material**: The deposits may be on the **endothelial or epithelial side of** GBM **or within the GBM** itself.
- **Thickening of the basement membrane** (e.g., in **diabetic glomerulosclerosis**).

Hyalinosis and Sclerosis

- **Hyalinosis:** It is the **accumulation of homogeneous and eosinophilic material in the glomeruli** and **consists of plasma proteins** that have leaked from the circulation into glomerular structures. It is usually due to endothelial or capillary wall injury.
- **Sclerosis:** It is characterized by **accumulations of extracellular collagenous matrix**, either in the **mesangium** (e.g., diabetic glomerulosclerosis) **or in the capillary loops, or both**.

BOX 20.1: Common glomerular diseases.

PRIMARY GLOMERULONEPHRITIS/GLOMERULOPATHIES

- Acute proliferative glomerulonephritis: Post-infectious, others
- Rapidly progressive (crescentic) glomerulonephritis (RPGN)
- Minimal-change disease (MCD)
- Membranous glomerulonephritis (MGN)
- Membranoproliferative glomerulonephritis (MPGN)
- C3 glomerulonephritis
- Dense deposit disease
- Focal segmental glomerulosclerosis (FSGS)
- Immunoglobulin A (IgA) nephropathy
- Chronic glomerulonephritis

SYSTEMIC DISEASES WITH GLOMERULAR INVOLVEMENT

- Systemic immunological diseases (e.g., systemic lupus erythematosus)
- Metabolic diseases (e.g., diabetes mellitus)
- Vasculitis: Microscopic polyarteritis/polyangiitis, Wegener granulomatosis, Henoch–Schönlein purpura
- Amyloidosis
- Goodpasture syndrome
- Bacterial endocarditis

HEREDITARY DISORDERS

- Alport syndrome
- Thin basement membrane disease
- Fabry disease

The cause in majority of the primary glomerulopathies is not known and they are classified by their microscopic features.

Nomenclature of glomerular injury is used to describe the distribution/pattern of injury in glomeruli **(Box 20.2)**.

Crescentic: Extensive proliferation of parietal epithelial cells and leukocyte infiltration.

Hyalinosis:

- Accumulation of homogeneous and eosinophilic material in the glomeruli
- Consists of plasma proteins.

Sclerosis: Accumulations of extracellular collagenous matrix, either in the mesangium or capillary loops or both.

PATHOGENESIS OF GLOMERULAR INJURY(FIG. 20.5)

Q. Write short essay on pathogenesis of glomerulonephritis.

Immune mechanisms are responsible for **most of primary** glomerulopathy and **many of the secondary** glomerular disorders **(Box 20.3)**.

ANTIBODY-MEDIATED INJURY

Antibody-mediated glomerular injury can be produced by **two mechanisms**.

1. **Circulating (antigen-antibody) complex** deposition in the glomerulus.
2. **In situ antibodies:** The antibodies may be against
   - **Fixed (intrinsic) glomerular antigens** [e.g., antiglomerular basement membrane (anti-GBM) antibody-induced glomerulonephritis] or
   - **Antigens** which are **planted** within the glomerulus.

Circulating Immune Complex Glomerulonephritis

Q. Write short essay on circulating immune complex glomerulonephritis.

Most of glomerulonephritis are immune complex-mediated. This type of glomerulonephritis is caused due to **trapping of circulating antigen–antibody complexes (type III hypersensitivity reactions) within glomeruli**. The **antibodies are not against any of glomerular constituents**, and the immune complexes localize within the glomeruli.

Antigens: They may be of endogenous or exogenous origin.

- **Endogenous:** Autoimmune diseases (e.g., systemic lupus erythematosus)

BOX 20.3: Various immune mechanisms involved in glomerular injury.

1. **Antibody-mediated injury**
   In situ immune complex deposition
   - Fixed intrinsic tissue antigens (e.g., anti-GBM nephritis)
   - Planted antigens
     - Exogenous (e.g., infectious agents, drugs)
     - Endogenous (e.g., DNA, nuclear proteins)

   Circulating immune complex deposition
   - Endogenous antigens (e.g., DNA, tumor antigens)
   - Exogenous antigens (e.g., infectious products)
2. **Cell-mediated immune injury**
3. **Activation of alternative complement pathway**

(GBM: glomerular basement membrane).

BOX 20.2: Terms used in glomerulopathies/glomerulonephritis.

- **Diffuse or generalized:** A lesion affecting/involving all of the glomeruli in the kidney
- **Global:** Involving the whole/entire individual glomeruli
- **Focal:** A lesion involving only a some/proportion of the glomeruli in the kidney but leaving others unaffected
- **Segmental:** Affecting a part/segment of each glomerulus
- **Capillary loop or mesangial:** Affecting predominantly capillary or mesangial regions.

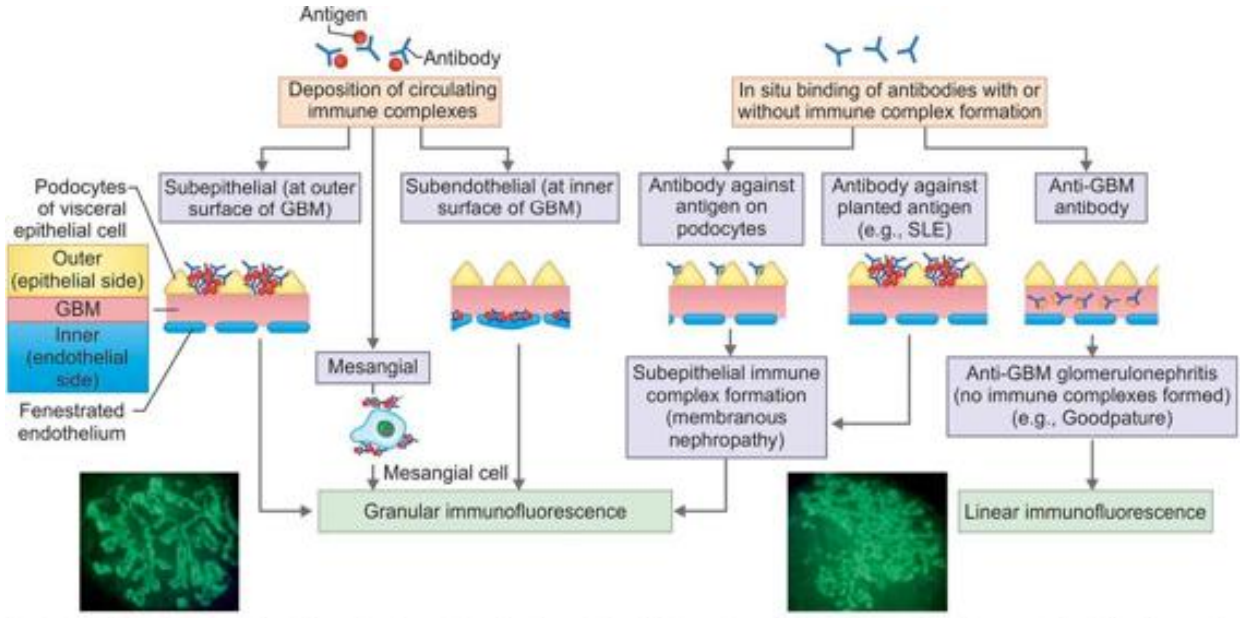


Fig. 20.5: Various immunological mechanisms of antibody-mediated glomerulonephritis. Glomerular injury can be either due to the deposition of circulating immune complexes or from antibody-binding to glomerular components followed by formation of complexes in situ. Deposition of circulating immune complexes produces a granular immunofluorescence pattern. Anti-glomerular basement membrane (anti-GBM) antibody glomerulonephritis produces a linear immunofluorescence pattern and its is not accompanied by immune deposit.

(GBM: glomrular basement membrane; SLE: systemic lupus erythematosus)

- **Exogenous:**
  - **Microbial antigens:**
    - Bacterial products (e.g., streptococci)
    - Viral antigen (e.g., hepatitis B virus, hepatitis C virus antigens)
  - **Spirochetal antigens** (of *Treponema pallidum)*
  - **Parasitic:** *Plasmodium falciparum*
  - **Tumor antigens**
  - **Unknown antigen**.

Mechanism of injury: Antibodies are produced against the antigens. They from **antigen–antibody complexes** in the **circulation** which gets **trapped in the glomeruli** and **produce injury**. The deposited antigen–antibody complexes in the glomeruli, produce injury by activating complement and recruiting leukocytes. Binding of immune complexes to Fc receptors on leukocytes also may contribute to activation of the cells and injury.

Morphology: The involved glomeruli show leukocytic infiltrates and variable proliferation of mesangial and parietal epithelial cells. The type of glomerular response to injury depends on localization of antigen, antibody, or immune complexes (*refer* page 662). If immune complexes are deposited in the endothelium or subendothelium, the glomeruli show infiltration of

leukocytes and exuberant proliferation of glomerular resident cells. If the antibodies are directed to the subepithelial region of glomerular capillaries, the glomeruli usually are free of inflammatory cells (e.g., primary membranous nephropathy).

Electron Microscopy (Fig. 20.6)

It shows the immune complexes as electron-dense deposits **(Fig. 20.6)** that may be found in any of these sites:

- **Mesangium** (mesangial deposits).
- **Subendothelial deposits** between the endothelial cells and the GBM.
- **Subepithelial deposits** between the outer surface of the GBM and the podocytes.
- **Other site may be in the glomerular basement membrane or it may be in more than one site mentioned above.**

Immunofluorescence Microscopy

Immunofluorescence microscopy is used for the demonstration of the presence of immunoglobulins and complement in glomerular deposits. The immune complexes appear as granular immunofluorescence deposits along the basement membrane, in the mesangium, or in both sites. These immune complex deposits gives rise to granular immunofluorescence. This is described as “lumpy-bumpy” deposits by pathologists.

Factors that Determine Localization

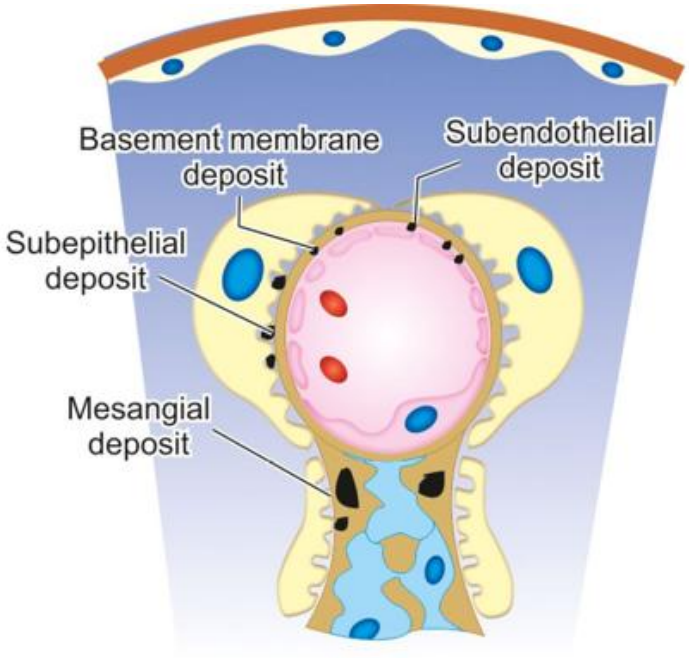


Fig. 20.6: Various locations of immune complex deposition in the glomerulus include: subepithelial, subendothelial, basement membrane and mesangial.

The localization of antigen, antibody, or immune complexes in the glomerulus mainly depend on: (1) the charge and (2) size of the molecules.

- **Molecular charge:**
  - **Highly cationic molecules** can cross the GBM and **appear in the subepithelial location**.
  - **Highly anionic molecules** cannot cross the GBM and are **trapped in subendothelial region**.
  - **Molecules of neutral charge** tend to accumulate **in the mesangium**.
- **Molecular size: Large circulating complexes are removed from the circulation by the mononuclear phagocyte system** and they do not enter the GBM in sufficient quantities. Thus, they **do not produce glomerulonephritis**. **Immune complexes** of **medium size** and with **slight antigen excess** are the **most pathogenic**.

Fate of Deposits

The immune complexes may be cleared by degradation or phagocytosis, mostly by infiltrating leukocytes and mesangial cells. Further changes depends on the inciting antigen.

- If the inciting antigen is short-lived and limited, the inflammatory reaction may subside (e.g., as in most cases of poststreptococcal or acute infection-related GN).
- If exposure to antigen is sustained, there will be repeated cycles of immune complex formation, deposition, and injury. This leads to chronic GN.

In Situ Antibodies

This type of injury is characterized by antibodies reacting directly with **intrinsic (fixed) tissue antigen, or antigens** which are **"planted"** in the glomerulus.

In Situ Antibodies against Fixed Antigen (Fig. 20.5)

- This is **an anti-GBM antibody-mediated glomerulonephritis**. In this type of injury, antibodies directly react with **intrinsic (fixed) tissue antigen** (that are normal components) of the GBM proper.
- Example in **humans** is **Goodpasture syndrome** and results from formation of **autoantibodies against fixed antigen present in the GBM**. This type of glomerulonephritis has an experimental counterpart known as Masugi or nephrotoxic nephritis.
- **Goodpasture syndrome:** It is characterized by conformational changes in the a_3 chain of type IV collagen of GBM. This fixed altered GBM antigen (Goodpasture antigen) can incite autoimmunity and produce **anti-GBM antibodies against Goodpasture antigen**. These antibodies may sometimes cross-react with not only renal GBM but also with basement membrane of lung alveoli, results in simultaneous production of lung and kidney lesions and this is known as Goodpasture syndrome (*refer* page 668).
- **Masugi or nephrotoxic nephritis:** It is an experimental model of nephritis produced in rats.
  - First, rat kidney tissue is injected into the rabbits to produce anti-rat kidney antibodies (anti-GBM antibody).
  - The anti-rat kidney antibodies from the rabbits are injected into the rat.

- The injected anti-rat kidney antibodies bind along the entire length of the GBM and produces glomerular damage.
- Injected anti-rat kidney antibody (i.e., anti-GBM antibody) is rabbit immunoglobulin (Ig). In the rat, this rabbit Ig is foreign protein and thus acts as an antigen eliciting anti-Ig antibody. The rat anti-Ig antibodies then react with the rabbit Ig. This further aggravates the glomerular damage.

- They produce **diffuse linear pattern of immunofluorescence**. This is in contrast to the granular lumpy pattern of immunofluorescent staining seen in circulating immune complexes (described below).

In Situ Antibodies against Planted Antigens(Fig. 20.5)

- Some antigens may not be normally present in the glomerulus but are "planted" (nonglomerular antigens) to the glomerulus.
- Such planted antigens may interact with various intrinsic components of the glomerulus.
- Planted antigens include nucleosomal complexes, mainly derived from breakdown of apoptotic cells (e.g., in systemic lupus erythematosus); bacterial products (e.g., endostreptosin, a protein expressed by group A streptococci); and large aggregated proteins [e.g., aggregated immunoglobulin G (IgG)], which tend to deposit in the mesangium. Antibodies produced against these planted antigens can react in situ in the glomerulus.
- **Immunofluorescence:** Most of them produce **granular fluorescence** under immunofluorescence microscopy, similar to the circulating immune complex nephritis.
- **Example** for this type of glomerulonephritis in humans is membranous nephropathy is discussed in pages 671-2.
- **Heymann nephritis:** It is experimental model for **in situ antibodies against planted antigens**.
  - It is induced in the rat by immunizing it with an antigen contained within preparations of proximal tubular brush border. This antigen is called as **Heymann antigen** which is a **protein called megalin**.
  - The rat develops antibodies to this antigen, and antibody reacts with an antigen complex located on the basal surface of visceral epithelial

cells. The rat develops a membranous nephropathy.

- *Electron microscopy*: It shows numerous discrete electron-dense deposits along the **subepithelial aspect of the basement membrane**.

Glomerular Diseases Caused by Complement Activation

In these type of glomerular diseases, the primary cause is unregulated activation of the alternative complement pathway. This may be due to acquired autoantibodies against complement components or inherited abnormalities of complement regulatory proteins. The complement-mediated injury may result in renal and systemic disease. This category of diseases include (i) two forms of GN (dense deposit disease and C3 GN) and (ii) one form of a systemic disease with significant renal manifestations [complement-mediated thrombotic microangiopathy (TMA) or atypical hemolytic uremic syndrome].

Antibody-mediated glomerular injury: Important mechanism of glomerular damage. Most commonly caused by formation of immune (antigen-antibody) complexes (in situ or circulating).

Immune complexes: Antigen may be exogenous or endogenous. These immune complexes are electron dense and give rise to granular pattern of immunofluorescence. The immune complexes activate complement → attracts leukocytes → damages tissues.

GBM: Size and charge dependent filtration.

Cytotoxic antibodies directed against glomerular cell components may rarely cause glomerular injury.

NON-IMMUNE MECHANISMS OF GLOMERULAR INJURY

Non-immune mechanisms may cause glomerular abnormalities in certain primary renal disorders.
These mainly include podocyte injury and nephron loss.

NEPHRITIC SYNDROME

Definition: Nephritic syndrome is **characterized by hematuria** (gross or microscopic), dysmorphic red cells, red cell casts in the **urine**, **azotemia** and **oliguria** (due to decreased glomerular filtration rate), and **mild to moderate hypertension**. Proteinuria and edema are common, but they are not as severe as in the nephrotic syndrome.

One of the causes of nephritic syndrome is acute proliferative glomerulonephritis.

Acute Proliferative Glomerulonephritis

- These are **immune complexes mediated** diseases. The **inciting antigen** may be:
  - **Exogenous** (e.g., postinfectious glomerulonephritis, which commonly follows streptococcal infection).
  - **Endogenous** [e.g., systemic lupus erythematosus (SLE)].
- Characterized histologically by **diffuse proliferation of glomerular cells, and infiltration by leukocytes**.

POSTSTREPTOCOCCAL (POSTINFECTIOUS) GLOMERULONEPHRITIS

Q. Classify glomerulonephritis. Discuss the etiopathogenesis of acute poststreptococcal glomerulonephritis. Describe its light microscopy, electron microscopy and immunofluorescence findings.

Poststreptococcal glomerulonephritis is a common disorder in developing countries.

Age group: Most frequent in **children between 6 and 10 years** of age, but may develop in adults.

Etiology and Pathogenesis

Q. Describe the etiopathogenesis of acute diffuse proliferative glomerulonephritis/poststreptococcal glomerulonephritis.

- It **follows streptococcal infection** (hence poststreptococcal) rather than direct primary infection of the kidney by streptococci.
- **Primary streptococcal infection** usually involves the **pharynx** (pharyngitis) or the skin (impetigo/pyoderma). Skin infections are usually associated with overcrowding and poor hygiene.
- Only **certain strains** of **group A β-hemolytic streptococci are nephritogenic**. More than 90% are due to **types 12**, **4**, **and 1**. These can be identified by typing of M protein of the streptococcal cell wall. Main streptococcal antigenic component responsible for immune reaction in poststreptococcal glomerulonephritis are streptococcal pyogenic exotoxin B (SpeB) and and streptococcal glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in most but not all cases.
- Manifests usually after a **latent period of 1–4 weeks** following primary streptococcal infection.
- It is an **immunologically mediated** disease. Both have an affinity for glomerular proteins and plasmin.

Evidences to Support Immunological Basis

- **Latent period:** It ranges from 1–4 weeks, between the streptococcal infection and beginning of glomerulonephritis and is compatible with the **time required for the production of antibodies and the immune complex formation**.
- **Antibodies against streptococcal antigens:** They are increased in majority of patients. These antibodies includes:
  - **Anti**-streptolysin O
  - **Anti**-deoxyribonuclease B (anti-DNase B)
  - **Anti**-streptokinase
  - **Anti**-hyaluronidase
  - **Anti**-nicotinyl adenine dinucleotidase.
- **Hypocomplementemia:** It is observed **in more than 90% of patients**, due to activation and utilization of complement components by immune complexes.

- **Immune-complex deposits:**
  - **Electron microscopy shows** glomeruli with **electron dense deposits** of immune complexes.
  - **Immunofluorescence shows granular fluorescence** to the immune deposits.
- **Streptococcal antigens in the glomeruli:** Many cationic antigens unique to nephritogenic strains of streptococci, can be demonstrated in the glomeruli. Examples:
  - Nephritis-associated streptococcal plasmin receptor **(NAPlR).**
  - Streptococcal pyogenic exotoxin B **(SpeB)** (main antigenic determinant responsible for immune reaction in most cases of poststreptococcal glomerulonephritis) and its zymogen precursor **(zSpeB).**
  - Streptococcal **GAPDH**.

Mechanism of Damage

- **Immune complexes** are **formed in the circulation** and gets **deposited within glomeruli**.
- These immune complexes **initiate inflammation** by **activating complement and** other humoral and **cellular mediators of inflammation** (*refer* page 153).
- The inflammatory mediators attract and **activate neutrophils and monocytes** and stimulate proliferation of mesangial and endothelial cell. The result is hypercellular glomerulus.

MORPHOLOGY

Q. Describe the morphology of kidney in acute diffuse proliferative glomerulonephritis.

Gross

The kidneys are enlarged and show pale capsular surface and cortex.

Q. Write a short answer on microscopy of kidney in acute post-streptococcal glomerulonephritis.

Microscopy (Figs. 20.7A and B)

1. **Glomeruli**:
   - **Enlarged hypercellular glomerulus:** It is the **classical diagnostic feature**, which is **seen in all glomeruli (**hence called **diffuse).** The hypercellularity is **due to**:
     - **Infiltration by leukocytes:** It includes both **neutrophils and monocytes**.
     - **Proliferation and swelling of endothelial and mesangial cells**.
     - R**arely** proliferation of **parietal cells** lining Bowman's capsule: It forms **crescent**. Presence of crescents is a **poor prognostic feature**.
   - **Obliteration of glomerular capillary lumen:** It is due to **swelling and proliferation of endothelial and mesangial cells + infiltration by leukocytes**.
2. **Tubules:** It may contain **red cell casts** in the lumen and the tubular epithelial cells may show degenerative changes.
3. **Interstitium:** It may show edema and inflammatory cell infiltrate.
4. **Blood vessels**: Unremarkable.

Immunofluorescence Microscopy (Fig. 20.8)

Granular deposits of **IgG, IgM, and C3** in the mesangium and along the GBM → **granular fluorescence**.

Electron Microscopic Findings (Fig. 20.9)

- **Subepithelial deposits** of **discrete**, **amorphous**, **electron-dense deposits** is **characteristic feature**. These appear as dome-shaped "humps" → termed as **'humps' or 'lumpy'** deposits. They correspond to the granular IgG and C3 demonstrated by the immunofluorescence.
- Subendothelial, intramembranous and mesangial deposits may also be present.

Clinical Course

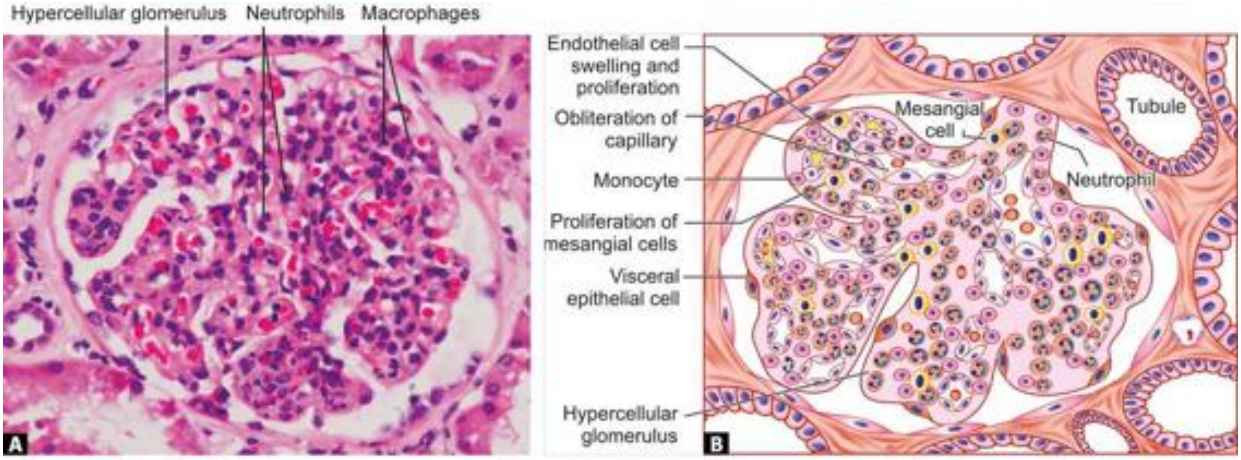
- Affected **child develops malaise**, **fever**, **nausea**, **oliguria**, **and hematuria** (**smoky or cola-colored urine**) **1–2 weeks** after recovery from a **sore throat**.
- **Periorbital edema**, and **mild to moderate hypertension** is usually observed.
- In adults, clinical features are atypical.

Laboratory Findings

Q. Laboratory findings in nephritic syndrome.

Blood Findings

- **Raised antistreptococcal antibody** titers [e.g., anti-streptolysin O (ASO) titer], but the level may be low in GN that follows streptococcal skin infection.



Figs. 20.7A and B: Light microscopic appearance of acute poststreptococcal glomerulonephritis: (A) (Hematoxylin and eosin stain); (B) (diagrammatic). Glomerulus is hypercellular is due to proliferation of intrinsic glomerular cells and infiltration by leukocytes.

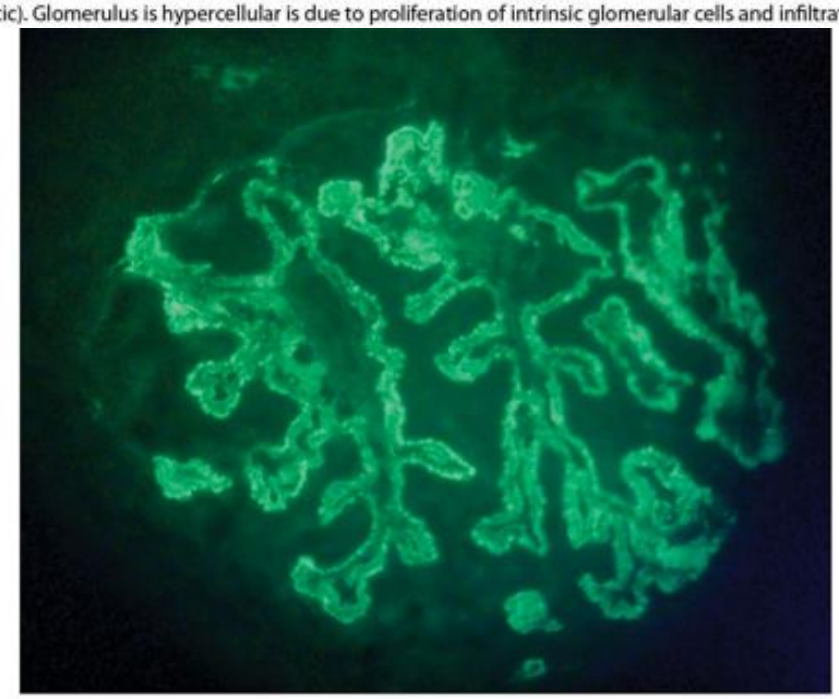


Fig. 20.8: Granular immunofluorescence characteristic of circulating or in situ immune complex nephritis.

- **Hypocomplementemia:** Significant **reduction in serum** concentration of **complement** (C3) components.
- Raised blood urea and serum creatinine indicate renal impairment.

Urinary Findings

Q. Write a short answer on urinary findings in acute nephritis.

- **Oliguria**
- **Mild** (variable degree) **proteinuria** (usually <1 g/day).
- **Hematuria** (**smoky or cola-colored urine):** RBCs in urine.
- **Microscopy: Red cell casts or dysmorphic red cells (red cells with cellular protrusions or fragmentation)**.

Prognosis

- **Children:** Prognosis is **good** in children and more than 95% totally recover.
- **Adults:** In adults, it is **less benign**. They may recover promptly or develop rapidly progressive glomerulonephritis or progress to chronic glomerulonephritis.

Q. A10-year-old boy was brought by the mother to pediatric outpatient with chief complaints of puffiness of face, particularly more marked in the morning, mild pitting edema on both legs, malaise with nausea and a slight fever for about one month. Mother also complained that he passes dark brown (red or cola) colored (smoky) urine and the urine output was reduced (oliguria). On enquiry, mother informed that about 2 weeks back the child had impetigo lesions on the face (or upper respiratory tract infection). On examination, the blood pressure was 140/100 mm Hg (moderate hypertension). Urine examination revealed mild (or moderate) albuminuria/proteinuria and microscopy showed RBC ++ and RBC cast +.

Laboratory investigation showed a raised serum anti-streptolysin O titer and low serum complement level.

(a) What is the most probable diagnosis? (Ans: Nephritic syndrome due to acute poststreptococcal glomerulonephritis)

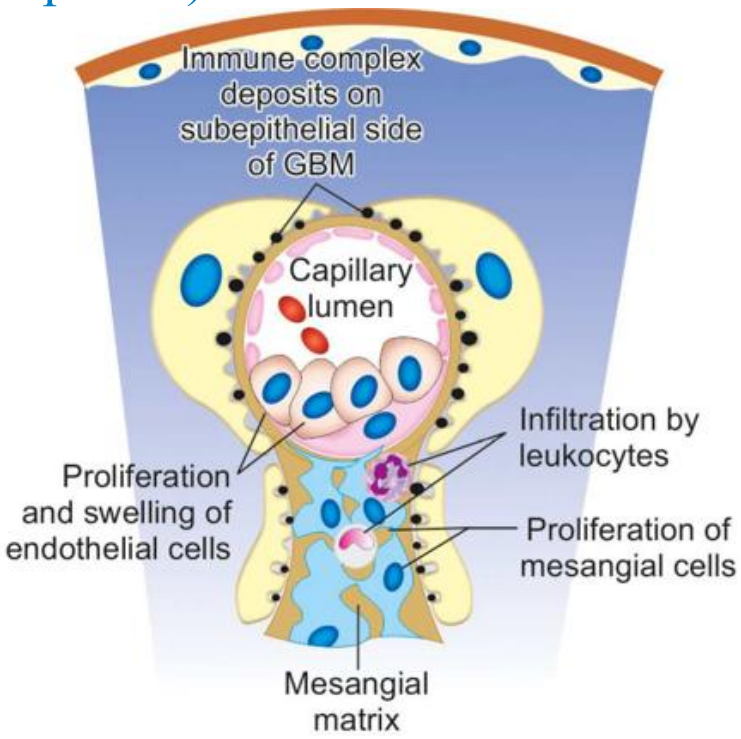


Fig. 20.9: Diagrammatic electron microscopic appearance of poststreptococcal glomerulonephritis. Subepithelial immune complexes appear as characteristic hump-like structures. Other features are endothelial and mesangial cell proliferation with narrowing of the lumen.

(b) Discuss the etiopathogenesis of these lesions (refer pages 664-5)

(c) Describe the light microscopy, immunofluorescence and electron microscopic findings (refer page 665)

(d) List the investigations. What is the prognosis of this disease? (refer pages 665-6)

Nephritic syndrome:
- Hematuria
- RBC casts/dysmorphic red cells in the urine
- Moderate proteinuria
- Oliguria
- Hypertension

Proliferative GN: Increased numbers of cells within the glomerulus due to proliferation of cells of the glomeruli and/or infiltration of polymorphs and macrophages.

Nonstreptococcal Acute Glomerulonephritis (Postinfectious Glomerulonephritis)

Acute proliferative glomerulonephritis may also occur in association with other infections:

- **Bacteria:** Examples, staphylococcal endocarditis, pneumococcal pneumonia, and meningococcemia.
- **Virus:** Examples, hepatitis (B and C), mumps, HIV infection, varicella, and infectious mononucleosis.
- **Parasite** (e.g., malaria, toxoplasmosis).

The morphological changes are similar to poststreptococcal glomerulonephritis.

Acute poststreptococcal glomerulonephritis: Develops after streptococcal infection in children and young adults. It is an immune-complex mediated disease.

Poststreptococcal glomerulonephritis:
- Diffuse cellular proliferation of endothelial and mesangial cells
- Infiltration by leukocytes
- Granular immunofluorescence
- Subepithelial deposits (humps) on EM

Acute proliferative glomerulonephritis:
- Periorbital edema
- Mild to moderate hypertension
- Cola-colored urine

Pitting edema: Does not distinguish nephritic from nephrotic syndrome.

Complement levels in poststreptococcal GN:
- Transient reduction during the disease
- Return to normal in 6–8 weeks

Hematuria with dysmorphic RBCs are seen in: Acute glomerulonephritis.

Poststreptococcal glomerulonephritis: Most affected children recover; in adults prognosis is worse.

RAPIDLY PROGRESSIVE (CRESCENTIC)GLOMERULONEPHRITIS

Q. Write short note on rapidly progressive glomerulonephritis.

Definition: Rapidly progressive glomerulonephritis (RPGN) is a syndrome, characterized by **rapid and progressive loss of renal function** associated with **severe oliguria** and signs of **nephritic syndrome**. If not treated, death occurs due to renal failure within weeks to months.

RPGN does not represent a specific etiologic type of glomerulonephritis. Most common microscopic features is the presence of crescents in most of the glomeruli. Hence the term crescentic glomerulonephritis.

Classification

Q. Write short essay on classification. Etiology and pathogenesis of rapidly progressive glomerulonephritis.

RPGN classified into three types based on immunological findings **(Box 20.4).** Each type may be idiopathic or associated with a known disorder.

BOX 20.4: Classification of rapidly progressive glomerulonephritides.

Type I (Anti-GBM antibody)
- Goodpasture syndrome

Type II (Immune complex)
- Idiopathic
- Postinfectious glomerulonephritis
- Lupus nephritis
- IgA nephropathy

Type III (Pauci-immune)
- Idiopathic
- Wegener granulomatosis
- ANCA-associated

(GBM, glomerular basement membrane; ANCA, antineutrophil cytoplasmic antibodies).

Type I (Anti-GBM Antibody)

Type I RPGN is an uncommon, aggressive type **caused by an anti-GBM antibody**. One of the examples is Goodpasture syndrome.

Type II (Immune Complex)

- It is due to **immune complex deposition** and may complicate any of the immune complex nephritides (e.g., postinfectious glomerulonephritis, lupus nephritis).
- **Immunofluorescence:** Shows **granular fluorescence** characteristic of immune complex nephritis.

Type III (Pauci-immune)

- It is also known as pauci-immune type.
- It **neither shows anti-GBM antibody nor immune complex** by immunofluorescence and electron microscopy.
- Majorities are **idiopathic** and have circulating **antineutrophil cytoplasmic antibodies (ANCAs)**.

- Some may be a component of systemic vasculitis, such as Wegener granulomatosis.

Pathogenesis

The RPGN is a syndrome caused by **primary glomerular diseases as well as systemic diseases**. Pathogenetic mechanism varies but most are immunologically mediated and it indicates severe glomerular damage.

RPGN: Clinical entity with features of nephritic syndrome and rapid loss of renal function.

GOODPASTURE SYNDROME

Q. Write short essay on Goodpasture syndrome.

In Goodpasture syndrome, **anti-GBM antibodies cross-react with pulmonary alveolar basement membranes** causing pulmonary hemorrhage associated with acute renal failure.

Pathogenesis

Genetic predisposition: High prevalence of certain HLA subtypes (e.g., **HLA-DRB1**) in these patients point towards genetic predisposition to autoimmunity.

- **Anti-GBM antibodies** are formed **against Goodpasture antigen**. This antigen results from conformational changes in the a3 chain of type IV collagen of GBM.
- **Anti-GBM antibodies cross-react** with the **target antigen,** which is also expressed on pulmonary **alveolar capillary basement membranes**. This results in **pulmonary hemorrhages** and **hemoptysis**, which may be life-threatening.

MORPHOLOGY

Gross

- Kidneys are enlarged and pale (large white kidney).
- May show **petechial hemorrhages on the cortical surfaces (flea bitten kidney)**.

Microscopy (Figs. 20.10A to C)

- **Glomeruli:** Its characteristic feature is **crescents** in most of the glomeruli. Hence, the term crescentic glomerulonephritis.
- **Crescents:**
  - **Components:** Crescents are formed by:
    - **Proliferation of the parietal epithelial cells** lining Bowman capsule.
    - Infiltration of **monocytes and macrophages** into the urinary space.
  - **Stimulus: Fibrin** leaked into the Bowman space is the stimulus and is found between the cellular layers. Fibrin is derived from fibrinogen, which escaped through the **ruptured GBM** into Bowman space.
  - **Significance:** It indicates **severe glomerular injury** and does not represent any specific etiological factor.
- **Tubules:** The tubular lumen may show casts and red cells.
- **Interstitium:** It shows edema with few inflammatory cells.
- **Blood vessels: Normal**.

Immunofluorescence Microscopy

Goodpasture syndrome show **linear GBM fluorescence (Fig. 20.11)** for IgG and C3 complement.

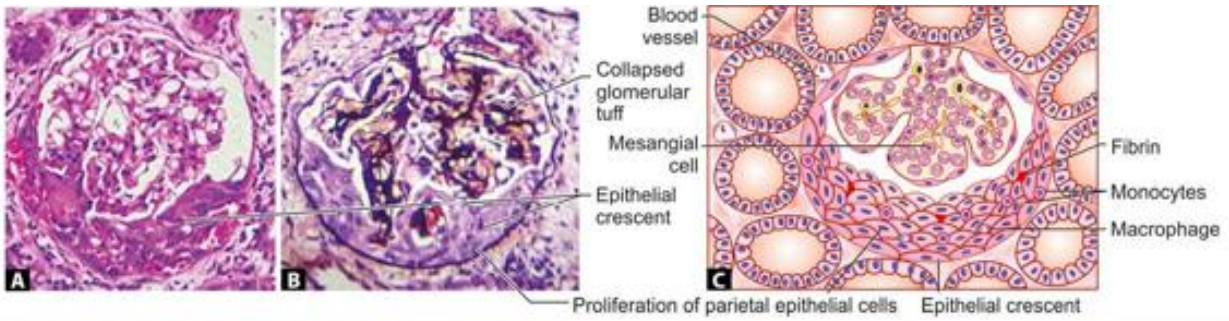
Electron Microscopy

Ruptures in the GBM: It allows **leukocytes, proteins**, and **inflammatory mediators to leak** into the urinary space and initiates crescent formation **(Fig. 20.12)**.

Clinical Course

In Goodpasture syndrome, patient may present with **recurrent hemoptysis** or life-threatening pulmonary hemorrhage. Other features are variable hypertension and edema.

Urine findings: (1) Hematuria, (2) RBC casts and dysmorphic RBCs, and (3) moderate proteinuria.



RPGN (crescentic GN): Prognosis depends upon number of crescents as it correlates with oliguria.

Figs. 20.10A to C: Crescentic glomerulonephritis showing crescent-shaped mass of proliferating parietal epithelial cells and leukocytes internal to Bowman capsule: (A) Hematoxylin and eosin stain (H & E stain); (B) Silver methenamine stain; (C) Diagrammatic representation.

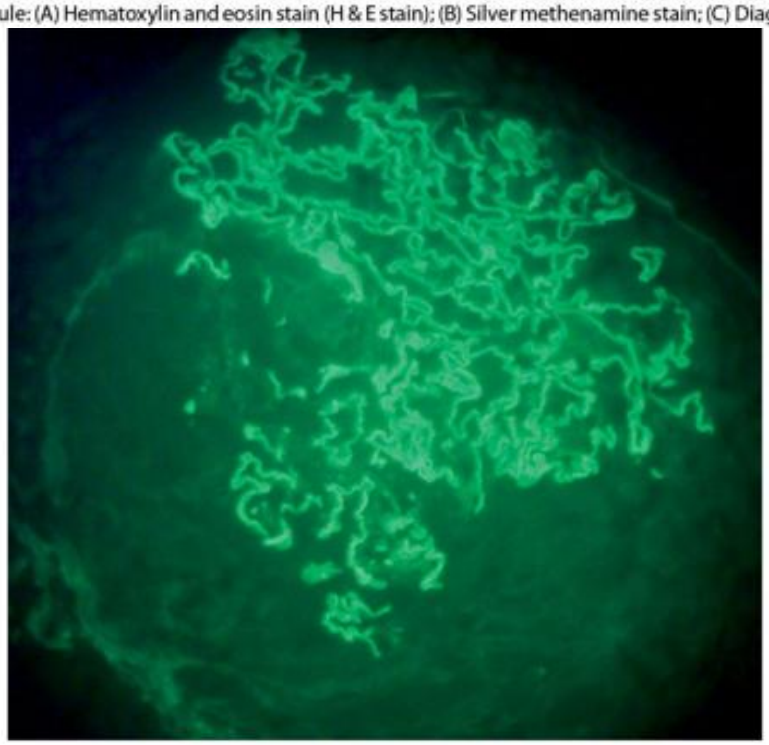


Fig. 20.11: Linear immunofluorescence for immunoglobulin G is seen along the GBM in Goodpasture syndrome.

Prognosis: Milder forms may recover, but the renal involvement usually progress within weeks and cause severe oliguria.

Goodpasture syndrome: Anti-GBM disease in which anti-GBM antibodies cross-react with alveolar basement membrane of lung.

Goodpasture antigen: A peptide within the noncollagenous portion of the α3 chain of collagen type IV of GBM.

Epithelial crescents: Proliferation of parietal epithelial cells + monocytes and macrophages. Formed due to fibrin leaked through ruptured GBM.

Uremia is the cause of death in Goodpasture syndrome.

Goodpasture syndrome:
- Anti-GBM disease
- Epithelial crescent
- Linear immunofluorescence

NEPHROTIC SYNDROME

Q. Define nephrotic syndrome. List the causes of nephrotic syndrome. Add a note on its pathophysiology. What are the causes of nephrotic syndrome in pediatric age group?

Definition: Nephrotic syndrome is characterized by:
- **Massive/heavy proteinuria (>3.5 g of protein/24 hours)**
- **Hypoalbuminemia**
- **Edema**
- **Hyperlipidemia and lipiduria**

Pathophysiology (Fig. 20.13)

In nephrotic syndrome, damage to the glomerular capillary walls causes increased permeability to plasma proteins.

1. **Massive proteinuria:** It is characterized by **daily loss of 3.5 g or more** of **protein** (less in children) in the **urine**.
   - Normally, the glomerular capillary wall acts as a size and charge dependent barrier for the plasma filtrate.
   - Proteinuria is **due to increased permeability of glomerular capillary wall to plasma proteins**.
   - The major proportion of protein lost in the urine is **albumin (low-molecular-weight proteins)**, and rarely globulins (high-molecular-weight proteins). The ratio between low- and high-molecular-weight proteins in the urine in various cases of nephrotic syndrome is due to selectivity of proteinuria. Types of glomerular proteinuria are presented in **Box 20.5**.

Albumin is the major protein lost in the urine, but globulins may also be excreted in some diseases.

2. **Hypoalbuminemia: Plasma albumin levels less than 3 g/dL.**
   - Massive proteinuria **decreases the serum albumin** levels (hypoalbuminemia).
   - Hypoalbuminemia **decreases the colloid osmotic pressure** of the blood.
3. **Generalized edema:**
   - **Causes:**
     - **Decreased colloid osmotic pressure of the blood:** It leads to accumulation of fluid in the interstitial tissues → generalized edema.
     - **Sodium and water retention:** Decreased plasma volume reduces glomerular filtration rate which in turn leads to **compensatory secretion of aldosterone**. It aggravates the edema by salt and water retention.
   - **Characteristics of edema:**
     - Soft and pitting
     - Most marked in the **periorbital regions (Fig. 20.14)** and **dependent portions of the body**
     - When massive associated with pleural effusions and ascites.
4. **Hyperlipidemia and lipiduria:**

   Hyperlipidemia:
   - Most patients with nephrotic syndrome have raised blood levels of cholesterol, triglyceride, very-low-density lipoprotein, low-density lipoprotein, Lp(a) lipoprotein, and apoprotein.

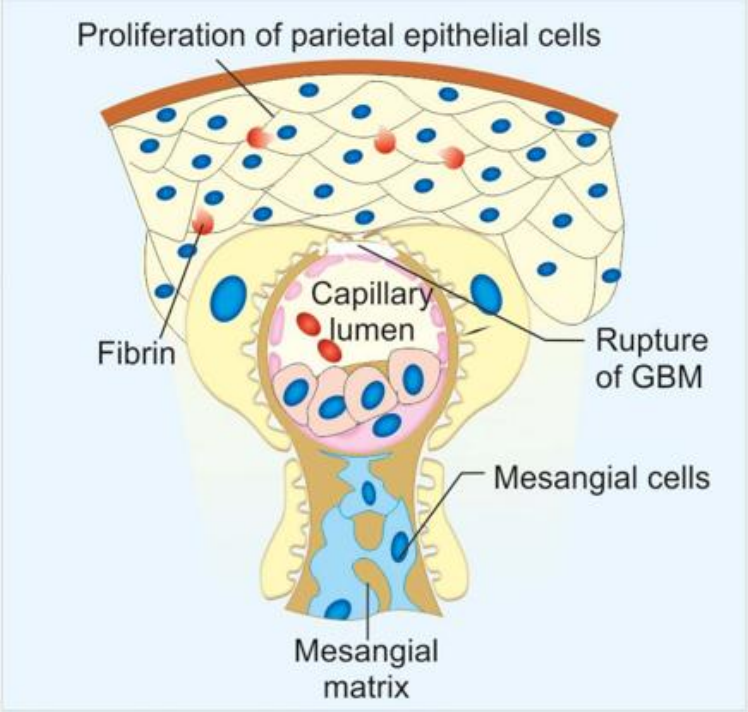


Fig. 20.12: Diagrammatic electron microscopic appearance of crescentic (rapidly progressive) glomerulonephritis. Shows disruption of GBM and proliferation of parietal epithelial cells to form crescent.

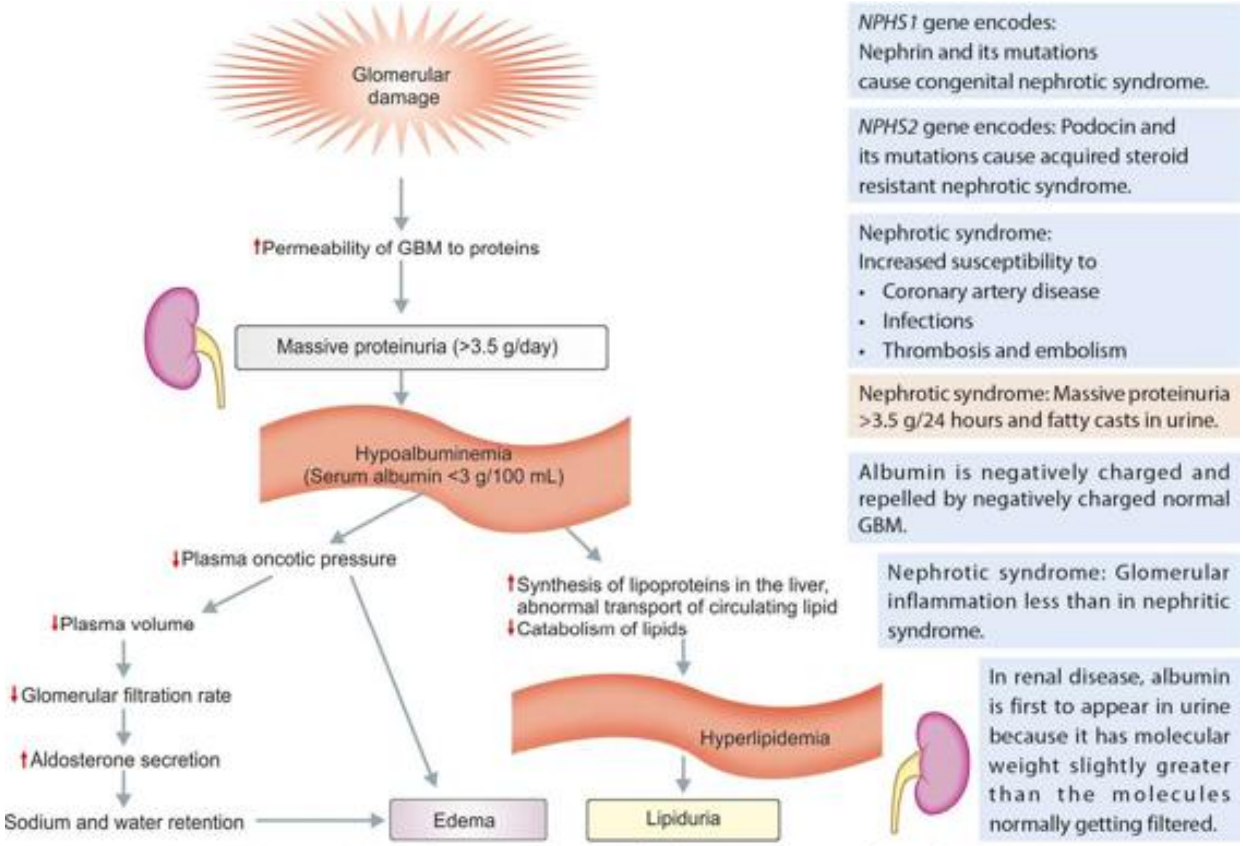


Fig. 20.13: Various characteristic features of nephrotic syndrome and its mechanism. (GBM: glomerular basement membrane).

Q. Write short note on selective and nonselective proteinuria.

BOX 20.5: Types of glomerular proteinuria.

- **Highly selective proteinuria:** In this type, only low-molecular weight/intermediate-sized (<100 kDa) proteins (such as albumin 70 kD, transferrin 76 kD molecular weight) leaks through the glomerulus, e.g., minimal-change disease
- **Nonselective (poorly-selective) proteinuria:** It is characterized by leakage of range of different proteins (proteins of any size) including larger/higher molecular-weight serum proteins (e.g., immunoglobulins) in addition to albumin through the glomerulus, e.g., membranous nephropathy



Fig. 20.14: Facial and periorbital puffiness in nephrotic syndrome.

- **Causes:** Hyperlipidemia due to:
  - Increased synthesis of lipoproteins in the liver
  - Abnormal transport of circulating lipid
  - Decreased catabolism of lipids.

Lipiduria:

- Hyperlipidemia is followed by **leakage of lipoproteins** across the glomerular capillary wall → leaked lipoprotein is reabsorbed by tubular epithelial cells → then shed along with the degenerated cells → appears **in urine either as free fat or as oval fat bodies**.

Major Complications of Nephrotic Syndrome

- **Coronary atherosclerosis:** Hyperlipidemia predisposes to atherosclerosis of coronary artery.

TABLE 20.2: Causes of nephrotic syndrome.

Primary glomerular diseases	*Systemic diseases*

TABLE 20.2: Causes of nephrotic syndrome.	
Primary glomerular diseases	***Systemic diseases***
Membranous glomerulopathy (~30% in adults)	Diabetes mellitus
Minimal-change disease (~75% in children)	Amyloidosis
Focal segmental glomerulosclerosis (~35% in adults)	Infections (malaria, syphilis, hepatitis B and C, HIV)
Membranoproliferative glomerulonephritis	Systemic lupus erythematosus
IgA nephropathy	Drugs (penicillamine, "street heroin")
	Malignancy (carcinoma, melanoma)
	Others (bee-sting allergy, hereditary nephritis)

- **Infections**: Nephrotic patients are s**usceptibility to infection**, especially staphylococci and pneumococci. It may be **due to loss of immunoglobulins in the urine**.
- **Thrombotic and thromboembolic complications:** This may be due to loss of endogenous anticoagulants (e.g., antithrombin III) and antiplasmins in the urine.

Causes of Nephrotic Syndrome (Table 20.2)

Q. Write short note on causes of nephrotic syndrome.

- Most common systemic causes of the nephrotic syndrome are **diabetes**, **amyloidosis**, **and SLE**.
- Most important primary glomerular lesions causing nephrotic syndrome are **minimal-change disease**, **membranous glomerulopathy**, and **focal segmental glomerulosclerosis**.

Nephrotic syndrome:
- Massive proteinuria
- Edema
- Lipiduria
- Hypoalbuminemia
- Hyperlipidemia

MEMBRANOUS NEPHROPATHY(MEMBRANOUS

GLOMERULOPATHY)

Q. Write short note membranous glomerulopathy.

Membranous nephropathy is characterized by **uniform diffuse thickening of the glomerular capillary wall**. This is due to the accumulation of **electron-dense deposits along the subepithelial side of the glomerular basement membrane**.

Membranous nephropathy is the **common cause (~30%) of the nephrotic syndrome in adults**.

Etiology

Q. Write short essay on etiology and pathology of membranous glomerulopathy.

Primary/idiopathic: No identifiable cause in about 85% of patients.

Secondary: Found in association with:

- **Therapeutic drugs** [e.g., penicillamine, captopril, nonsteroidal anti-inflammatory drugs (NSAIDs)]
- **Malignant neoplasms** (e.g., carcinomas of the lung and colon, lymphomas and melanoma)
- **Autoimmune disease** [e.g., SLE (systemic lupus erythematosus), Hashimoto thyroiditis]
- **Infections** (e.g., chronic hepatitis B, hepatitis C, malaria, syphilis, schistosomiasis).
- **Exposure to inorganic salts** (e.g., gold, mercury).

Pathogenesis

Membranous glomerulopathy is a **chronic immune complex-mediated** disease:

- **Idiopathic:** It is an **autoimmune disease** caused by antibodies reacting **in situ** to an endogenous or planted glomerular antigen. Autoimmune reaction by auto-antibodies are most often directed against phospholipase A2 receptor antigen (PLA2R) on the podocyte (autoantibodies against podocyte antigen). It results **in situ formation of immune complex**.

Heymann nephritis is one of the experimental model of membranous nephropathy/glomerulopathy.

- **Secondary:** It results from **circulating immune complex** and the antigens may be:
  - **Exogenous antigens** (e.g., hepatitis B, *Treponema*).
  - **Endogenous antigens** [e.g., nonrenal antigens (e.g., thyroglobulin) or renal antigens].

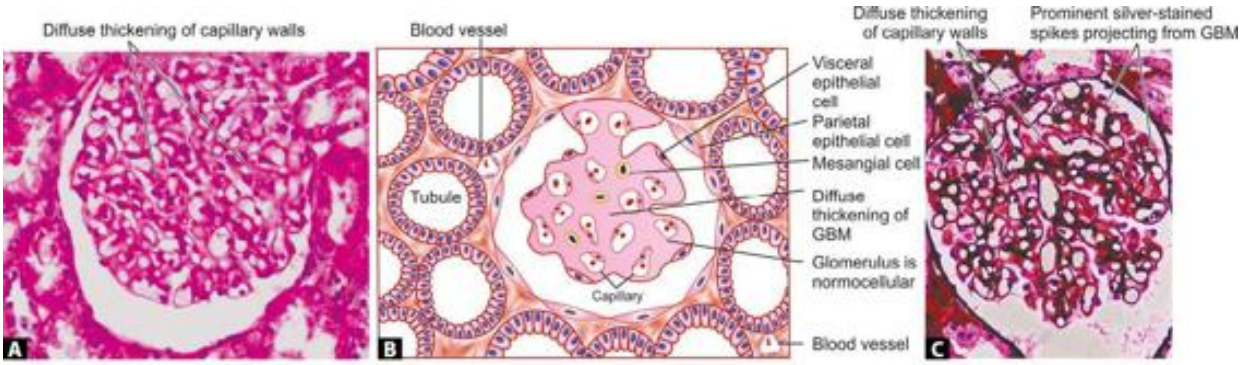
Mechanism of Glomerular Injury

- **Glomeruli do not show** any **inflammatory cells** (like neutrophils, monocytes), or platelets.
- There is formation of subepithelial immune deposits which activates the complement system on the surface of podocytes and generates **C5b-C9** (membrane attack complex).
- The **C5b-C9** damages the podocyte of glomerular epithelial cells and mesangial cells → **liberates proteases and oxidants → cause capillary wall injury** → leakage of protein.

MORPHOLOGY

Light Microscopy (Figs. 20.15A to C)

1. **Glomeruli:**
   - Show **uniform**, **diffuse thickening of the glomerular capillary wall**.
   - **Normocellular glomeruli:** There is n**either proliferation nor inflammation**.
   - **Special stains**: **Silver stains (black color) or periodic acid-Schiff stain (pink color)** show **characteristic spikes** in all glomeruli, **between the deposits of immune complex**.
   - As the disease progresses glomeruli may become totally sclerosed.
2. **Tubules:** The **epithelial cells of the proximal tubules** show **droplets of reabsorbed protein**. Later tubules undergo atrophy.



Figs. 20.15A to C: Light microscopy (A) Hematoxylin and eosin stain (H & E); (B) Diagrammatic of membranous nephropathy showing marked diffuse thickening of the GBM without an increase in the number of cells; (C) Silver methenamine stain showing marked thickening of capillary walls and prominent silver-stained spikes projecting from GBM.

3. **Interstitium:** It shows mononuclear cell inflammatory cells and interstitial fibrosis in late stages.
4. **Blood vessels**: Early stages vessels appear normal.

Electron Microscopy (Fig. 20.16)

- **Thickening of the glomerular capillary wall due to irregular electron-dense deposits of immune complexes** between the basement membrane and the overlying visceral epithelial cells (**subepithelial)**. The **visceral epithelial cells show effaced foot processes**.
- In **between these immune deposits, basement membrane material is laid down**, which appear as **irregular spikes protruding from the GBM (spike and dome pattern).**

Changes due to subepithelial immune complexes are divided into stages:

- **Stage I:** Capillary walls are normal or show mild thickening and subepithelial dense deposits.
- **Stage II:** Projections of GBM material around dense deposits, that appear as spikes on the epithelial surface of the basement membrane, which stain with silver.
- **Stage III:** As the disease progresses, the basement membrane encircles the deposits and is laid over the immune deposits. This will bury (incorporate) them within a markedly thickened, irregular membrane. In silver stain, they appear dome-like protrusions.
- **Stage IV:** Later in the disease, the incorporated immune deposits undergo degradation and lysis leaving cavities within the GBM.
- If the deposition of basement membrane matrix continues, it causes progressive thickening of basement membranes. This may result in sclerosis of glomeruli.

Immunofluorescence Microscopy

It shows **granular deposits** of both **immunoglobulins and complement (IgG and C3)** in the **subepithelial** (between the visceral epithelial cell and the GBM) region. There is also intense staining for membrane attack complex.

Clinical Features

- Present as sudden onset **nephrotic syndrome**
- **Nonselective proteinuria** (which is selective in minimal-change disease).
- **Does not respond to corticosteroid** therapy.

Course: Variable but generally indolent.

Q. A 45-year-old man complained of swelling in the extremities for 2 months and face especially eyelids (facial puffiness) for the past 3 months. Protein estimation of a 24-hour urine sample revealed4.1 g of protein (albumin and globulins). His serum albumin level was 2.0 g/dL. Urine microscopy showed fat globules. A course of corticosteroid therapy was given and there was no response. A renal biopsy was performed and microscopic examination showed diffuse thickening of the glomerular basement membrane. Immunofluorescence staining with antibody to the C3 component of complement was positive in a granular pattern in the glomerular capillary loops.

(a) What is the probable diagnosis? (Ans: One of the most common causes of nephrotic syndrome in adults is membranous nephropathy).

(b) Discuss the etiopathogenesis of this disease (refer page 671).

(c) Describe the light microscopic, electron microscopic and immunofluorescence findings in this condition (refer pages 671-2).

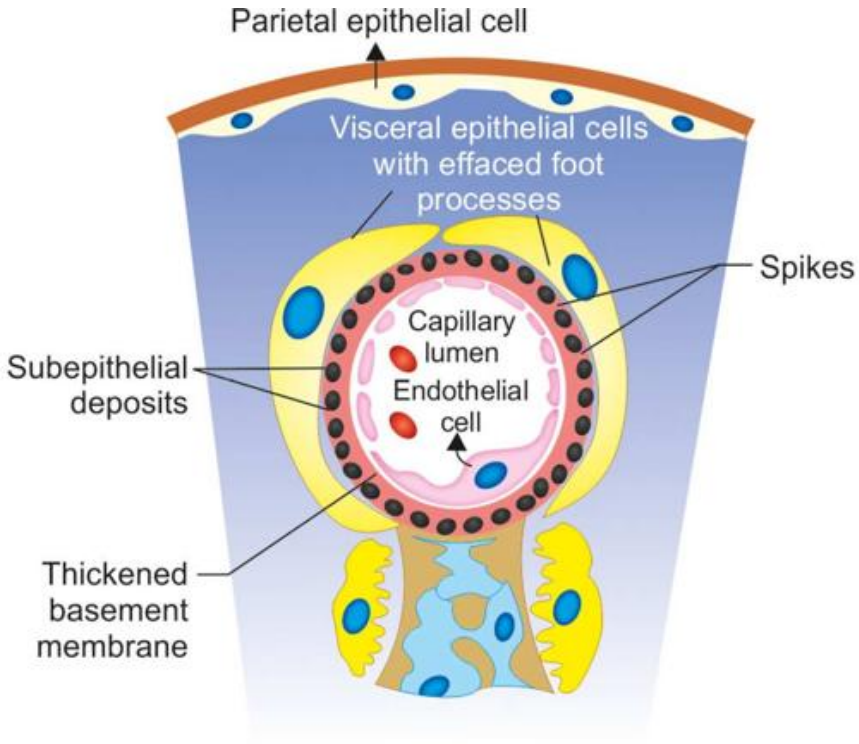


Fig. 20.16: Diagrammatic representation of electron microscopy of membranous glomerulopathy. Electron-dense deposits are seen along the epithelial side of the basement membrane. The visceral epithelial cells show effacement of foot processes overlying deposits.

Membranous nephropathy: Common primary cause of the nephrotic syndrome in adults.

Membranous nephropathy: Autoantibodies are directed against phospholipase A2 receptor on the podocyte.

Membranous glomerulopathy: Nephrotic syndrome, diffuse, uniform, GBM thickening due to expansion of the basement membrane. The glomerulus is normocellular with little or no inflammation.

Membranous glomerulopathy: Characteristic spikes between the deposits of immune complex better appreciated in PAS or silver stains.

Membranous nephropathy:

- Granular IF
- Subepithelial deposits on EM

MINIMAL-CHANGE DISEASE

Minimal-change disease (MCD) is named so because the **glomerular changes are absent or minimal and glomeruli appear normal under light microscopy**. But under electron microscopy, it shows **diffuse effacement (loss) of foot processes of visceral epithelial cells** (podocytes).

MCD is the **major cause of nephrotic syndrome in children**, but it is less common in adults. MCD is relatively benign disorder.

Age: Peak incidence between 2 and 6 years of age.

Etiology and Pathogenesis

Q. Write short note on etiology and pathogenesis of minimal-change disease.

The pathogenesis of MCD is unknown. The electron microscopic changes indicate that **primary injury is to the visceral epithelial cells**, which may be caused by either immunological or nonimmunological mechanism.

1. Immunological Mechanism

Lack of immune deposits in the glomerulus that excludes the possibility of immune complex mechanism. However, several features suggest immunological mechanism:

- Sometimes **follows a respiratory infection or routine prophylactic immunization**.
- Dramatically **responds to corticosteroids** and/or other **immunosuppressive therapy**.
- **Associated with atopic/allergic disorders** (e.g., eczema, rhinitis).
- **Increased incidence** in patients **with Hodgkin lymphoma**, which has defects in T-cell–mediated immunity.
- Increased prevalence of certain **HLA haplotypes** suggesting a genetic predisposition.

Immune dysfunction causes production of a **cytokine** by T-cells. The cytokine **damages visceral epithelial cells** and increases glomerular permeability causing **proteinuria**.

2. Nonimmunological Mechanism

It may cause injury to visceral epithelial cell.

- **Mutations** in structural proteins namely: **nephrin and podocin (**localized to the slit diaphragm) causes structural defects of the podocyte leading to marked proteinuria.
- **Mutation in the nephrin gene** is found in hereditary type of **congenital nephrotic syndrome (Finnish type)** with minimal-change glomerular morphology.

Mechanism of Proteinuria

- MCD is accompanied by a **loss of glomerular polyanions** on the GBM.
- **Defects in the charge barrier** allow anionic proteins, particularly **albumin**, to pass more easily through the GBM → **massive proteinuria**.

MORPHOLOGY

Q. Write short note on morphology of minimal-change disease.

Gross

- Kidneys are mildly enlarged with smooth capsular surface.
- Cut section shows pale to yellow cortex.

Light Microscopy

- **Glomeruli:** Appear normal by light microscopy **(Fig. 20.17A)**.
- **Tubules:** The **cytoplasm of epithelial cells** lining the **proximal tubules contain lipid vacuoles and glassy (hyaline) protein droplets** (hence, the historical term lipoid nephrosis for this disease). These are **due to the tubular reabsorption of lipoproteins** passing through damaged glomeruli.
- **Interstitium:** May show mild edema.
- **Blood vessels:** Appear normal.

Electron Microscopy (Fig. 20.17B)

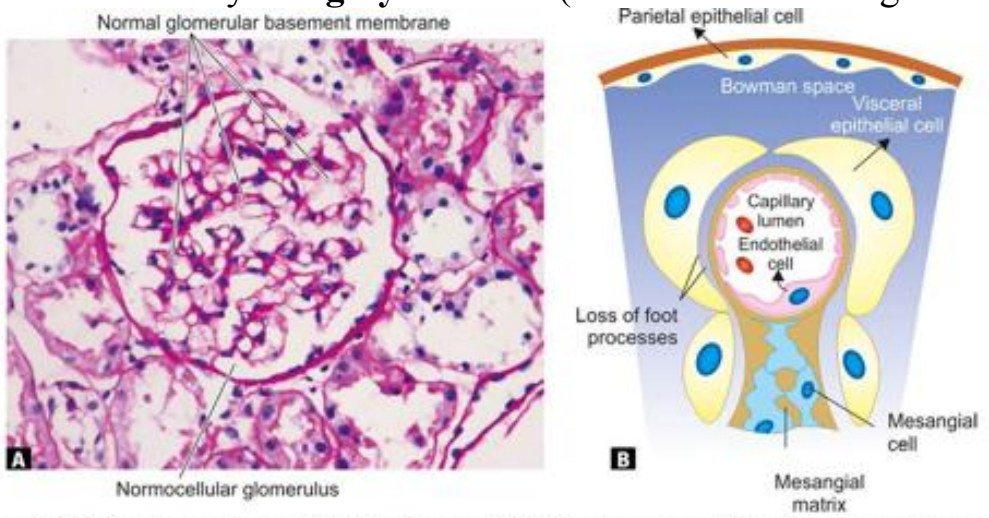
- **GBM appears normal** without any electron-dense deposit.
- **Changes in the visceral epithelial cells:** Most **characteristic feature is uniform and diffuse effacement of foot processes in the visceral epithelial cells**. This change is wrongly called as "fusion" of foot processes. The cytoplasm shows vacuolization and swelling.

Immunofluorescence

Does not show immunoglobulin or complement deposits.

Clinical Features

- Classically, it present as **nephrotic syndrome**.
- Onset of nephrotic syndrome may be **preceded by upper respiratory tract infection or immunization**.
- **Proteinuria** usually is **highly selective** (albumin more than globulins).



Figs. 20.17A and B: Minimal-change disease: (A) Light microscopy. PAS stain shows normal GBM without any cellular proliferation; (B) Diagrammatic electron microscopic appearance. It shows effacement of the foot processes of visceral epithelial cells. All other glomerular structures appear normal.

- **Other features:** Generalized edema, hypoalbuminemia, and hyperlipidemia.
- In spite of massive proteinuria, renal function remains good.

Urine findings: Proteinuria, oval fat bodies and, fatty casts.

Prognosis: Excellent.

Q. A 6-year-old child was brought to the hospital for the complaint that he has become less active over the past 10 days. On physical examination, the boy had facial puffiness which is more prominent around his eyes and also had swelling of both ankles. There was neither history of fever nor hypertension. Urinalysis showed 4+ proteinuria and there was no blood,

glucose, or ketone bodies. Microscopic examination of urine showed fat globules and there were no cells, casts or crystals. The protein in a 24-hour urine sample revealed 3.8 g of protein. The serum creatinine and blood urea levels were normal. He responded well to corticosteroid therapy.

(a) What is the most probable diagnosis? (Ans: Steroid-responsive proteinuria in a child is feature nephrotic syndrome due to minimal change disease)

(b) Discuss the etiopathogenesis of this disease (refer page 673).

(c) Describe the light microscopic, electron microscopic and immunofluorescence findings in this condition (refer page 673)

MCD: Common cause of nephrotic syndrome in children.

MCD: Historical original name was lipoid nephrosis, because the most noticeable feature by light microscopy was fat in the renal tubular epithelial cells.

Steroid resistant nephrotic syndrome: Mutation in the gene encoding podocin.

Finnish type of congenital nephrotic syndrome: Mutation in the nephrin gene.

MCD:

- Glomeruli appear normal by light microscopy

- Proximal tubules contains lipid vacuoles in the cytoplasm.

MCD: EM shows uniform and diffuse effacement/loss of foot processes in the visceral epithelial cells.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Q. Write short answer on focal segmental glomerulosclerosis.

Focal segmental glomerulosclerosis (FSGS) is characterized by the **sclerosis** that **involves only part of the capillary tuft** (i.e., segmental) **of some glomeruli** (i.e., focal).

Classification

- **Primary (idiopathic)**.
- **Secondary:**
  - Viruses (e.g., HIV infection)
  - Drugs (e.g., heroin addiction)
  - Sickle-cell disease
  - Massive obesity
  - **Congenital** (e.g., unilateral agenesis) and acquired (e.g., reflux nephropathy) reductions in renal mass.
- **Hereditary forms:** Autosomal recessive FSGS due to mutations in the *NPHS2* gene, which maps to chromosome 1q25-q31 and encodes the protein product podocin. **Inherited mutations** in genes that encode podocyte proteins, e.g., **podocin, α-actinin-4**. Adult-onset FSGS with mutations in the gene encoding TRPC6.

Pathogenesis

Probably primary FSGS is caused due to an unknown injury to podocytes. According to some, FSGS and minimal-change disease are part

of a continuum and that minimal-change disease may transform into FSGS. Both show similar **visceral epithelial damage, i.e., diffuse effacement of foot processes.** In FSGS, the deposition of hyaline in the glomeruli is due to the entrapment of plasma proteins and lipids in foci of injury where sclerosis develops. The recurrence of proteinuria in patients undergoing renal transplantion for FSGS, sometimes within 24 hours of transplantation, suggests the possibility of a circulating mediator which leads to podocyte damage in some cases.

MORPHOLOGY

Light Microscopy

Glomerular involvement: Primary FSGS initially involves only the juxtamedullary glomeruli and with progression of the disease it affects all levels of the cortex. The lesions occur in only some tufts within a glomerulus while other tufts appear normal on light microscopy.

Microscopic features: The affected glomeruli show: **(i) increased mesangial matrix, (ii) obliterated capillary lumina, (iii) deposition of hyaline (hyalinosis)** [which is characterized by extracellular accumulation of **homogeneous and eosinophilic material** in the glomeruli. It represents **leaked plasma proteins** due to endothelial or capillary wall injury] and **(iv) foamy (lipid-laden) macrophages.**

Advanced stages: As the FSGS progresses, the sclerosis of the glomeruli involve other glomeruli (global sclerosis), there will be pronounced atrophy of the tubules, and fibrosis of the interstitium. At this stage, it is difficult to differentiate it from other forms of chronic glomerular disease.

Collapsing glomerulopathy: It is a morphologic variant of FSGS characterized by collapse of the glomerular tuft and epithelial cell hyperplasia. This severe form of FSGS may be idiopathic or may be seen with HIV infection, drug-induced toxicities, and some microvascular injuries. It has poor prognosis.

Electron Microscopy

Both sclerotic and nonsclerotic areas show **diffuse effacement of foot processes** of podocytes of visceral epithelial cells of glomeruli as in minimal-change disease.

Immunofluorescence Microscopy

It usually shows nonspecific trapping of immunoglobulins, usually IgM, and complement in the areas of hyalinosis.

Clinical Course

It is necessary to distinguish FSGS from minimal-change disease, because of markedly different clinical courses and responses to therapy. Both presents

with nephrotic syndrome, but hematuria and hypertension is more frequent in FSGS. In contrast to minimal change disease, FSGS-associated proteinuria is nonselective, and it shows poor response to corticosteroid therapy. About 50% of FSGS patients progress to end-stage renal disease within 10 years of diagnosis.

Urine findings: Proteinuria, oval fat bodies and, fatty casts.

Prognosis: Children have better prognosis than adults.

FSGS: Epithelial damage consists of degeneration and focal disruption of visceral epithelial cells with effacement of foot processes is the hallmark of FSGS.

Collapsing glomerulopathy:
- Variant of FSGS
- Retraction and/or collapse of entire glomerular tuft
- Proliferation and hypertrophy of visceral epithelial cells
- Idiopathic or HIV associated

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

Membranoproliferative glomerulonephritis (MPGN) is histologically characterized by:

- **Thickening** of the **glomerular basement membrane**.
- **Proliferation** of mainly **mesangial cells**.
- **Leukocyte infiltration**.

Because the proliferation is mainly mesangial cells and also involves the capillary loops, it is also known as **mesangiocapillary glomerulonephritis**.

Etiology

Idiopathic (primary): Depending on pathogenesis, electron microscopic, and immunofluorescent appearance, idiopathic MPGN was subdivided into two major types:

- Type I (immune complex deposit)
- Type II now termed as dense-deposit disease. It belongs to a group of disorders termed as C3 glomerulopathies and discussed in pages 677-78.

Secondary: MPGN can develop in association with systemic disorders.

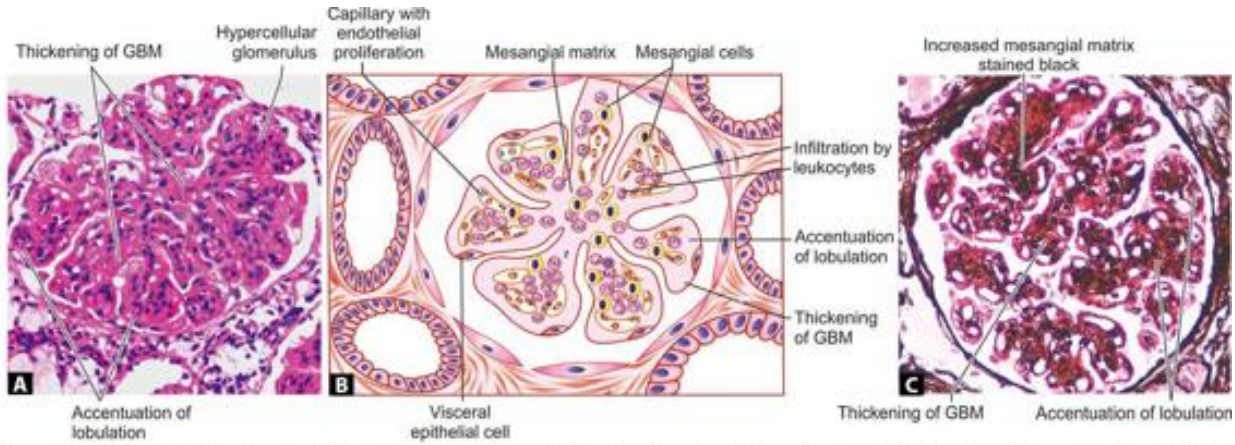
Pathogenesis of MPGN

- **Previously known as type I MPGN. Immune complexes** are deposited in the **subendothelial** zone of glomerulus.
- Origin of **nephritogenic antigen is not known**.
- Type I MPGN may be caused due to the deposition of circulating immune complexes or formation of in situ immune complex against a planted antigen.

MORPHOLOGY

Light Microscopy (Figs. 20.18A to C)

- **Glomeruli**
  - **Large and hypercellular glomeruli:** It is due to:
    - **Proliferation of (mesangial and endothelial) cells**
    - Infiltration of **leukocytes**
    - **Epithelial crescents** are seen **in many cases**.



Figs. 20.18A to C: Membranoproliferative glomerulonephritis: (A) Hematoxylin and eosin (H & E); (B) Diagrammatic; (C) Silver methenamine stain. The glomerulus shows mesangial cell proliferation, increased mesangial matrix and thickening of GBM, accentuation of lobular architecture and infiltration by leukocytes.

- **Accentuation of lobular architecture (lobular distortion or hypersegmentation):** It is due to the proliferating mesangial cells as well as increased mesangial matrix.
- **Thickening of GBM:** It may be segmental and most prominent in the peripheral capillary loops.
- **Subendothelial immune complex** deposits stimulates → synthesis of new basement membrane→ produces **"duplication" of the basement membrane** (commonly called as splitting)→ produces **"double-contour" or "tram-track"** appearance. This is better appreciated with **special stains such as silver or PAS**. The "splitting" of the

GBM is due to: (i) extension of mesangial processes and inflammatory cells into the peripheral capillary loops, (ii) the deposition of mesangial matrix and (iii) subepithelial immune complexes.

- **Tubules**: Epithelial cells of tubules may show vacuolation and droplets of hyaline.
- **Interstitium:** It may show mild focal chronic inflammatory cells.
- **Blood vessels**: During early stages appear normal but later may show changes due to hypertension.

Electron Microscopy (Fig. 20.19)

Shows discrete **subendothelial electron-dense deposits**.

Immunofluorescence

Its appearance **suggests activation of classic complement pathway** by immune complex.

- C3 is deposited in a **granular pattern**
- IgG and early complement components (C1q and C4) are often seen.

Clinical Features

Age: Mostly found in adolescence or young adults.

Presentation: It presents with **nephrotic syndrome**, some only with hematuria or proteinuria in the non-nephrotic range, but many have a combined **nephrotic-nephritic picture**.

Urine findings: Proteinuria, oval fat bodies and, fatty casts.

Course: Slowly progressive course and about 50% develop chronic renal failure. Some may develop crescents and develop RPGN.

MPGN:
- Thickening of GBM
- Proliferation of mainly mesangial cells
- Leukocyte infiltration

MPGN: Responsible for about 10–20% of nephrotic syndrome in children and young adults.

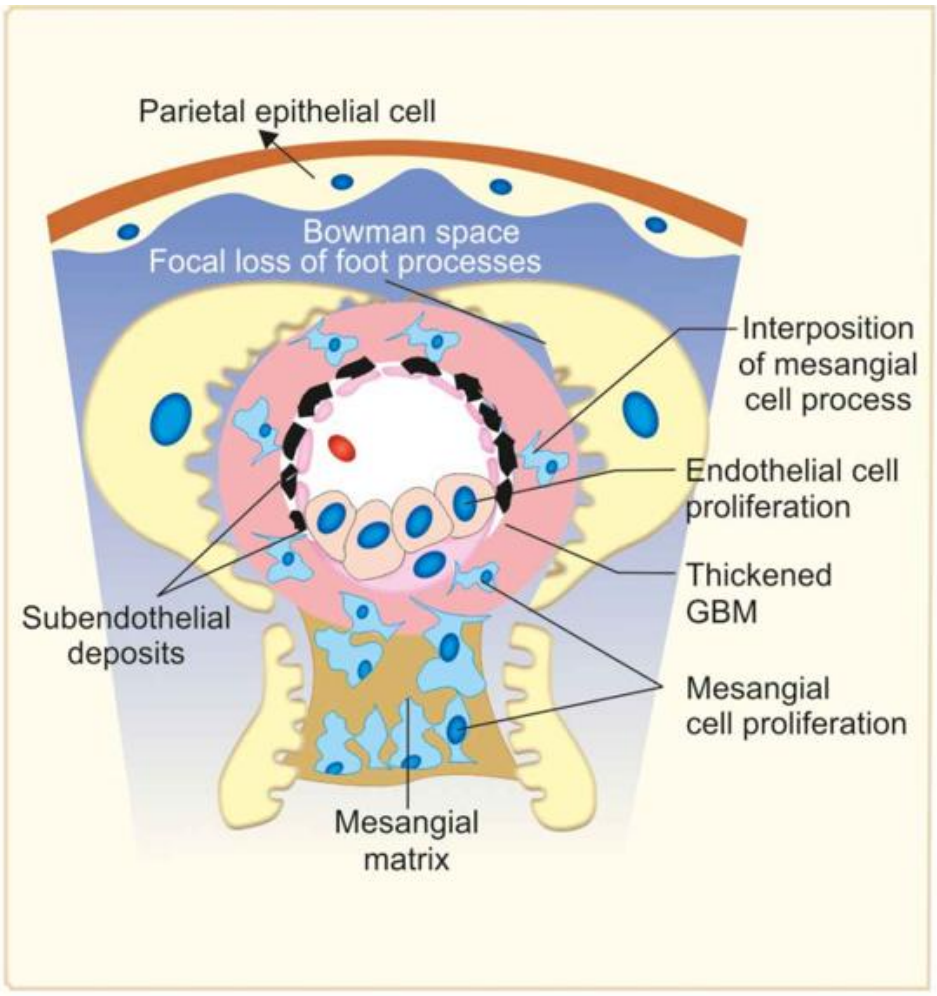


Fig. 20.19: Type 1 MPGN. Diagrammatic electron microscopic appearance of membranoproliferative glomerulonephritis characterized by hypercellular glomeruli (proliferation of endothelial and mesangial cells), thickened GBM, narrowing of the capillary lumen. Type I MPGN shows large subendothelial deposits of immune complexes, which extend along the inner border of the basement membrane.

MPGN is a histological pattern of immune-mediated injury rather than a specific disease.

MPGN:
- Primary (two types I and II) dense deposit disease
- Secondary.

Membranoproliferative GN: A combination of the thickening of basement membrane and proliferation.

MPGN:
- Large hypercellular glomeruli

- Accentuation of lobular architecture
- Thickening of GBM

GBM: Periodic acid–Schiff (PAS) stain highlights thickening of glomerular basement membrane better than H&E.

MPGN: Subendothelial immune complex deposits, duplication of GBM → produces "double-contour" or "tram-track" appearance and nephrotic/nephritic syndrome.

C3 GLOMERULOPATHY

C3 glomerulopathy consists of two conditions namely: (i) dense deposit disease (formerly called MPGN, type II) and (ii) C3 glomerulonephritis (C3 GN). Both are relatively rare and share certain clinical, morphologic, and pathogenic features. However, they differ in the electron microscopic appearance.

Pathogenesis

Both dense deposit disease and C3 GN are caused **due to complement dysregulation.** This may **either due to acquired or hereditary abnormalities of the alternative pathway of complement activation (Fig. 20.20)**. The supporting evidences for this are:

- **Decreased serum C3 but normal C1 and C4**. The early components of complement (C1 and C4) are used only in the activation of classical complement pathway.
- **Decreased serum levels** of **factor B and properdin**, which are the **components of the alternative complement pathway**.
- In the alternative complement pathway, C3 is directly cleaved to C3b. The glomeruli show deposits of C3 and properdin but not IgG.

Sequence of Events (Fig. 20.20)

- Substances like **bacterial polysaccharides**, **endotoxin**, **and aggregates of IgA cleave C3 to C3b**.
- **C3b in the presence of factors B and D generates C3bBb (**alternative pathway C3 convertase).
- **C3bBb is labile** and **degraded by factors I** and **H**, but **stabilized by properdin**.

- Some patients with C3 glomerulopathy have an IgG autoantibody against C3 convertase in the serum known as **C3 nephritic factor (C3NeF)**.
- **C3NeF** acts at the same step as properdin and **stabilizes C3bBb and prevent its degradation**.
- This results in persistent and prolonged C3 activation (uncontrolled cleavage of C3 by the alternative complement pathway) and hypocomplementemia by C3NeF. In addition, decreased synthesis of C3 by the liver, also responsible for hypocomplementemia.

In other patients, the cause of unregulated activation of the alternative pathway of complement may be due to mutations in various complement regulatory proteins. These include mutations in Factor H, Factor I, and membrane cofactor protein (MCP). It may also be due to autoantibodies to Factor H.

MORPHOLOGY

Light Microscopy

Dense deposit disease and C3 GN shows wide spectrum of microscopic changes. Some of them show features of MPGN described earlier.

The glomeruli are hypercellular and, the capillary walls show duplicated basement membranes, and the majority show mainly mesangial proliferative pattern of injury, with increase

in mesangial matrix while others may show an inflammatory and focally crescentic appearance. Some cases show dense deposits of a cellular material in the glomerular basement membranes.

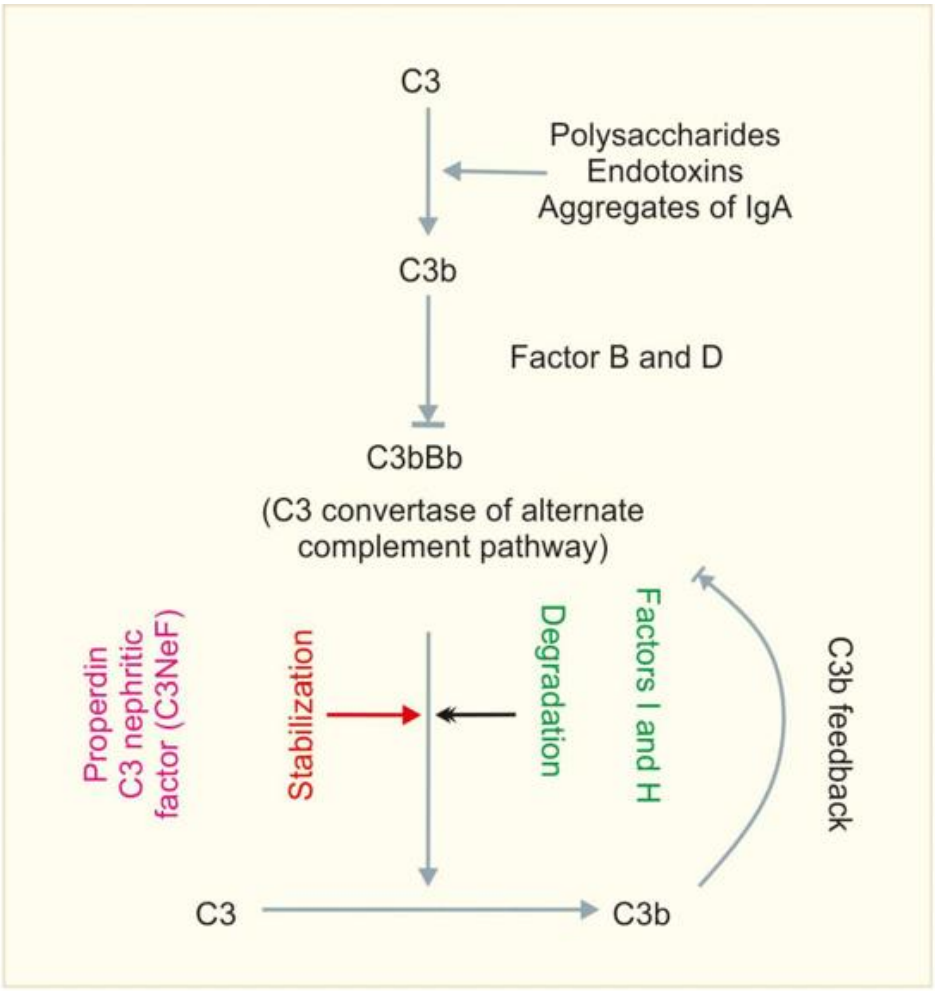


Fig. 20.20: The alternative complement pathway in C3 glomerulopathy. Patients show C3NeF antibody in the serum which acts at the same step as properdin and stabilize the alternative pathway C3 convertase. This enhances C3 activation and consumption, resulting in hypocomplementemia.

Election Microscopy

- **C3 GN:** It shows mesangial and subendothelial electron-dense "waxy" deposits. Similar deposits also may be seen along the basement membranes of renal tubules.
- **Dense deposit disease (Fig. 20.21):** In this, the lamina densa (central zone of GBM) and the subendothelial space of the GBM are transformed into an irregular, ribbon-like, extremely electron-dense structure, resulting from the deposition of C3-containing material.

Immunofluorescence

Its appearance suggests activation of alternate complement pathway.

- **Bright C3 staining is seen in the mesangial** (forms a characteristic circular aggregates termed as mesangial rings) **and glomerular capillary wall** in both dense deposit disease and C3 GN. In dense deposit disease, C3 staining also may be seen along the tubular basement membranes.
- IgG and early complement components (C1q and C4) are absent in both conditions.

Clinical Features

- Patients with dense deposit disease are usually younger and more likely to have low serum C3 levels than patients with C3 GN.
- Present as nephritic syndrome with hematuria and/or nephrotic syndrome with proteinuria similar to MPGN.
- Both dense deposit disease and C3 GN have **poor prognosis**.

Dense deposit disease and C3 GN: Due to activation of alternative complement pathway.

Differences Between Nephritic and Nephrotic Syndrome (Table 20.3)

Alport syndrome: EM is diagnostic and shows "basket weave appearance" of the GBM.

Alport syndrome:
- Hereditary nephritis
- Abnormal α3 (COL4A3), α4 (COL4A4), or α5 (COL4A5) chain of type IV collagen
- Hematuria with progression to chronic renal failure
- Nerve deafness
- Eye disorders
- Foamy cells in interstitium

IgA NEPHROPATHY (BERGER DISEASE)

CC: Define and describe the etiology, pathogenesis, pathology, laboratory, urinary findings, progression and complications of IgA nephropathy.

IgA nephropathy is one of the most common causes of recurrent microscopic or gross hematuria. It is the most common type of primary

glomerular disease **revealed by renal biopsy. The microscopic characteristic feature is the deposition of IgA in the mesangium.**

Etiology and Pathogenesis

IgA is the main immunoglobulin (Ig) present in mucosal secretions. In plasma, it is present at low concentrations. **An abnormally glycosylated IgA1 [i.e., galactose-deficient IgA1 (Gd-IgA1)] immunoglobulin probably involved in its pathogenesis.**

Genetic susceptibility: IgA nephropathy can occur in families and in HLA identical siblings. There may be increased frequency in certain HLA and complement genotypes.

Pathogenesis (Flowchart 20.1):

- **Formation of IgA:** In a genetically susceptible individuals, upper respiratory tract or gastrointestinal exposure to microbial or other antigens (e.g., viruses, bacteria, food proteins) may triggers to increased synthesis of IgA, some of which is abnormally glycosylated.
- **Deposition of IgA in mesangium:** Abnormal aberrantly glycosylated IgA1 is either deposited by itself in glomeruli or it elicits an autoimmune response to produce IgG autoantibodies.
  - The abnormal IgA1 is deposited directly in the mesangium may form immune complexes in situ.
  - Autoantibodies form large immune complexes with abnormal IgA molecules in the circulation. These immune complexes get deposit in the glomerular mesangium. This unusual location in mesangium may be due to physicochemical characteristics of IgA and may be facilitated by an IgA1receptor (CD71) on mesangial cells.

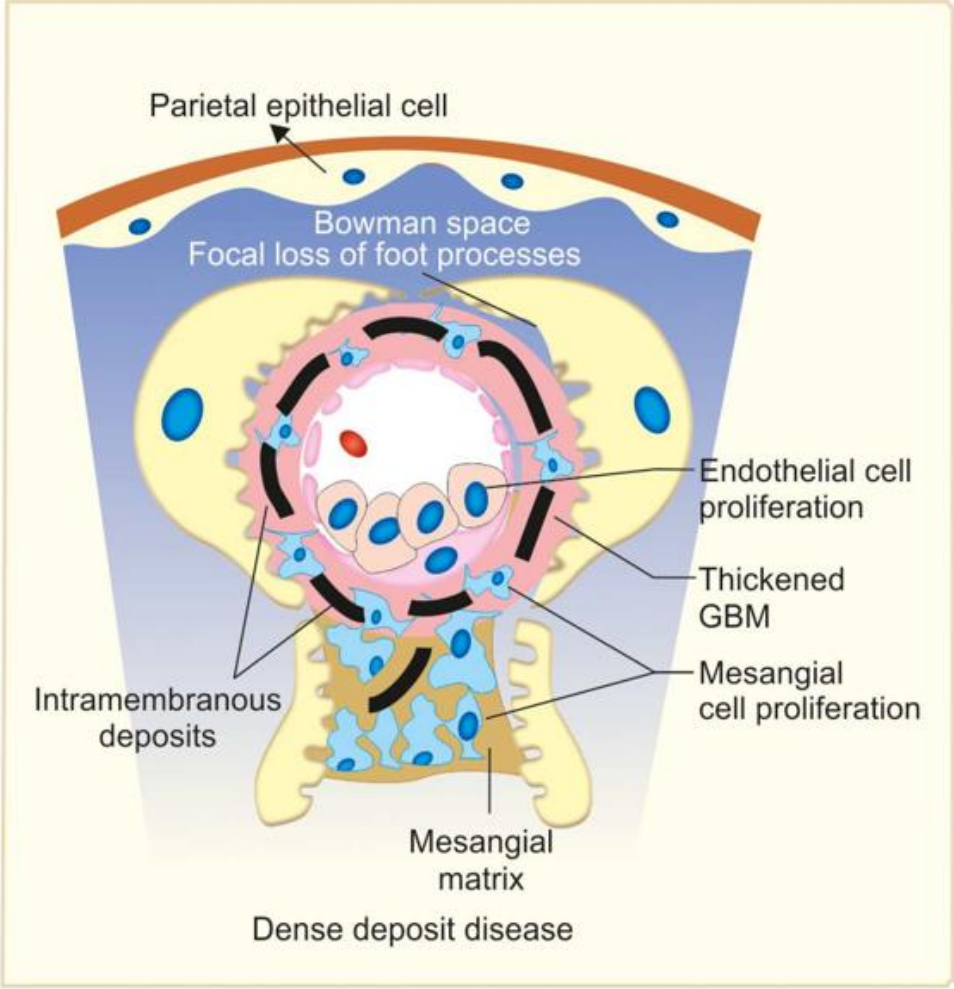
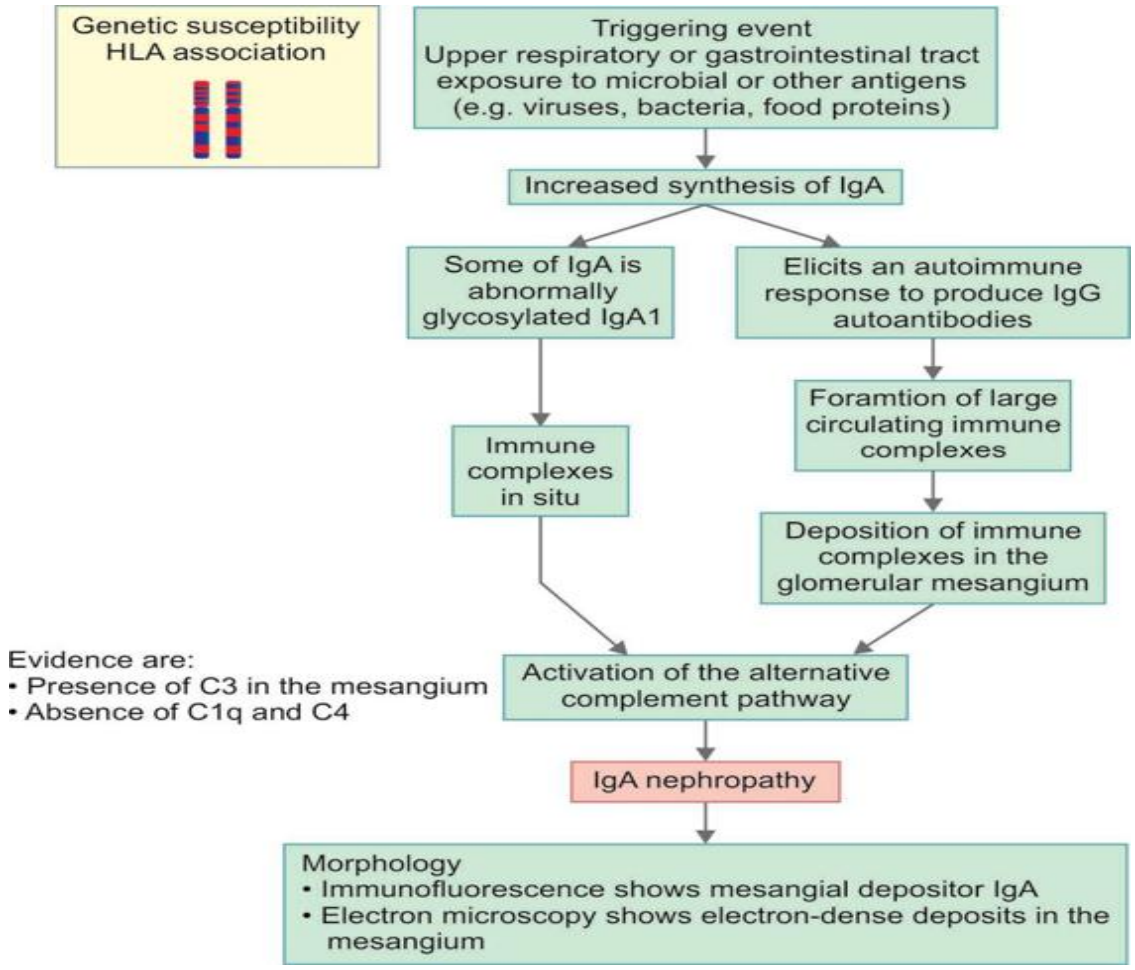


Fig. 20.21: Dense deposit diseases. Diagrammatic electron microscopic appearance of dense deposit disease (formerly called type II MPGN) intramembranous deposits.

TABLE 20.3: Differences between nephritic and nephrotic syndrome.

Features	***Nephritic syndrome***	***Nephrotic syndrome***
Proteinuria	1–2 g/day	Massive; >3.5 g/day
Hematuria	Present	Absent
Hypertension	Present	Absent
Hypoalbuminemia	Absent	Present; serum albumin <3g/dL
Hyperlipidemia and lipiduria	Not seen	Present
Edema	Mild	Moderate to severe
Azotemia and oliguria	Present	Absent
Microscopy of urine	RBC and RBC casts	Fatty casts

Flowchart 20.1: Pathogenesis of IgA nephropathy.



- **Activation of the alternative complement pathway**: The immune complexes activate the alternative complement pathway and initiate glomerular injury. The presence of C3 in the mesangium and the absence of C1q and C4 points to activation of the alternative complement pathway in the pathogenesis.

This concept is supported by the fact that increased frequency of IgA nephropathy occurs in individuals with celiac disease (there is defect in intestinal mucosa), and in liver disease (where there is defective hepatobiliary clearance of IgA complexes).

Few considered IgA nephropathy as a localized variant of *Henoch–Schönlein purpura* (which also shows IgA deposition in the mesangium). However, Henoch–Schönlein purpura is a systemic syndrome and also involves the skin (purpuric rash), gastrointestinal tract (abdominal pain), and joints (arthritis).

MORPHOLOGY

Microscopic changes are variable. The glomeruli may appear normal or may show mesangial widening and segmental inflammation confined to some glomeruli (focal proliferative GN); diffuse mesangial proliferation (mesangioproliferative GN); or (rarely) frank crescentic GN.

Immunofluorescence: It is essential for diagnosis of IgA nephropathy. It shows **mesangial deposit of IgA**, usually with C3 and properdin and smaller amounts of IgG or IgM. Early components of the classical complement pathway are usually not seen.

Electron microscopy (Fig. 20.22): Shows electron-dense deposits in the mesangium.

Clinical Features

- Occurs in **children and young males**.
- Presents with asymptomatic/painless microscopic hematuria or recurrent macroscopic hematuria generally within 1–2 days after a nonspecific upper respiratory or gastrointestinal viral infection. The hematuria usually lasts for several days and initially subsides. Then hematuria periodically recurs usually in association with a viral infection.
- Proteinuria occurs and in 5% it can be in the nephrotic range.
- Occasionally, it may present as acute renal failure (ARF) or nephritic syndrome.

Diagnosis by renal biopsy: Immunofluorescence microscopy shows prominent **IgA deposits** in the mesangial regions.

Course and prognosis: Course are highly variable.

- **Prognosis is usually good** and many patients maintain normal renal function for decades.
- **Risk of development of** slowly progresses **end-stage renal failure** in about 25% of patients over a period of 20 years.
- Poorer prognostic indicators are presence of diffuse mesangial proliferation, segmental sclerosis, endocapillary proliferation, or tubulointerstitial fibrosis in renal biopsy.

CHRONIC GLOMERULONEPHRITIS

Q. Write short note on causes of chronic glomerulonephritis.

Q. Write short note on end stage kidney.

Chronic glomerulonephritis is an **end-stage of many types of glomerulonephritis (Table 20.4).**

MORPHOLOGY

Gross (Figs. 20.23A to C)

- **Both kidneys** show **diffusely granular** cortical surfaces and **symmetrical contraction**.
- Cut section: It shows **thinned cortex** and an **increase in peripelvic fat**. The **capsule is difficult to remove** because of adhesions.

Microscopy

- **Glomeruli:** The glomerular changes depend on the stage of the disease:
  - **Early stages:** It may show features of the **primary disease** (e.g., membranous nephropathy or MPGN).
  - **Late stages: Obliteration of glomeruli,** which appear as **acellular eosinophilic masses** consisting of plasma proteins, increased mesangial matrix, basement membrane-like material, and collagen.
- **Tubules:** They show **marked atrophy**.
- **Interstitium: It shows fibrosis and mononuclear leukocytic infiltration**.
- **Blood vessels: Hypertension develops** in patients with chronic glomerulonephritis and the **vascular changes of hypertension** such as **arterial and arteriolar sclerosis** may be seen (*refer* page 47).

Uremic Complications

Patients of chronic renal failure may show complications due to uremia (for features of uremia, *refer* **Table 20.1**).

These include:

- Uremic **pericarditis**
- Uremic **gastroenteritis**
- **Secondary hyperparathyroidism** with nephrocalcinosis and renal osteodystrophy
- **Left ventricular hypertrophy** due to hypertension
- **Diffuse alveolar damage** (uremic pneumonitis).

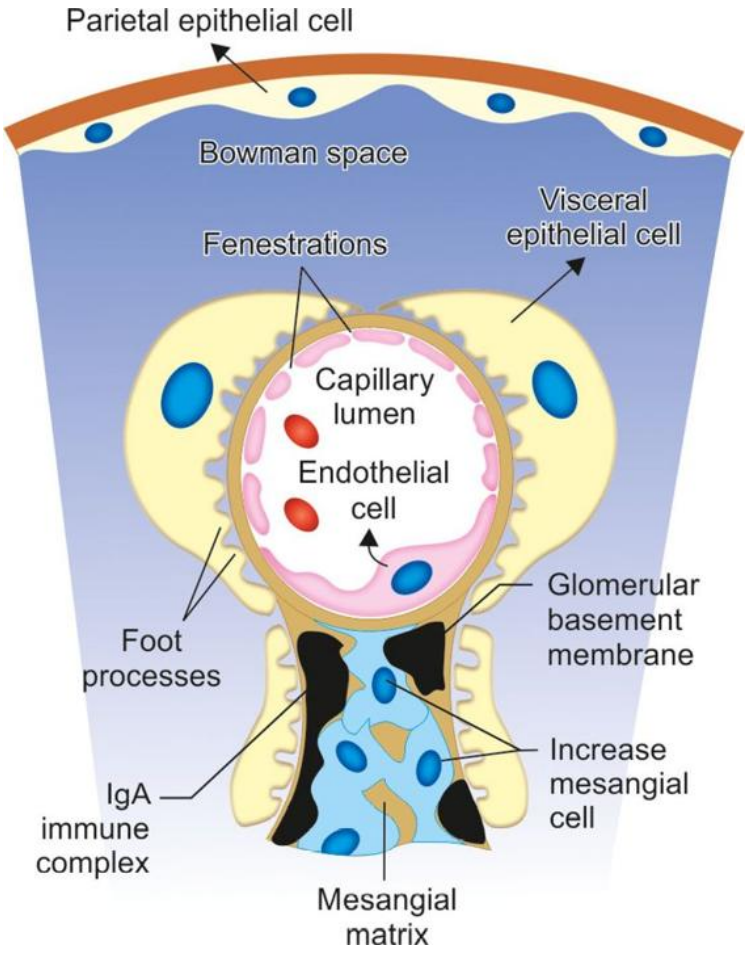
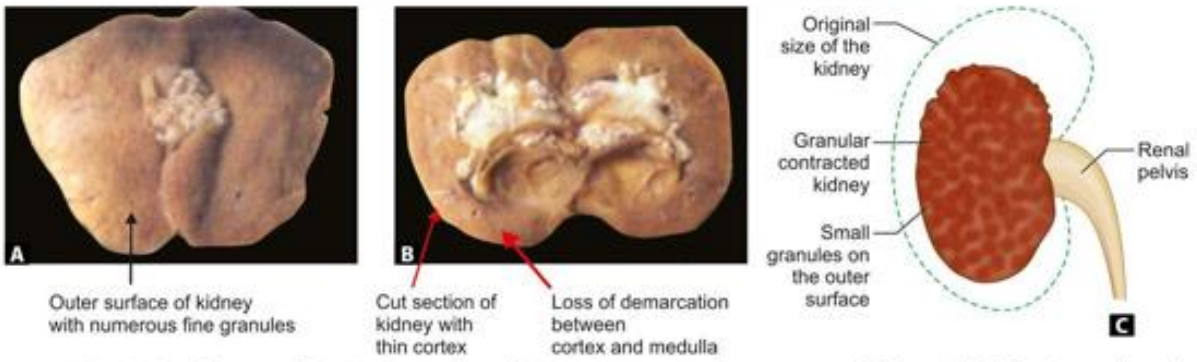


Fig. 20.22: Diagrammatic appearance of electron microscopic findings in immunoglobulin A (IgA) nephropathy. It shows significant accumulation of IgA in the mesangium, most commonly between the mesangial cells and the glomerular basement membrane.

TABLE 20.4: Causes of chronic glomerulonephritis.

Crescentic glomerulonephritis (RPGN)	Focal segmental glomerulosclerosis (FSGS)
Membranoproliferative glomerulonephritis (MPGN)	IgA nephropathy
Membranous nephropathy	Poststreptococcal glomerulonephritis—rare
Others	

Nonproliferative glomerulonephritis:
- Membranous glomerulonephritis
- Diabetic glomerulosclerosis
- Amyloidosis



Figs. 20.23A to C: Gross features of chronic glomerulonephritis: (A) Outer aspect shows contracted kidney with diffusely granular surface; (B) Cut section shows thinned cortex and loss of demarcation between cortex and medulla; (C) Diagrammatic appearance of chronic GN.

Clinical Course

Insidious onset: It may **progress to renal insufficiency or uremia** during a span of years. Most patients have **hypertension** and may present with hypertension induced cerebral or cardiovascular diseases.
Nonspecific complaints: These include loss of appetite, anemia, vomiting, or weakness.
It **may be detected during routine medical examination or** during the course of investigation of edema or urinary abnormalities.

Azotemia: Elevation of blood urea nitrogen (BUN) and creatinine levels.

Chronic glomerulonephritis:
- End-stage of any GN
- Granular contracted kidney
- Hyalinized glomeruli
- Progresses to renal failure

Chronic GN: Urine shows broad waxy casts.

GLOMERULAR LESIONS ASSOCIATED WITH SYSTEMIC DISEASES

CC: Enumerate and describe the findings in glomerular manifestations of systemic disease.

Lupus Nephritis

Q. Write short note on lupus nephritis.

- Renal (kidney) changes in SLE are known as lupus nephritis and are seen in up to 50% of SLE patients.
- It is an **immune complex mediated** (type III hypersensitivity reaction) disease.
- The main mechanism of renal injury is by immune complex, which consist of **DNA and anti-DNA antibodies**.
- Morphological features are presented in page 164.

DIABETIC NEPHROPATHY

Diabetic nephropathy is the term used for **collective lesions that often occur together in the diabetic kidney**. Diabetic nephropathy can develop in both insulin-dependent type 1 diabetes and type 2 diabetes.

Pathogenesis

Diabetic glomerulosclerosis represents a part of the generalized diabetic microangiopathy that involves small vessels throughout the body (*refer* page 689-91).

In diabetes, **insulin deficiency, hyperglycemia, (or glucose intolerance) and metabolic defects** cause **biochemical alterations**.

- **Nonenzymatic glycosylation and formation of advanced glycation end-products:** In diabetics, glucose binds to protein nonenzymatically to form advanced glycation end-products (AGEs). These AGEs produce their effects either by binding to its specific receptors (RAGE) or by direct action and includes: **increased synthesis of extracellular matrix, increased production of reactive oxygen species and direct cross-linking with extracellular matrix proteins**.
  - **Consequences**: **Cross-linking with collagen type IV** in glomerular basement membrane (GBM) results in increased collagen type IV and fibronectin and decreased heparin sulfate proteoglycans. This results in **thickening of GBM and increased mesangial matrix**.
- **Hemodynamic changes:** They cause **glomerular hypertrophy** and may also be responsible for the initiation and progression of diabetic glomerulosclerosis.

  Both **advanced glycosylated end-products and hemodynamic changes** contribute to the **loss of podocytes**. This may occur due to apoptosis of podocytes in response to the metabolic abnormalities and exposure to reactive oxygen species.
- **Disturbances in polyol pathways: This increases** the glomerular cells **susceptibility** to oxidative stress.

Diabetic Nephropathy—Renal Changes in Diabetes

Q. Write short note on renal changes in diabetes mellitus (diabetic nephropathy).

- The **kidneys** are **main targets** of diabetes.

- **Renal failure** is second only to myocardial infarction as a **cause of death** in diabetes.

MORPHOLOGY

Renal lesions in diabetes can involve any component. Four varieties of lesions are encountered namely:

1. **Glomerular lesions**
2. **Renal vascular lesions (mainly arteriolosclerosis)**
3. **Pyelonephritis (including necrotizing papillitis)**
4. Tubular lesions.

1. Glomerular Lesions (Figs. 20.24 and 20.25)

They are most **important and common renal lesions**. These include: (a) Thickening of capillary basement membrane (GBM), (b) diffuse mesangial sclerosis and (c) **n**odular glomerulosclerosis (Kimmelstiel–Wilson nodules).

a. **Diffuse widespread thickening glomerular capillary basement membrane**: It occurs in almost all cases of diabetic nephropathy. It represent part and parcel of the diabetic microangiopathy.

b. **Diffuse mesangial sclerosis**: It is characterized by **diffuse increase in PAS-positive mesangial matrix and mesangial cells**. As the disease progresses, the mesangial areas can expand to assume nodular appearance.

c. **Nodular glomerulosclerosis**: It is also called as **intercapillary glomerulosclerosis or Kimmelstiel–Wilson disease** and **are pathognomonic**.

Q. Write short note on Kimmelstiel–Wilson (KW) lesion.

i. **Develops in about 15–30%** of long-term diabetes and is **mostly associated with renal failure**.

ii. Glomerular lesion consists of **ovoid or spherical**, often laminated, **nodules of mesangial matrix** seen within the mesangial core and **situated in the periphery of the glomerulus**. These nodules can be surrounded by peripheral capillary loops with patent or dilated lumen. The nodules are **PAS-positive (Fig. 20.25C)**. As the disease advances, the individual nodules increase in size → compress the surrounding capillaries.

d. **Insudative lesions**:

i. **Fibrin caps**: These are **prominent subendothelial** accumulations of **hyaline material** in/along capillary loops.

ii. **Capsular drops:** They consist of **round nodules adherent to Bowman's capsules** and are **seen between Bowman's capsule and the parietal epithelium**.

2. Renal Vascular Lesions

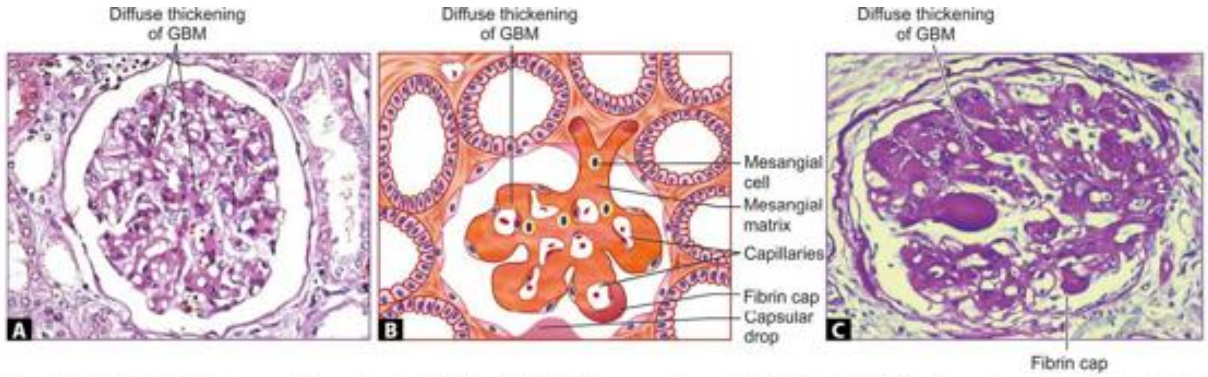
a. **Hyaline arteriosclerosis** (hyalinosis): It involves **both the afferent and the efferent arteriole** at the hilum of glomeruli. **Arteriolosclerosis of efferent arteriole is rare in nondiabetics**.
b. **Renal atherosclerosis:** It involves arteries and arterioles and are similar to those found in other parts of the body.

Glomerular and arteriolar lesions together produce **renal ischemia** → leads to atrophy of tubules, interstitial fibrosis, and contraction of kidney.

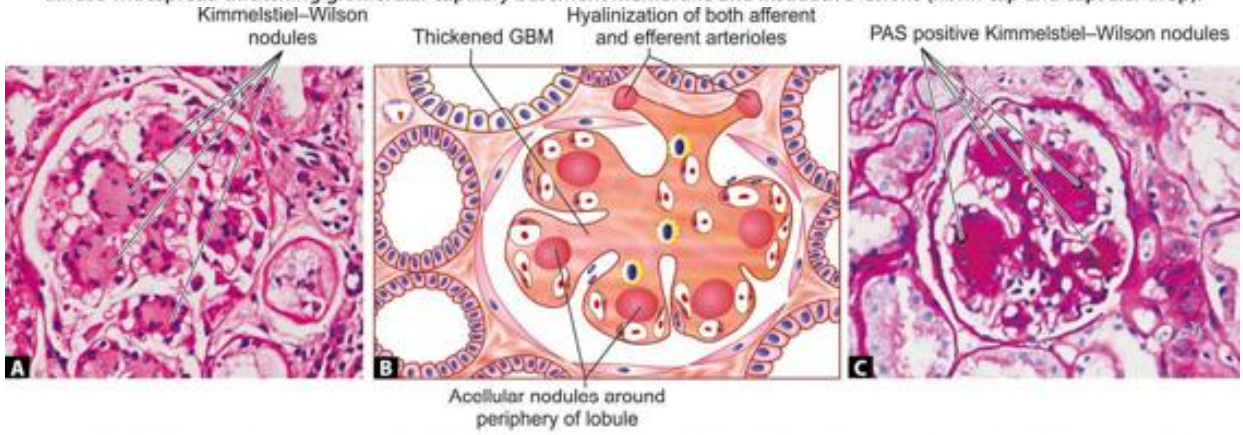
3. Pyelonephritis

It is a tubulointerstitial inflammation of the kidneys.

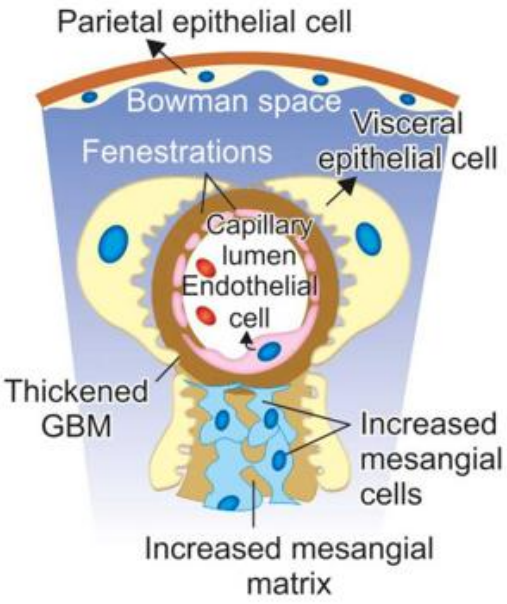
a. Both the **acute and chronic pyelonephritis** occurs in nondiabetics as well as in diabetics. It is **more common** and tends to **more severe** in diabetics than in the general population.



Figs. 20.24A to C: (A) Hematoxylin and eosin (H & E stain); (B) diagrammatic; and (C) PAS stain. Diabetic nephropathy glomeruli with diffuse widespread thickening glomerular capillary basement membrane and insudative lesions (fibrin cap and capsular drop).



Figs. 20.25A to C: Diabetic nephropathy: (A) Hematoxylin and eosin (H & E stain); (B) Diagrammatic; and (C) PAS stain. Nodular glomerulosclerosis shows oval nodules of mesangial matrix at the periphery of glomerulus, (C) Nodules are PAS positive.



Liver-fatty acid binding protein (L-FABP) is a biomarker of diabetic nephropathy and chronic kidney disease.

Microalbuminuria: Urinary excretion of 30–300 mg of albumin/24 hours.

Diabetic glomerulopathy: Microalbuminuria is first laboratory sign.

Fig. 20.26: Diagrammatic electron microscopic appearance of diabetic glomerulosclerosis showing thickened glomerular basement membrane, increased mesangial cells and mesangial matrix.

b. **Necrotizing papillitis** (or papillary necrosis): It is one **special pattern of acute pyelonephritis** and is much more frequent in diabetics compared to nondiabetics. **Hyaline arteriolosclerosis** narrows blood vessels → **reduces the blood supply** to the renal medulla → ischemia → causes **necrosis of the tips of papillae** (papillary necrosis).

4. Tubular Lesions

The basement membrane of the tubules show thickening. In patients with high blood sugar level, the **epithelial cells of the proximal convoluted tubules show extensive deposits of vacuoles of glycogen**. These are known as **Armanni–Ebstein lesions**.

Electron Microscopy (Fig. 20.26)

- **Thickening of the lamina densa** of the glomerular basement membrane.
- **Increase in the mesangial matrix**, particularly in nodular lesions.
- **Insudative lesions** appear as electron-dense masses.

Immunofluorescence Microscopy

It reveals IgG, albumin, fibrinogen, and other **plasma** proteins in the GBM.

Amyloidosis

Kidney is the most common and the most serious form of organ involvement in amyloidosis (Renal amyloidosis—*refer* page 185).

Diabetes: Glomerular lesions may present with:
- Non-nephrotic proteinuria,
- Nephrotic syndrome, and
- Chronic renal failure

Diabetic nephropathy: Nodular glomerulosclerosis (Kimmelstiel–Wilson nodules) is pathognomonic lesion.

Causes of necrotizing papillitis:
- Diabetes mellitus
- Analgesic nephropathy
- Sickle cell anemia
- Obstructive uropathy

Armani–Ebstein lesion: Proximal tubular epithelial cells with glycogen accumulation seen in uncontrolled diabetes mellitus.

PYELONEPHRITIS AND URINARY TRACT INFECTION

CC: Enumerate and classify diseases affecting the tubular interstitium.

Classification of Urinary Tract Infections

- **Lower urinary tract infections:** These include **cystitis** (bladder) and **urethritis**. Bacterial infection of the lower urinary tract may be completely asymptomatic (asymptomatic bacteriuria). Most of the cases, it remains localized to the bladder. However, lower urinary tract infection always carries the risk of spread to the kidney.
- **Pyelonephritis:** It involves **kidneys and their collecting systems** (pyelonephritis).

PYELONEPHRITIS

Q. Write short essay on pyelonephritis.

Definition: Pyelonephritis is **inflammatory disease** of kidney **affecting the tubules**, **interstitium**, **and renal pelvis**.

It is one of the most common diseases of the kidney.

Classification of Pyelonephritis

- **Acute pyelonephritis:** It is caused by bacterial infection and is associated with urinary tract infection.
- **Chronic pyelonephritis:** It is a more complex disorder. However, bacterial infection plays a major role, but other factors (vesicoureteral reflux, obstruction) are also involved in its pathogenesis.

Acute Pyelonephritis

Acute pyelonephritis is an **acute suppurative inflammation** of the kidney affecting the tubules, interstitium, and renal pelvis.

Etiology and Pathogenesis (Fig. 20.27)

Q. Write short note on etiopathogenesis of acute pyelonephritis.

Causative Organisms

Majority (~85%) of urinary tract infection are caused by **gram-negative bacilli,** which are normal inhabitants of the intestinal tract (enteric origin).

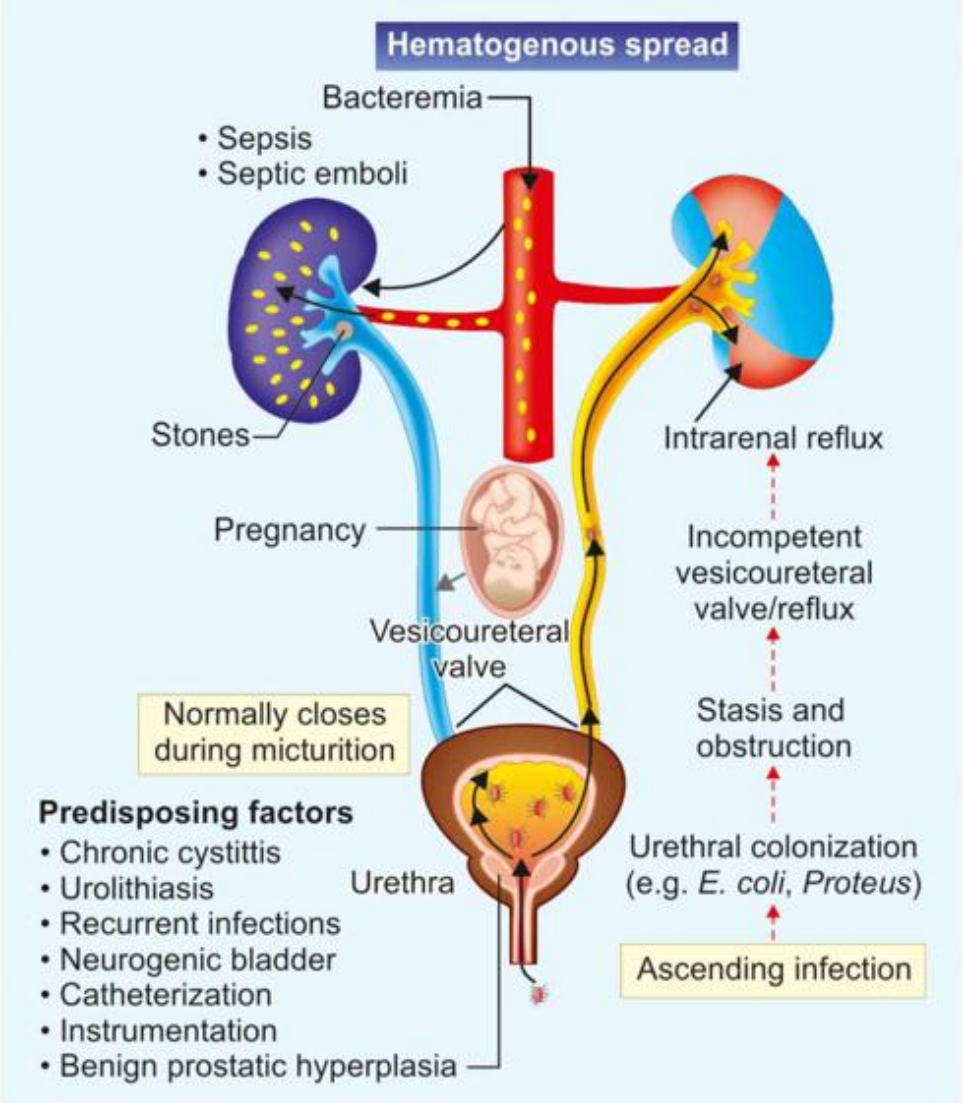
- **Most common pathogens**: ***Escherichia coli***, ***Proteus, Klebsiella, and Enterobacter***.
- **Less common:** *Streptococcus faecalis*, staphylococci, and fungi.
- **In immunocompromised patients: Viruses** (polyomavirus, cytomegalovirus, and adenovirus).

Source and Route of Infection

Bacteria can reach the kidney by **two routes**:

A. **Ascending infection:** It is the **most common route** of infection of pyelonephritis. It is a form of endogenous infection, where the source of infecting organisms is the **patient's own fecal flora**. The infection ascends from the lower urinary tract into the renal parenchyma. **Different steps** in the pathogenesis of pyelonephritis are:

1. **Colonization of the distal urethra and introitus** (in the female): By enteric or coliform bacteria from the perineum due to poor hygiene and hormonal effects.
2. **Entry from the urethra to the bladder:** Organisms may enter the bladder during **urethral catheterization or other instrumentation**. Urinary infections are **more common in females**, because of:
   - Shorter urethra
   - Absence of prostatic fluid, which has antibacterial properties.
   - Hormonal changes in women, which affect the adherence of bacteria to the mucosa.
   - Trauma to the urethra during sexual intercourse.
3. **Urinary tract obstruction and stasis of urine:**
   - **Obstruction/bladder dysfunction:** It causes **incomplete emptying** and **increased residual volume of urine**. For example:
     - **Lower urinary tract obstruction** (e.g., Benign prostatic hypertrophy, tumors, or calculi).
     - **Neurogenic bladder dysfunction** (e.g., diabetes or spinal cord injury).
   - **Stasis: Organisms** introduced into the bladder **can multiply** when there is stasis.
4. **Vesicoureteral reflux:** It **propels infected bladder urine into the renal pelvis** through ureter. Incompetent vesicoureteral orifice/valve may be congenital or acquired.
   - **Congenital vesicoureteral reflux** (e.g., congenital absence or shortening of the intravesical portion of the ureter)
   - **Acquired vesicoureteral reflux** (e.g., persistent bladder atony caused by spinal cord injury).
5. **Intrarenal reflux:** It is most common at both the poles of the kidney and allow the organism to enter from the renal pelvis into the renal parenchyma through open ducts at the tips of the papillae.



Pyelonephritis: Ascending infection is the most common route of infection by most bacteria.

Acute pyelonephritis: Caused by ascending bacterial infection or hematogenous spread.

Fig. 20.27: Pathogenesis of acute pyelonephritis. More common mode is ascending infection. Hematogenous infection results from bacteremic spread.

B. **Hematogenous route:** It is **less common route of infection**. Because of rich blood supply, bacteria can seed the kidneys during the **course of septicemia or infective endocarditis** through the bloodstream. It occurs with **nonenteric organisms** (e.g., staphylococci), fungi and viruses. Hematogenous infections occur in:

- The presence of ureteral obstruction
- Debilitated patients
- Patients receiving immunosuppressive therapy.

MORPHOLOGY

Gross

- **Unilateral or bilateral**.
- **Focal abscesses:** It may be seen on the **subcapsular surface** that appear small and **white**. In pyelonephritis associated with reflux, they are **most common in the lower and upper poles**.
- **Pelvic and calyces:** They may be **hyperemic and covered by purulent exudate**.

Microscopy

- **Interstitium: It shows patchy interstitial neutrophilic infiltration**, which may later become extensive.
- **Tubules: Intratubular aggregates of neutrophils** forms abscess with the destruction of the involved tubules → **tubular necrosis**.
- **Glomeruli:** They appear normal because they are relatively resistant to the infection.

Complications

Q. Write short note on complications of acute pyelonephritis.

1. **Papillary necrosis:** It is the **necrosis of the tips of the renal papillae**.
   - Seen mainly in **diabetics** and with **urinary tract obstruction**.
   - **Usually bilateral** but may be unilateral. One or all of the pyramids of the affected kidney may be involved.
   - **Cut section**: The **tips or distal two-thirds of the renal pyramids** show areas of gray-white to yellow **necrosis**.
   - **Microscopy**: The necrotic area shows **coagulative necrosis**, with preservation of tubular outline.
2. **Pyonephrosis:** It is characterized by accumulation of **pus** (suppurative exudates) **within the renal pelvis**, **calyces**, **and ureter** → kidney distended with pus. This complication develops when there is total or complete obstruction to the urinary drainage high in the urinary tract near the kidney.

3. **Perinephric abscess**: It develops when the **suppurative infection breaks the renal capsule** and spreads into the perinephric tissue.

Clinical Features

- **Sudden onset of pain at the costovertebral angle, fever and malaise**.
- Dysuria, frequency and urgency.

Urinary Findings

Microscopy

- **Pus cells:** Urine shows many leukocytes (**pyuria**); but it does not differentiate upper from lower urinary tract infection.
- **WBC casts:** Casts are formed only in tubules and the finding of leukocyte casts, which is rich in neutrophils (pus cells), **indicates renal involvement** (i.e., pyelonephritis).

Culture and sensitivity: It can **establish the causative organism**.

Course of the disease: Usually, follows a benign course.

Acute pyelonephritis: Bacterial infection of renal tubules, interstitium and renal pelvis.

Acute pyelonephritis: Predisposing conditions
- Urinary tract obstruction
- Instrumentation of the urinary tract
- Pregnancy
- Female gender
- Diabetes mellitus
- Immunosuppression and immunodeficiency
- Urinary catheters

Normal urine in the bladder is sterile.

If organisms enter into the bladder, they are normally cleared by the continual flushing or voiding and by antibacterial mechanisms.

Normal ureteral insertion into the bladder is in such a way that it prevents retrograde flow of urine during micturition or whenever there is raised pressure in the bladder.

Renal papillae in the midzones are convex pointed type and do not allow urine to flow back into the collecting tubules.

Renal papillae in the upper and lower poles tend to have flattened or concave tips and can allow urine to flow back into the collecting tubules.

Tuberculosis: Involves kidney by hematogenous spread.

Complications of acute pyelonephritis:
- Papillary necrosis
- Pyonephrosis
- Perinephric abscess

WBC casts in urine indicates pyelonephritis because casts are formed only in tubules.

Sterile pyuria: White blood cells in urine in the absence of significant bacterial growth.

Chronic Pyelonephritis and Reflux Nephropathy

CC: Describe the etiology, pathogenesis, pathology, laboratory findings, distinguishing features progression and complications of acute and chronic pyelonephritis and reflux nephropathy.

Q. Discuss the etiopathogenesis, gross and microscopy of chronic pyelonephritis.

Definition: Chronic pyelonephritis is a **chronic inflammation of tubulointerstitial tissue** leading to scarring of calyces, pelvis and renal parenchyma.

Chronic pyelonephritis is an **important cause of end-stage renal disease**.

- **Reflux nephropathy (chronic reflux-associated pyelonephritis)**
  - **D**evelops due to **superimposition of a urinary infection on congenital vesicoureteral reflux and intrarenal reflux**.
  - Reflux may be unilateral or bilateral and accordingly causes scarring of one or both kidney, leading to chronic renal insufficiency.
- **Chronic obstructive pyelonephritis**: Develops due to **recurrent infections superimposed on obstructive lesions**, which lead to renal inflammation parenchymal atrophy and scarring.

MORPHOLOGY

Q. Describe gross and microscopy of chronic pyelonephritis.

Gross (Figs. 20.28A to C)

- May be **unilateral or bilateral**. When bilateral, the involvement is **asymmetric**.
- **Size**: Kidney is **reduced** in size due to **irregular scars**.
- **Nature of scars:** May affect one or both kidneys.
  - Most are seen in the **upper and lower poles** due to intrarenal reflux.
  - Scars are **coarse**, **well-defined** and can vary from one to several in number.
- **Calyces and renal pelvis:** It may appear **thickened and irregular** with **scarring of the mucosal surface**. **Calyces below the scars are dilated**, **blunted**, **or deformed**. **Papillae are flattened**.

Microscopy (Figs. 20.29A to C)

Q. Write short note on microscopic features of chronic pyelonephritis.

Changes are predominant in the interstitium and tubules.

- **Interstitium:** It shows dense **chronic inflammatory infiltrate** of lymphocytes and macrophages and fibrosis. Few neutrophils may be found with active infection.
- **Tubules**: It may show **atrophy** in some places and hypertrophy or dilation in others.
  - **Thyroidization: Dilated tubules** with flattened epithelium may be **filled with eosinophilic hyaline material and resemble colloid** containing thyroid follicles.
  - **Blood vessels:** They may show **endarteritis obliterans** and in the presence of hypertension may show **hyaline arteriosclerosis**.
- **Glomeruli:** It may appear normal except for **periglomerular fibrosis**.
- **Calyces:** Shows calyceal epithelium surrounded by **fibrosis** and infiltrated by **dense chronic inflammatory infiltrate**.

Xanthogranulomatous Pyelonephritis

Q. Write note on xanthogranulomatous pyelonephritis.

It is an **unusual and rare form of chronic pyelonephritis** associated with ***Proteus*** infections.

MORPHOLOGY

Gross

Cut surface shows large, yellowish-orange nodules, which may be **confused with renal cell carcinoma**.

Microscopy

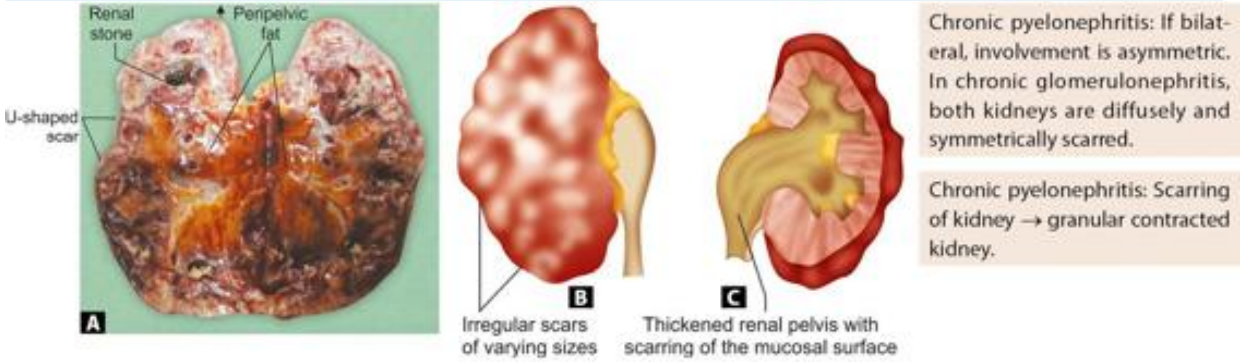
Interstitium shows foamy **macrophages, plasma cells and lymphocytes (Fig. 20.28C)**.

Clinical Features

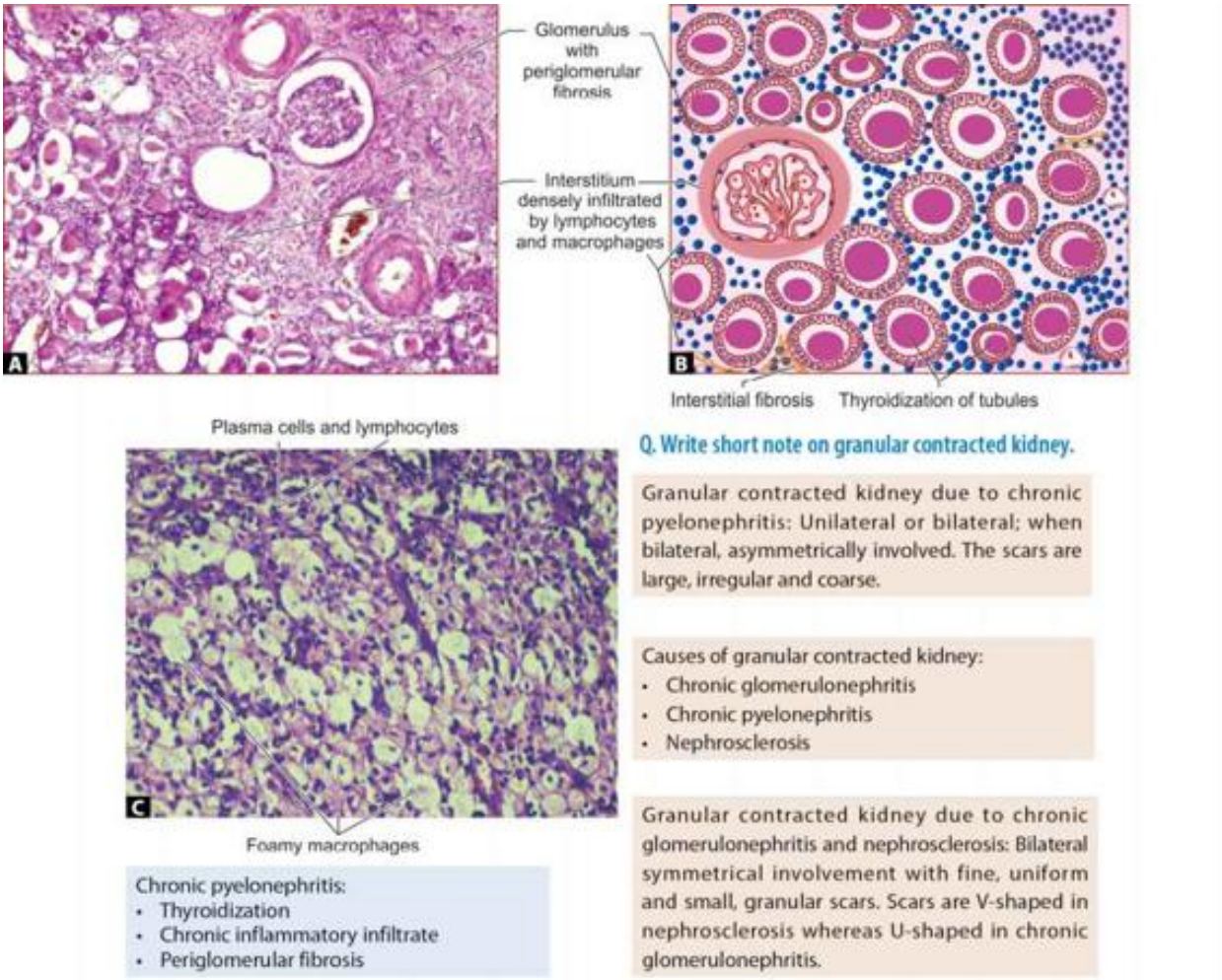
Chronic pyelonephritis associated with reflux **may be silent** onset. Usual symptoms are **back-pain, fever, pyuria and bacteriuria**.

Types of chronic pyelonephritis: Chronic reflux-associated pyelonephritis or chronic obstructive pyelonephritis.

Periglomerular fibrosis: Characteristic of chronic pyelonephritis.



Figs. 20.28A to C: Gross appearance of chronic pyelonephritis: (A) Cut section of kidney showing scars and a renal stone in the calyces; (B) Outer aspect (diagrammatic); (C) Cut section (diagrammatic).



Figs. 20.29A to C: Microscopic appearance of chronic pyelonephritis. Tubules containing hyaline material resembling the colloid of thyroid follicles (thyroidization), interstitium with chronic inflammatory cell infiltrate and periglomerular fibrosis: (A) Shows glomerulus with periglomerular fibrosis; (B) Diagrammatic; (C) Xanthogranulomatous pyelonephritis showing foamy macrophages, plasma cells and lymphocytes.

VASCULAR DISEASES OF KIDNEY

CC: Define classify and describe the etiology, pathogenesis, pathology, laboratory, urinary findings, distinguishing features progression and complications of vascular disease of the kidney.

Almost all kidney diseases secondarily affect the renal blood vessels, as well as systemic vascular diseases (e.g., various vasculitis) can also involve renal blood vessels. The kidney is also involved in the pathogenesis of both essential and secondary hypertension and kidney can both be a cause and consequence of increased blood pressure.

Classification (Box 20.6)

BOX 20.6: Classification of vascular disease of the kidney.

- Nephrosclerosis (previously known as benign nephrosclerosis)
- Malignant nephrosclerosis
- Thrombotic microangiopathies
- Renal artery stenosis
- Other vascular disorders
  - Atherosclerotic ischemic renal disease
  - Atheroembolic renal disease
  - Sickle-cell nephropathy
  - Diffuse cortical necrosis.
  - Renal infarcts

Nephrosclerosis

Q. Write short note on benign nephrosclerosis.

Definition: Nephrosclerosis is defined as the **renal pathology associated with sclerosis of renal arterioles and small arteries**.

It was previously termed as benign nephrosclerosis. Sclerosed vessels with thickened walls → cause narrowing of lumens → result in focal ischemia of renal parenchyma → glomerulosclerosis + chronic tubulointerstitial injury → reduction in functional renal mass.

Causes

Nephrosclerosis is associated with:

- Hypertension } increases the incidence and severity of the lesions
- Diabetes mellitus
- Increasing age and may be seen in the absence of hypertension.

Pathogenesis

Two processes which results in the arterial lesions are:

- **Medial and intimal thickening:** It develops as **a response to hemodynamic changes, aging, genetic defects**, or any combination of these factors.
- **Hyaline deposition in arterioles:** It is **partly due to extravasation of plasma proteins** through injured endothelium and **partly due to increased deposition of basement membrane matrix**.

MORPHOLOGY

Q. Write short note on kidney changes in hypertension.

Gross

- Involvement: Bilateral.
- **Size:** Kidneys are either **normal or smaller** (atrophic).
- **Weight:** Between 110 and 130 g (normal 150 g).
- **Outer surface:** It shows a **fine**, **even granularity** resembling grain leather.
- **Cut section:** It shows **thinned cortex with scarring**.

Microscopy

1. **Blood vessel:** Changes in the kidney depend on the size of the vessel.
   - **Arterioles and small arteries:** They show thickening and hyalinization of the walls causing narrowing of their lumens called as **hyaline arteriolosclerosis** (*refer* **Figs. 16.7A and B**).
   - **Interlobular and arcuate arteries:** They show a characteristic **fibrotic thickening of intima** with **reduplication of the elastic lamina**, **medial hypertrophy** known as **fibroelastic hyperplasia** → narrow the lumen.

   Consequences of vascular narrowing → foci of tubular atrophy and interstitial fibrosis + glomerular alterations.
2. **Tubules:** They show atrophy.
3. **Interstitium:** Fibrosis and chronic inflammatory infiltrate.
4. **Glomeruli:** Most of them appear normal and others may show.
   - **Thickening of glomerular capillaries:** It is due to **thickening**, **wrinkling and collapse of GBMs**.
   - **Sclerosis of glomeruli**.

Clinical Features

Uncomplicated nephrosclerosis does not cause renal insufficiency or uremia. There are usually associated with moderate reductions in renal blood flow,

but the GFR is either normal or slightly reduced.

Laboratory findings: Mild proteinuria, hematuria (no RBC casts), increase in serum blood urea nitrogen (BUN) and creatinin (renal azotemia).

Hypertension is closely linked with the kidney. Kidney can be both a target and a cause of hypertension.

Benign nephrosclerosis:
- Hyaline arteriolosclerosis
- Fibroelastic hyperplasia of blood vessels

Primary disease of the kidney is the most common cause of secondary hypertension.

Malignant Nephrosclerosis

Definition: Malignant nephrosclerosis is defined as **renal disease associated with the malignant or accelerated phase of hypertension**.

- Malignant hypertension is defined as blood pressure usually >200/120 mm Hg. Malignant or accelerated phase of hypertension is relatively uncommon.
- It is often superimposed on pre-existing **essential benign hypertension**, **secondary forms of hypertension**, or an underlying chronic renal disease, particularly glomerulonephritis or reflux nephropathy.
- Occasionally, it may develop in previously **normotensive** individuals.
- **Age and sex:** Pure form usually seen in younger age and more often in men.

Pathogenesis

Exact pathogenesis is not known, but the following sequence of events is suggested.

- **Vascular damage to the kidneys:** It may be by the initial insult (e.g., long-standing benign hypertension) and may result in:
  - **Increased permeability of the small vessels** (arterioles and small arteries) to fibrinogen and other plasma proteins → leads to **fibrinoid necrosis**.
  - **Endothelial injury and deposition of platelets** leads to **swelling of the vascular intima and intravascular thrombosis**.

- **Hyperplastic arteriolosclerosis: Mitogenic factors from platelets** (e.g., PDGF), **plasma and other cells** cause **hyperplasia (proliferation) of intimal smooth muscle → hyperplastic arteriolosclerosis characteristics of malignant hypertension → narrow the vascular lumen** → results in **ischemia of the kidneys**.
- **Raised plasma rennin:** Severe **narrowing of renal afferent arterioles** → stimulation of **renin-angiotensin system → raised levels of plasma renin**.
- **Raised blood pressure:** Elevated aldosterone levels → salt retention → elevation of blood pressure → **changes in blood vessels throughout the body known as malignant arteriosclerosis**.

MORPHOLOGY

Q. Write short answer on kidney changes in malignant hypertension.

Gross

- The size of the kidney depends on the duration and severity of the hypertension.
- **Flea-bitten kidney:** It is characterized by the presence of **small, pinpoint petechial hemorrhages on the cortical surface** due to **rupture of arterioles or glomerular capillaries**.

Microscopy

Histological changes of blood vessels → narrowing of vascular lumens→ ischemic atrophy.

- **Fibrinoid necrosis of arterioles:** It is characterized by **eosinophilic granular change in the wall of the blood vessel**. It stains positively for fibrin by histochemical or immunofluorescence techniques.
- **Onion-skinning (**hyperplastic arteriolitis) (*refer* Figs. 16.7C to E)**:** It is seen in **the interlobular arteries and arterioles**. It is characterized by **intimal thickening due to concentric proliferation of smooth muscle cells + fine concentric layering of collagen, proteoglycans and plasma proteins**.

Clinical Features

- Malignant hypertension is characterized by systolic pressures >200 mm Hg and diastolic pressures >120 mm Hg, papilledema, retinal hemorrhages, encephalopathy, cardiovascular abnormalities and renal failure.

- Initial symptoms are due to raised intracranial pressure and include headaches, nausea, vomiting and visual impairments.
- **Laboratory findings:** Urine examination may show marked proteinuria and hematuria with RBC casts without any significant alteration in renal function. Increase in serum BUN and creatinine.
- Later renal failure may develop.

Malignant nephrosclerosis: Renal changes due to malignant hypertension.

Renin: Major source is juxtaglomerular (JG) cell of the kidney.

Malignant nephrosclerosis: Fibrinoid necrosis and onion-skinning of arterioles.

Q. Write short answer on flea bitten kidney and its causes.

Causes of flea bitten kidney:
- Malignant hypertension
- Subacute bacterial endocarditis
- Rapidly progressive GN
- Henoch–Schönlein purpura

Thrombotic Microangiopathies

NC: Describe the etiology, genetics, pathogenesis, pathology, presenting features and progression of thrombotic angiopathies.

Thrombotic microangiopathy (TMA) is a spectrum of clinical syndromes characterized by **widespread formation of platelet-fibrin thrombi (**mainly composed of platelet aggregates) **in the microcirculation (capillaries and arterioles) in various tissue beds (including kidney)**.

Classification: TMA is classified as primary and secondary forms.

Primary forms: In this form of TMA, etiology is known (well-defined) and these include:

- **Shiga toxin-mediated hemolytic uremic syndrome (HUS)** is and acquired due to Shiga toxin-producing *E. coli, Shigella dysenteriae* serotype 1.

- **Atypical HUS** (complement-mediated TMA in which excessive complement activation is an important pathogenic mechanism). It may be inherited or acquired.
- **Thrombotic thrombocytopenic purpura** (TTP) and may be inherited or acquired.
- **Drug-mediated TMAs:** Mitomycin, cisplatin, cyclosporine, tacrolimus, anti-VEGF therapy.

Secondary forms: They develop in the background of other diseases or various underlying conditions without a well-defined etiology. Examples include TMA associated with malignant hypertension, scleroderma, pregnancy, chemotherapy, anti-phospholipid antibodies, and transplant rejection.

Thrombotic microangiopathy includes two closely related entities namely thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS).

The early and timely diagnosis of thrombotic microangiopathy must be made because the delay carries the attendant risk of sudden death.

Pathogenesis

The major pathogenetic factors involved in the pathogenesis of thrombotic microangiopathies are endothelial cell injury and platelet activation and aggregation. The various insults that lead to TMA include external toxins, drugs, autoantibodies, and inherited mutations.

The discussion is limited to three major primary forms of thrombotic microangiopathies.

Pathogenesis of *Shiga* Toxin-mediated HUS(Fig. 20.30)

In about 75% patients, it follows intestinal infection with *Shiga* toxin-producing *E. coli*. This infection follows ingestion of contaminated ground meat (e.g., in hamburgers) and infections with *Shigella dysenteriae* type I. The pathogenesis is due to the *Shiga* toxin. The thrombus formation in small vessels (particularly in the kidney) is probably due to endothelial damage. Renal glomerular endothelial cells express the membrane receptor for *Shiga*

toxin. At low doses, the toxin activates endothelial cells. This causes increased production of endothelium-derived endothelin and decreased production nitric oxide (both favors vasoconstriction) and promotes platelet adhesion and activation. At high doses, the toxin causes endothelial cell death. All of these alterations lead to formation of thrombi. These thrombi are most prominent in glomerular capillaries, afferent arterioles, and interlobular arteries. **Red cells** get trapped in the formed thrombi, **undergo fragmentation** resulting in schistocytes. Splenic trapping of the fragmented red cells causes **extravascular hemolysis.**

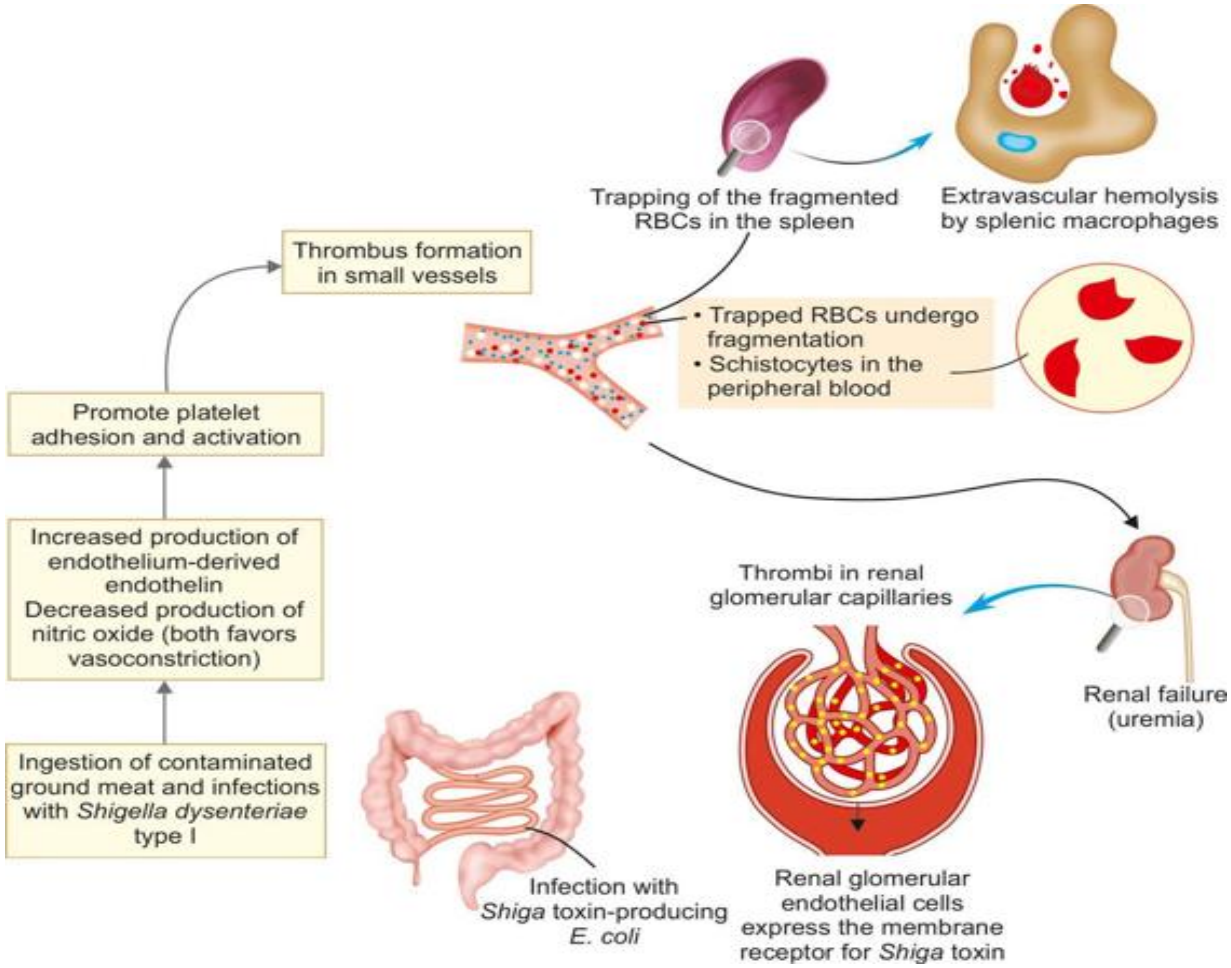


Fig. 20.30: Pathogenesis of hemolytic-uremic syndrome.

Pathogenesis of Atypical HUS

It is caused by either acquired or hereditary abnormalities of factors that weaken the activation of complement by the alternative pathway. This in turn leads to excessive activation of complement and produces

microvascular injury and microvascular thrombosis. The C5-9 membrane attack complex of complement pathway plays a central role. This is supported by the evidence that use of therapeutic antibody that inhibits this C5b-9 complex stops consumption of platelets and improves renal function. Abnormalities of the alternative pathway of complement activation may lead to C3 glomerulonephritis and dense deposit disease (discussed earlier on pages 676-7).

Pathogenesis of Thrombotic Thrombocytopenic Purpura (TTP)

It is caused either due to acquired or inherited deficiencies in ADAMTS13.

- **Acquired defects in ADAMTS13:** They are caused by inhibitory autoantibodies directed against ADAMTS13
- **Inherited deficiencies:** They are due to mutations in the ADAMTS13 gene.

Normally endothelial cells and megakaryocytes secrete normal von Willebrand factor (vWF) **multimers** into the plasma. These vWF **multimers** spontaneously develop into **unusually large multimers** which are quite effective in **mediating platelet adhesion**. A plasma protease enzyme called **ADAMTS 13** ("vWF metalloprotease") **regulates** the activity of **vWF** by cleaving the hemostatically active unusually large multimers into normal multimers of smaller sizes. Thus, ADAMTS 13 regulates the size of vWF multimers and **prevents platelet adhesion**. ADAMTS 13 is not related to tissue metalloproteases which cleave extracellular matrix.

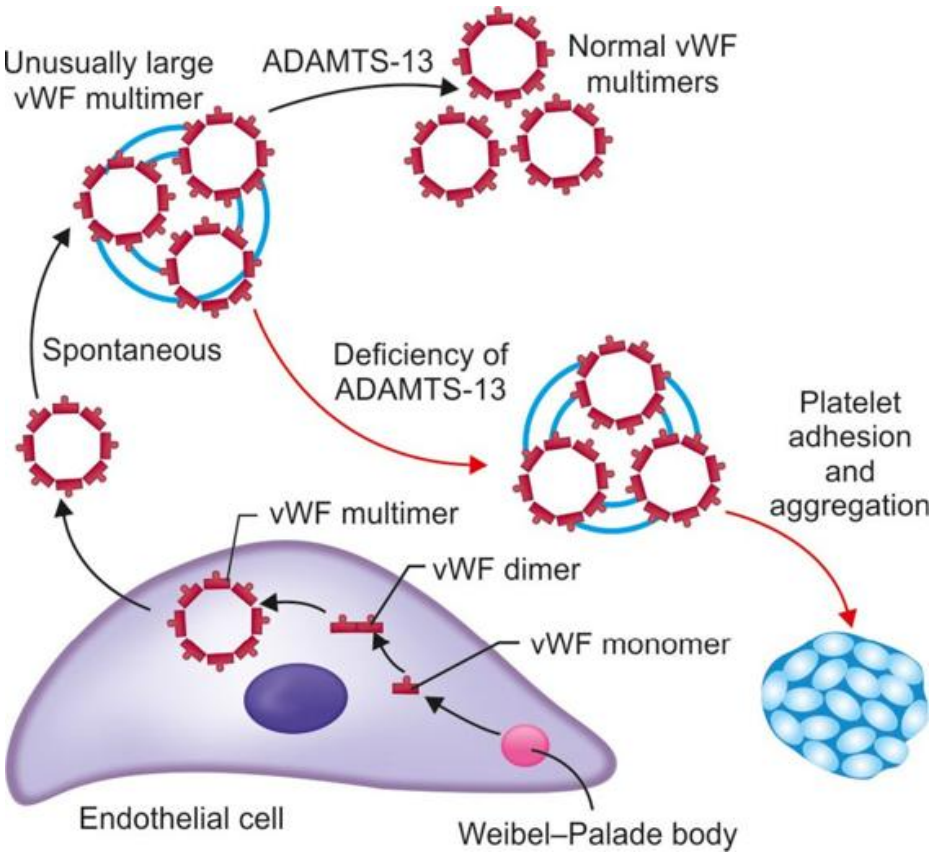


Fig. 20.31: Pathogenesis of thrombotic thrombocytopenic purpura.

When there is deficiency of ADAMTS13 (inherited or acquired), there is formation of abnormally large vWF multimers **(Fig. 20.31)**. These multimers may adhere to the endothelial cell surface andactivate platelets spontaneously and produces platelet aggregation. This results in **platelet-fibrin thrombi throughout the microcirculation**in multiple organs (including the kidney), leading to tissue **ischemia and infarction** that are characteristic of TTP.

MORPHOLOGY

Morphology is very similar in all forms of TMA regardless of the etiology.

- **Thrombi in glomerular capillaries,** arterioles and sometimes the larger arteries in more severe cases.
- **Additional glomerular changes:** These include widening of the subendothelial space, duplication or splitting of GBMs, and lysis of mesangial cells. Cortical necrosis may be found in severe cases.

Consequences

- It is accompanied by widespread consumption of platelets that leads to **thrombocytopenia.**
- Excessive small vessel (in the capillaries and arterioles) intravascular thrombi in various organs.
- Result in **microangiopathic hemolytic anemia**.
- **P**roduce microvascular occlusions that cause ischemic injury to tissue and consequent organ damage (e.g., *renal failure* in certain instances).

Laboratory Findings

- Thrombocytopenia due to excessive platelet consumption.
- Microangiopathic hemolytic anemia due to mechanical injury (shearing) of red cells as they pass through vascular channels narrowed by thrombi.

Clinical Features

The clinical course depends on the etiology and the severity of the morphological changes. Prognosis is less favorable in patients with severe vascular changes.

- **Shiga toxin-associated HUS:** It is sudden in onset with bleeding manifestations (hematemesis and melena), severe oliguria, hematuria, microangiopathic hemolytic anemia, and in few patients with prominent neurologic changes. It usually follows a gastrointestinal or flulike prodromal episode. It is *one of the main causes of acute kidney injury in children.*

  Prognosis: Most patients recover within weeks, if the acute kidney injury is managed properly with dialysis. The long-term prognosis (over 15–25 years) is not uniformly favorable. About 25% of children develop renal insufficiency.
- **Atypical HUS:** It also develops suddenly but without prodromal diarrhea. Plasma exchange will help in temporarily restoring the missing factors (patients with inherited disease) or remove pathogenic autoantibodies.
  - **Liver transplantation**: It can be curative because it synthesizes most complement factors.
  - **Antibody inhibitors of the membrane attack complex:** They have been found to reduce thrombosis and improve renal function. Hence, they are used as the first-line therapy in "complement-mediated" forms of HUS.

  Prognosis: It is significantly poorer than in *Shiga* toxin-mediated HUS
- **Thrombotic thrombocytopenic purpura:** Its onset is sudden. There is dominant involvement of the central nervous system and less involvement of the kidneys than in *Shiga* toxin and atypical HUS. Plasma exchange (plasmapheresis) therapy is used to replace the deficient ADAMTS13.

  Prognosis: Without treatment, it follows a rapid fatal course. The survival rate is about 10%. Prognosis has improved with plasma

exchange treatment. Patients who survive usually have no residual renal abnormalities.

CHRONIC KIDNEY DISEASE

CC: Define and describe the etiology, precipitating factors, pathogenesis, pathology, laboratory urinary findings, progression and complications of chronic renal failure.

Chronic kidney disease (CKD) previously termed chronic renal failure or insufficiency.

Definition: Chronic kidney disease (CKD) is a broad term used to describe the final common pathway of progressive nephron loss resulting from any type of kidney disease. It refers to a spectrum of **longstanding** (>3 months), usually **progressive** processes associated with **irreversible worsening of renal function** and decline in glomerular filtration rate (GFR). **CKD spectrum ranges from abnormalities detectable only by laboratory testing to uremia.**

BOX 20.7: Important causes of chronic kidney disease.

- **Glomerulopathies***
  - *Proliferative GN*
  - *Crescentic GN*
  - *Membranoproliferative GN*
  - *Mesangiocapillary GN*
- **Systemic and metabolic diseases**
  - *Diabetes**
  - *Systemic lupus erythematosus (SLE)*
  - *Polyarteritis nodosa (PAN)*
  - *Amyloidosis*
  - *Gout*
- **Interstitial**
  - *Chronic interstitial nephritis**
  - *Chronic pyelonephritis**
  - *Analgesic*
  - *Tuberculosis*
  - *Nephrocalcinosis*
- **Obstructive***
  - *Calculus*
  - *Tumors*
  - *Retroperitoneal fibrosis*
  - *Prostatic enlargement*

- **Vascular**
  - *Essential hypertension (accelerated)**
  - *Renovascular vasculitis (SLE, PAN, scleroderma)*
- **Congenital**
  - *Polycystic kidney**
  - *Medullary cystic disease*
  - *Alport's syndrome*

* Common causes of chronic renal failure.

Important causes of chronic kidney disease are listed in **Box 20.7.**

Pathogenesis

In chronic kidney disease there is progressive renal damage and it destroys more and more nephrons as the disease progresses. There will be initiation of adaptive mechanisms that try to maintain renal function.

Adaptive mechanisms: There will be **glomerular hyperfiltration** which tends to compensate for decreased glomerular filtration rate resulting from the loss of nephrons. Other adaptive mechanisms include an **increase in the rate of excretion of solutes per nephron** through increased plasma concentrations (for creatinine), decreased tubular reabsorption (for sodium, phosphate, and calcium), or increased tubular secretion (for potassium and hydrogen ions). These adaptive mechanism helps to maintain homeostasis till the late stages of chronic kidney disease.

Deterioration of renal function: Renal function usually deteriorates progressively even when the original cause is controlled. Uncontrolled hypertension in chronic kidney disease produces more rapid decline in the renal function.

MORPHOLOGY

Gross

Kidneys are **symmetrically contracted.** The outer surface is red-brown and **diffusely granular** when the underlying disorder

affects blood vessels (benign nephrosclerosis) or glomeruli. Their granules are small, regular and fine. Kidneys damaged by chronic pyelonephritis are usually unevenly (asymmetrically) involved and have deep, irregular, coarse and large scars.

Microscopy

Glomeruli: Common to all is the advanced scarring of the glomeruli. The **obliteration of the glomeruli** is the end-point of many diseases, and it is not possible to determine the nature of the initial lesion.

Tubules: Atrophy and dropout of many of the tubules in the cortex.

Interstitium: Shows marked **interstitial fibrosis.** Lymphocytic (and, rarely, plasma cell) infiltrates are present in the fibrotic interstitial tissue.

Blood vessels: The small- and medium-sized arteries show **thickening of wall with narrowing of lumina**, secondary to hypertension.

As damage progresses, it may become difficult to identify whether the primary lesion was glomerular, vascular, tubular, or interstitial. Such markedly damaged kidneys are termed as end-**stage kidneys.**

Clinical Features

Chronic kidney disease may develop insidiously and often symptomatic. Frequently, it is first suspected when proteinuria, hypertension, or azotemia was detected during on routine medical examination. Some degree of proteinuria is present in almost all cases and hypertension is very common. Microscopic hematuria is usually present.

Laboratory Findings

Urinalysis

Physical examination: Fixed specific gravity around 1.010 (isosthenuria) is seen in CRF.

Chemical examination:

- Hematuria may indicate glomerulonephritis.
- Proteinuria, if heavy, is strongly suggestive of glomerular disease.
- Glycosuria with normal blood glucose level is common in CRF.

Urine microscopy:

- **White cells** in the urine usually indicate **bacterial urinary infection**, sterile pyuria suggests papillary necrosis or renal tuberculosis.
- **Eosinophils** indicate **allergic tubulointerstitial nephritis** or cholesterol embolization.
- **Red cells** source may be from anywhere in the urinary tract between the glomerulus and the urethral meatus.
- **Red-cell casts** are suggestive of **glomerulonephritis**.
- **Granular casts** indicate **active renal disease**.
- **Broad casts** are seen in CRF.

Urine culture should be performed. Early-morning urine samples should be cultured for tuberculosis.

Urine Biochemistry

- **24-hour creatinine clearance** is useful to know the severity of renal failure.
- **Urine osmolality** is a measure of concentrating ability.
- **Urine electrophoresis** and immunofixation for the detection of light chains in myeloma.

Serum Biochemistry

- **Serum urea and creatinine:** The most consistent abnormalities in CRF are **elevated** levels of urea and creatinine. The level of serum creatinine correlates with the degree of renal damage.
- **Electrophoresis and immunofixation for myeloma**.
- Extreme elevations of creatine kinase and a disproportionate elevation in serum creatinine and potassium compared to urea suggestive of rhabdomyolysis.
- Other biochemical abnormalities include hypocalcemia, hyperphosphatemia, hyperuricemia and hyperkalemia.

Hematology

- **Anemia**
- **Eosinophilia** suggestive of allergic tubulointerstitial nephritis, vasculitis, or cholesterol embolism.
- **Peripheral smear** with **fragmented red cells (Burr cells) and/or thrombocytopenia** suggestive of **intravascular hemolysis** due to accelerated hypertension, hemolytic uremic syndrome or thrombotic thrombocytopenic purpura.
- **Markedly raised ESR** is suggestive of **myeloma**.

Immunology

- **Low complement components** may be seen in **active glomerular disease** (e.g., SLE, poststreptococcal glomerulonephritis).
- **Autoantibody screening** helpful autoimmune diseases (e.g., SLE).

- **Antibodies to streptococcal antigens** (ASOT, anti-DNase B) if post-streptococcal glomerulonephritis is suspected.
- **Antibodies to hepatitis B and C.**
- **Antibodies to HIV** when HIV-associated renal disease is suspected.
- **Malaria** can cause glomerular disease in the tropics.

Renal Biopsy

Aim: To establish the nature and extent of renal disease, which helps in treatment and predicting prognosis. It should be performed in patient with unexplained renal failure and normal-sized kidneys (exception diabetic glomerulosclerosis), unless there are strong contraindications.

Prognosis of chronic kidney disease: Without treatment, the prognosis is poor. They progress to uremia and death.

Complications of Chronic Renal Failure

- **Anemia of chronic disease**
- **Metabolic bone disease (Renal osteodystrophy):** It includes (1) hyperparathyroid bone disease (osteitis fibrosa cystica), (2) osteomalacia, (3) osteoporosis, (4) osteosclerosis, and (5) adynamic bone disease.
- **Gastrointestinal complications:** (1) reduced gastric emptying and increased risk of reflux esophagitis, (2) increased risk of peptic ulceration and acute pancreatitis, (3) constipation (especially in patients on continuous ambulatory peritoneal dialysis (CAPD), and (4) gastrointestinal bleed.
- **Metabolic abnormalities:**
  - **Gout:** Urate retention is a common in CRF.
  - **Insulin requirement and resistance:** Insulin is catabolized by and to some extent excreted via the kidneys. Thus, insulin requirements in diabetic patients reduce as renal failure progresses. By contrast, end-organ resistance to insulin is observed in advanced renal impairment.
  - **Lipid metabolism abnormalities:** Include (1) impaired clearance of triglyceride-rich particles and (2) hypercholesterolemia.
- **Endocrine abnormalities:** Include (1) hyperprolactinemia, (2) increased luteinizing hormone (LH) levels in both sexes, (3) decreased serum testosterone levels, (4) absence of normal cyclical changes in female sex hormones, resulting in oligomenorrhea or amenorrhea, (5) abnormalities

of growth hormone secretion and action, and (6) abnormal thyroid hormone levels.

- **Muscle dysfunction proximal myopathy:**
- **Nervous system:** Median nerve compression in the carpal tunnel due to β2-microglobulin-related amyloidosis.
- **Cardiovascular disease: Increased (16-fold) incidence of cardiovascular disease,** particularly myocardial infarction, cardiac failure, sudden cardiac death and stroke, uremic pericarditis or dialysis pericarditis. Hypertension develops in about 80% of patients with CRF.
- **Malignancy: Raised** incidence of malignancy. Malignant change can occur in polycystic kidney disease. Lymphomas, primary liver cancer and thyroid cancers can also develop.

HORSESHOE KIDNEYS

Q. Write short note on horseshoe kidney.

- Horseshoe kidneys are **common congenital anomaly** in which there is fusion of the upper (10%) or lower poles (90%) of the kidneys.
- It gives a horseshoe appearance to kidneys **(Fig. 20.32)**, which are continuous across the midline anterior to the great vessels.



Fig. 20.32: Horseshoe kidney showing fusion of the lower poles.

CYSTIC DISEASES OF THE KIDNEY

CC: Define classify and describe the genetics, inheritance, etiology, pathogenesis, pathology, laboratory, urinary findings, distinguishing features, progression and complications of cystic disease of the kidney.

Q. Write short note on cystic disease of kidney.

Definition: Cystic diseases of the kidney represent a heterogeneous group of hereditary, developmental, and acquired disorders characterized by the presence of unilateral or bilateral renal cysts.

Classification of Renal Cystic Disease (Box 20.8)

BOX 20.8: Classification of renal cystic disease.

1. Polycystic kidney disease
   - Autosomal dominant (adult) polycystic disease
   - Autosomal recessive (childhood) polycystic disease
2. Medullary cystic disease
   - Medullary sponge kidney
   - Nephronophthisis
3. Multicystic renal dysplasia
4. Acquired (dialysis-associated) cystic disease
5. Localized (simple) renal cysts
6. Renal cysts in hereditary malformation syndromes (e.g., tuberous sclerosis)
7. Glomerulocystic disease
8. Extraparenchymal renal cysts (pyelocalyceal cysts, hilar lymphangitic cysts)

Autosomal Dominant (Adult) Polycystic Kidney Disease

Q. Write short note on polycystic kidney disease.

Definition: Autosomal-dominant (adult) polycystic kidney disease (ADPKD) is a **hereditary disorder** characterized by **multiple cysts in both kidneys**. These cysts expand and cause destruction of kidney parenchyma and leads to renal failure.

Genetics and Pathogenesis

Genetics

- The ADPKD is **genetically heterogeneous disorder** caused by **mutations in *PKD1*** (85% of cases) **and *PKD2* gene** (15%).
- Germline mutations in *PKD1* gene are present in all renal tubular epithelial cells of affected individuals. Cyst only develop in some tubules.
- Like all tumor suppressor genes, *PKD* gene also requires a second hit for the expression of disease.

- Mutations in other genes involved in polycystic kidneys include gene, which produce protein fibrocystin/polyductin and nephrocystin.

Pathogenesis (Fig. 20.33)

The exact pathogenesis of ADPKD is unknown.

- Protein **products of** *PKD* **genes** are **polycystin-1** and **polycystin-2**. The polycystins are mainly extracellular molecules present in the primary cilia of tubular epithelial cells that are **involved in cell-cell or cell-matrix adhesion**. Cilia are hair-like organelles present on the apical surface of tubular epithelial cells. They **act as mechanosensors of fluid flow in the lumen of the tubules**.
- Renal cystic disease is considered as a type of **ciliopathy**.
- **Mutations of the** *PKD* **genes** lead to **defects in mechanosensing**. This causes **disrupted calcium signaling** (cilium is essential for maintenance of epithelial cell differentiation and normally inhibits renal tubule growth) and leads **to dysregulation of cell polarity, abnormal cell proliferation and cell-matrix adhesion**.
- **Evolution of cysts from renal tubules**
  - **Abnormal proliferation of tubular epithelium:** It follows disrupted calcium signaling and tubule becomes covered by an undifferentiated epithelium. **Repeated cycles of cell proliferation lead to dilatation/expansion of the tubule wall into a cyst**.
  - **Abnormal extracellular matrix and defect in basement membrane:** The cystic epithelium is associated with **thickening of the basement membrane of the affected tubule**.
  - **Fluid secretion:** Initially, the cyst fluid is derived from the glomerular filtrate. Later, the cystic segment separates (becomes disconnected) from the original tubule, and the fluid is secreted by epithelial cells lining the cysts into the cyst cavity.
- **Progression of cyst size:** The abnormal proliferation of tubular epithelial cells and the expanding volume of intraluminal fluid result in progressive increase in the size of the cysts.
  - **Consequences:** The cyst fluids contain mediators derived from epithelial cells, which further promotes fluid secretion, damage glomerulus, stimulate inflammation and interstitial fibrosis.

MORPHOLOGY

Q. Write short answer on gross features of adult polycystic kidney disease.

Gross (Figs. 20.34A and B)

- **Bilateral**.
- **Size: Markedly enlarged**; weight is increased and each may reach up to 4 kg.

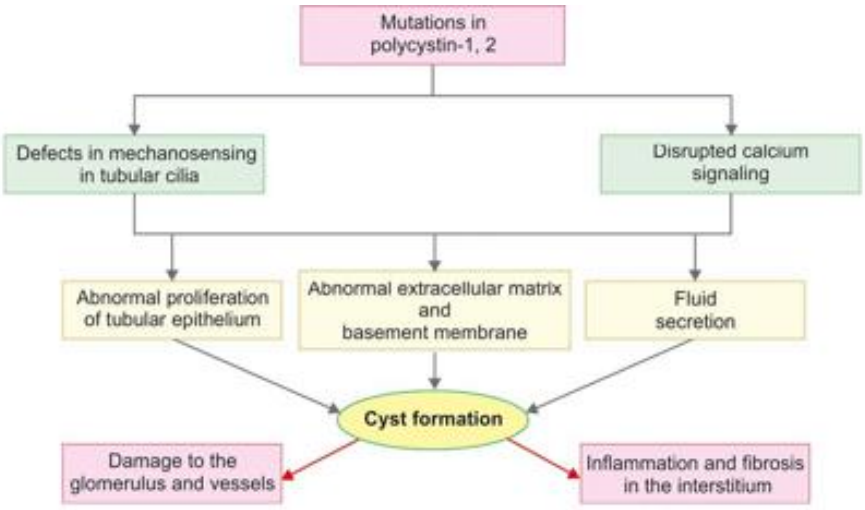


Fig. 20.33: Mechanisms of cyst formation in polycystic kidney disease.

- **External surface: Bosselated** with **numerous cysts of varying sizes**, measuring up to 3–4 cm in diameter, with no intervening parenchyma.

Microscopy

- **Numerous cysts** with functioning nephrons between these cysts.
- **Cysts:**
  - **Origin**: Cysts can **arise in all segments of the nephron** including glomeruli, proximal tubules, distal tubules, and collecting ducts, and therefore, they **show variable lining epithelia**. Occasionally, Bowman capsules may be involved in cyst formation.
  - **Lining**: **Cuboidal and columnar** epithelium.
  - **Content**: Cysts may show a **clear**, **serous** fluid **or more commonly** contain **turbid**, **red to brown**, sometimes hemorrhagic fluid.
- **Intervening renal parenchyma**: It may be normal or may show foci of interstitial fibrosis and pyelonephritis.

Clinical Features

- **Majority** of patients remain **asymptomatic until the fourth decade** of life.
- It may present with **flank pain** (heaviness or dragging sensation), **bilateral abdominal masses**, and **renal colic** (due to passage of blood clots in the urine), and **hypertension**. When sufficient quantity of nephrons are destroyed, patient develops **renal failure**.

Extrarenal Associated Congenital Anomalies

- Cysts in other organs such as liver (**polycystic liver disease**).
- Intracranial **berry aneurysms** in the **circle of Willis** which may rupture and cause subarachnoid hemorrhages.
- **Mitral valve prolapse** and other cardiac valvular anomalies.

ADPKD: Autosomal dominant mode of inheritance.

ADPKD: Mutations in:
- *PKD1* gene on chromosome 16p13.3 code for polycystin-1, or
- *PKD2* gene on chromosome 4q21code for polycystin-2

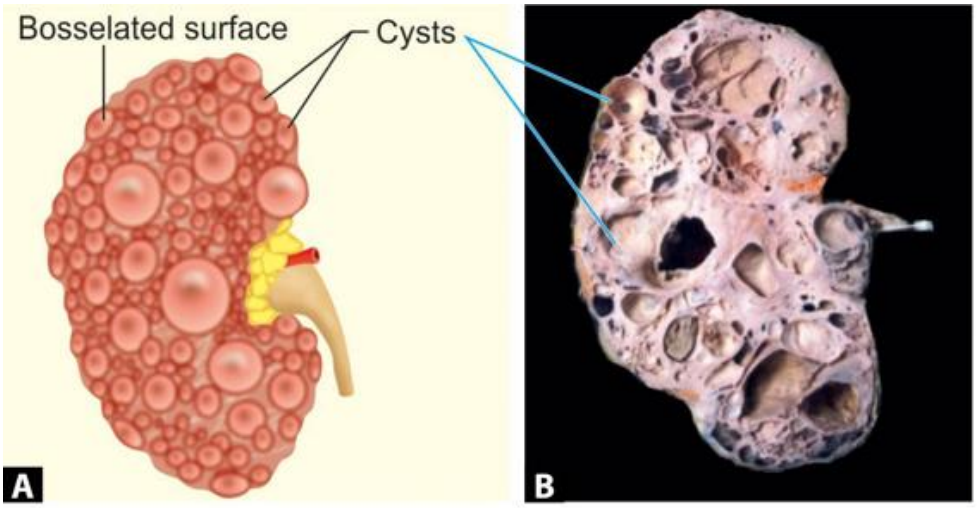
ADPKD: Chronic renal failure develops between ages of 40–60 years.

ADPKD:
- Large multicystic kidneys, liver cysts
- Berry aneurysms
- Mitral valve prolapse

Autosomal Recessive (Childhood)Polycystic Kidney Disease

Autosomal recessive (childhood) polycystic kidney disease (ARPKD) is **genetically different from adult polycystic kidney disease** (ADPKD).

Subcategories: ARPKD can be categorized as **perinatal**, **neonatal**, **infantile**, **and juvenile**, depending on the time of presentation. The perinatal and neonatal categories are most common and are usually fatal.



Figs. 20.34A and B: Autosomal-dominant adult polycystic kidney disease (ADPKD) shows markedly enlarged kidney with numerous dilated cysts: (A) External surface (diagrammatic); (B) Cut section.

Etiology

- In **majority** of the cases, ARPKD is caused by **mutations of the polycystic kidney hepatic disease (*PKHD1*) gene**.
- PKHD1 **encodes for a protein called fibrocystin (polyductin)**.
- It is highly expressed in the epithelial cells of the collecting ducts and thick ascending loops of Henle.

MORPHOLOGY

Gross

- **Both kidneys** are **markedly enlarged** and have a **smooth external appearance**. Thus, they retain their **reniform configuration**.
- **Cut section (Fig. 20.35)**:
  - **Medulla and cortex** are completely **replaced by numerous small cysts**.
  - The **cysts** tend to be **linear and radiate from the medulla to the outer cortex** (right angles to the cortical surface). It gives a **sponge-like appearance to the kidney**.

Microscopy

- **Shape: Cyst is cylindrical** and appears as dilated tubular structure or, less commonly saccular.

- **Lining: All the cysts are lined** by **cuboidal epithelial cells**, reflecting their **origin from the collecting ducts**.

Features of ARPKD

- All patients also have cysts in the liver.
- Patients who survive infancy (infantile and juvenile forms), may develop **congenital hepatic fibrosis**.
- It is characterized by bland periportal fibrosis and the proliferation of well-differentiated biliary ductules.
- Older children may develop portal hypertension with splenomegaly.

Childhood polycystic kidney disease: Autosomal recessive.

ARPKD: Mutations in gene PKHD1, which codes for fibrocystin.

ARPKD: Large, cystic kidneys at birth.

ARPKD: Usually death in infancy or childhood.

Medullary Diseases with Cysts

Q. Write short answer on medullary sponge kidney.

There are two major types: (1) medullary sponge kidney and (2) nephronophthisis-medullary cystic disease complex.

1. **Medullary sponge kidney:** It is relatively common and usually harmless. It may be occasionally associated with nephrolithiasis.
2. **Nephronophthisis-medullary cystic disease complex:** It usually begins in childhood. It is associated with renal dysfunction and progress to chronic kidney disease.
   - **Variants:** Depending on the time of onset, it is divided into: (i) infantile, (ii) juvenile (most common), (iii) adolescent nephronophthisis, and (iv) medullary cystic disease developing later in adult life.

MORPHOLOGY

Kidneys are small and contracted. Numerous small cysts are seen at the corticomedullary junction. These cysts are lined by flattened or cuboidal epithelium. Other nonspecific

findings include a chronic tubulointerstitial nephritis, tubular atrophy, thickening of the tubular basement membranes and interstitial fibrosis.

Clinical Features

It usually manifests with polyuria and polydipsia due to reduced tubular function. Over a period of 5–10 years it progresses to end-stage renal disease.

ACUTE KIDNEY INJURY

CC: Define and describe the etiology, precipitating factors, pathogenesis, pathology, laboratory urinary findings, progression and complications of acute renal failure.

Definition: Acute kidney injury (AKI) is a **clinicopathologic entity, characterized clinically by acute** (abrupt, over days to few weeks) **reduction of renal function** (decreased glomerular filtration rate [GFR]) and excretory **function**.

The deterioration in renal function is sufficiently severe to result in retention of nitrogenous wastes in the body (uremia) and other waste products normally cleared by the kidneys (elevation of serum creatinine).

Etiology and Pathophysiology

Acute kidney injury is a clinical diagnosis and not a structural one. It is not a single disease but a heterogeneous group of conditions that share common diagnostic features namely an increase in serum creatinine often associated with a reduction in urine volume. It is important to recognize that AKI. Patient may have AKI with or without injury to the kidney parenchyma. Its severity can range from asymptomatic to rapidly fatal condition.

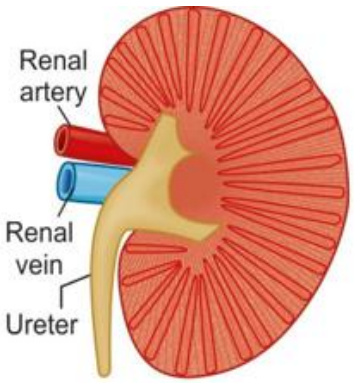


Fig. 20.35: Diagrammatic appearance of cut section of autosomal-recessive childhood PKD, showing smaller cysts and dilated channels at right angles to the cortical surface.

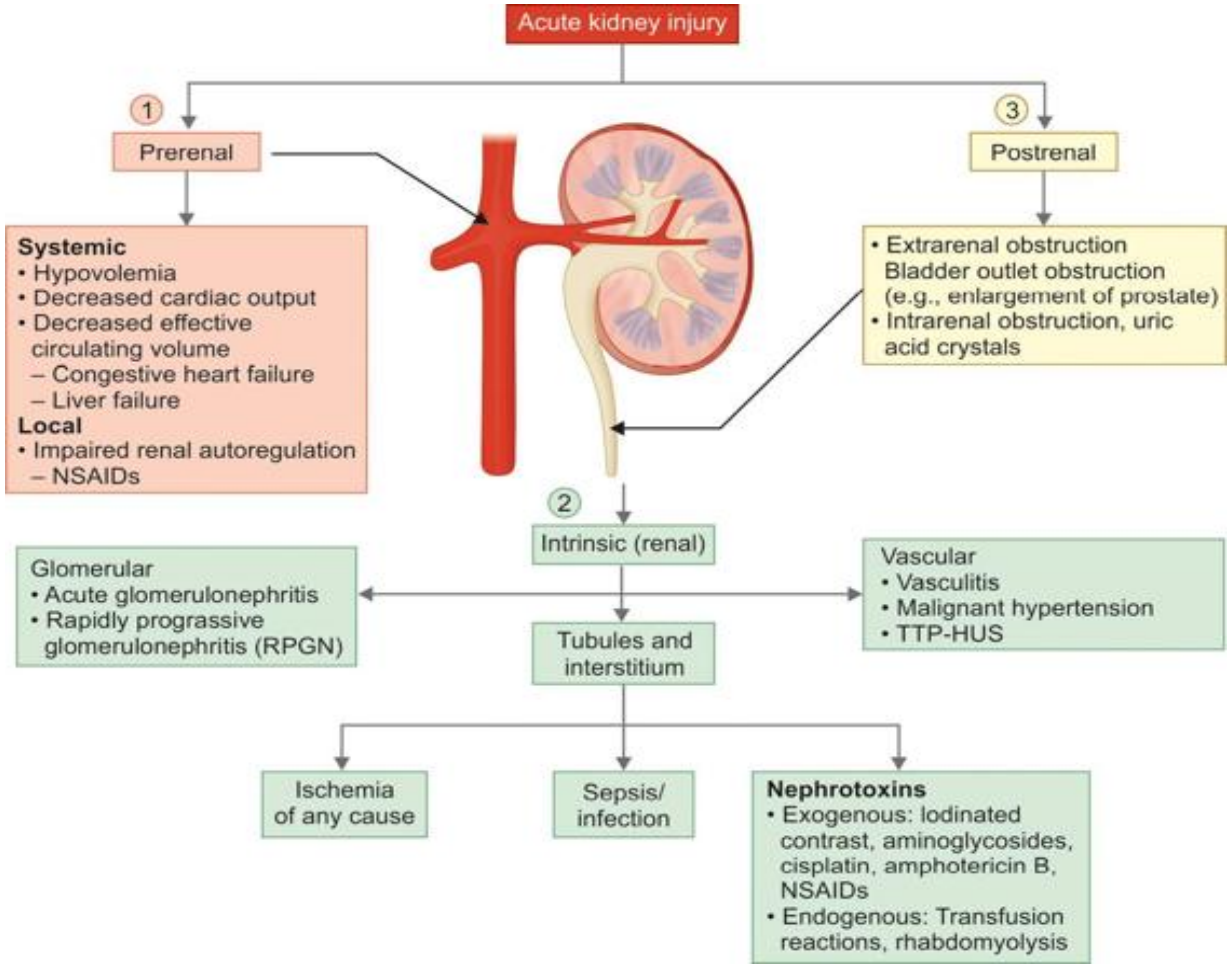


Fig. 20.36: Classification of causes of acute kidney injury.

(NSAIDs: nonsteroidal anti-inflammatory drugs; TTP-HUS: thrombotic thrombocytopenic purpura-hemolytic-uremic syndrome).

Classification of Causes of Acute Kidney Injury (Fig. 20.36)

The causes of AKI are traditionally divided into three broad categories: (i) prerenal azotemia (prerenal causes), (ii) intrinsic renal parenchymal disease (intrarenal/renal causes), and (iii) postrenal obstruction (postrenal causes). Most common cause of AKI (acute kidney injury) is **acute tubular necrosis** (ATN) and **acute tubular injury** (ATI) **followed by prerenal causes.** ATI may produce oliguria (defined as urine output of <400 mL/day).

Prerenal Causes

Kidneys are inadequately perfused and the GFR is markedly reduced and produces oliguria (urine output <400 mL/day).

Prerenal azotemia ["azo," meaning nitrogen, and "-emia," meaning in the blood (*refer* **Table 20.1**)] is characterized by a rise in serum creatinine or blood urea nitrogen (BUN) concentration. Prerenal azotemia does not produce damage to renal parenchymal and is rapidly reversible once parenchymal blood flow and intraglomerular hemodynamics are restored.

- **Systemic causes:** Precipitating event is **renal hypoperfusion** (e.g., hypovolemia, decreased cardiac output) which may be due to **reduction in the volume of extracellular fluid** or disease states associated with decreased effective arterial volume or other causes.
- **Local causes:** These include medications that interfere with renal autoregulatory responses such as nonsteroidal anti-inflammatory drugs (NSAIDs) and inhibitors of angiotensin II. Prolonged periods of prerenal azotemia may lead to ischemic injury, often termed acute tubular necrosis (ATN).

Renal Causes (intrinsic AKI)

Intrinsic diseases of kidney causing AKI (intrarenal causes) are classified according to the primary histologic site of injury: t**ubules, interstitium, vasculature, or glomerulus**.

- **Renal tubular epithelial cell injury**, commonly known as **acute tubular necrosis** (ATN): It occurs more commonly due to **ischemia** of any cause. Other causes include **sepsis and nephrotoxins** (specific renal

toxins). Nephrotoxins may be endogenous or exogenous. ATN results in ischemia and necrosis of the tubular epithelial cells. However, when the causative factors are removed the tubular cells can regenerate.

- Acute tubulointerstitial nephritis: Hypersensitivity reaction to drugs
  - Glomerular causes: e.g., Acute glomerulonephritis, rapidly progressive glomerulonephritis (RPGN).
- **Vascular causes: These include vasculitis, malignant hypertension and TTP**-HUS (thrombotic thrombocytopenic purpura-hemolytic-uremic syndrome).

Postrenal Causes (Postrenal AKI)

These include obstruction (functional or structural) of the urinary tract at any point in its course from the tubule to the urethra. Obstruction may be extrarenal or intrarenal.

Obstruction to urinary flow may be caused by derangements anywhere from the renal pelvis to the tip of the urethra. Examples include benign nodular hyperplasia of prostate, ureteral obstruction (e.g., ureteral stones, invasive cervical cancer) and bladder cancer. Postrenal AKI occurs when the normally unidirectional flow of urine is acutely blocked either partially or totally. This leads to increased retrograde hydrostatic pressure and interferes with glomerular filtration.

Urinary findings in different types of acute kidney injury is presented in **Table 20.5.**

TABLE 20.5: Urine finding in different types of acute kidney injury.

Type of acute kidney injury	*Urine findings*
Prerenal	Normal or hyaline casts
Intrarenal • Tubular cell injury	Muddy-brown, granular, epithelial casts
• Interstitial nephritis	Pyuria, hematuria, mild proteinuria, granular and epithelial casts, eosinophils
• Glomerulonephritis	Hematuria, marked proteinuria, red blood cell casts, granular casts
• Vascular disorders	Normal or hematuria, mild proteinuria
Postrenal	Normal or hematuria, granular casts, pyuria

Complications of AKI (Box 20.9)

BOX 20.9: Complications of acute kidney injury.

- **Metabolic:** Hyperkalemia, hypocalcemia, hyperphosphatemia, hypermagnesemia, hyperuricemia, metabolic acidosis (increased anion gap)
- **Cardiovascular:** Cardiac arrhythmias, pulmonary edema, pericarditis/pericardial effusion
- **Gastrointestinal:** Gastrointestinal hemorrhage
- **Neurologic:** Encephalopathy, neuropathy, seizures
- **Hematologic:** Anemia, bleeding—platelet dysfunction
- **Miscellaneous:** Infections (pneumonia, urinary tract infection, septicemia)

ACUTE TUBULAR INJURY/NECROSIS

CC: Define and describe the etiology, pathogenesis, pathology, laboratory, urinary findings, progression and complications of acute tubular necrosis.

Definition: Acute tubular injury (ATI) is a clinicopathologic entity characterized by damage to renal tubular epithelial cells and an acute decline in renal function. It is usually associated with shedding of granular casts and tubular cells into the urine.

Frank necrosis is rarely observed in a kidney biopsy in patient with *acute tubular injury/necrosis*. Hence, the preferred term is *acute tubular injury.* ATI is the most common cause of acute kidney injury (AKI described above) and may produce oliguria (urine output of <400 mL/day).

Types of ATI: There are two types of ATI that differ in the underlying causes.

Ischemic ATI:

Prerenal Causes

It usually develops when there is a period of inadequate blood flow to all or some peripheral organs such as the kidney. This may occur when there is marked hypotension and shock (*refer* prerenal causes in **Fig. 20.36**). This can develop due to various conditions such as severe trauma, blood loss, acute pancreatitis and septicemia.

- **Glomerular causes:** Acute glomerulonephritis, rapidly progressive glomerulonephritis (RPGN).
- **Vascular causes:** Ischemia to tubules may also occur due to vascular causes that lead to reduced intrarenal blood flow, as in microscopic polyangiitis, malignant hypertension, and thrombotic microangiopathies.

Nephrotoxic ATI: Toxin may be endogenous and exogenous. Exogenous toxins include many poisons, including heavy metals (e.g., mercury); organic solvents (e.g., carbon tetrachloride); and many drugs (e.g., gentamicin and other antibiotics), and radiographic contrast agents. It may also be produced by endogenous toxins such as mismatched blood transfusions and other hemolytic crises, as well as myoglobinuria.

Pathogenesis (Figs. 20.37A and B)

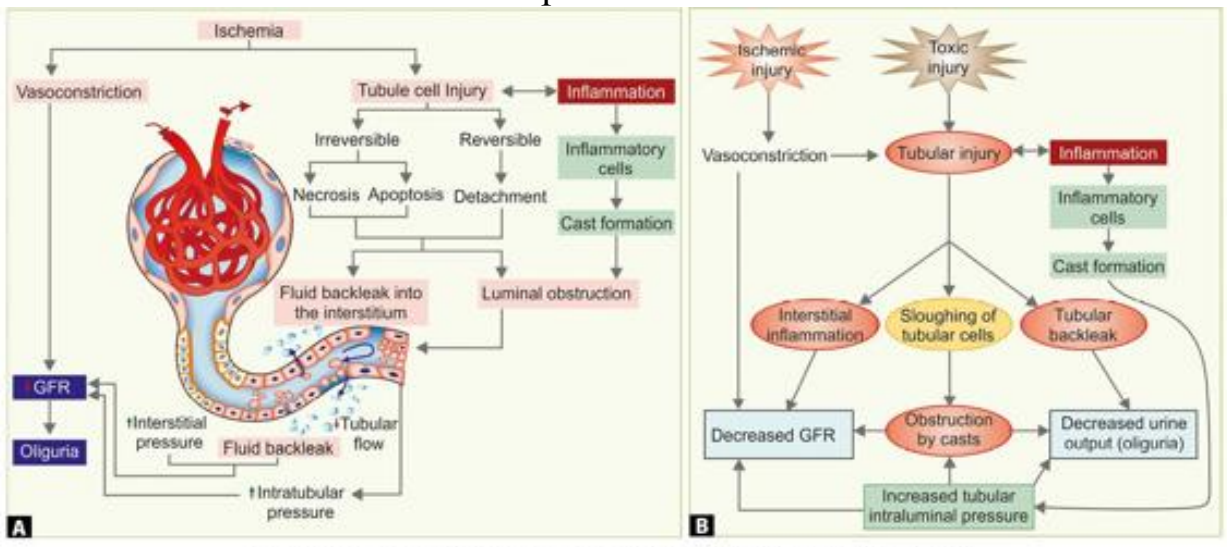
Two important features of ATI (in both ischemic and nephrotoxic) are: (1) Tubular cell injury, and (2) Disturbances in blood flow.

1. Injury to the Tubular Epithelial Cells

Proximal tubular epithelial cells are sensitive to hypoxemia and vulnerable to nephrotoxins.

Factors that predispose tubular epithelial cells to toxic and ischemic injury are:

- Elevated intracellular concentrations of various molecules that are resorbed or secreted across the proximal tubule.

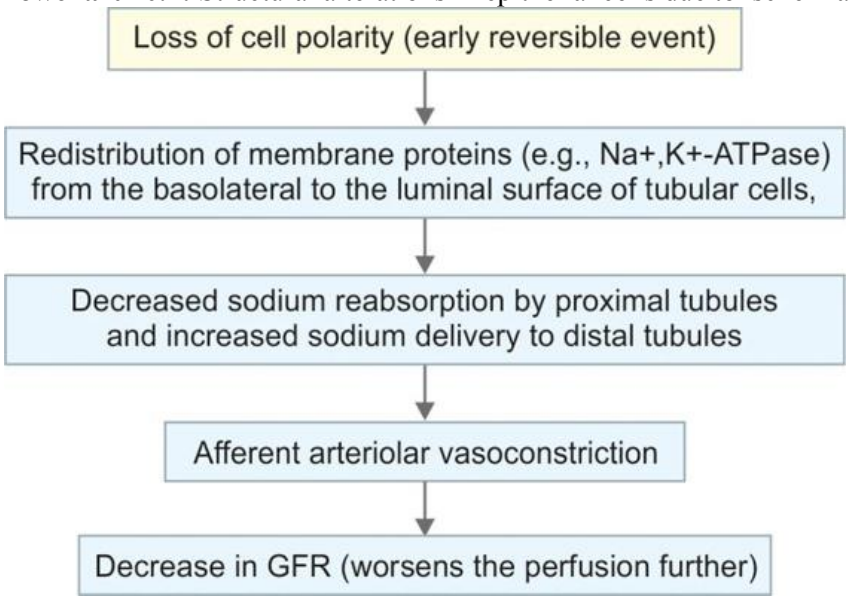


Figs. 20.37A and B: Pathogenesis of acute ischemic or toxic tubular injury (ATI).

- Exposure to high concentrations of luminal solutes. These are concentrated because of the resorption of water from the glomerular filtrate.
- High rate of oxygen consumption needed for the generation of the ATP required for transport and reabsorption functions.

Structural alterations in epithelial cells due to ischemia are presented in **Flowchart 20.2**.

Flowchart 20.2: Structural alterations in epithelial cells due to ischemia.



Consequences of Tubular Injury

- **Luminal obstruction by casts:** Injury to the tubular epithelial cells causes detachment of the damaged cells from the basement membranes. They are shed into the urine. When there is sufficient build-up of tubular debris, it can block the outflow of urine (obstruction by forming casts) in the tubules. **Obstruction results in: (1) increased intratubular pressure**, **(2)** further **decrease in GFR,** and (3) **decreased urine outflow.**
- **Back-leakage of fluid from lumen into the interstitium:** It **occurs in the damaged tubules** → results in decreased urine output, **interstitial edema (due to** back leak into the interstitium) → causes **increased interstitial pressure**, and **further damage** and collapse **of the tubule**.
- **Interstitial inflammation**: Ischemic tubular cells also produce chemokines, cytokines, and adhesion molecules (e.g., P-selectin). They attract leukocytes and produce interstitial inflammation. This in tun also causes decreased GFR.

2. Disturbances in Blood Flow

Ischemia also causes **vasoconstriction** (intrarenal) and **reduces both glomerular blood flow** and **oxygen supply to tubules**.

Recovery: The tubular necrosis is patchy and there is maintenance of the integrity of the basement membrane along many segments. This allows repair of the injured foci of tubular epithelial cells and recovery of function if the precipitating cause is removed.

MORPHOLOGY

Ischemic ATI

Gross

Both kidneys are **swollen** and show a **pale cortex** and a **congested medulla**.

Microscopy

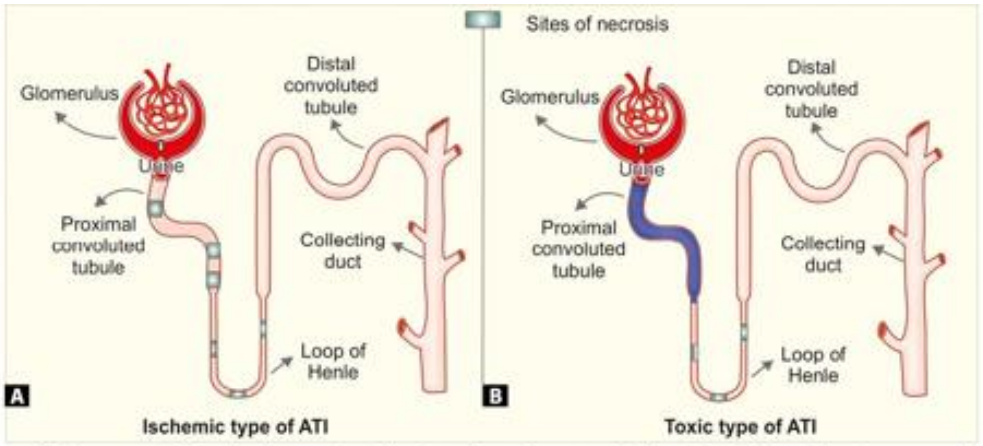
- **Glomeruli**: Appear normal.
- **Tubules**:
  - **Areas involved (Fig. 20.38A):** Tubules show **focal and multiple areas of damage** along the nephron, **with large skip areas** in between. The lesions are **most marked in the proximal tubules** and the **ascending thick limbs of the loop of Henle** in the **outer medulla**.
  - **Tubular epithelial injury:**
    - **Epithelial simplification:** It is characterized by **focal flattening** and **loss of proximal tubule brush borders**.
    - **Sloughing** of the apical cytoplasm of non-necrotic tubular cells into the lumen of the tubules.
    - **Necrosis of tubular epithelial cells (Fig. 20.39).**
    - **Formation of casts:** Sloughed apical cytoplasm, non-necrotic and necrotic cells form **hyaline casts and brown pigmented granular cast**. The casts are seen in the lumen of distal tubules and in the urine. These casts are **composed of Tamm–Horsfall protein** (a urinary glycoprotein normally secreted by the cells of ascending thick limb and distal tubules) **and other plasma proteins**.
    - **Occlusion of tubular lumens:** The casts occlude the lumen and result in d**ilation of the lumen**.
    - **Rupture of basement membranes** (tubulorrhexis): It can occur in later stages.
  - **Epithelial regeneration:** If the precipitating cause is removed, the tubular epithelium can regenerate and tubules may return to normal without any residual evidence of damage.
- **Interstitium:** It shows edema and accumulations of leukocytes.

- **Blood vessels**: Normal.

Nephrotoxic ATI

Microscopy

- Areas involved **(Fig. 20.38B):** Most **common site of toxic injury** is seen in the **proximal convoluted tubules**. The **necrosis** of tubular epithelium is **more-extensive** in toxic ATN than seen in ischemic ATN.
- Tubular necrosis is limited to certain segments of tubules that are most sensitive to the particular toxin.
- **ATN due to hemoglobin or myoglobin** show many **red-brown colored** (due to heme pigments) **tubular casts**.



Figs. 20.38A and B: Patterns of tubular damage in ischemic and toxic acute tubular injury: (A) In ischemic type, tubular necrosis is patchy, relatively short lengths of tubules are affected, and most marked in the straight segments of proximal tubules and ascending limbs of loop of Henle; (B) In toxic acute tubular injury, extensive necrosis involves the proximal convoluted tubule segments.

Q. Write short answer on Tamm–Horsfall protein.

Urinary casts in urine sediment provide important clues for differentiating renal diseases. Casts are formed in the tubule of the nephron. Cast are composed of a protein meshwork with entrapped cells or cell fragments. Cells in the ascending thick limb of Henle and distal tubules produce and secrete small amounts of a glycoprotein called *Tamm–Horsfall* protein into the tubular lumen. Tamm–Horsfall protein forms the meshwork that entraps cells in the tubule to form casts and is found in normal urine. Normal protein casts that do not have cells in them are called *agranular casts*.

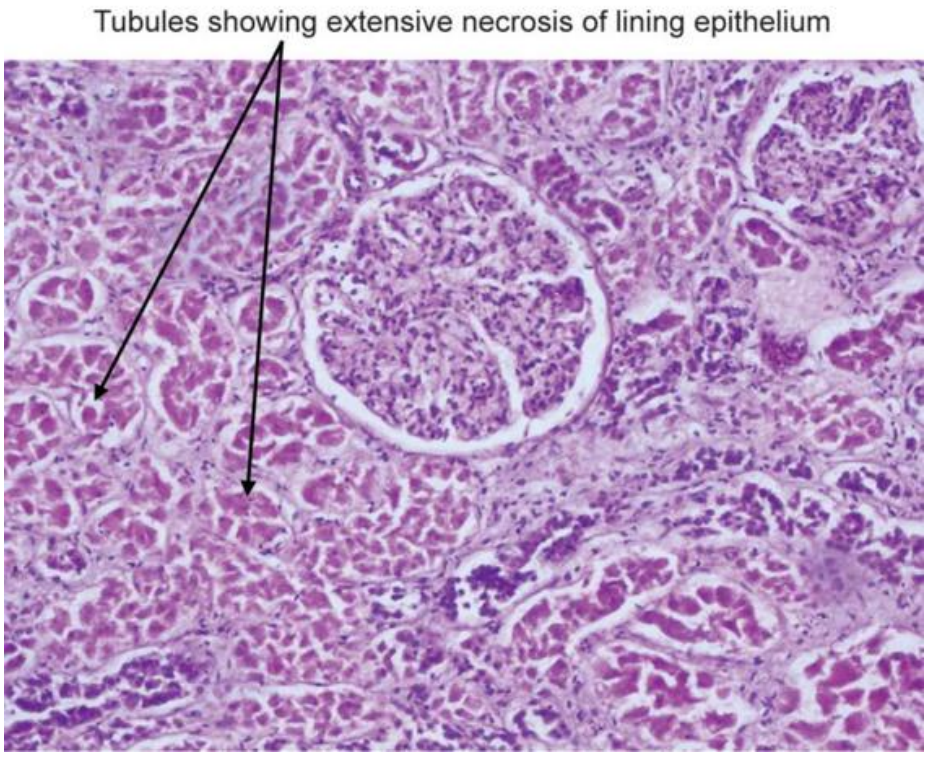


Fig. 20.39: Photomicrograph of acute tubular necrosis showing tubules with necrosis of tubular epithelial cells. The glomerulus appears normal.

TABLE 20.6: Tubular epithelial changes in direct tubular toxicity by poison and organic solvents.

Poison/organic solvent	*Changes in tubular epithelial cells*
• Mercuric chloride	• Large acidophilic inclusions
• Carbon tetrachloride	• Accumulation of neutral lipids
• Ethylene glycol	• Ballooning and vacuolar degeneration of cells of proximal convoluted tubule • Calcium oxalate crystals in tubular lumen

Changes in tubular epithelial cells induced by few poisons and organic solvents are presented in **Table 20.6**.

Clinical Course

The clinical course of ATI may be divided into three stages namely: (1) initiation, (2) maintenance, and (3) recovery phases.

1. Initiation Phase

Clinical features depend on the initiating cause of ATI. There is a mild reduction of urine output and increase in BUN.

2. Maintenance Phase

During this phase, there is sustained decrease in urine output in the range of 40–400 mL/day (oliguria), salt and water overload, rising BUN level, hyperkalemia, metabolic acidosis, and other features of uremia.

3. Recovery Phase

During this phase, there is a steady increase in urine volume, which may reach up to 3 L/day. There is loss of large amounts of water, sodium and potassium (leading to hypokalemia) in the urine. Once the renal tubular function returns to normal, BUN and creatinine levels also return to normal.

Prognosis: ATN is a **potentially reversible condition** and with modern therapy most patients recover.

Common causes of acute renal failure are shown in **Table 20.7**.

TABLE 20.7: Common causes of acute renal failure and associated urinary findings.

Causes of acute renal failure	***Urinalysis findings***
Acute tubular necrosis	Dirty brown casts and epithelial cells
Acute glomerulonephritis	RBC casts and proteinuria
Acute tubulointerstitial nephritis	WBC casts and pyuria

ATI/ATN: Most common cause of acute renal failure.

ATI/ATN: Most common is ischemic type.

AKI: Acute reduction of renal function and mostly with morphological feature of tubular injury.

Pathogenesis of ATI:
- Tubular cell injury
- Disturbances in blood flow

Tamm–Horsfall protein: Urinary glycoprotein normally secreted by the cells of ascending thick limb of Henle and distal tubules.

Three phases of acute tubular injury

1. Initiation
2. Maintenance
3. Recovery

URINARY TRACT OBSTRUCTION(OBSTRUCTIVE UROPATHY)

CC: Define classify and describe the etiology, pathogenesis, pathology, laboratory, urinary findings, distinguishing features progression and complications of renal stone disease and obstructive uropathy.

Urinary tract obstruction is caused by **structural or functional abnormalities** and results in **obstruction to the flow of the urine (Fig. 20.40)**.

Consequences:

- Unrelieved obstruction almost always leads to:
  - Renal dysfunction **(obstructive nephropathy)** and permanent renal atrophy.
  - Dilation of the collecting system (hydronephrosis).
- Increased **susceptibility to infection** and to **stone formation**.

Hydronephrosis

Q. Write short note on hydronephrosis.

Definition: Hydronephrosis is defined as an aseptic **dilation of the renal pelvis and calyces due to obstruction of urinary outflow,** associated with **progressive atrophy of the kidney**.

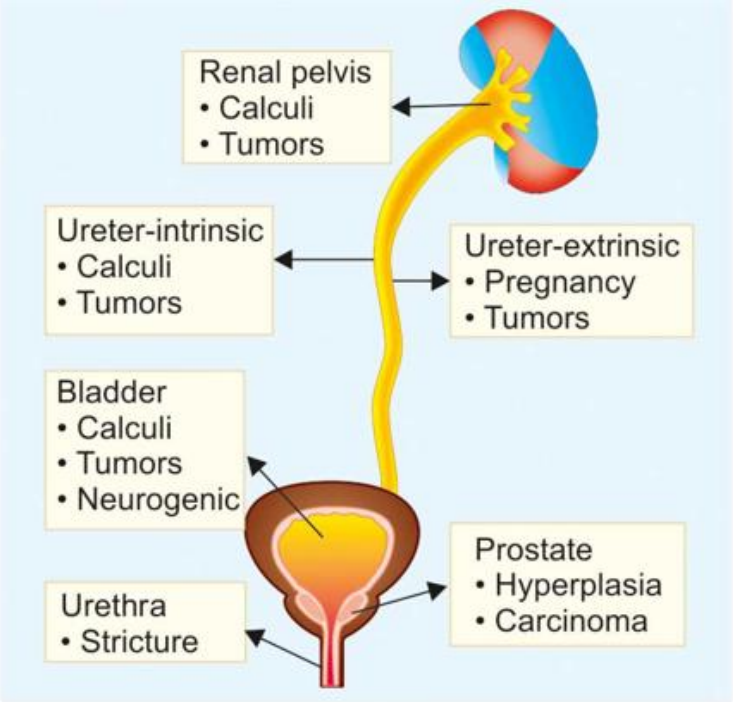
Causes (Fig. 20.40)

Q. Write short note on causes (etiology) and pathogenesis of hydronephrosis.

It may be classified as structural or functional disorders.

1. **Structural disorders:**
   - **Urinary calculi**
   - **Tumors:** Carcinoma of the prostate, bladder tumors, carcinoma of the cervix or uterus

- **Benign prostatic hypertrophy**
- **Congenital anomalies:** Urethral strictures, meatal stenosis, bladder neck obstruction



Hydronephrosis: Nature of obstruction may be:
- Congenital or acquired
- Sudden or insidious
- Partial or complete
- Unilateral or bilateral
- Intrinsic lesions or extrinsic lesions
- May occur at any level of the urinary tract from the urethra to the renal pelvis

Fig. 20.40: Various common obstructive lesions of the urinary tract.

- **Inflammation:** Prostatitis, urethritis, retroperitoneal fibrosis
- Pregnancy, uterine prolapse and cystocele.

2. **Functional disorders:** Neurogenic bladder (spinal cord damage or diabetic nephropathy).

Pathogenesis

- **Obstruction:** It may be **complete or incomplete**. Obstruction in the urinary tract → leads to **accumulation of urine proximal to the obstruction**.
- **Dilatation:** Even with complete obstruction, **glomerular filtration** does not stop but **continues for some time** and → leads to **accumulation of urine** → causes **dilatation of affected calyces and pelvis** due to back pressure. Raised pressure in the renal pelvis → transmitted back through the collecting ducts into the renal parenchyma and its consequences are:
  - **Renal atrophy**
  - **Compresses the renal vasculature of the medulla** → reduces the blood flow to the medulla with **diminished tubular function**.
- **Reduced GFR:** If obstruction persists, the functional alterations of tubule result in loss of concentrating function and later **reduce the glomerular filtration rate**.
- **Interstitial inflammation:** Obstruction also initiates an **interstitial inflammatory reaction** and interstitial **fibrosis**.

MORPHOLOGY

Q. Write short answer on morphology (gross and microscopy) of kidney in hydronephrosis.

Type of Obstruction and its Consequence

- **Sudden and complete obstruction:** It reduces the glomerular filtration and leads to **mild dilation** of the pelvis and calyces.
- **Subtotal or intermittent obstruction:** It does not suppress glomerular filtration, and **produces progressive dilation**.

Level of Obstruction

Depending on the level of urinary obstruction, the dilation may first affect the bladder, or ureter and then the kidney.

Gross

- Depending on the level of obstruction, it may be **unilateral or bilateral** and **may be accompanied by dilatation of ureter** (**hydroureter**).
- Depending on the degree and the duration of the obstruction, kidney may show **slight to massive enlargement**.
  - **Early stage**: Simple **dilation of the pelvis and calyces**
  - **Late stage**: Progressive **blunting of the apices of the pyramids**

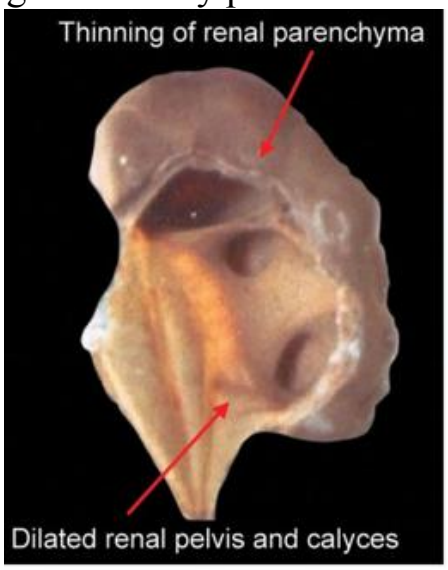
- **Advanced stage**:
  - **Kidney may appear** like a **thin-walled**, **cystic structure** having a diameter of up to 15–20 cm.
  - **Renal parenchyma shows destruction due to severe pressure atrophy and thinning of the cortex**. A kidney destroyed by longstanding hydronephrosis appear as a thin-walled, lobulated, fluid-filled sac **(Fig. 20.41).**

Microscopy

- **Tubules:** Dilatation and atrophy.
- **Glomeruli:** Partial or complete sclerosis.
- **Interstitium:** Marked chronic inflammatory infiltrate and fibrosis.

Clinical Features

- Clinical features **depend on the cause of obstruction**. For example, calculi in the ureters may present with renal colic, and prostatic enlargements may present with bladder symptoms.



Hydronephrosis: Pelvicalyceal dilatation causes atrophy and destruction of renal parenchyma.

Fig. 20.41: Marked hydronephrosis of the kidney showing marked dilation of the pelvis and calyces and thinning of the renal parenchyma.

- Unilateral complete or partial hydronephrosis may be silent due to maintenance of adequate renal function by the unaffected kidney.
- **Bilateral partial obstruction** may manifest as **polyuria and nocturia** due to inability to concentrate the urine (tubular dysfunction).
- **Complete bilateral obstruction** present with **oliguria or anuria**.
- **Ultrasonography** is **useful** in the diagnosis of obstructive uropathy.

Hydronephrosis: Dilation of the renal pelvis and calyces due to obstruction of urinary outflow.

Hydronephrosis: Most common complication of upper urinary tract obstruction.

Urolithiasis (Renal Calculi, Stones)

Q. Write short note on renal stones.

Stones may be formed anywhere in the urinary tract, but most are found in the renal pelvis and calyces of kidney.

- **Age**: **Peak is between 20 and 30 years**.
- **Sex: M**ore common in men than in women.

Terminology

- **Nephrolithiasis (renal stones)**—stones within the collecting system of the kidney.
- **Urolithiasis (urinary calculi/stones)**—stones anywhere in the collecting system of the urinary tract.
- A primary bladder stone is one that develops in sterile urine; it often originates in the kidney.
- A secondary stone occurs in the presence of infection, outflow obstruction, impaired bladder emptying or a foreign body.

Etiology

Q. Write short note on etiopathogenesis of renal stones.

- **Familial and hereditary predisposition** to stone formation is well-known. Many inborn errors of metabolism (like gout, cystinuria, and primary hyperoxaluria) are characterized by excessive production and excretion of stone-forming substances.
- **Other factors:**
  - Individual factors
  - Geography
  - **Diet:** Deficiency of vitamin A causes desquamation of epithelium and these cells may form a nidus on which a stone can be deposited.
  - Metabolic alterations

- **Altered urinary solutes and colloids:** Dehydration increases the concentration of urinary solutes and are liable to precipitate.
- **Infection:** It favors the formation of calculi. Stone formation are common when urine is infected with urea-splitting streptococci, staphylococci and, especially *Proteus*.
- **Decreased urinary citrate:** Citrate in urine present as citric acid and is under hormonal control. It tends to keep otherwise relatively insoluble calcium phosphate and citrate in solution. Urinary excretion of citrate is decreased during menstruation.
- Changes in urinary pH.
- Urinary stasis: It favors stone formation.

Pathogenesis of Renal Stones

Q. Write short note on pathogenesis of renal stones.

There are two main steps involved in stone formation: Initiation and propagation of stones.

1. Supersaturation

Initiation starts with the supersaturation of urine.

- **Increased urinary concentration of stone constituents:** It is the most important factor in stone formation → exceeds their solubility (supersaturation).
- **Decreased urine volume:** It may also favor supersaturation.

2. Precipitation of Crystals

- **Deficiency in inhibitors of crystal formation in urine enhances precipitation of crystals**. These inhibitors include: pyrophosphates, citrates, glycosaminoglycans, osteopontin, and a glycoprotein called **nephrocalcin**.
- All calculi consist of an organic mucoprotein matrix, which makes up 1–5% of the stone by weight. The mucoproteins in the urine provide the organic nidus on which the crystals form.

General Features of Renal Stones

- Stones are **unilateral** in **about 80%** of patients.

- **Number:** It may be **single or multiple**.
- **Sites:** Renal **calyces** and **pelvis** and in the **bladder**.
- **Shape:** Stones may have **smooth contours or** may be **irregular**, **jagged mass of spicules**.

Types of Renal Stones (Box 20.10)

Q. Write short note on types of renal stones.

1. Calcium Stones (Oxalate Calculus/Calcium Oxalate)

Most (80%) renal stones are **composed of calcium complexed with oxalate** (calcium oxalate) or **phosphate** (calcium phosphate) or a mixture of these (calcium oxalate + calcium phosphate). These stones are radiopaque.

Etiology (Box 20.10)

- **Hypercalciuria without hypercalcemia:** It is **observed in about 50% of patients**. Causes include: Hyperabsorption of calcium from the intestine (absorptive hypercalciuria), an intrinsic impairment in renal tubular reabsorption of calcium (renal hypercalciuria) or idiopathic.
- **Hypercalcemia and hypercalciuria:** It is found in about 10% of patients and may be due to hyperparathyroidism, diffuse bone disease, or sarcoidosis.

Q. Write short answer on causes of renal stones (conditions in which it occurs).

BOX 20.10: Types and causes of renal stones.

Types

1. **Calcium oxalate and/or calcium phosphate (~80%)**
   - Idiopathic hypercalciuria—most common
   - Hypercalciuria and hypercalcemia
   - Hyperoxaluria: Enteric, primary
   - Hyperuricosuria
   - Idiopathic
2. **Struvite (magnesium, ammonium, phosphate) (~10%)**
   - Urinary tract infection
3. **Uric acid (~7%)**
   - Associated with hyperuricemia
   - Associated with hyperuricosuria
   - Idiopathic
4. **Cystine (~2%)**
5. **Others/Unknown (~1%)**

Birefringent crystals in urine is seen in: Calcium oxalate stones.

- **Hyperuricosuria:** It causes "nucleation" of calcium oxalate in the collecting ducts.
- **Hyperoxaluria***:* It may be either hereditary (primary oxaluria) or acquired.
- **Idiopathic:** These stones develop without any identifiable cause.

MORPHOLOGY

- **Calcium oxalate** stone **(Fig. 20.42A)**: It is irregular in shape, **hard** and covered with **sharp projections**. **Hemorrhage** from the mucosa of the renal pelvis may be produced by its sharp edges and blood may cover the stone **making it to appear black**. It is radiodense.
- **Calcium phosphate** stone **(Fig. 20.42B): Soft and pale**.

2. Struvite Stones or (Triple Stones/Magnesium, Ammonium, Phosphate Stones)

Q. Write short answer on struvite stones.

They are composed of calcium phosphate often with magnesium and ammonium phosphate, and are **known as struvite stones or triple phosphate stones**.

Etiology

They **develop after infections of the urinary tract by urea-splitting bacteria** (e.g., ***Proteus***), which convert urea to ammonia → produces alkaline pH + slowing of urine flow → precipitation of magnesium, ammonium, phosphate (struvite), and calcium phosphate (apatite).

MORPHOLOGY

Q. Write short answer on staghorn calculus.

- **Yellow-white** and **solitary**
- **Hard to soft** and **friable**
- **Largest stones**, because normally large amounts of urea is excreted
- **Sometimes fill the pelvis and calyces** to **form a cast** of these spaces → referred to as a **staghorn calculus (Figs. 20.42C and D)**
- Even a very large staghorn calculus may be clinically silent for years until it produces hematuria, urinary infection or renal failure
- Easily seen on radiographic films.

Complications: Intractable urinary tract infection, pain, bleeding, and perinephric abscess.

3. Uric Acid and Urate Stones

Etiology

- Commonly found in patients **with hyperuricemia** (e.g., gout) and **diseases involving rapid cell turnover** (e.g., leukemias).
- However, more than 50% of patients have neither hyperuricemia nor increased urinary excretion of uric acid.
- **Uric acid is insoluble in acidic urine** and **urine pH below 5.5** may **predispose** to uric acid stones.

MORPHOLOGY

- Uric acid stones are **radiolucent;** this is in contrast to calcium stones, which are radiopaque.
- **Smooth**, **hard**, **and** vary from yellow to reddish-brown. Sometimes have multifaceted appearance.
- Usually **multiple** and are **less than 2 cm** in diameter.
- Show **lamination on cut section**.



Figs. 20.42A to D: (A) Calcium oxalate stone; (B) Calcium phosphate stone; (C) Staghorn calculus; (D) X-ray shows staghorn calculus (arrow).

4. Cystine Stones

Etiology

- Cystine stones are uncommon and associated with **cystinuria**, which is due to genetic defects in the renal reabsorption of cystine or other amino acids.
- Stones form at **low urinary pH** (acidic urine).

MORPHOLOGY

- Cystine stones are **small**, **round**, **smooth** and usually **multiple**
- **Yellow and waxy**.
- They are very hard and radiopaque because of their sulfur content.

Clinical Features of Renal Stones

- Stones may be **asymptomatic** or **may obstruct urinary flow or produce ulceration and bleeding**.
- Small stones **may pass into the ureters**, producing **colic** and ureteral obstruction.
- **Larger stones** cannot enter the ureters and are likely to **remain silent** within the renal pelvis. Larger stones may present with **hematuria**.

- Stones also **predispose to superimposed infection** and may also **cause significant renal damage**.

Complications of Renal Stones

- **Hematuria**
- **Hydronephrosis** due to obstruction
- **Pyelonephritis and pyonephrosis**
- **Carcinoma:** Stones can cause squamous metaplasia and later squamous cell carcinoma.

Renal stones: Most common cause of upper urinary tract obstruction.

Renal stones: Most common cause of upper urinary tract hematuria.

There are **four main types** of calculi:
1. **Calcium stones** (~80%): Composed of calcium oxalate or calcium phosphate or mixture of both.
2. **Triple stones or struvite stones** (~10%): Composed of magnesium, ammonium and phosphate.
3. **Uric acid stones** (~7%).
4. **Cystine stones** (~2%).

Renal stones: Calcium stones are most common.

Calcium renal stones: Most common metabolic cause is hypercalciuria.

Calcium oxalate:
- Needle-shaped/square envelope-shaped
- Radiopaque

Struvite stones: Develop after urinary tract infections by urea-splitting bacteria (e.g., *Proteus*).

Struvite (triple) stone:
- Coffin lid shaped
- Radiolucent

Uric acid:
- Diamond/barrel shape
- Radiolucent

Renal stones: 80% are radiopaque.

Cystine stone:
- Hexagonal
- Radiopaque

Renal stones: Ultrasound can detect only hydronephrosis and not the renal stone.

MALIGNANT TUMORS OF THE KIDNEY

CC: Classify and describe the etiology, genetics, pathogenesis, pathology, presenting features, progression and spread of renal tumors.

Both benign and malignant tumors can occur in the kidney. Most common malignant tumors are renal cell carcinoma and Wilms tumor. WHO classification (abridged) of tumors of the kidney is presented in **Box 20.11.**

Q. Classify tumors of kidney. Describe the etiopathogenesis and morphology of renal cell carcinoma.

Renal Cell Carcinoma (Adenocarcinoma of the Kidney, Hypernephroma, Grawitz Tumor)

Q. Write short note on renal cell carcinoma.

Renal cell carcinoma (RCC) is a **malignant tumor** of kidney.

- **Cell of origin:** The tumors arise from renal tubular epithelium.
- Because of their **gross yellow color** and the **microscopic resemblance of the tumor cells to clear cells of the adrenal cortex**; these tumors were **originally** thought to arise from embryonic adrenal rests and **were called hypernephroma**.

BOX 20.11: WHO classification of tumors of the kidney.

Renal cell tumors

- Clear cell renal cell carcinoma

- Papillary renal cell carcinoma
- Chromophobe renal cell carcinoma
- Carcinoma of the collecting ducts of Bellini
- Papillary adenoma
- Oncocytoma

Nephroblastic tumors

- Nephroblastoma (Wilms tumor)

Metanephric tumors

- Metanephric adenoma

Mesenchymal tumors

- Occurring mainly in children
  - Clear cell sarcoma
  - Rhabdoid tumor
- Occurring mainly in adults
  - Leiomyosarcoma (including renal vein)
  - Angiosarcoma
  - Rhabdomyosarcoma

Others

Metastatic tumors

- **Age:** Usually in the **sixth and seventh decades** of life.
- **Sex: More common in males** with male to female ratio of 2:1.

Etiology

Risk Factors

- **Tobacco:** Cigarette and pipe smoking
- Obesity (mainly in women)
- Hypertension
- Unopposed estrogen therapy
- Exposure to asbestos, petroleum products, and heavy metals
- Chronic renal failure and acquired polycystic disease
- Tuberous sclerosis.

Types

- **Sporadic: Most (96%) of RCC are sporadic**.
- **Hereditary/inherited/familial:** About **4% of RCC are inherited**. All hereditary RCC are **multifocal** and **bilateral**, and **occur at a younger age** than sporadic RCC. It **occurs in four distinct syndromes**.

1. **Von Hippel–Lindau (VHL) syndrome: *VHL* gene is a tumor suppressor gene** and is **involved in** the development of **both familial and sporadic clear cell carcinoma**. VHL patients develop bilateral renal cysts and multiple renal cell carcinomas.
2. **Hereditary leiomyomatosis and renal cell cancer syndrome:** It is an autosomal dominant disease due to mutations of the FH gene (expresses fumarate hydratase). It is characterized by leiomyomata (cutaneous and uterine) and an aggressive type of papillary carcinoma with increased tendency for metastatic spread.
3. **Hereditary papillary carcinoma:** It is **autosomal dominant** form with **multiple bilateral tumors** showing **papillary growth pattern**. These tumors show a several cytogenetic abnormalities and **mutations in the *MET* proto-oncogene**.
4. **Birt–Hogg–Dubé syndrome:** It is an autosomal dominant disease is due to mutations of the BHD gene (which expresses folliculin). It is characterized by lesions of skin (fibrofolliculomas, trichodiscomas, and acrochordons), lung (cysts or blebs), and renal tumors of various histological subtypes.

Classification of Renal Cell Carcinoma (Fig. 20.43)

Q. Write a short note on classification of renal cell carcinoma.

Both sporadic and familial forms of renal cell carcinoma can be **classified depending on the cytogenetics**, **genetics**, **and microscopic features** into **five major types**.

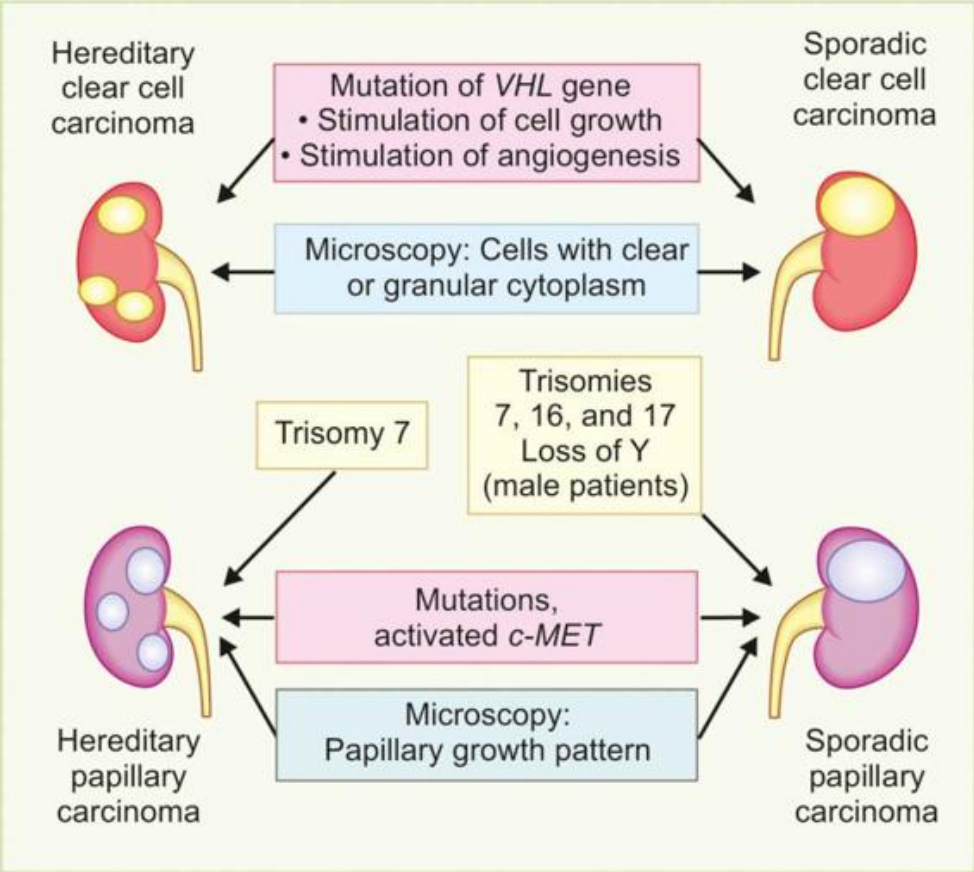
1. Clear Cell Carcinoma

- **Most common** type (~70%).
- **Most** (95%) are **sporadic**; but they **can be familial**.
- **Cell of origin:** Arise from **proximal tubular epithelial** cells.
- Usually occur as **solitary** and **unilateral lesions**.
- **Genetic mechanism**: *VHL* gene is localized to 3p, and acts as a tumor suppressor gene in both sporadic and familial forms of cancers. **Effects of loss or mutation of *VHL* gene are**:

- **Stimulation of cell growth**
- **Stimulation of angiogenesis:** *VHL* gene encodes a protein that degrades other proteins like hypoxia-inducible factor-1 (HIF-1).
  - Mutated *VHL* → HIF-1 is not degraded → HIF-1 levels remain high → increases the production of proangiogenic proteins such as VEGF, PDGF, TGF-α, and TGF-β → stimulates angiogenesis.

2. Papillary Carcinoma

- **~10–15%** of renal carcinomas.
- Occurs in both **familial** and **sporadic** forms **(Figs. 20.46A and B)**.
- **Cell of origin:** Arise from **distal convoluted tubule epithelial** cells.
- Frequently **multifocal** and **can be bilateral**.
- **Hereditary papillary RCC** is **not associated with deletions of** *VHL* **gene**.



Both sporadic and familial RCC are associated with: Loss of sequence on chromosome 3 either by translocation (3:6, 3:8, 3:11) or deletion. This region harbors the VHL gene.

Familial RCC is associated with: Von Hippel–Lindau syndrome.

Fig. 20.43: Cytogenetics, genetics and microscopic features of clear cell and papillary renal cell carcinoma.

- **Mutations in the** c-***MET***, **a proto-oncogene** (*MET*) is **found in both the sporadic and familial** forms.
- **Cytogenetic abnormalities**:
  - **Sporadic** form**: (1) trisomies 7**, **16**, **and 17, and (2) loss of Y in male** patients
  - **Familial/**hereditary **form: Trisomy 7**.

3. Chromophobe Renal Carcinoma

- ~5% of renal cell carcinomas

- **Cytogenetic abnormalities**: Multiple chromosome losses and extreme hypodiploidy
- **Cell of origin:** Arise from **intercalated cells of renal collecting ducts**
- **Excellent prognosis** compared to clear cell and papillary cancers.

4. Collecting Duct (Bellini Duct) Carcinoma

- ~ 1% or less of renal carcinoma
- **Cell of origin:** Arise from the **medullary collecting duct** and **occur in the medullary region**. Medullary carcinoma is a morphologically similar tumor developing in patients of sickle cell trait.
- **Cytogenetic abnormalities: No distinct pattern** but several chromosomal losses and deletions have been described.

5. Xp11 Translocation Carcinoma

It is a genetically distinct subtype of renal cell carcinoma that develops in young individuals. It is associated with translocations of the *TFE3* gene located at Xp11.2 with a number of partner genes that lead to overexpression of the TFE3 transcription factor. The tumor cells have clear cytoplasm and papillary architecture.

MORPHOLOGY

Q. Write short note on morphology of renal cell carcinoma.

Gross

A. General Features of RCC

- **Site:** May arise in **any part** of the kidney. More commonly affects the poles, **mostly upper pole**
- **Size varies**
- **Outer surface**: It is usually **bosselated and** may distort the renal outline.

B. Cut Surface (Figs. 20.44A and B)

- Tumor is **solid**, **bright yellow-gray-white** and shows **areas of hemorrhage**, **necrosis** and **cystic change**, which give a **variegated appearance**. The **yellow color** is due to **prominent lipid** in the tumor cells.
- Tumor may **appear as circumscribed**, **spherical masses with well-demarcated margins**. But may show some breach in the renal capsule and invasion into the perinephric fat.

C. Venous Invasion

- **One of the characteristics of RCC** is its **tendency to invade the renal vein**.
- Sometimes, the tumor may grow as a continuous cord in the inferior vena cava and rarely may extend into the right atrium.

Microscopy

Q. Write a short answer on microscopic variants of renal cell carcinoma.

1. Clear Cell Carcinoma (Figs. 20.45A and B)

- **Growth pattern**: Varies from **solid to trabecular (cord-like) or tubular** (resembling tubules or glands).
- **Tumor cells:** Tumors are **composed of cells with clear or granular cytoplasm** and are **nonpapillary**.
  - **Shape:** The tumor cells are **round or polygonal** in shape.
  - **Cytoplasm**:
    - **Abundant** either **clear or granular**.
    - **Clear cells** have sharply outlined cell membrane ("vegetable cells"). The clear cytoplasm is due to **glycogen and lipids**; which are removed by the water and solvents (xylene) used during processing of tissue for histopathological examination.
    - **Special stains: Glycogen may be demonstrated with PAS and fat by oil red O stains**.
  - **Nucleus:** It is **centrally located** and **small,** which falsely mask the malignant nature of this tumor.
- **Stroma: Consists of branching vasculature**.

  Most tumors are well-differentiated with **little cellular or nuclear pleomorphism**.

2. Papillary tumors (Fig. 20.46A)

It shows a **papillary growth pattern**.

- **Papillary configuration**: Tumor is composed of cuboidal or low columnar tumor cells arranged in papillary formations. Interstitial foam cells are common in the fibrovascular stalks/cores of papillary projections.

- **Cytoplasm** may be **eosinophilic or basophilic**.
- **Psammoma bodies may be seen**.
- Stroma is usually scanty and highly vascularized.

3. Chromophobe Renal Carcinoma (Fig. 20.46B)

It consists of **granular cells arranged in solid sheets** with a concentration of the largest cells around blood vessels. The tumor cells have prominent cell membranes, pale eosinophilic cytoplasma and a halo around the nucleus (perinuclear halo) .

4. Collecting Duct Carcinoma

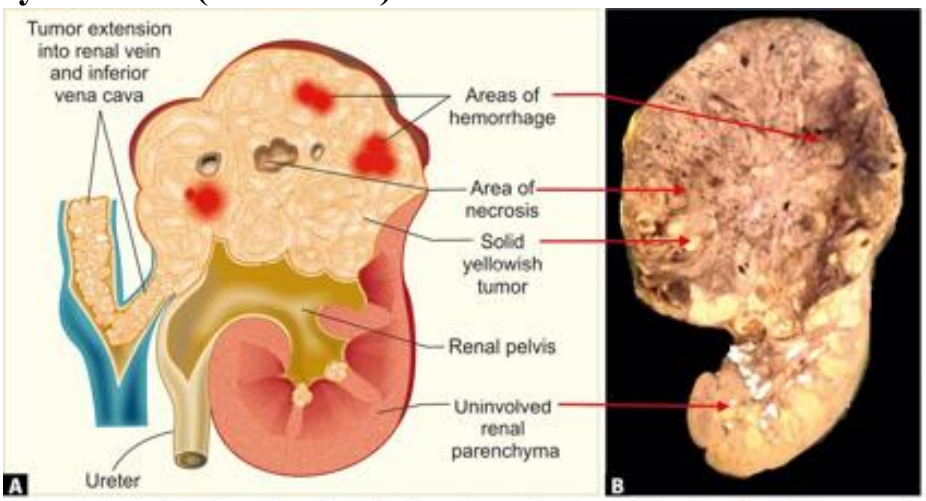
It consists of **nests of malignant cells separated by prominent fibrotic stroma**. It shows **irregular channels lined by highly atypical epithelium with a hobnail pattern**.

Clinical Features

Classical diagnostic triad (seen in only 10% of cases) of renal cell carcinoma are:

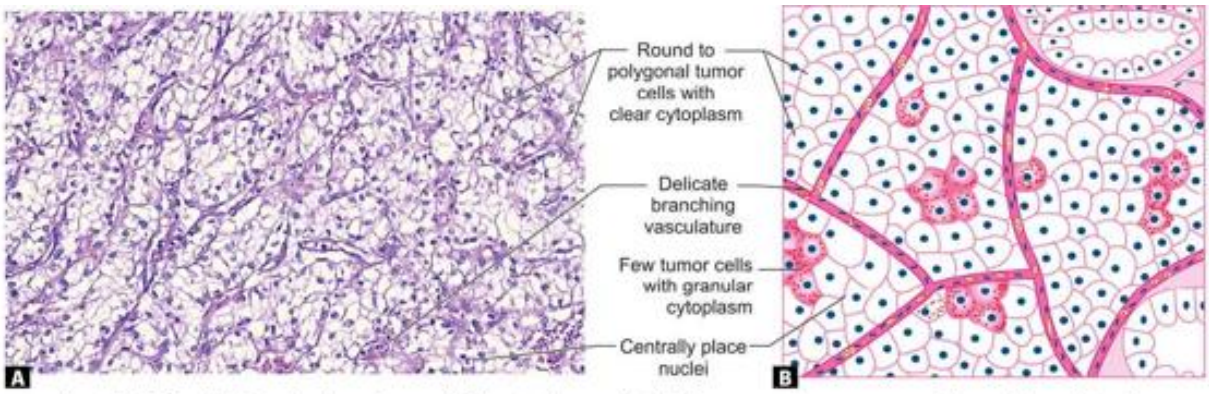
- **Costovertebral or flank pain**
- **Palpable abdominal mass**
- **Hematuria (most reliable)**.

Renal cell carcinoma **can produce ectopic hormones and paraneoplastic syndromes (Table 20.8).**

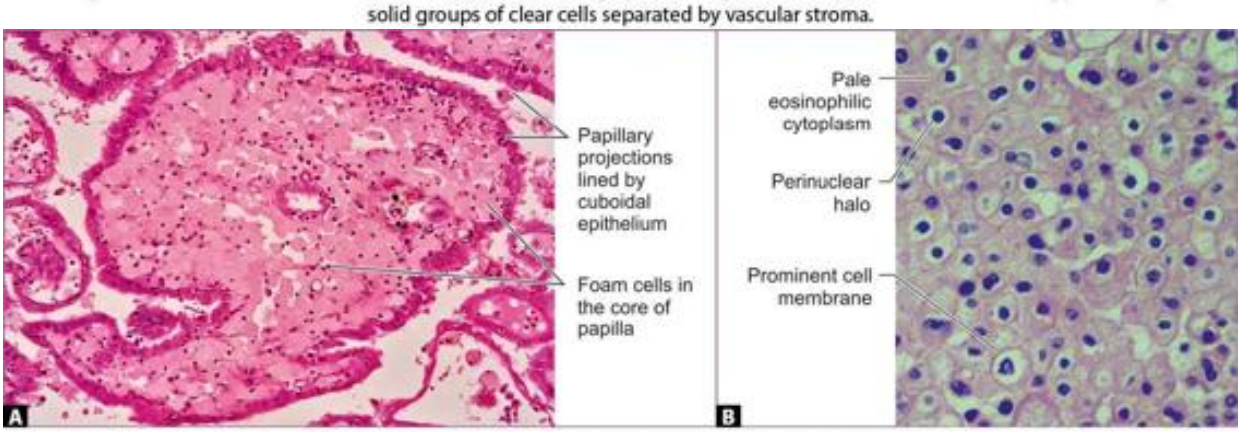


Papillary carcinoma of the kidney:
- Strong tendency to invade the renal vein and grow as solid column of cells.
- Tumor may even extend to the inferior vena cava and right side of the heart.

Figs. 20.44A and B: Cut surface of renal cell carcinoma showing a yellowish, spherical, circumscribed, variegated tumor at the upper pole of kidney: (A) (Diagrammatic); (B) Gross specimen.



Figs. 20.45A and B: Renal cell carcinoma: (A) Photomicrograph; (B) Diagrammatic appearance of clear cell type showing solid groups of clear cells separated by vascular stroma.



Figs. 20.46A and B: (A) Papillary carcinoma showing papillae lined by cuboidal tumor cells and fibrovascular core with foam cell; (B) Chromophobe renal carcinoma showing tumor cells with prominent membrane, pale eosinophilic cytoplasm and perinuclear halo.

Q. Write a short note on paraneoplastic syndromes produced by renal cell carcinoma.

Spread

Patients may **develop wide metastasis** before giving rise to any local symptoms or signs.

- **Local spread:** It invades perinephric fat.
- **Lymphatic spread:** It spreads to regional lymph nodes and occurs when tumor extends beyond renal capsule.
- **Hematogenous spread:** Most common route. Most common sites are lungs (cannon ball deposits and pulsating secondaries) → bones → liver → adrenal → brain.

Prognosis: Depends on the extent of tumor.

Q. A 55-year-old man presents to urology department with complaints of feeling of fullness and pain in right flank of his abdomen, and gross painless hematuria. He also informed that hr lost about 7 kg weight over the last 6 months.

On physical examination, a right-sided flank mass was palpable on bimanual examination. Urine examination showed 3+ hematuria, but no protein, glucose, or leukocytes. Abdominal CT scan showed a 9-cm mass in the upper pole of the right kidney. A right-sided nephrectomy was done.

(a) What is the probable diagnosis? [Ans: Renal cell carcinoma (RCC)].

(b) How do you classify this disease of the kidney? (refer pages 705-6 and Box 20.11).

(c) Describe the gross and microscopic appearance of the kidney in this disease (refer pages 707-8).

(d) List the paraneoplastic syndromes produced by this disease (refer Table 20.8).

Most common type of RCC is sporadic.

Most common histological type of RCC: Clear cell carcinoma.

Most common type of RCC seen with dialysis associated cystic disease: Papillary carcinoma.

VHL syndrome: Autosomal dominant cancer syndrome, characterized by:

- Cerebellar hemangioblastomas
- Retinal angiomas
- Clear cell RCC
- Pheochromocytoma
- Cysts in various organs

Triad of VHL syndrome:
- Cerebellar hemangioblastoma
- Bilateral renal cell carcinoma
- Retinal angiomatosis

Clear cell carcinoma: Associated with homozygous loss of tumor suppressor gene VHL. VHL gene is localized to 3p.

Papillary carcinoma of kidney: Most common cytogenetic abnormalities are trisomies 7,16, and 17.

Papillary carcinoma of kidney: Trisomy in familial form due to activating mutations of MET oncogene on chromosome 7.

RCC associated with best prognosis: Chromophobe carcinoma.

Renal cell carcinoma: Yellow tumor with variegated appearance.

RCC: Yellow color is due to lipid in the tumor cells.

Renal cell carcinoma: Tendency to invade renal vein.

Clear cell RCC: Nucleus has raisin appearance.

Notable features of RCC:
- Encapsulated in spite of being malignant (pseudocapsule)
- Spontaneous regression
- Prolonged period of stable disease
- Refractoriness to cytotoxic agents

Sarcomatoid changes develop infrequently in all types of renal cell carcinoma and are of poor prognostic feature.

Clinical features of RCC: Triad of
1. Flank pain
2. Abdominal mass
3. Hematuria

Classical traid is seen in only 10% of cases.

Renal cell carcinoma: Paraneoplastic syndrome with ectopic production of erythropoietin and PTH-related peptide.

Erythropoietin: Major source is interstitial cells in peritubular capillaries and tubular epithelial cells.

Wilms Tumor (Nephroblastoma)

Q. Write short note on Wilms tumor.

- Wilms tumor is **most common primary renal tumor of childhood**.
- **Highly malignant primary embryonal tumor**.

TABLE 20.8: Paraneoplastic syndromes produced by renal cell carcinoma.

• Polycythemia (due to erythropoietin)	• Hepatic dysfunction (due to IL-6 leading to increased ALP, PT and bilirubin)
• Hypercalcemia (due to PTH-rp)	• Feminization or masculinization
• Hypertension (due to renin)	• Cushing's syndrome

Canon ball pulmonary metastases: Characteristic features of:
- RCC (produces spherical or round cannon-ball metastases)
- Testicular cancer

- **Age group**: Most common between **2 and 5 years of age**, and more than 95% occur below 10 years of age.

Pathogenesis and Genetics

- In **most (90%)** cases, the Wilms tumor is **sporadic and unilateral**.
- About 5–10% are bilateral which involve either simultaneously (synchronous) or one after the other (metachronous).
- **Mutation in tumor suppressor genes** is associated with Wilms tumor. These include **Wilms tumor associated genes 1 (*WT1*) and** *WT2*. ***WT1* gene is located in the chromosome 11p13**.
- **In about 5% of cases**, Wilms tumor **arises in three congenital syndromes** at an early age and often bilaterally. These syndromes are:
  1. **WAGR (**for **W**ilms tumor, **a**niridia, **g**enital anomalies, and mental **r**etardation) **syndrome:** It has germline deletions of 11p13, region where Wilms tumor-associated gene *(WT1)* is located.
  2. **Denys–Drash syndrome (**Wilms tumor, intersexual disorders, glomerulopathy**)** is associated with mutations of the *WT1* gene.
  3. **Beckwith–Wiedemann syndrome (BWS)** is associated with Wilms tumor and *WT2* gene imprinting abnormalities.
- Mutations of the β-*catenin* gene were found in few sporadic cases of Wilms tumors. Other recurrent mutations may be seen in genes encoding proteins involved in microRNA processing (DROSHA, DGCR8, and DICER1).

- **Nephrogenic rests** (small foci of persistent primitive blastemal cells, which are precursor lesions of Wilms tumors) are seen in the renal parenchyma adjacent to bilateral Wilms tumors.
- Wilms tumor was **also found in association with other malignancies** (e.g., osteosarcoma, retinoblastoma, hepatocellular carcinoma and neuroblastoma).

MORPHOLOGY

Q. Write short note on morphology of Wilms tumor.

Gross (Fig. 20.47)

- Wilms tumor is **usually large**, **single**, **round**, **well-circumscribed** mass.
- **Usually unilateral** but 10% is either bilateral or multicentric.
- **Cut section**:
  - Tumor is **soft**, **bulging**, **homogeneous**, **and tan to gray**.
  - Foci of hemorrhage, cyst formation, and necrosis may be seen.

Microscopy (Figs. 20.48A and B)

- Tumor shows **three major components**, which resemble normal fetal tissue. These cells attempt to recapitulate different stages of nephrogenesis.

- The **three types of cells** are:
  1. **Blastemal component:** It consists of **small**, **round to oval blue cells** with **scanty cytoplasm**. These cells are arranged in sheets, nests and trabeculae.
  2. **Immature stromal** (mesenchymal) **component:** It consists of **undifferentiated fibroblast-like spindle cells or myxoid tissue**. They **may show smooth muscle, skeletal muscle or fibroblast differentiation**.
  3. **Immature epithelial component: Epithelial cells show differentiation** in the form of **small abortive (embryonic) tubules or immature glomeruli**.

Classically, the tumor shows **triphasic** (all three cell types) combination, although the percentage of each component varies. Occasionally, they contain only two elements (biphasic) or even only one (monophasic).

- **Anaplasia:** It is defined as the **presence of cells with large, hyperchromatic, pleomorphic nuclei and atypical mitotic figures**. Anaplasia is **associated with acquired *TP53* mutations** and such tumors **do not respond to chemotherapy**.

Clinical Features

- Most children present with an **abdominal mass**, when large it may extend across the midline and down into the pelvis.
- Others: **Hematuria**, **pain in the abdomen**, intestinal obstruction, and pulmonary metastases are other patterns of presentation.

Spread

- **Local spread:** It spreads to perirenal soft tissues.
- **Lymphatics:** It spreads to regional lymph nodes.
- **Hematogenous:** Lungs, liver and peritoneum.

Prognosis

- **Clinical parameter:** Children **younger than 2 years** of age have a **better prognosis**.

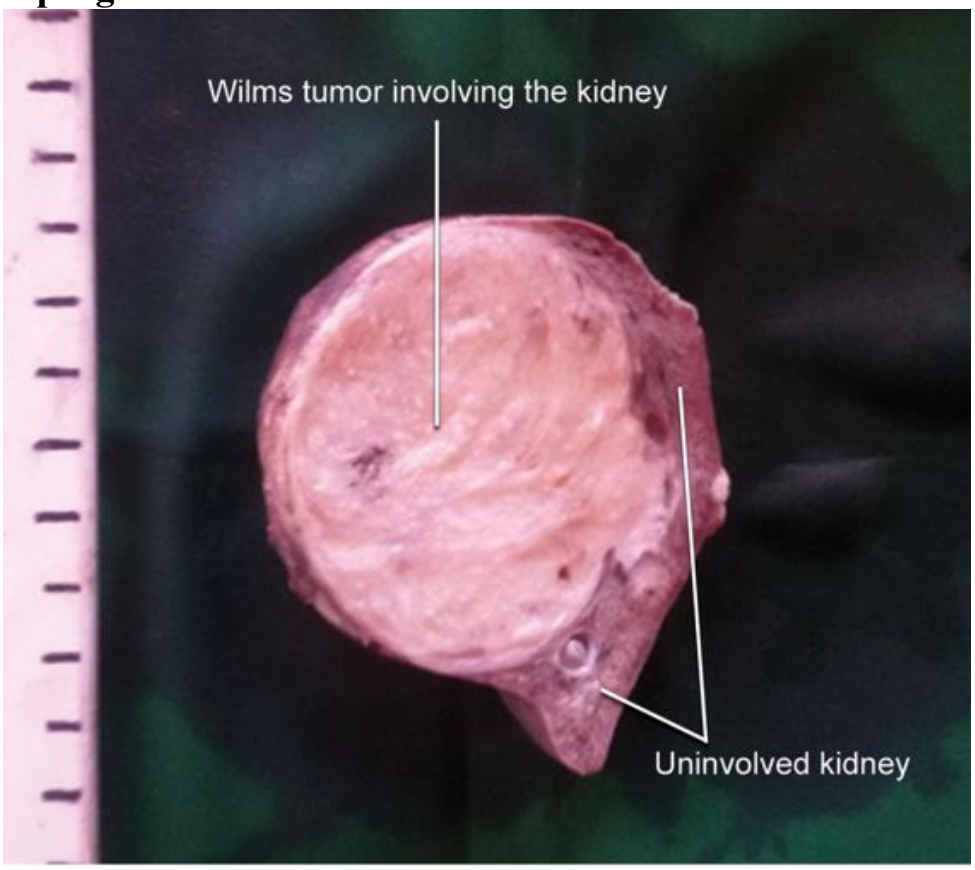
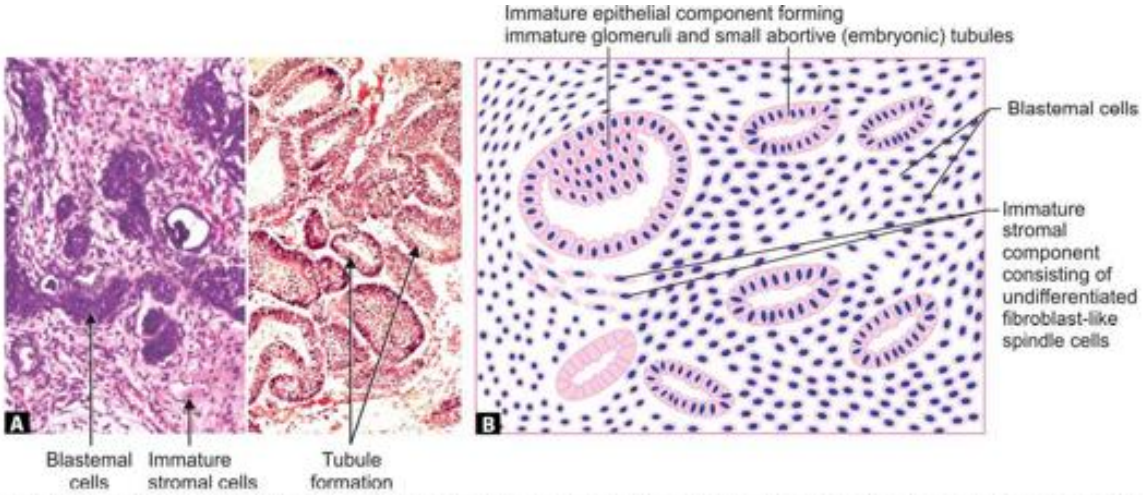


Fig. 20.47: Wilms tumor of the kidney with well-circumscribed margins.



Figs. 20.48A and B: (A) Hematoxylin and eosin (H & E); (B) Diagrammatic. Wilms tumor shows highly cellular areas composed of tightly packed blue cells (undifferentiated blastema) separated by loose stroma containing undifferentiated mesenchymal cells, and immature (primitive) tubules.

- **Histological parameter:**
  - **Invasion of the renal capsule** is associated with **poor prognosis**.
  - **Anaplasia** indicates a **poorer prognosis**.

Wilms tumor: Most common primary kidney tumor in children (2–5 years). Second most common malignant abdominal tumor in children.

WT1 gene: Tumor suppressor gene located in the choromosome 11p13.

Wilm tumor: Deletion of short arm of choromosome 11p13.

Wilms' tumor: Associated congenital syndromes
- WAGR syndrome
- Denys–Drash syndrome
- Blackwith–Wiedmann syndrome

WT1 and PAX6 genes are located at chromosome 11p13.

Wilms tumor microscopy: Three components:
1. Blastemal
2. Immature stromal
3. Immature epithelial

Anaplasia in Wilms tumor: Associated with:
- *TP53* mutations
- Resistance to chemotherapy

Wilms tumor:
- Abdominal mass
- Microscopic hematuria

UROTHELIAL TUMORS

NC: Describe the etiology, genetics, pathogenesis, pathology, presenting features and progression of urothelial tumors.

Q. Write short note on transitional cell carcinoma of urinary bladder.

- **About 95% of bladder tumors are of epithelial origin**. Most of the epithelial tumors of the bladder are composed of urothelial (transitional) cell type and are known as **urothelial (transitional) tumors**.
- Urothelial tumors form about 90% of all bladder tumors. These tumors may range from small **benign lesions to aggressive cancers**.
- Many of urothelial tumors are **multifocal** and are most commonly seen in the **bladder**. But they may develop at any site where there is urothelium, from the renal pelvis to the distal urethra.

Precursor Lesions

Two precursor lesions of invasive urothelial carcinoma are:

1. **Noninvasive papillary tumors:** They show a range of atypical changes in the urothelial cells, and are graded according to their biological behavior.
2. **Flat noninvasive urothelial carcinoma:** It is known as **carcinoma in situ or CIS** and is characterized by cytologic changes of malignancy.

Epidemiology

- **More common in developed** than in developing countries, and in **urban** than in rural dwellers.
- These tumors are usually **not familial**.
- **Sex:** Higher in **males** than in females (male-to-female ratio is 3:1).
- **Age:** Most between **50–80 years** of age.

Pathogenesis

Q. Write short answer on causes of urinary bladder (urothelial) tumors.

A. Risk Factors of Urothelial Carcinoma

- **Cigarette smoking:** It is the **most important risk factor** and risk depends on the amount of smoking and smoking habits. Most are associated with the use of cigarettes. Cigars, pipes and smokeless tobacco are associated with a minor risk.
- **Industrial exposure to arylamines**: The **aromatic amines (β-naphthylamine) and azo dyes** that were widely used in the past **in the aniline dye and rubber industries** are associated with bladder carcinoma. The cancers develop 15–40 years after the first exposure.

Mechanism of action (Flowchart 20.3):

- **Schistosoma hematobium** infection: It is a risk factor in endemic areas (Egypt, Sudan). More than three-fourth of the cancers are **squamous cell type**, and remaining urothelial type.
- **Analgesics:** Long-term use of analgesics is a risk factor.
- **Cyclophosphamide:** It produces hemorrhagic cystitis, and increases the risk of carcinoma of the bladder.
- **Radiation:** Previous exposure of the bladder to irradiation (for other pelvic malignancies).

B. Genetic Alterations in Urothelial Carcinoma

- **Chromosome 9 monosomy or deletions:** It frequently occurs in superficial papillary tumors and rarely in noninvasive flat tumors. The 9p deletions (9p21) involve 2 tumor suppressor genes: *p16 (INK4a)* and *p15*.
- **Mutations in** *p53* are found in CIS and urothelial carcinoma.

MORPHOLOGY

Q. Write short answer on morphology of urothelial tumors.

General morphological features of urothelial tumors are:

Site: Most urothelial tumors arise from the **lateral or posterior walls at the bladder base**.

Number: Single or multicentric producing separate tumors.

WHO classification (2016) of urothelial tumors (abridged) is presented in **Box 20.12.**

Gross (Figs. 20.49A to D)

- **Purely papillary:** These tumors appear as **red, elevated excrescences**. Size varies from small (less than 1 cm in diameter) to large masses (up to 5 cm in diameter). Majority of papillary tumors are low grade.
- **Nodular**.
- **Flat**.

Microscopy

Noninvasive Urothelial Lesions

Urothelial papilloma

It is uncommon (<4% of noninvasive bladder neoplasms) and consists of two histological forms: Classical exophytic papilloma and inverted papilloma.

Exophytic papillomas

They appear as a **delicate**, **finger-like papillary structures.** They have a central **core of loose fibrovascular tissue** covered by **urothelium that is microscopically have normal appearance and thickness**.

Inverted papillomas

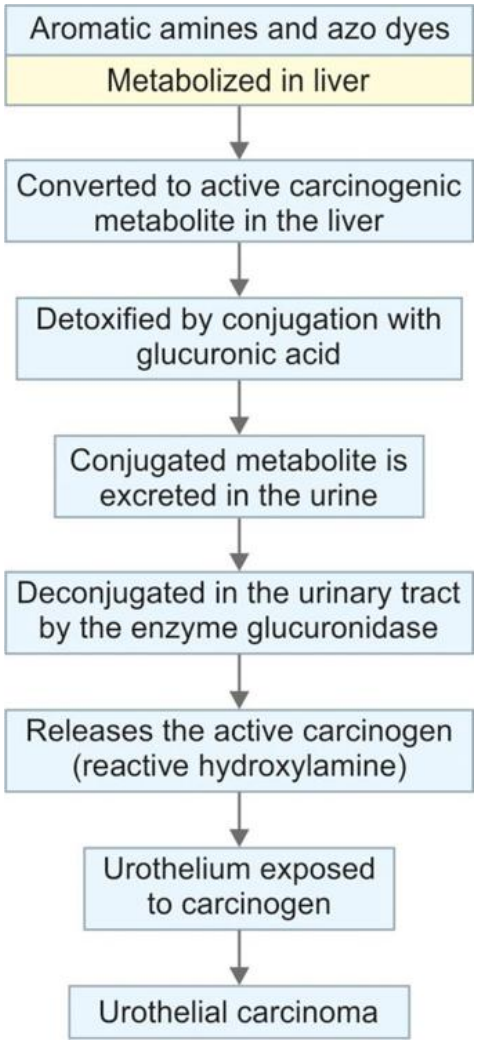
These are rare and appear as nodular lesion in the mucosa of the urinary bladder, usually in the trigone area. They consists of **invagination of inter-anastomosing cords of normal transitional epithelium, down into the lamina propria**.

Papillary Urothelial Neoplasms of Low Malignant Potential (PUNLMPs)

Definition: PUNLMP is a papillary urothelial tumor with minimal atypia.

- Cystoscopically, they present as single or multiple discrete exophytic papillary lesions of variable size.

Flowchart 20.3: Mechanism of action of aromatic amines and azo dyes.

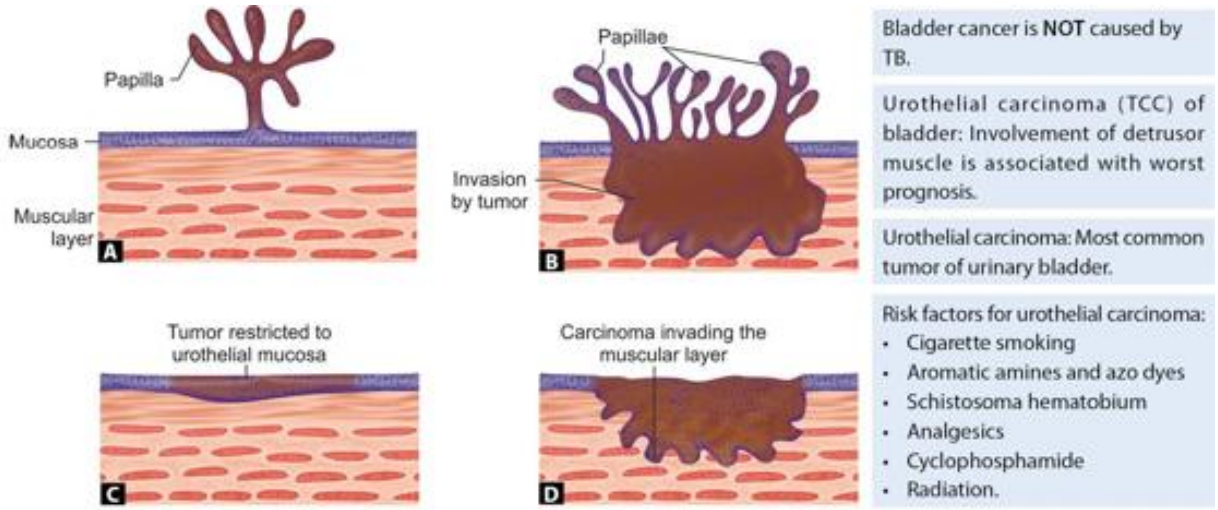


BOX 20.12: WHO classification (2016) of urothelial tumors (abridged).

Noninvasive urothelial lesions

- Papilloma
  - Urothelial (exophytic) papilloma
  - Inverted urothelial papilloma
- Papillary urothelial neoplasm of low malignant potential
- Noninvasive papillary urothelial carcinoma
  - Low-grade
  - High-grade
- Urothelial carcinoma in situ

Infiltrating urothelial carcinoma



Figs. 20.49A to D: Four morphologic patterns of tumors of urinary bladder: (A) Papilloma-papillary carcinoma; (B) Invasive papillary carcinoma; (C) Flat noninvasive carcinoma (CIS); (D) Flat invasive carcinoma.

(CIS: carcinoma in situ).

- **Papillae:** They consist of papillae with a **fibrovascular core** covered by urothelium. The lining **urothelium is thicker and/or more cellular than seen in normal urothelium and in papilloma**.
- **Cytological features:** The cells have a monotonous appearance (cells virtually identical to each other). The nuclei may be slightly enlarged and more crowded relative to normal urothelial nuclei. Nucleoli are either absent or inconspicuous. The chromatin is uniformly even, without the scattered hyperchromatic nuclei seen in low-grade papillary carcinoma. **Mitotic figures are rare**. There is no loss of polarity.
- **May recur** but have a lower risk of recurrence than low-grade urothelial carcinoma.

Noninvasive Papillary Urothelial Carcinoma

Definition: Papillary urothelial carcinoma is a papillary urothelial neoplasm with some cytological and architectural disorder seen at low to intermediate magnification. There is no invasion beyond the basement membrane (Ta).

Low-grade noninvasive papillary urothelial carcinomas (Figs. 20.50A and B)

- **Papillae:** It consists of **branching and delicate papillae**.
- **Cytological features:**
  - **Papillae** are **lined by neoplastic transitional epithelium** with **orderly appearance,** both architecturally and cytologically.
  - **Polarity is maintained**.
  - **Minimal nuclear atypia:** Mild variation in nuclear size and shape **(nuclear pleomorphism)**, **scattered hyperchromatic nuclei and occasional mitotic figures (usually non-atypical) may be** seen, mainly away from the basement membrane.
- Can recur.

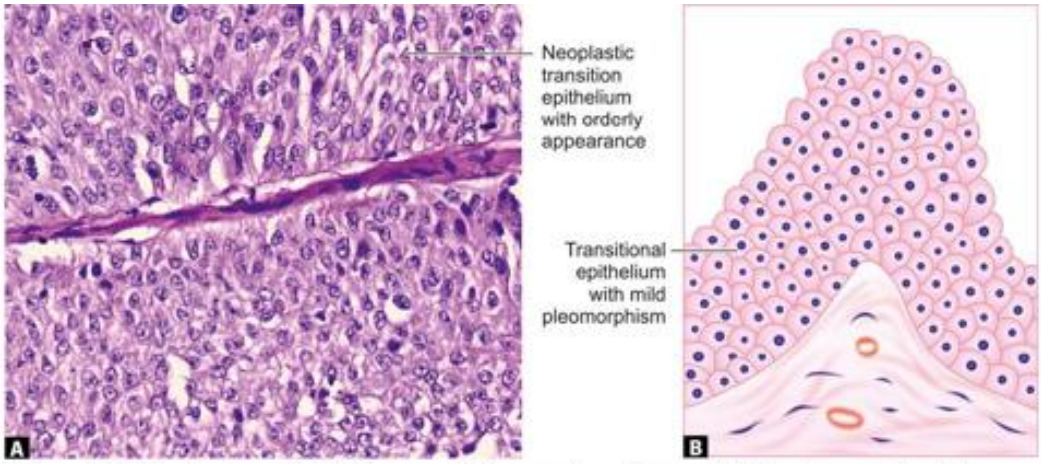
High-grade noninvasive papillary urothelial carcinoma(Fig. 20.51)

- **Papillae:** It consists of **fused**, **branching papillae** giving them a more solid exophytic appearance.
- **Cytological features:**
  - **Loss of polarity:** Architecturally, the epithelium is disorganized and consists of discohesive cells with frequent loss of polarity. Some of the tumor cells show frank anaplasia.
  - **Moderate to severe nuclear atypia** and irregular prominent nucleoli.
  - **Significant** variation in nuclear size and shape **(moderate to marked nuclear pleomorphism)**.
  - **Significant nuclear hyperchromasia**.
  - **Frequent mitotic figures** in all layers, including atypical mitotic figures.
- **Invasion:** About 80% of high-grade urothelial carcinomas show invasion into the lamina propria, muscular layer or entire thickness of the bladder wall.
- **Metastasis:** About 40% of invasive tumors may metastasize.
  - Regional lymph nodes
  - Hematogenous dissemination to liver, lungs, and bone marrow.
- **Prognosis:** About 60% recurr and may progress to ivade lamina propria and invasion of muscularis propria in 25% and 5%, respectively.

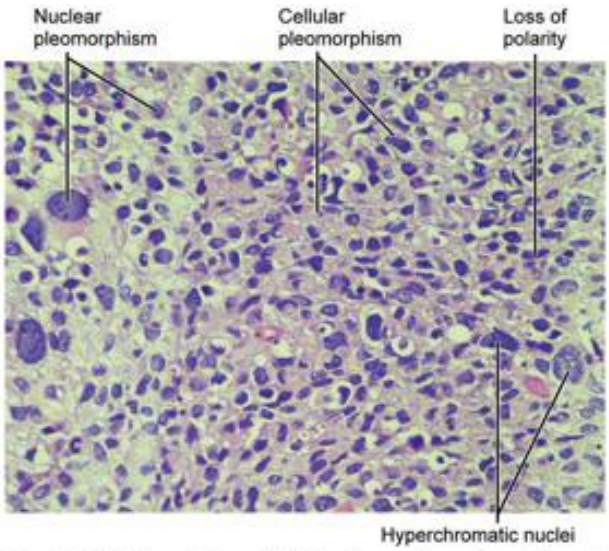
Urothelial Carcinoma In Situ

Definition: Urothelial carcinoma in situ (CIS) is a flat urothelial lesion without papillary structures. The lining urothelium consists of cytologically malignant cells is of variable thickness.

Gross: On cystoscopy, it appears as areas of erythema (may be focal, multifocal, or diffuse) and may be difficult to identify. The mucosa may appear slightly raised, with a velvety, granular appearance.



Figs. 20.50A and B: (A) Photomicrograph; (B) Diagrammatic. Low-grade papillary urothelial carcinoma with orderly appearance of transitional cells.



Exfoliated markers for detection of bladder cancer:
- Newer markers: BTA test, urinary nucler matrix protein (NMP22)-detects cancer specific proteins in urine (BTA/NMP22)
- Hyaluronidase, Lewis-X antigen on exfoliated urothelial cells
- Determination of telomerase activity in exfoliated cells

Urothelial carcinoma (TCC): Painless hematuria is the most common symptom.

Urothelial carcinoma (TCC): Multifocal tumor and may recur.

Carcinoma of bladder: Most common lymph node involved is obturator.

Urothelial carcinoma of bladder: Most common noninfectious cause of lower urinary tract painless hematuria.

Fig. 20.51: High-grade urothelial carcinoma showing nuclear and cellular pleomorphism, hyperchromatic nuclei and loss of polarity.

Microscopy: Urothelial CIS is characterized by the presence of tumor cells without invasion of basement membrane. The tumor cells need not occupy the entire thickness of the urothelium and the presence of isolated malignant cells (pagetoid spread) is enough for a diagnosis of CIS.

Cytological features

Cells: Appearance of **individual tumour cells may be quite variable**.

- Some tumor cells may be **large** (about 5–6 times the size of a lymphocyte) having **abundant eosinophilic cytoplasm**. They may have an either **pleomorphic or uniformly large hyperchromatic nuclei.**
- In some cases, the tumor cells may be **small with relatively scanty basophilic cytoplasm.** They may contain atypical, hyperchromatic nuclei.
- **Loss of cell polarity** is found.

Nuclei: Large **pleomorphic and hyperchromatic nuclei** with one or more irregular nucleoli. May show **irregular nuclear crowding**. Mitoses may or may not be present with occasional atypical forms.

CIS may be associated with foci of microinvasion.

Prognosis: A significant number of patients with CIS respond to intravesical instillation of BCG, but almost all cases eventually recur, and as about 25% of cases progress to invasive disease.

Infiltrating Urothelial Carcinoma

It is common malignant neoplasm of the urinary bladder.

Definition: Infiltrating urothelial carcinoma is characterized by divergent differentiation of lining urothelial cells and invasion beyond the basement membrane. It was **formerly called as transitional cell carcinoma** (not recommended).

Gross: Can be unifocal or multifocal. Most are polypoid, sessile, or ulceroinfiltrative. Sometimes they may show papillary architecture.

Microscopy: **Remarkable for its diversity of morphological manifestations** and can present with a wide range of architectural patterns.

Architectural patterns include variably· sized **nests** with smooth borders, **sheets, trabeculae, cords**, and **single cells**. There is often a **mixture of patterns**. In larger nests, the tumour cells may show stratified architecture, with the nuclei lined up perpendicular to the basement membrane and with some maturation towards the centre **recapitulating the structure of urothelium**. However, most tumours, there is no resemblance to normal urothelium.

Cytological features:

- **Nuclei**: Characteristically, they show **striking nuclear pleomorphism** with variably sized and shaped **hyperchromatic nuclei**. Nuclei are irregular.
- **Mitotic figures**: **Numerous** mitotic figures and **abnormal mitotic figures** are present.
- **Cytoplasm**: Usually pale to eosinophilic and moderate to abundant. This can produce a squamoid appearance, which should be distinguished from true squamous differentiation.

Grade: Majority of invasive urothelial carcinomas are **high-grade**. Since significant **outcome differences have been found between low- and high-grade invasive tumors,** it is necessary to identify the grade of tumor.

Pattern of invasion: It has prognostic significance. Tumors with the **more infiltrative cords and single cell patterns have worse prognosis.**

Clinical Course of Bladder Cancer

- **Painless hematuria**: Sometimes, it may be the only clinical feature. Sometimes, the hematuria may be associated with frequency, urgency and dysuria.
- **Complications:** When the tumor obstructs the ureteral orifice, it may lead to pyelonephritis or hydronephrosis.
- **Recurrences:** Urothelial tumors, irrespective of their grade may recur, usually at different sites than the original tumor.
- **Prognosis:** It depends on the histologic grade and the stage at the time of diagnosis.

Laboratory diagnosis: Cytologic examinations of urine for malignant cells and biopsy of the tumor.

CC: Identify and describe common urinary abnormalities in a clinical specimen.

Various components of normal urine with their reference value is presented in **Table 20.9**.

TABLE 20.9: Various components of normal urine with their reference value.

TABLE 20.9: Various components of normal urine with their reference value.

Component	*Reference value*
Volume—24 hours	600–1800 mL (variable)
pH	5.0–9.0
Specific gravity, quantitative (random)	1.002–1.028 (average 1.018)
Protein—24 hours urine	<150 mg/day
Protein, qualitative (random)	Negative
Glucose, quantitative—24 hours urine	50–300 mg/day
Glucose, qualitative (random)	Negative
Urobilinogen—24 hour urine	1.0–3.5 mg/day
Microalbuminuria (24 hours)	0–30 mg/24 hours (0–0.03 g/day) (0–30 µg/mg creatinine)(0–0.03 g/g creatinine)

Case 1

History: A 8-year-old boy was brought by the mother to pediatric outpatient with chief complaints of puffiness of face, particularly more marked in the morning, mild pitting edema on both legs, malaise with nausea and a slight fever for about one month. Mother also complained that he passes dark brown (red or cola) colored (smoky) urine and the urine output was reduced (oliguria). On enquiry, mother informed that about 2 weeks back the child had upper respiratory tract infection. On examination, the blood pressure was 140/100 mm Hg (moderate hypertension).

Urine Examination

Urine color	Brown (smoky)
Quantity	700 mL/day (reduced)
Proteinuria	+
Glucose	Nil
Microscopy	
RBC	++
RBC cast	+
WBC (leukocyte)	Occasional

Serological examination: Raised anti-streptolysin O titer and low serum complement level.

Questions

1. What is the most probable diagnosis?
2. Describe the light microscopy, immunofluorescence and electron microscopic findings.
3. What is the prognosis of this disease?
4. What are the causes of hematuria?

Answers

1. Nephritic syndrome due to acute post-streptococcal glomerulonephritis. The points in favors of nephritic syndrome are hematuria (gross or microscopic), RBC casts in the urine, and oliguria (due to decreased glomerular filtration rate), and mild to moderate hypertension. Two weeks back the child had upper respiratory tract infection, favors post-streptococcal disease.
2. Describe the light microscopy, immunofluorescence and electron microscopic findings (*refer* page 665)

.3What is the prognosis of this disease? (*refer* page 666)

4. Causes of hematuria (*refer* **Box 14.14** on page 400).

Case 2

History: A 6-year-old child was brought to the hospital for the complaint of increased puffiness around his eyes and he has become less active over the past 10 days.
Examination: On physical examination, the boy had facial puffiness which is more prominent around his eyes and also had swelling of both ankles. There was neither history of fever nor hypertension.

Urine Examination

Albuminuria/proteinuria	++++
Glucose	Nil
Microscopy	
RBC	-

RBC cast	-
Fat globules/ oval fat bodies	+
Protein in a 24-hour urine sample	3.8 g of protein

Serological examination: Serum creatinine and blood urea levels were normal.

He responded well to corticosteroid therapy.

Questions

1. What is the most probable diagnosis?
2. Describe the light microscopic, electron microscopic and immunofluorescence findings in this condition.
3. What are the causes of proteinuria?

Answers

1. Steroid-responsive proteinuria in a child is feature nephrotic syndrome due to minimal change disease and is the most common cause for nephrotic syndrome in children. Points favoring nephrotic syndrome are presence of massive/heavy proteinuria (>3.5 g of protein/24 hours), generalized edema (due to hypoalbuminemia) and presence of fat globules in urine (due to lipiduria). The leakage of large amounts of protein (>3.5 g per day) in urine results in hypoalbuminemia. The subsequent fall in plasma oncotic pressure leads to redistribution of extracellular fluid to the interstitial compartment, producing edema. Treatment by steroids achieve a complete remission in the majority of children. *Nephrotic* syndrome should be distinguished from *nephritic* syndrome (characterized by hematuria, uraemia and a degree of renal failure).
2. In *minimal change disease, glomeruli l*ook normal under light microscope. However, on electron microscopy, shows effacement of podocyte foot processes (fusion of podocyte foot processes) in the glomerulus and is the only pathologic finding found on electron microscopy.
3. Causes of proteinuria (*refer* **Box 14.6** on page 396).

Case 3

History: A 57-year-old female admitted to the hospital with a history of increasing back pain and fevers for the last 3 days. She is a type 2 diabetic on oral hypoglycaemic agents and has mild hypertension. There is no history of burning micturition or colicky abdominal pain or chronic ingestion of analgesics.
Examination: The patient is febrile (37.9°C). [Normal body temperature is about 37°C (98.6°F)]. On palpation, there is tenderness over the left loin without any tenderness on anterior abdomen.

Investigations

Type of investigation	*Value (normal range)*
Serum creatinine	0.9 mg/dL (0.6–1.2 mg/dL)
Serum urea	26 mg/dL (20–40 mg/dL)
Urine analysis	
Protein	+
Sugar	++
Ketone bodies	–
Urine microscopy	
Leukocytes	Plenty
RBCs	Occasional
Leukocyte casts	+
Granular cast	+

Questions

1. What is the likely diagnosis in this patient?
2. What is the significance of casts on examination of the urinary sediment?
3. What other investigation will be helpful in confirming the diagnosis?

Answers

1. History and urine findings suggest the it as an episode of acute pyelonephritis. Diabetic and immunocompromised patients are predisposed to urinary tract infection. The symptoms of increasing back pain and fevers without burning micturition suggests an upper tract rather than lower urinary tract infection.

2. The presence of casts indicates renal parenchymal disease. There are different types of cast and their significance are presented in **Figure 14.5** on page 401. The leukocytic casts are indicative of an infective renal parenchymal episode.
3. Urine for culture and sensitivity will help in identify the causative organism and sensitivity of the organism to the antibiotics.

Case 4

History: A 29-year-old female complains of fever and burning sensation when passing urine for the last 3 days. She also complains that she voids urine more often than usual without any sense of urgency and suffering from severe lower back pain that started 1 day back. She has not noticed any blood in her urine. She has never had this problem in the past and she is not taking any regular medications. There is no history of vaginal discharge or urinary incontinence except an episode of cystitis 3 months ago. She is sexually active and has two children and is currently not pregnant.

Examination: Her temperature is 39.4 °C [normal body temperature is about 37°C (98.6°F)]. Her pulse is 120 beats/min, and blood pressure is 106/70 mm of Hg. Abdominal examination shows suprapubic tenderness and marked tenderness in both loins. There was no tenderness at renal angle on either side. Examination of the cardiovascular and respiratory systems is unremarkable. Bowel sounds are normal.

Investigations

Type of investigation	Value (normal range)
Hemoglobin	13.5 g/dL (12.0–15.0 g/dL)
WBC count	22,000/µL (4,000–11,000/µL)
Platelets	334,000/µL (150,000–450,000/µL)
Serum creatinine	0.8 mg/dL (0.6–1.2 mg/dL)
Serum urea	24 mg/dL (20–40 mg/dL)
Urine analysis	
Protein	+
Sugar	–
Blood	+
Ketone bodies	–
Urine microscopy	Midstream urine was collected for microscopy, culture and sensitivity
Leukocytes	Plenty
RBCs	Occasional

Leukocyte casts	+
Granular cast	+
Urine culture and sensitivity	
E. coli grew after one day	Sensitivity to nitrofurantoin, trimethoprim, gentamicin and co-amoxiclav

Blood sent for culture and sensitivity.

Questions

1. What is the probable diagnosis?
2. List the common organisms causing this disease?
3. What are the complications of this disease?
4. What is Tamm Horsfall protein?

Answers

1. This patient is probably suffering from an uncomplicated urinary tract infection (UTI). UTIs are classified by anatomy into lower and upper UTIs. Upper UTIs refer to infection above the bladder, and include the ureters and kidneys. Infection of the kidney and renal pelvis is known as pyelonephritis. Lower UTIs refer to infections at or below the level of the bladder, and include cystitis, urethritis, prostatitis, and epididymitis. In this patient the recent onset of fever and back pain suggest an upper UTI. Plenty of leukocytes and leukocyte in the urine favors acute pyelonephritis. Acute pyelonephritis is much more common in females than males and occurs due to ascending bacterial infection from lower to upper urinary tract.
2. *Escherichia coli* causes 80–90% of UTI. For other causative organisms include other enteric gram-negative bacteria such as *Proteus, Klebsiella, Enterobacter and Pseudomonas* species.
3. Complications of acute pyelonephritis (*refer* page 685).
4. Tamm–Horsfall protein (*refer* page 700).

CC: Describe and interpret the abnormalities in renal function tests.

Case 5

History: A 65-year-old male complaints of vague history of generalized weakness anorexia, bony pains and impotence for the past few months. He informs that he had hypertension for the past 20 years which was poorly controlled. He was not visiting any medical professionale for the past 6 years. Irregularly he used to take diuretic for his blood pressure.
Examination: Physical examination reveals conjunctival pallor and his blood pressure is 180/100 mm of Hg.

Investigations

Type of investigation	*Value (normal range)*
Hemoglobin	10.2g/dL (13.0–17.0 g/dL
WBC count	8,000/µL(4,000–11,000/µL)
Platelets	210,000/µL (150,000–450,000/µL)
Mean cell volume	92 fL (82–100 fL)
Sodium	132(136–146 mEq/L)
Potassium	5.6 mEq/L (3.5–5.0 mEq/L
Urea	62 mg/dL (20–40 mg/dL)
Creatinine	37 mg/dL (0.6-1.2 mg/dL)
Urine analysis	
Urine specific gravity	1010 fixed specific gravity
Protein	+++
Urine microscopy	Granular casts and broad casts

Ultrasound shows both kidneys smaller than normal

Questions

1. What is the likely diagnosis?
2. What are the common causes of this condition?
3. What are the likely complications in this condition?
4. What is the mechanism of fixed specific gravity of 1010?

Answers

1. The blood tests show high serum creatinine and urea. The renal ultrasound shows small kidneys bilaterally, which suggests that this condition is long-standing. The probable diagnosis is chronic kidney

disease (CKD). It develops due to progressive impairment of renal function with a decrease in the number of functioning nephrons. Usually the patients are asymptomatic until late stage of CKD. In this patient, the blood pressure is 180/100 and there is associated pallor [normocytic anemia due to reduced erythropoietin (EPO) production by kidney in chronic kidney disease] most likely underlying cause of CKD is hypertension. The symptoms of generalized weakness anorexia, bony pains and impotence are the consequences of complications of chronic kidney disease.

2. Common causes of CKD are: (1) diabetes mellitus, (2) hypertension, (3) glomerulonephritis (*refer* **Box 20**.7, page 692, Important causes of chronic kidney disease).
3. Complications of chronic renal failure (*refer* page 693)
4. When specific gravity of urine is low and fixed at 1.010, this is known as isosthenuria. In this condition, the urine whose specific gravity (concentration) is neither greater (more concentrated) nor less (more dilute) than that of protein-free plasma (1.008–1.012). It is indicative of severe renal damage (chronic kidney disease) with disturbance of both the concentrating and diluting abilities of the kidney. It reflects damage to the kidney's tubules or the renal medulla.

CHAPTER
21

Male Genital Tract Disorders

PENIS

PRECURSOR LESIONS

Q. Write short note on premalignant lesions of penis.

Penile Intraepithelial Neoplasia

Definition: Penile intraepithelial neoplasia (PelN) is a histological alteration of the penile squamous epithelium characterized by dysplastic changes with an intact basement membrane. PelN is a putative precursor of invasive squamous cell carcinoma (SCC) of penis. It was also termed as **squamous cell carcinoma in situ**, **erythroplasia of Oueyrat** and **Bowen disease**.

Classification

Classification is discussed in **Box 21.1**.

MORPHOLOGY

Gross: Differentiation between the types and subtypes of PelN is not easy on gross appearance.

- **Differentiated PelN:** They commonly affect the foreskin appear as a solitary white or pink macule or plaque that may be slightly elevated.
- **Warty and basaloid PelN:** Usually affects the glans appear as flat to slightly elevated, velvety, moist erythematous, dark brown or black macules, papules, or plaques. Lesions may be smooth, warty, granular, or villous.

However, both types may affect any compartment of penis.

MICROSCOPY

Non-HPV-related PelN–Differentiated (simplex) PelN

It is characterized by a **thickened epithelium**, **elongation and anastomosis of rete ridges**, **abnormal maturation** (enlarged keratinocytes with abundant eosinophilic cytoplasm), **whirling and keratin pearl** formation in deep rete ridges, and **atypical basal layer cells** (with nuclear enlargement, hyperchromatic nuclei, and prominent nucleoli). Differentiated PelN is usually negative for HPV.

HPV-related PelN

- **Basaloid (undifferentiated) PeIN:** The full thickness of the squamous epithelium is replaced by immature cells. These immature cells are small, monotonous and are basophilic

with round to oval nuclei; inconspicuous nucleoli; and scant cytoplasm. Apoptosis and mitotic figures are prominent. They are positive for HPV16.

- **Warty (Bowenoid) PeIN:** This variant is characterized by a mild papillomatous surface. There is prominent cellular pleomorphism, koilocytosis (characterized by multinucleation, nuclei with irregular contours, perinuclear halo, and dyskeratosis), and mitoses. They are usually positive for HPV, but unlike in basaloid PelN, the genotypes are heterogeneous.
- **Warty-basaloid PeIN:** This variant shows a variable mixture of warty and basaloid cells observed in above two types. They have a spiking surface with koilocytic changes, whereas the lower half of the epithelium is mainly consisting of small basaloid cells. HPV is usually positive.

Most warty PelNs and basaloid PelNs fall within the high-grade category. When the atypia involves the full-thickness of the epithelium, it constitutes carcinoma in situ.

Bowenoid Papulosis

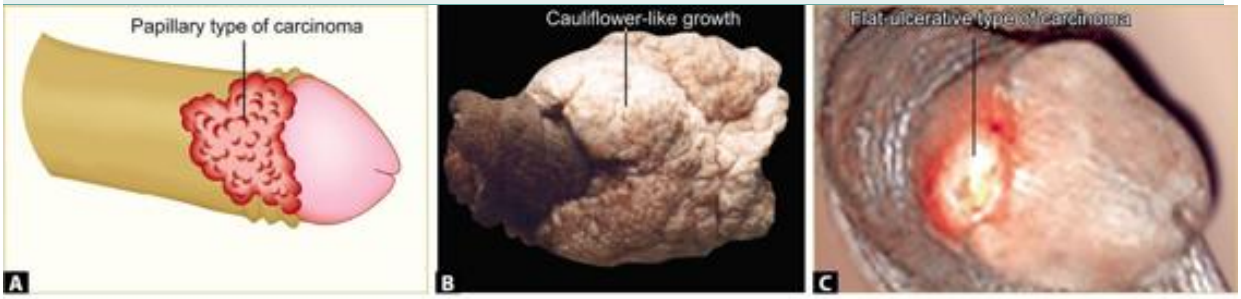
- **It occurs in sexually active adults**. In contrast to Bowen disease, it occurs at a **younger age** and the **lesions are multiple** (rather than solitary) **reddish-brown papular lesions**.

MORPHOLOGY

- **Sites involved:** In men—glans and shaft of penis and in women—perineal and vulvar areas.
- **Microscopy:** Bowenoid papulosis is **indistinguishable from Bowen disease** and is also related to HPV type 16.

BOX 21.1: WHO (2016) pathological classification of penile intraepithelial neoplasia (PelN).

1. **Non-HPV-related PelN:** Differentiated (simplex) PelN: Most commonly associated with usual and low- grade subtypes of SCC (non-HPV-related variants). It is usually associated with lichen sclerosus and other chronic inflammatory conditions.
2. **HPV-related PelN (most commonly HPV type 16):** These are associated with warty basaloid invasive carcinoma (HPV-related variants).
   - Basaloid PelN
   - Warty PelN
   - Warty-basaloid PelN
3. **Other rare patterns of PeIN:** Pleomorphic, spindle, clear cell, Pagetoid



Figs. 21.1A to C: Carcinoma of the penis: (A) Diagrammatic; (B) Papillary type of carcinoma (specimen). Shows glans penis deformed by a cauliflower-like growth; (C) Flat-ulcerative type of carcinoma.

- **Fate of the lesion:** (1) Tendency toward spontaneous resolution, (2) spontaneous regression, and (3) very rarely invasive squamous cell carcinoma.

INVASIVE CARCINOMA

CC: Describe the pathogenesis, pathology, presenting and distinguishing features, diagnostic tests, progression and spread of carcinoma of the penis.

Q. Write short note on carcinoma of penis.

- Squamous cell carcinoma (SCC) of the penis is not a common malignancy.
- **Age:** Carcinomas are commonly found in between **40 and 70 years** of age.

Etiology

- **Circumcision:** It has a **protective role**, and hence very rare among Jews and Muslims.
  - Circumcision is associated with **better genital hygiene**, and **reduces exposure to carcinogens** that may be concentrated **in smegma**.
  - It also **decreases** the likelihood of **infection with** potentially **oncogenic types of HPV** (HPV type 16 and HPV 18).
- **HPV DNA** can be detected in squamous cancer of penis in about 50% of patients.
- **Cigarette smoking** raises the risk of carcinoma of the penis.

MORPHOLOGY

Site: Squamous cell carcinoma of the penis usually develops on the **glans or inner surface of the prepuce** near the coronal sulcus.

Gross (Figs. 21.1A to C)

Two macroscopic patterns of carcinoma are: Papillary and flat.

1. **Papillary lesions:** They appear similar to condylomata acuminata and usually produce a **cauliflower-like fungating mass**.

2. **Flat lesions:** They appear as **areas of epithelial thickening** accompanied by fissuring of the mucosal surface. **With progression**, it appears as an **ulcerated papule**.

Microscopy (Fig. 21.2)

Both the papillary and the flat lesions show squamous cell carcinomas with varying degrees of differentiation. Microscopic features are similar to squamous cell carcinoma in other regions of the body (*refer* page 868).

Clinical Features

- Invasive squamous cell carcinoma of the penis present as a slowly growing and locally invasive lesion.
- They usually do not produce pain until they undergo ulceration and infection.
- They may metastasize to inguinal lymph nodes. The prognosis depends on the stage of the tumor.

Spread and Metastases

- **Local spread:** It can occur from one epithelial compartment to the other and from superficial to deep anatomical levels. Spread may be horizontal or vertical. Local spread into the urethra is common.

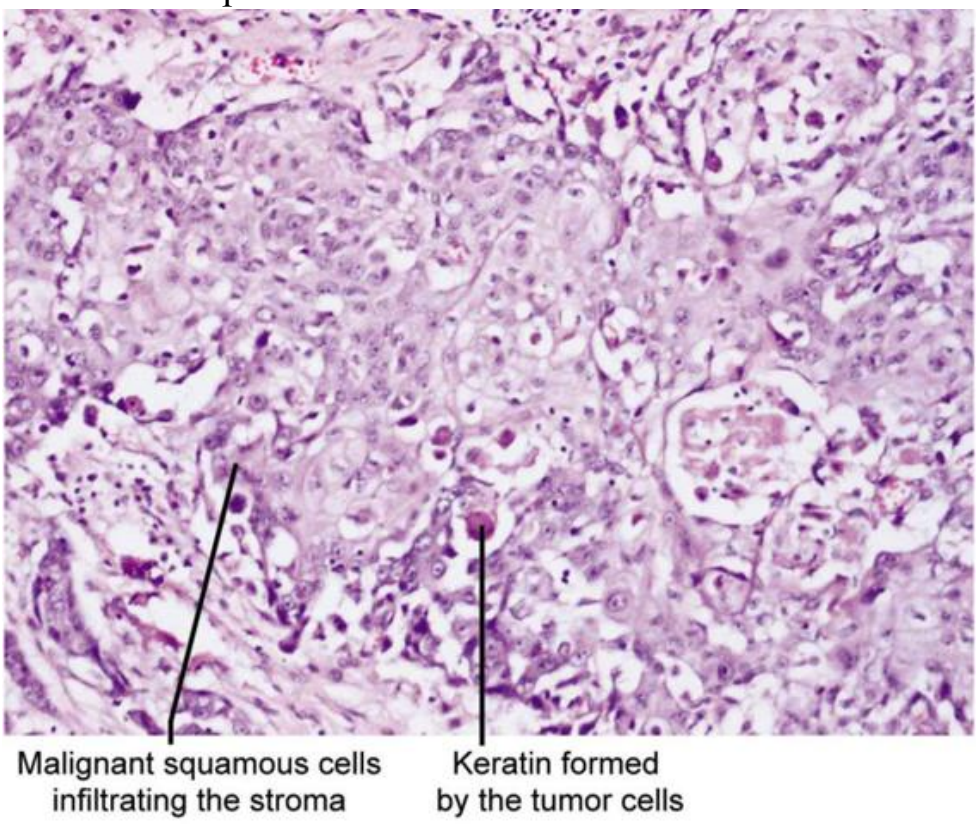


Fig. 21.2: Microscopic appearance of squamous cell carcinoma of penis.

- **Lymphatic spread**: It usually first spread to superficial inguinal lymph nodes ("sentinel node"), then to deep inguinal and pelvic lymph nodes, and last to the periaortic nodes in the retroperitoneum. Inguinal lymph node metastases are found in about 15% of cases. Since carcinomas of penis are often associated with secondary infection, it commonly produces enlargement of inguinal nodes. Lymph node metastases are usually not common in exophytic tumors whereas it is common in deeply invasive tumors.
- **Hematogenous spread:** It develops only as a late event. The usual sites of metastatic deposits are liver and lungs.

Penis: Squamous cell carcinoma and its precursor lesions may be associated with HPV infection.

Carcinoma penis: Squamous cell carcinoma.

Circumcision: Protective role against carcinoma of penis; decreases infection with HPV 16 and 18.

Penis: Squamous cell carcinoma occurs on the glans or inner surface of the prepuce.

Squamous cell carcinoma of penis
Two gross patterns: (1) Papillary, (2) Flat.

Verrucous carcinoma: Exophytic well-differentiated variant of squamous cell carcinoma which has low malignant potential.

Verrucous carcinoma: Locally invasive, but they rarely metastasize.

Carcinoma of penis: Most common lymph node involved is inguinal.

PROSTATE

PROSTATITIS

NC: Describe the etiology, pathogenesis, pathology and progression of prostatitis.

Definition: Prostatitis is inflammation of the prostate.

Classification: Prostatitis can be classified into three categories.

1. **Acute bacterial prostatitis:** It is caused by the organisms (coliform uropathogens such as *E. coli, Klebsiella, Proteus, Pseudomonas, Enterobacter,* gonococci, staphylococci and streptococci) that produce acute urinary tract infections. It constitutes 2–5% of cases. **Usually it develops as a complication of other urinary tract infections. It results from reflux of infected urine into the prostate.**
2. **Chronic bacterial prostatitis:** It is also caused by common pathogens involved in urinary tract infections and they constitute 2–5% of cases. ***It may or may not be preceded by an episode of acute prostatitis.***
3. **Chronic nonbacterial prostatitis:** ***Sometimes in chronic prostatitis, no causative organism is identified.*** Some of them may be due to *Chlamydia trachomatis, Mycoplasma* or *Ureaplasma urealyticum.* It is usually diagnosed after excluding the other causes.

4. **Granulomatous prostatitis:** It is usually caused due to instillation of Bacille Calmette-Guérin (BCG) within the bladder for treatment of superficial bladder cancer. BCG is an attenuated tuberculosis strain that produces a granulomatous immune reaction. Microscopically it is indistinguishable from tuberculosis. Other causes of granulomatous prostatitis are tuberculosis*(M. tuberculosis)* and fungal (*Histoplasma capsulatum*) granulomatous prostatitis (in immunocompromised hosts).
   - **Nonspecific granulomatous prostatitis:** It may develop as a foreign-body reaction to fluids that leak into tissue from ruptured prostatic ducts and acini or following surgery (postsurgical prostatic granulomas).
5. **Chronic pelvic pain syndrome:** It form majority of prostatitis (90–95% of cases). It can be subdivided into (i) inflammatory (associated with leukocytes in prostatic secretions), and (ii) noninflammatory cases (no leukocytes in prostatic secretion). The etiology is not known and its diagnosis is by exclusion.

Diagnosis: It is usually not diagnosed based on biopsy, because the microscopic findings are nonspecific and if biopsy is performed on an infected prostatitis, it can result in sepsis. However, in *granulomatous prostatitis,* which may cause induration of prostate and biopsy should be done to rule out prostate cancer.

MORPHOLOGY

Microscopy

Acute prostatitis: Shows acute inflammatory infiltrate in the prostatic acini and stroma.
Chronic bacterial prostatitis: It shows infiltration by lymphocytes, plasma cells and macrophages.

Clinical Features

- **Acute bacterial prostatitis:** It presents with sudden onset of fever, chills, dysuria (intense discomfort on urination), perineal pain, and bladder outlet obstruction. It may be complicated by sepsis. If acute prostatitis is suspected, digital rectal examination should not be done because it can produce bacteremia. Most patients respond well to antibiotics.

- **Chronic bacterial prostatitis:** It usually presents with recurrent urinary tract infections interspaced by asymptomatic periods. Presenting features include low back pain, dysuria, perineal and suprapubic discomfort and burning at the urethral meatus. The urine usually contains bacteria. Diagnosis is made by detection of more than 10–12 leukocytes/high power fields in expressed prostatic secretions, and by positive culture of urine specimen. It is treated also with antibiotics.
- **Chronic pelvic pain syndrome:** It is characterized by chronic pain localized to the perineum, suprapubic area, and penis. Prominent feature is pain during or after ejaculation. Therapy for chronic pelvic pain syndrome is empirical and depends on the nature of the symptoms.

BENIGN PROSTATIC HYPERPLASIA ORNODULAR HYPERPLASIA

CC: Describe the pathogenesis, pathology, hormonal dependency presenting and distinguishing features, urologic findings and diagnostic tests of benign prostatic hyperplasia.

Q. Describe the pathogenesis of nodular hyperplasia/benign hyperplasia of prostate.

- Benign prostatic hyperplasia (BPH) is characterized by **hyperplasia of both prostatic stromal and epithelial cells. They form** large, fairly discrete (separate) nodules in the **periurethral region of the prostate**.
- When the nodules become sufficiently large, they compress and narrow the urethral canal and cause **partial or complete obstruction of the urethra and urinary outflow**.
- **Age:** Very common disorder in men **50 years of age**.

Etiology and Pathogenesis (Fig. 21.3)

Q. Etiopathogenesis of nodular hyperplasia of prostate.

In BPH, there are **increased number of epithelial cells and stromal cells** in the **periurethral area** of the prostate.

- **Epithelial cells: Increased number** of epithelial cells is not due to increased epithelial cell proliferation but mainly **due to reduction of the**

rate of cell death → results in the **accumulation of senescent epithelial cells**.

- **Stromal cells: Increased** due to **proliferation.**

Role of Androgen

- Androgens are **required for the development of BPH**.
- It **increases cellular proliferation** and also **inhibits cell death**.
- **Source: Main source of androgen** (90% of total prostatic androgens) in the prostate is **dihydrotestosterone (DHT)**.
- **Synthesis:** DHT is **formed** in the prostate **from the conversion of testosterone** by the **type 2 5α-reductase enzyme** present **in the stromal cells** and **few basal cells**. This **enzyme** (type 2 5α-reductase) is **not present in the epithelial cells** of the prostate. **Thus**, **stromal cells are responsible for androgen-dependent growth of the prostate**.
- **Actions of DHT: DHT binds to the nuclear androgen receptor** (AR) present in **both epithelial and stromal cells** of the prostate. **DHT** is **more potent than testosterone** because: (1) **i**t has a **higher affinity for AR** and (2**) forms a more stable complex with AR**.

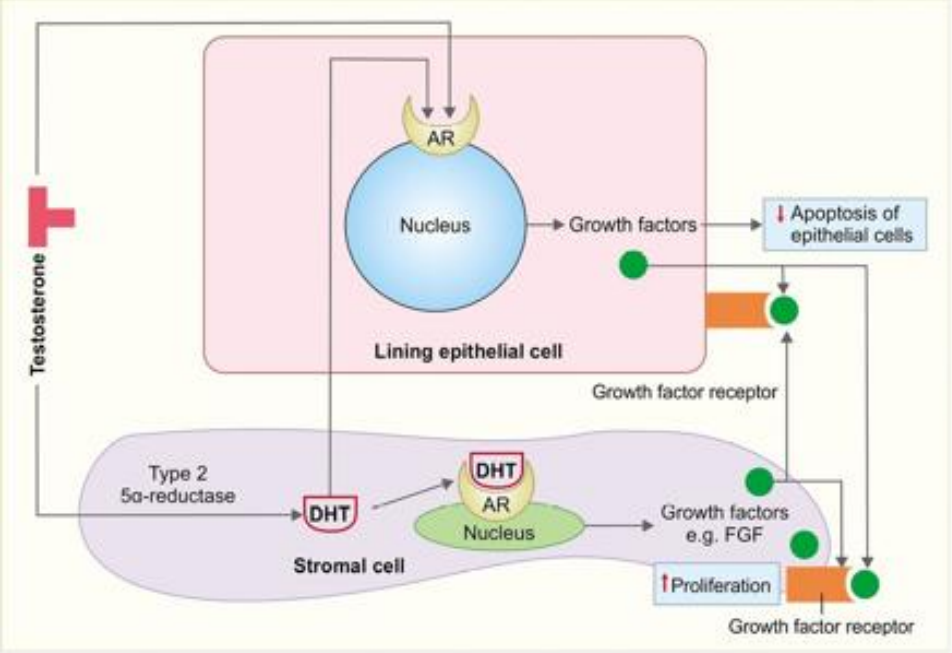


Fig. 21.3: Pathogenesis of prostatic hyperplasia. The stromal cells play a central role in the synthesis of dihydrotestosterone (DHT) from testosterone by type 2 5α-reductase. (AR: androgen receptor; FGF: fibroblast growth factor).

- DHT binding to AR **activates the transcription of androgen-dependent genes**, which results in the **increased production of**

many growth factors and their receptors. DHT-induced growth factors increase the proliferation of stromal cells and decrease the death of epithelial cells. Most important growth factors are:

- Fibroblast growth factor (FGF) family: FGF-7 (keratinocyte growth factor) is an important growth factor produced by stromal cells and mediate prostatic growth by paracrine mechanism.
- Other growth factors: FGFs 1 and 2, and TGF-β cause fibroblast proliferation.

MORPHOLOGY

Q. Describe the morphology of nodular hyperplasia of prostate.

Gross (Figs. 21.4A to C)

- **Weight:** Usually weighs **2 to 4 times the normal weight** and ranges from 60 g–100 g.
- **Sites of BPH:** Nodular hyperplasia begins in the **submucosa (the inner portions of the prostate) of the proximal urethra (transition zone)**.
- **Consequences:**
  - As the hyperplastic **nodules enlarge**, they may **compress** the centrally located **urethra** to a slit-like orifice and also the more peripherally located normal prostate.
  - Sometimes, hyperplastic **nodule may project up into the floor of the urethra** as a hemispheric mass directly beneath the mucosa of the urethra. This is called by the clinicians as **median lobe hypertrophy** (**Figs. 21.4A to C** and *refer***Fig. 2.28**). It does not correspond to the anatomical middle lobe.
- **Cut-section:** It shows **multiple circumscribed nodules** without any true capsules and compresses the surrounding prostatic tissue, which creates a plane of cleavage between nodule and the normal prostatic tissue. The nodules may appear solid or contain cystic spaces (represents dilated glands). **Nodules vary in color and consistency** depending on the predominant component.
  - Nodule **with predominant glands:** They appears **yellow-pink**, **honeycombed and have soft consistency**. Milky-white prostatic fluid oozes out from these areas.
  - Nodule with **predominant fibromuscular stroma:** They appear **pale gray**, **and are firm/tough in consistency**. These do not exude fluid.

Microscopy (Figs. 21.5A and B)

Q. Write a short note on microscopy of benign hyperplasia of prostate.

Nodularity: It is the characteristic feature of BPH.

Composition of the Nodules

Proliferation of three types of cells in variable proportions.

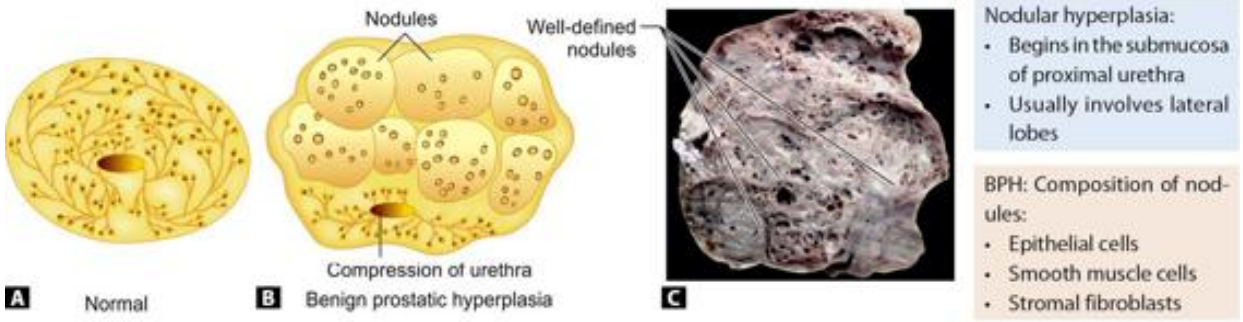
1. **Epithelial cells** (Acini and ductules): Their proliferation (adenomatous/glandular) leads to formation of small to large to cystically-dilated glands or acini.
   - **Glands are lined by two layers of cells**, an inner tall columnar and an outer basal layer of cuboidal or flattened epithelium.
   - Characteristic feature is **papillary structures,** which project into the glandular lumen and are lined by columnar epithelial cells.
   - **Corpora amylacea** (eosinophilic-laminated concretions) are commonly seen within the acini.
2. **Smooth muscle cells.**
3. **Stromal fibroblasts.**

Types of Nodules

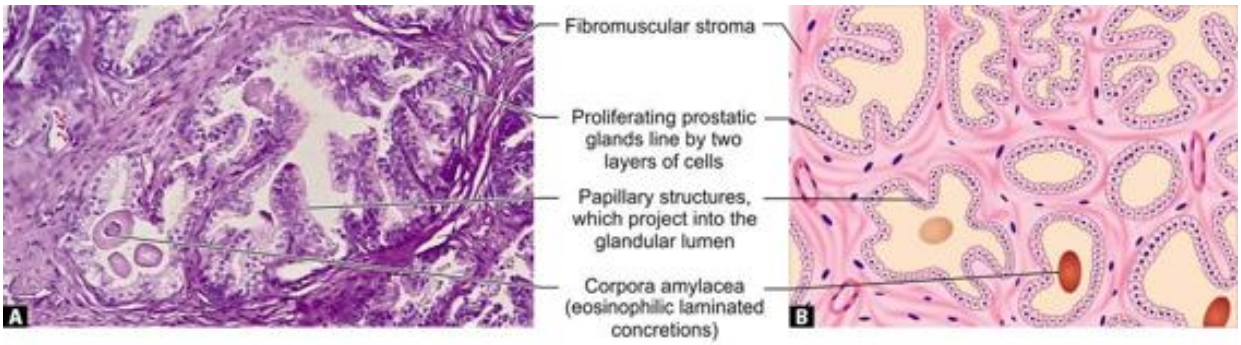
- Five types are recognized and depend on the proportion of type of cells: **(1) stromal (fibrous), (2) fibromuscular, (3) muscular, (4) fibroadenomatous, and (5) fibromyoadenomatous (most common)**.
- **Other findings**: (1) foci of lymphocyte infiltration, (2) areas of infarction, and (3) foci of squamous metaplasia at the edges of the infarcts.

Clinical Features

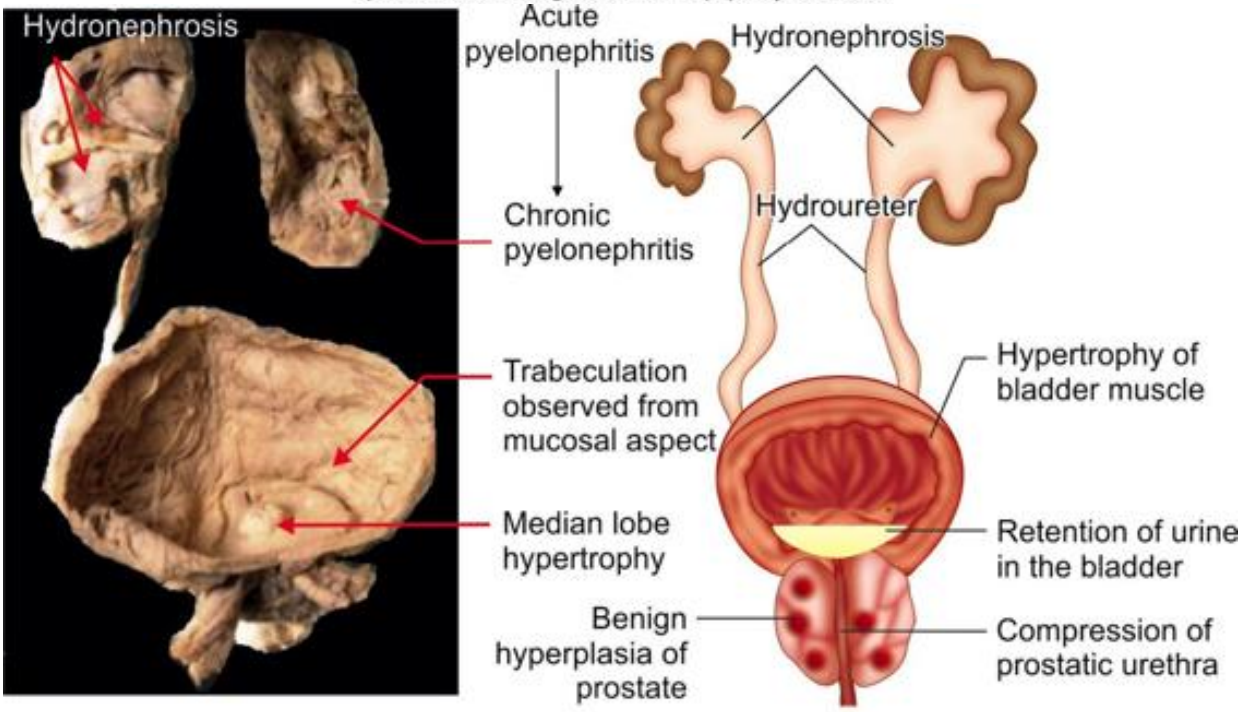
Clinical manifestations of prostatic hyperplasia occur in only about 10% of men with BPH. Nodular hyperplasia compresses the prostatic urethra and results in bladder outlet obstruction.



Figs. 21.4A to C: Schematic representation of cut-section of prostate: (A) Normal; (B) Benign prostatic hyperplasia consisting of well-defined nodules; (C) Nodular prostatic hyperplasia. Cut section shows numerous well-defined nodules which compressed the urethra into a slit-like lumen.



Figs. 21.5A and B: (A) Photomicrograph; (B) Diagrammatic appearance of benign prostatic hyperplasia showing proliferation of epithelial cells forming acini, few with papillary structures.



Complications of BPH:

- Hypertrophy of bladder
- Cystitis
- Pyelonephritis
- Hydronephrosis
- Bladder diverticula

5α-reductase inhibitors (e.g., finasteride) and α1a receptor antagonists (e.g., tamsulosin) can be used for treatment of BPH.

BPH: Hyperplasia of both prostatic stromal and glandular epithelial cells.

BPH: Incidence is age related.



Figs. 21.6A and B: Complications of benign hyperplasia of prostate: (A) Gross appearance of hypertrophied bladder with a nodule projected up into the floor of the bladder. Both kidneys show hydronephrosis and chronic pyelonephritis; (B) Diagrammatic representation.

- Increasing urinary frequency
- Difficulty in urination
- Urine retention.

Complications (Figs. 21.6A and B)

Q. Write short note on complications (secondary effects) of nodular hyperplasia of prostate.

- **Hypertrophy of bladder:** Obstruction to urinary outflow leads to hypertrophy and distension of the bladder accompanied by **retention of urine**. Trabeculation of bladder wall may develop, which can lead to **diverticula**.
- **Infection:** Inability to empty the bladder completely leads to residual urine in the bladder which is liable for infection (**cystitis**, and consequent ascending infection may cause **pyelonephritis**).

- **Consequences of obstruction:** Prolonged severe obstruction with back-pressure (in the absence of appropriate treatment) results in **hydroureter**, **hydronephrosis**, and ultimately death due to renal failure.

Treatment: Initial treatment is use of targeted therapeutic agents that inhibit the formation of DHT from testosterone (such as 5-a reductase inhibitors) or that relax prostatic smooth muscle by blocking α1-adrenergic receptors. Surgical treatment is reserved for severely symptomatic cases that do not respond to medical therapy.

BPH: Most common cause of prostate enlargement in males above 50 years of age.

BPH mostly originates from transitional zone of prostate whereas carcinoma mostly arises from peripheral zone. BPH is not considered as a premalignant lesion.

BPH: Microscopically shows nodules composed of variable proportion of proliferating stroma and glands. The hyperplastic glands are lined by two cell layers (i) inner columnar, and (ii) outer flat basal cells.

ADENOCARCINOMA OF PROSTATE

CC: Describe the pathogenesis, pathology, hormonal dependency presenting and distinguishing features, diagnostic tests, progression and spread of carcinoma of the prostate.

- Acinar adenocarcinoma is an invasive carcinoma consisting of neoplastic prostatic epithelial cells with secretory differentiation arranged in a variety of histomorphological patterns. These patterns include glands, cords, single cells, and sheets. Basal cells are typically absent.
- Ductal adenocarcinoma is a subtype of prostatic adenocarcinoma with large glands lined by tall pseudostratified columnar cells.

Adenocarcinoma of the prostate is mainly two histological types namely acinar and ductal. Acinar adenocarcinoma is the **most common malignant tumor in males**.

Etiology and Pathogenesis

Factors Involved in Carcinoma of Prostate

- **Age:** Most are detected in men aged >60 years.

- **Environmental factors**: Infections such as sexually transmitted infections (e.g., trichomoniasis, chlamydia, and gonorrhoea), oxidative stress may initiate chronic inflammation of the prostate. Epithelial damage and accompanying inflammation is the common pathogenic link between environmental carcinogens and development of prostate cancer.
- **Dietary factors:** Evidences indicate that glandular epithelial cell injury may be caused by dietary carcinogens.
  - **Increased consumption of red meat and animal fat increases the risk.**
  - **Diets which prevent or delay prostate cancer**: **Lycopenes** (found in tomatoes), **marine omega-3 fatty acids (in fish), soya products, and cruciferous vegetables (e.g., cauliflower, broccoli, Brussels sprouts), selenium and vitamin D.**
- **Androgens:** Prostatic **cancer growth depends on androgens**.
  - Androgens bind to the androgen receptor (AR) and express **pro-growth and pro-survival genes**.
  - **Castration** or treatment with **antiandrogens induces** disease **regression**.
  - Tumors which are resistant to antiandrogen therapy usually acquire androgen receptor gene amplifications or mutations. This permits androgen receptors to activate the expression of their target genes in spite of therapy.
- **Race:** Differences in prostate cancer risk among races were observed.
- **Family history:** Men with strong family history of prostate cancer have **two-fold risk** of prostate cancer and develop cancer at an earlier age.
- **Hereditary factors:** Germline **mutations of** the tumor suppressor gene ***BRCA*2** and germline mutation in *HOXB13* (a homeobox gene encoding a transcription factor that regulates prostatic development) is associated with **increased risk** of prostate cancer.

Somatic Mutation in Prostate Cancer

Prostate carcinoma develops as a product of combination of acquired somatic mutations *(genetic aberrations)* and epigenetic changes.

- ***TPRSS2-ETS* fusion gene:** This fusion genes consists of the androgen regulated promoter of the *TMPRSS2* gene and the coding sequence of ETS family transcription factors. They are observed in 40–50% of prostate cancer and **occur relatively early in tumorigenesis**.

- **Over-expression of ETS transcription factors:** It results in upregulation of matrix metalloproteases and makes normal prostate epithelial cells more invasive.
- **Epigenetic changes: Hypermethylation of glutathione *S*-transferase (*GSTP1*) gene** can predispose to cancer of prostate. ***GSTP1* gene** (located on chromosome 11q13) **prevents damage from a wide variety of carcinogens**.
- **Activation of oncogenes:** They activate PI3K/AKT pathway of cell proliferation.
- **Mutation that inactivate tumor suppressor gene *PTEN*:** *PTEN* acts as a brake on cell proliferation by PI3K.

MORPHOLOGY (FIGS. 21.7A TO C)

Carcinomas detected clinically are usually not grossly visible. More advanced cancers appear as firm, gray-white lesions with ill-defined margins that infiltrate the adjacent gland.

Gross

- **Site:** Carcinoma of the prostate arises in the **peripheral zone of the gland** (70%), usually in a **posterior location**, (posterior/posterolateral peripheral zone) and makes it **palpable on rectal examination**. However, there is relative increase of dominant anterior prostatic tumors (located in the anterior peripheral zone or transition zone).
- **Cut-section**: Tumor tissue is **gritty and firm**. When tumor is embedded within the prostate, it may be more readily **appreciated on palpation than by visualization**.

Microscopy

Most prostate cancers are **adenocarcinomas** and **consist of well-defined glandular patterns**.

Archilectural Features

- The finding of crowded small glands is suspicious but not diagnostic of carcinoma. A more specific feature is the presence of either a linear row of atypical glands or small atypical glands on both sides of a benign gland.
- Neoplastic **glands** are **usually smaller than benign glands—microacinar**.
- In contrast to benign glands, prostate cancer glands are **more crowded**, and **lack branching and papillary infolding**.
- Glands are **lined by a single uniform layer of cuboidal or low columnar** cells.
- **The outer basal cell layer, which is seen in benign glands is absent** in cancer. This is **used as criteria to distinguish benign and malignant prostate glands**.

Tumor Cells

Cytoplasmic features

- Uniform **cuboidal or low columnar type.**
- **Cytoplasm** ranges from **pale-clear** (similar to benign glands) **to** a distinctive **amphophilic** (dark purple) **appearance** than the surrounding benign glands, which have pale to clear cytoplasm. Abundant cytoplasm with straight luminal borders in larger glands is also a helpful feature of carcinoma.

Nuclear features

- **Nuclei** are **large** and **may contain** one or more large **nucleoli**. The presence of prominent nucleoli is a important cytological feature for the diagnosis of prostate adenocarcinoma. However, it should not be used as the sole criterion to establish the diagnosis.
- Mild variation in nuclear size and shape may be seen, but **pleomorphism is not marked**.

Intraluminal contents

- Prostatic crystalloids in the lumen are dense eosinophilic crystal-like structures and appear in various geometric shapes (such as rectangular, hexagonal, triangular, and rodlike structures) can also help in the diagnosis of prostatic adenocarcinoma. Crystalloids, although not diagnostic are more common than in benign glands.

Mitotic figures are more common in high-grade prostate carcinoma.

As the grade increases, irregular or ragged glandular structures, cribriform glands, sheets of cells, or infiltrating individual cells are observed. In about 80% of cases, there is precursor lesions, called as high-grade prostatic intraepithelial neoplasia (HGPIN) seen. Many of the molecular changes of invasive cancers are also found in HPIN.

Immunohistological Markers

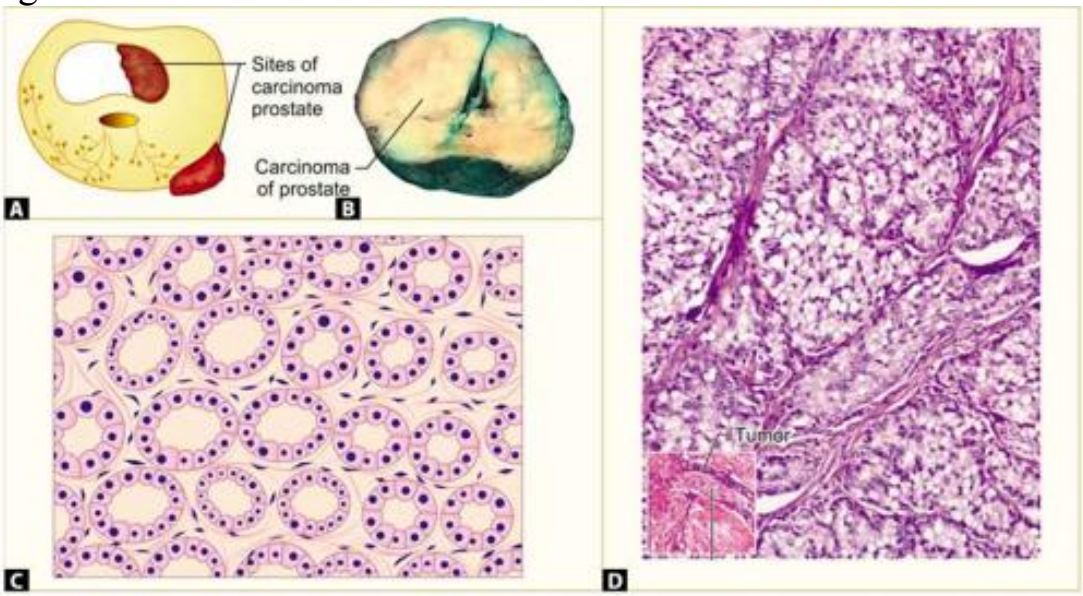
Differentiation of benign from malignant prostate glands is that benign glands have basal cells, whereas they are absent in glands in cancer. This can be detected by using various immunohistological markers to label basal cells. One of the immunohistochemical markers namely, α-methylacyl-coenzyme A-racemase (AMACR) is upregulated in prostate cancer. Most of the prostate cancers are positive for AMACR.

High-grade Prostatic Intraepithelial Neoplasia (HGPIN)

Definition: High-grade prostatic intraepithelial neoplasia (HGPIN) constitutes the preinvasive end of the continuum of neoplastic proliferations of secretory cells lining the prostatic ducts and acini. The term prostatic intraepithelial neoplasia is obsolete.

Q. Write a short answer on (high-grade) prostatic intraepithelial neoplasia.

- HGPIN consists of **benign prostatic acini** lined **by cytologically atypical cells with prominent nucleoli**. There is neoplastic proliferation of secretory cells within preexisting ducts and acini, with cytological changes resembling those seen in cancer, including enlargement of nuclei and numerous prominent nucleoli. The basal cell layer is often fragmented or lost.



Carcinoma of prostate:
Hematogenous spread occurs:
- Mostly to bone (axial skeleton is the most common site with lumbar spine being most frequently involved)—osteoblastic in nature
- Visceral metastasis most commonly to lung → liver → adrenal glands.

Most common primary site of bone secondaries:
- In males: Carcinoma prostate
- In females: Carcinoma of breast.

Feature that differentiate benign and malignant prostate gland:
- Benign glands have two layers: Basal cells and columnar cells
- Cancer: Single layer and absence of basal cells.

Figs. 21.7A to D: (A and B) Carcinoma of prostate: (C and D) microscopy; prostate cancer consisting of back-to-back uniform-sized malignant glands. Inset in (D) shows perineural invasion [green color in (B) is due to dye used to paint the margins of tumor].

- HGPIN is a **precursor of invasive cancer**.
- **Location:** HGPIN is most commonly found in the peripheral zone of the prostate.

Spread

Q. Write short note on spread of carcinoma of prostate.

- **Local spread**:
  - Invasion of prostatic **capsule.**
  - **Perineural tumor invasion (Fig. 21.7D inset)** both in the prostate and adjacent tissues.

- Spreads into periprostatic tissue (periprostatic fat), seminal vesicles, and the base of the urinary bladder.

- **Lymphatic spread**: Three pathways of lymphatic spread are: (1) laterally to the iliac nodes, (2) inferiorly to the pudendal and iliac nodes, and (3) posteriorly to the presacral nodes.
  - First to the obturator nodes.
  - Later to iliac and to the para-aortic lymph nodes.
  - Metastases to the lung are due lymphatic spread through the thoracic duct and through the prostatic venous plexus to the inferior vena cava.
- **Hematogenous spre**ad:
  - Mainly to the **bones of the axial skeleton**.
  - The bony metastases are **osteoblastic** and strongly point towards prostatic cancer.
  - The **bones involved, in descending order of frequency, are lumbar spine, proximal femur, pelvis, thoracic spine, and ribs**.
  - Massive visceral dissemination is rare.

Grading and Staging

Q. Write short note on grading and staging of carcinoma of prostate.

Grading and staging are the **best prognostic predictors** of carcinoma of prostate. **Gleason system** of grading was created in 1967 and was updated in 2014.

Gleason Grading

- This is the most **widely used microscopic grading system** for adenocarcinoma of prostate.
- It is **based on the degree of glandular architectural differentiation** and **the growth patter**n of the tumor in relation to the stroma as identified at relatively low magnification.

Architectural patterns: It can be divided into two patterns:

1. **Primary pattern:** It is the **predominant tumor pattern**.
2. **Secondary pattern:** It is the **second most prevalent pattern** (if present).

Grading

- Both the primary and secondary architectural patterns are **graded from 1 to 5**, with 1 being the most differentiated and 5 being undifferentiated.
  - **Grade 1 is the well-differentiated** tumor and consists of uniform and round neoplastic glands which form well-circumscribed nodules.
  - By contrast, **grade 5** tumors consist of cords, sheets, and nests of tumor cells infiltrating the stroma **without glandular differentiation**.
  - The other grades fall in between these two.
- If a tumor has only one histological pattern, then, both the primary and secondary patterns are given the same grade.

Gleason Score or Sum

- The **combined Gleason grades** is called as Gleason score or sum. It is obtained by adding the two numbers of primary and secondary patterns.
- It ranges from 2 (1 + 1 = 2), which represents tumors uniformly composed of Gleason pattern 1 tumor, to 10 (5 + 5 = 10), which represents totally undifferentiated tumors.

WHO grading: A new grading system also based on glandular pattern was recently accepted by the World Health Organization (WHO). It is used initially in conjunction with the Gleason system. It ranges from 1 (excellent prognosis) to 5 (poor prognosis).

Staging of Prostatic Cancer

- **TNM system** is used for staging.
- Staging is not only important for **predicting prognosis**, but also important in the selection of the **appropriate form of therapy**.

Clinical Course

- **Early stages**, it is **asymptomatic**. They are usually discovered during rectal examination or elevated serum PSA level.
- **Advanced** prostatic cancer **may present** with **urinary symptoms**, like dysuria, frequency, or hematuria.
- **Vertebral metastases may present** as **back pain** and has fatal outcome. **Detection of osteoblastic metastases by skeletal surveys or by**

radionuclide bone scanning is virtually diagnostic of prostatic cancer.

- Digital rectal examination, transrectal ultrasonography and other imaging modalities (MRI) has both low sensitivity and specificity.
- **Methods of tissue diagnosis:** These include: fine-needle aspiration cytology (FNAC), needle core biopsy, transurethral resection, open (simple) prostatectomy/ enucleation and radical prostatectomy.

Tumor Markers

Prostate-specific Antigen (PSA)

Q. Write short note on prostate-specific antigen.

- Most **important tumor marker used for diagnosis and management**.
- **PSA is serine protease produced by prostatic secretory cells in the prostatic ducts and acini.** And **only minute amounts of PSA circulate in the serum** (serum level of 4 ng/mL is cutoff point between normal and abnormal). Serum PSA levels correlate strongly with the risk of prostate cancer.
- **Raised blood PSA levels occur with localized as well as advanced prostatic cancer**. But, PSA value may be normal or less.
- PSA is **organ-specific**, but **not cancer-specific**. PSA has limitations, because it is commonly elevated in benign prostatic hyperplasia, prostatitis, and with mechanical manipulation of the prostate gland.
- Serum PSA is used for screening, for diagnostic, therapeutic, and prognostic purposes. **Serial measurements** of PSA are **useful for assessing the response to therapy**. For example, a rising PSA after radical prostatectomy or radiotherapy indicates recurrence or dissemination.

Prostate-specific Antigen Derivatives

- **PSA density**: It is the **ratio between the serum PSA value and volume of prostate gland**.
- **PSA velocity**: It is the **rate of change in PSA value with time**.
- **Age-specific reference ranges.**
- **Ratio of free and bound PSA in the serum.**

Prostatic Acid Phosphatase (PAP)

- It is secreted by prostatic epithelium. Its activity is 1,000 fold greater in prostate than in any other tissue.
- It is elevated when the prostatic cancer has spread beyond prostate or metastasized.
- Not prostate specific and is increased in renal, liver and bone malignancies.

Urine Markers

PCA is a noncoding RNA that is overexpressed in 95% of prostate cancers. Quantification of urine PCA3A is also used as diagnostic additional biomarker in patients suspected to have prostate cancer (because of raised PSA, but without cancer on prostate biopsy). The combination of urinary PCA3 with screening of urine for TMPRSS2-ERG fusion DNA improves the prostate cancer diagnosis.

Carcinoma prostate: Most common cancer in males above 50 years. Most common cause of bone secondaries in males. Produces osteoblastic metastases to axial skeleton (e.g., lumbar spine, pelvis). Most other carcinoma bone metastases is osteolytic.

Carcinoma of prostate most commonly arises in the outer peripheral zone and may be palpable by rectal examination.

Carcinoma prostate: DTH dependent. *TPRSS2-ETS* fusion gene.*GSTP1* gene: Located on chromosome 11q13 prevents damage from a wide variety of carcinogens.

Carcinoma of prostate: Adenocarcinoma consists of neoplastic glands lined by single layer of tumor cells. Most are acinar type.

1. Neoplastic glands
   - Smaller
   - More crowded
   - Lack branching and papillary folding
2. Diagnosis is based on the absence of outer basal cell layer.

Carcinoma prostate: Gleason grading system is used which correlates stage and prognosis.

Grading of carcinoma prostate: Gleason system (score or sum).

- Gleason grade: Ranges from 1 to 5 with 5 having the worst prognosis
- Gleason score: Ranges from 2 to 10. It is used for grading of prostate cancer.

Minimum Gleason score is 2 (1 + 1) and is most differentiated whereas maximum score is 10 (5 + 5), least differentiated.

Transrectal needle biopsy is needed to confirm the diagnosis.

Raised PSA:
- Carcinoma prostate
- BPH
- Prostatitis
- Infarct of prostate
- Instrumentation of the prostate
- Ejaculation

PSA: Organ-specific, but not cancer-specific. Great value in assessing the response to therapy and detect recurrence.

PSA velocity of 0.75 ng/mL/year best distinguishes between cancer and benign lesions of prostate.

TESTIS

CRYPTORCHIDISM

Q. Write short note on cryptorchidism.

Cryptorchidism is an anomaly in which there is a complete or incomplete **failure of the intra-abdominal testes to descend into the scrotal sac.** It usually occurs as an isolated anomaly or may be associated with other malformations of the genitourinary tract.

Normal descent of testis: Testicular descent occurs in two phases.

- **Transabdominal phase:** In this first phase, the testis comes from the abdomen cavity to lie within the lower abdomen or brim of the pelvis.
- **Inguinoscrotal phase:** During this phase, the testes descend through the inguinal canal into the scrotal sac.

Normally, the testes descend from the abdominal cavity into the pelvis by the third month of gestation and then through the inguinal canals into the scrotum during the last 2 months of intrauterine life. It can be diagnosed

with certainty after 1 year of age, particularly in premature infants, because testicular descent into the scrotum is not always complete at birth.

Sites of arrest of testis: Testes may be arrested anywhere along their pathway of descent **(Fig. 21.8)**. In most of the patients, the undescended testis is palpable in the inguinal canal. Cryptorchidism is asymptomatic, and it is found by the patient or the examining physician only when no testis is found while examining the scrotal sac.

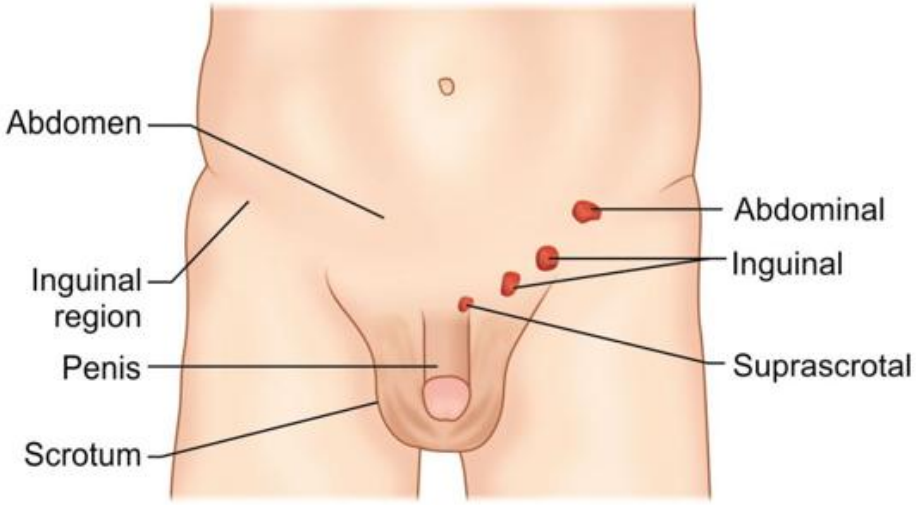


Fig. 21.8: Various sites of arrest of testicular descent resulting in cryptorchidism.

MORPHOLOGY

Gross

Cryptorchidism is unilateral in most of the cases, but it may be bilateral. The cryptorchid testis may be of normal size early in life, but some degree of atrophy is seen by the onset of puberty **and testis appears small in size**.

Microscopy

Atrophy of seminiferous tubules begins to appear by 5 to 6 years of age, and is usually advanced by the time of puberty. These **atrophied tubules** arrest in the development of germ cells. The **tubular basement membrane** shows marked **hyalinization and thickening**.

Increase in interstitial stroma and **prominent Leydig cells**.

- *Germ cell neoplasia in-situ* may develop in cryptorchid testes and is a likely precursor of subsequent germ cell tumors.

Complications

Q. Write short note on complications of undescended testis.

- **Atrophy and infertility/sterility:** Undescended testes usually become atrophic. Hence, bilateral cryptorchidism leads to sterility. Even unilateral cryptorchidism may be associated with atrophy of the contralateral descended testis.
- **Susceptibility to trauma and crushing** against the ligaments and bones, may be accompanied by an **inguinal hernia**.
- **Testicular cancer:** It is associated with a **3- to 5-fold increased risk for testicular cancer** than the descended testis. Even unilateral cryptorchidism is also at increased risk for the development of cancer in the contralateral, normally descended testis. This suggests the possibility of some intrinsic abnormality, rather than simple failure of descent, being responsible for the increased cancer risk. Hence, surgical placement of the undescended testis into the scrotum (orchiopexy) is recommended by 18 months of age to reduce the likelihood of testicular atrophy, infertility, and testicular cancer.

Other causes of testicular atrophy: Apart from cryptorchidism other conditions associated with testicular atrophy are: chronic ischemia, trauma, irradiation, and anti-cancer chemotherapy, and conditions associated with chronically elevated estrogen levels (e.g., cirrhosis).

Cryptorchid testis: Incomplete descent of testis into scrotal sac.

Cryptorchid testis: Risk factor for seminoma (more for abdominal than for inguinal testis). Risk of testicular malignancy in undescended testes is **not reduced** by orchiopexy.

Cytogenetic abnormality in testicular germ cell tumor: Isochromosome *p12*.

TESTICULAR TUMORS

CC: Classify testicular tumors and describe the pathogenesis, pathology, presenting and distinguishing features, diagnostic tests, progression and spread of testicular tumors.

Q. Classify testicular tumors. Describe the morphology of seminoma. Add a note on role of tumor markers in testicular tumors.

Classification (Box 21.2)

Testicular neoplasms are divided into **two major categories**:

1. **Germ cell tumors:** They arise from germ cells and **constitute about 95% of testicular tumors**. **Most of them are aggressive** and can rapidly disseminate, but with current therapy most can be cured.
2. **Sex cord–stromal tumors:** These tumors arise from stroma and are generally benign.

Testicular tumors: Most common cause of painless enlargement of testis.

Most common tumor of testis:

- Seminoma
- Mixed germ cell tumor

Most common testicular tumor:

- In infant and children up to 3 years: Yolk sac tumor
- In prepubertal children: Teratoma
- >60 years: Lymphoma

Most common bilateral tumor:

- Primary is seminoma
- Secondary is lymphoma

GERM CELL TUMORS

Etiology and Pathogenesis

BOX 21.2: WHO classification (abridged) of testicular tumors (2016).

I. **Germ cell tumors**
   A. **Germ cell tumors derived from germ cell neoplasia in situ**
      1. **Non-invasive germ cell neoplasia:** Germ cell neoplasia in situ (GCNIS), specific forms of intratubular germ cell neoplasia
      2. **Tumors of a single histological type (pure forms)**
         - **Seminoma**
      3. **Non-seminomatous germ cell tumors**
         - Embryonal carcinoma
         - Yolk sac tumor, postpubertal-type
         - Trophoblastic tumors: Choriocarcinoma
         - **Teratoma, postpubertal-type**
         - Teratoma with somatic-type malignancy
      4. **Non-seminomatous germ cell tumors of more than one histological type**
         - Mixed germ cell tumors: Polyembryoma, diffuse embryoma
   B. **Germ cell tumors unrelated to germ cell neoplasia in situ**
      - Spermatocytic tumor
      - **Teratoma, prepubertal-type**
      - Mixed teratoma and yolk sac tumor prepubertal-type
      - Yolk-sac tumor, prepubertal-type
II. **Sex cord-stromal tumors**
   A. **Pure tumors:** Leydig cell tumor, Sertoli cell tumor, NOS
   B. **Mixed and unclassified sex cord-stromal tumors**

The exact etiology of testicular tumors is not known.

- **Environmental factors:** They may play role in testicular germ cell tumors. The incidence of testicular tumors shows a geographic variation.
- **Cryptorchidism:** About 10% of testicular germ cell tumors are associated with **undescended testis and is the most important risk factor**. There is also increased risk for cancer in the contralateral descended testis. The high incidence of testicular germ cell tumors may be due to its exposure to high temperature in the abdomen or inguinal region compared to that in the scrotum.
- **Testicular dysgenesis syndrome (TDS):** It constitutes a spectrum of disorders and is one of the known risk factor for testicular germ cell tumors. This syndrome **includes cryptorchidism, hypospadias,** intersex syndromes (including androgen insensitivity syndrome and gonadal dysgenesis) **and poor sperm quality**. These conditions might be related to in utero exposures to pesticides and estrogens.
- **Genetic/family predisposition:** There is a strong family or genetic predisposition with the testicular germ cell tumors. The **cytogenetic abnormality observed is an additional fragment of chromosome 12 (isochromosome p12) [i(12p)]**.

- **Klinefelter syndrome:** It is **associated with 50 times greater risk** (than normal) **for the development of mediastinal germ cell tumors**, but they **do not develop testicular tumors**.
- **Time of malignant transformation:** It may occur **during fetal development** or in the **peripubertal period**.
- **Oncogenic mutations in KIT:** Point mutations producing oncogenes are relatively rare in germ cell tumors, and develops at a lower frequency than in any other solid tumors in adults. Oncogenic mutations in KIT may be found in up to 25% of tumors.

Classification

Germ cell tumors may be divided into: (1) germ cell tumors derived from germ cell neoplasia in situ and (2) germ cell tumors unrelated to germ cell neoplasia in situ.

GERM CELL TUMORS DERIVED FROM GERM CELL NEOPLASIA IN SITU

Germ Cell Neoplasia In Situ (GCNIS)

- GCNIS (formerly known as intratubular germ cell neoplasia-ITGCN) is **preinvasive form** of most invasive germ cell tumors.
- **Progress:** About 50–70% of patients with GCNIS **subsequently develop invasive tumors**.

MORPHOLOGY

Gross: GCNIS **involves testes in a patchy manner.** No grossly visible lesion seen.

Microscopy

- **Seminiferous tubules** are lined by malignant germ cells and they are located within the seminiferous tubules between Sertoli cells.
- **Tumor cells:**
  - **Size:** Tumor cells are **larger than normal spermatogonia**.
  - **Nuclei: Large, irregular,** and **centrally placed, with finely dispersed chromatin** and **prominent nucleoli**.
  - **Cytoplasm: Abundant, distinct cell membrane** and **clear or vacuolated cytoplasm**, which contains large amounts of glycogen.
- **Immunohistochemistry: Placental alkaline phosphatase** (PLAP) **positive** on the plasma membrane. Other markers include OCT3/4 (also called POU5F 1) and NANOG.

Specific Forms of Intratubular Germ Cell Neoplasia

- **Intratubular seminoma:** In this, there is complete filling of seminiferous tubules by neoplastic cells that are morphologically identical to those of GCNIS and seminoma. lntratubular seminoma is found adjacent to about 30% of seminomas and 15% of non-seminomas.
- **Intratubular non-seminoma:** Intratubular non-seminoma consists almost exclusively of pure embryonal carcinoma. Probably they result from reprogramming of a GCNIS cell. They usually show distortion and enlargement of the seminiferous tubules. There may be necrosis and calcification. The tumour cells are more pleomorphic than of GCNIS cells. lntratubular non-seminoma is only seen adjacent to tumours with non-seminomas.

GCNIS: PLAP positive.

Tumors of a Single Histological Type

Seminoma

Q. Write short note on seminoma.

Definition: Seminoma (previously known as classical seminoma) is a **malignant germ cell tumor** (GCT) whose cells are considered the neoplastic counterparts of the primordial germ cells/gonocytes present during early embryonic development.
Seminomas were previously classified as: (i) classical or typical, (ii) anaplastic and (iii) spermatocytic tumor (initially known as spermatocytic seminoma.

- Seminomas are the **most common type of germ cell tumor (**~ 50% of germ cell tumors).
- **Age group:** Peak incidence is during **third decade (between 30 and 40 years)** and almost never found in infants or prepubertal children.
- **Female counterpart** occurs in the ovary is known as **dysgerminoma**.
- **Origin:** Classical seminoma arises from **undifferentiated germ cells**.

Genetic Alterations

- Seminomas contain an **isochromosome 12p** (gain of additional fragment short arm of chromosome 12), and express *OCT3/4 and NANOG*.

- **Mutations of *KIT*** (oncogene) in about 25% of tumors. ***KIT* amplification** and overexpression may also occur without genetic defects.

MORPHOLOGY

Q. Write short note on morphology of seminoma.

Gross (Figs. 21.9A and B)

Seminomas are **bulky**, **solid**, **rubbery-firm**, and **bosselated tumors.**

Size: It varies from **2 to 6 cm** and **testis is enlarged**, sometimes up to 10 times of the normal testis.

Cut Surface

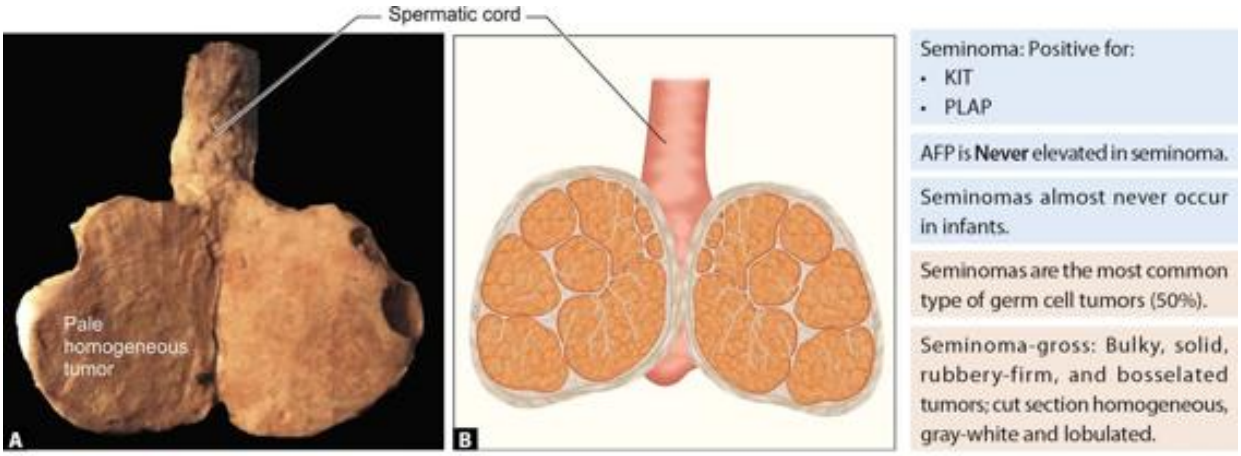
- Tumor is **homogeneous**, **gray-white** or grayish-yellow, and **lobulated**. Their cut surface bulge above the surrounding parenchyma.
- Tumor is **sharply demarcated from normal testis**, which may be compressed, and atrophic.
- Usually, the **tunica albuginea** is **not penetrated**.
- **Areas of necrosis or hemorrhage** are **usually not seen**.

Microscopy (Figs. 21.10A and B)

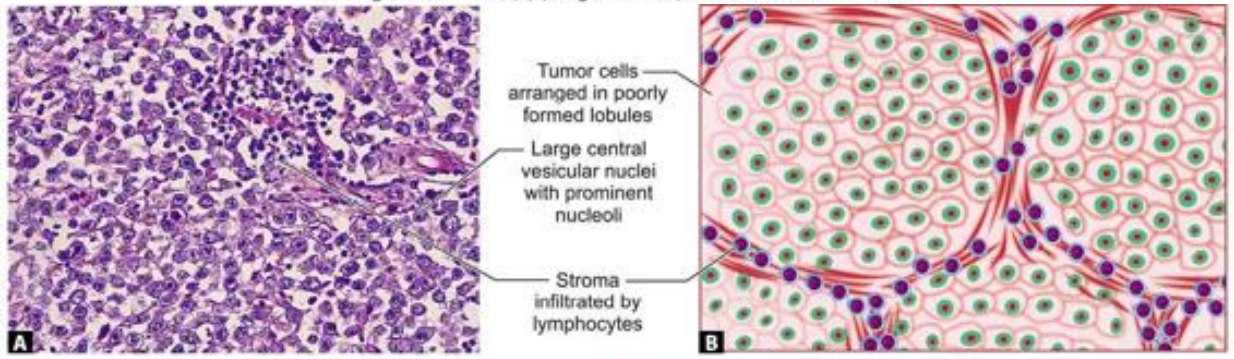
Seminoma appears **microscopically similar to dysgerminoma** of ovary.

1. **Pattern:** Tumor is composed of **sheets or nests or cords of uniform** population of **seminoma cells**
2. **Seminoma (tumor) cells**: They have following features:
   - **Uniform single population** of cells that **resemble spermatogonia**.
   - Cells are **large**, **round to polyhedral** and have a **distinct cell membrane**.
   - **Cytoplasm:** May appear **pale and eosinophilic or clear (watery)**. It contains varying amounts of **glycogen (stain positive with periodic acid-Schiff stain)** and **some lipid**.
   - **Nucleus: The nuclei are polygonal with finely granular chromatin** with **one or two large, centrally located prominent nucleoli**.
   - Mitoses vary in number. GCNIS can be found in the residual seminiferous tubules in about 85–90% of cases.
3. **Stroma:**
   - Seminoma **cells divided into poorly demarcated lobules** by **delicate fibrous septa**.
   - Fibrous **septa are infiltrated with** moderate amount of **lymphocytes and plasma cells**.
4. **Rare features:**
   - **Syncytiotrophoblasts (~15%)** → minimally elevated serum **hCG** (in about 10% of cases).
   - Ill-defined granulomas with giant cells in the stroma.

5. **Immunohistochemistry:** Seminoma cells are **diffusely positive for:** (i) cytoplasmic/plasma membrane include KIT and placental alkaline phosphatase (PLAP) and (ii) nuclear include OCT3/4 and SALL4.



Figs. 21.9A and B: Gross appearance of seminoma of the testis: (A) Testis shows circumscribed, pale, fleshy, homogeneous mass; (B) (Diagrammatic) shows a lobulated tumor.



Figs. 21.10A and B: Microscopy of seminoma shows seminoma cells divided into lobules separated by delicate septa with lymphocytic infiltrate.

Spread

- **Local spread:**
  - Invasion of the testicular parenchyma.
  - Spread into rete testis.
  - Invasion of the epididymis.
- **Lymphatic spread:** To abdominal lymph nodes.

Prognosis of Seminoma

- Extremely **radiosensitive.**

- **Remain localized** to the testis for long time (clinical stage I).
- **Metastases** mainly involve **lymph nodes**.
- **Hematogenous** spread occurs **late**.
- **Best prognosis.**

Genetic alterations in seminoma:

- Isochromosome p12
- Mutations of *KIT* (oncogene)
- *KIT* amplification.

Q. A 26-year-old male complains of a vague feeling of painless heaviness in the right scrotum for the last 3 months. There is no history of antecedent trauma. Physical examination shows that the right testis was enlarge and measured 5 cm in size, firm and non-tender. The swelling is negative for transillumination. Ultrasound scan reveals a solid, circumscribed, mass in the right testis. Regional lymph nodes were not palpable. Right orchiectomy was done.

(a) What is the probable diagnosis? (Ans: Seminoma of right testis).

(b) Discuss the morphological features of this condition. (refer pages 731-2).

(c) How this lesion does spreads and what is its prognosis? (refer page 732).

Non-seminomatous Germ Cell Tumors

Q. Write short essay on non-seminomatous germ cell tumors of testis.

Embryonal Carcinoma

Definition: Embryonal carcinoma (EC) is a malignant germ cell tumor (GCT) composed of tumor cells resembling embryonic stem cells.

- **More aggressive than seminomas.**
- **Age group:** 20–30 years.

MORPHOLOGY

Gross

- **Size:** Small or quite often enlarge the testis.
- **Cut surface**:
  - Has a **variegated appearance.**
  - **Poorly demarcated** at the margins.
  - **Areas of hemorrhage or necrosis** are common.
  - **Tumor extends into tunica albuginea, epididymis or spermatic cord**.

Microscopy

- **Pattern**:
  - The tumor cells are arranged in **alveolar or tubular or papillary** patterns. But they lack the well-formed glands with basally situated nuclei and apical cytoplasm.
  - More undifferentiated tumor show sheets of cells.
- **Tumor cells:**
  - Cells have an **epithelial appearance** and are **large and anaplastic**. The cytoplasm is dense amphophilic with poorly defined cytoplasmic membranes.
  - **Cell borders** are usually **indistinct**.
  - Considerable **variation in cell and nuclear size and shape**.
  - **Nuclei** are large, pleomorphic, vesicular with single or multiple macronucleoli.
  - **Mitotic figures** and **tumor giant cells** are **frequent**. Nuclear crowding, overlapping, and marked pleomorphism are helpful features to distinguish it from other GCT types.
- **Immunohistochemistry: Positive for cytokeratin and CD30**, and **negative for KIT**.

Embryonal carcinoma:
Positive for: (1) Cytokeratin, (2) CD30; Negative for KIT.

Teratoma, Postpubertal-type

Q. Write short note on teratoma of testis.

Definition: Postpubertal-type teratoma is a **malignant germ cell tumor** (GCT) composed of several types of tissue representing one or more of the germinal layers (endoderm , mesoderm, and ectoderm). It may be composed exclusively of well-differentiated, mature tissues or have immature, embryonic-type tissues. Teratoma may contain syncytiotrophoblastic giant cells.

- **Classification:** Previously teratomas were **classified as (i) mature, (ii) immature**, and (iii) teratoma with somatic-type malignancy **(Teratoma with malignant transformation). Presently WHO (2016) classify it as: (i) teratoma, post pubertal type (includes both mature and immature teratoma of earlier classification) and (ii) teratoma with somatic-type malignancy**.
- Pure teratomas constitute about 2–3% of germ cell tumors, but teratomas mixed with other germ cell tumors form about 45% of germ cell tumors.
- **Age:** Most postpubertal-type teratomas are seen in young adults.

MORPHOLOGY

Q. Write short note on morphology of teratoma of testis.

Gross (Fig. 21.11)

- **Size:** Usually **large**, and range from **5 to 10 cm** in diameter.
- **Appearance:** The tumors are nodular and firm. The cut surfaces have heterogeneous appearance with solid and cystic areas depending on the tissue types present histologically. Cartilage, bone, and pigmented areas may be recognizable.
- Presence of hemorrhage and necrosis usually point towards the mixed tumors like embryonal carcinoma, choriocarcinoma, or both.

Microscopy (Figs. 21.12A and B)

Types of tissue: Virtually any epithelial or mesenchymal tissue type derived **from more than one germ cell layer** can be seen including neural tissue. The tissues may be mature and/or fetal or embryonic-type derived from more than one germ cell. These **heterogeneous tissues are arranged in a Helter–Skelter pattern. Tissue derivatives of three germ cell layers include:**

- **Ectoderm: The structures include** skin (clusters of squamous epithelium, sweat and sebaceous glands, hair), tooth enamel, brain substance (neural tissue, glia). Immature neuroectodermal structures resembling those of the early embryonic central nervous system **(neuroepithelial rosettes and immature glia)**, are particularly common.

- **Mesoderm:** Mesenchymal tissues such as smooth muscle bundles, islands of cartilage, bone, fat, blood vessels and lymphatics.
- **Endoderm:** Respiratory tract (bronchial or bronchiolar epithelium) till here, gut (bits of intestinal wall) structures reminiscent of thyroid gland.

Organoid arrangements characteristic of the skin, respiratory tract, gastrointestinal tract, and genitourinary tract are more often seen prepubertal type of teratoma (*refer* page 735), but may be found in smaller proportion of postpubertal-type teratomas. They often contain multiple cysts lined by glandular or squamous epithelium, neuroectodermal tissue, non-cystic glands, and mesenchymal tissues (e.g., adipose tissue and cartilage).

Cytological atypia: It is not uncommon for various elements to show cytological atypia. For example, mature glandular tissue may show features of high-grade dysplasia or in situ carcinoma, and cartilage may resemble chondrosarcoma.
Stroma: Commonly these tumors have a primitive, mitotically active, cellular stroma cuffing glands.
Invasion: Teratoma can invade paratesticular tissue and may invade intra- and extratesticular vessels.
Changes in uninvolved region of testis: In about 90% of cases, they show germ cell neoplasia in situ.
Mixed GCTs: In these tumors, teratoma is often found along with yolk sac tumor or embryonal carcinoma.
Metastasis: These tumors, when they metastasize, follow the same routes of spread as other GCTs.

Teratoma with Somatic-type Malignancy

- **Definition:** It is a teratoma that **develops** a distinct secondary component that resembles a somatic-type malignant neoplasm **(malignant non-germ cell tumors)**, as seen in other organs and tissues.
- It was previously termed as "teratoma with malignant transformation" which is not recommended. This is because it may lead to a misconception that teratomas lacking somatic-type malignancy are benign.
- Somatic-type malignancy may occur in any tissue derived from one or more germ cell layers. For examples, **squamous cell carcinoma**, **mucin-secreting adenocarcinoma and sarcoma** (e.g., sarcomas and carcinomas).
- They are **rare**.

MICROSCOPY

The tumor usually consists of teratoma mixed with a pure population of atypical mesenchymal or epithelial cells and occupies at least one low-power field. Sarcomas are the most common histological

type (most commonly rhabdomyosarcomas and others are rare such as leiomyosarcoma, angiosarcoma, and others). Most carcinomas are adenocarcinomas, usually the NOS type.

- **Significance:** When the non-germ cell malignancy spreads outside of the testis, it does not respond to chemotherapy.

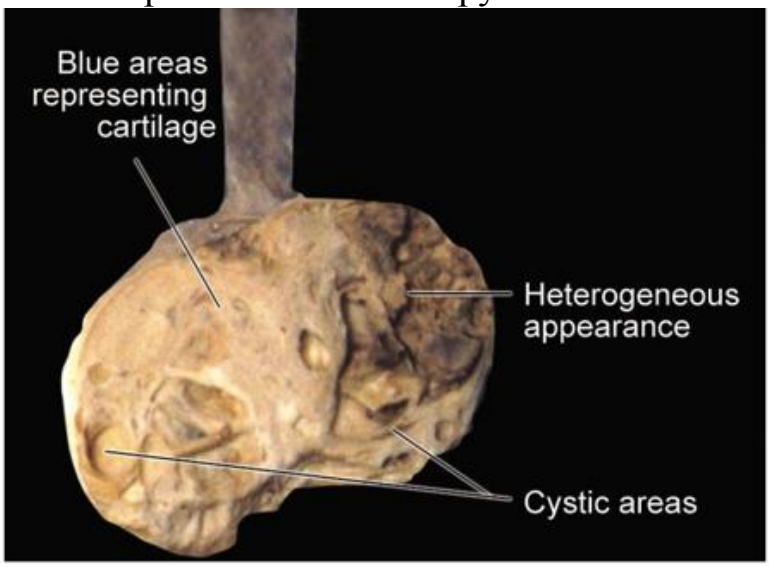
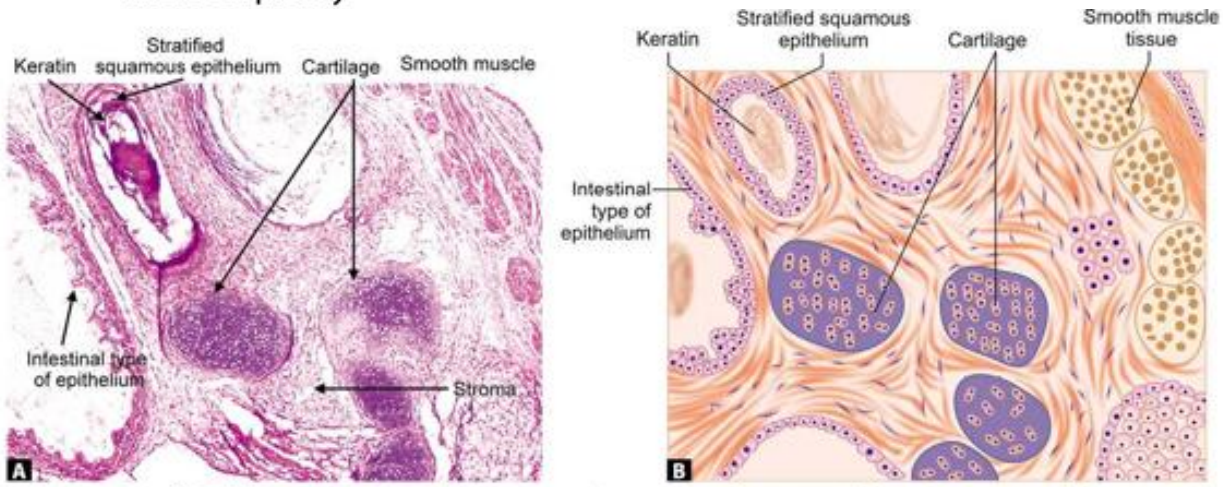


Fig. 21.11: Teratoma of testis. Cut section of testis has a variegated appearance with cysts, reflects the multiplicity of tissue found microscopically.



Figs. 21.12A and B: (A) Photomicrograph and (B) diagrammatic appearance of teratoma of the testis consisting of a disorganized collection of glands, cartilage, smooth muscle, and stroma.

Prognosis of Teratoma

All postpubertal teratomas (both mature and immature) **are considered malignant** and **are capable of metastasis**. Metastases can develop in 22–37% of cases. Presence of epithelium or mesenchymal elements with cytological atypia or immaturity has no effect on prognosis.

Differences between seminomatous and nonseminomatous tumors of testis are presented in **Table 21.1**.

Teratoma: Germ cell tumor arising from totipotent cells, which has capacity to differentiate into any of the germ cell layer (ectoderm, endoderm and mesoderm).

Teratoma:

- Mature
- Immature
- Teratoma with malignant transformation.

Dermoid cyst: A form of teratoma which are common in the ovary, rare in the testis.

All postpubertal teratomas (both mature and immature):

Considered malignant and are capable of metastasis.

Mixed Germ Cell Tumors

- Mixed germ cell tumors (GCTs) are malignant tumors with more than one GCT component. All are clinically regarded as non-seminoma, regardless of the presence or absence of a seminoma component.
- Constitute ~ 60% of testicular tumors.
- **Common mixtures** are:
  - Teratoma, embryonal carcinoma, and yolk sac tumor.
  - Seminoma with embryonal carcinoma.
  - Embryonal carcinoma with teratoma (teratocarcinoma).
- **Prognosis:** Depends on the more aggressive component in the mixed tumor.

GERM CELL TUMORS UNRELATED TO GERM CELL NEOPLASIA IN SITU

Spermatocytic Tumor

Definition: Spermatocytic tumor is a germ cell tumor (GCT) derived from postpubertal-type germ cells. The tumor cells resemble spermatogenic cells, most commonly spermatogonia or early

primary spermatocytes.

- Spermatocytic tumor was **formerly called as spermatocytic seminoma** (not recommended) is a distinctive tumor of testis both clinically and morphologically
- It is rare tumor (~1 to 2% of all testicular germ cell tumors).
- **Origin:** Spermatocytic tumor originates **from germ cells undergoing spermatogenesis**. Represent more differentiated type of germ cell tumor.
- **Age:** The tumor occurs over a wide patient age range (19–92 years), with a peak incidence in the sixth decade of life (usually above 65 years of age). Most present with a painless testicular mass.

Q. Differences between seminoma and nonseminomatous germ cell tumors.

TABLE 21.1: Differences between seminomatous and non-seminomatous tumors of testis.

Characteristics	*Seminomatous*	*Nonseminomatous*
Characteristic of tumor	Remain localized to testis for long time	Some of them spread rapidly
Aggressiveness	Not very aggressive	Aggressive
Common mode of spread	By lymphatics	By hematogenous
Metastasis	Does not occur early	Early metastasis
Radiosensitivity	Highly radiosensitive	Radioresistant
Prognosis	Good	Poor

MORPHOLOGY

Gross

- Usually 3–5 cm (but may be larger). Lobular to multinodular. Soft and pale gray.
- Cut surface varies from gray-white to hemorrhagic and from fleshy to myxoid. May show mucoid cysts.

Microscopy

Arrangement: The tumor cells are arranged in diffuse to multinodular patterns. Cystic change is common.

Tumor cells: The hallmark is a polymorphous population of tumor cells often described as tripartite (composed of three types). These three types are:

1. **Smaller lymphocyte-like cells**: These cells have round, uniformly dense nuclei and scant, thin rim of eosinophilic cytoplasm. They resemble secondary spermatocytes.
2. **Medium-sized intermediate cells**: They show dense eosinophilic to amphophilic cytoplasm, round nucleus having finely granular to filamentous chromatin and variably prominent nucleoli. The chromatin in some tumor cells is filamentous in appearance, similar to that seen in the meiotic phase of nonneoplastic spermatocytes (spireme chromatin). They are the most numerous cells seen. The cytoplasmic membranes are often (but not always) poorly defined.
3. **Large/giant cells**: Scattered giant cells with single or multiple nuclei having similar nuclear features of the intermediate-sized cells,

Mitotic figures, including atypical forms, are usually frequent

Stroma: It is usually scant and finely fibrous.

In contrast to classical seminomas, spermatocytic tumors have these features namely: (i) interstitial lymphocytic infiltration is usually absent or may be scant; (ii) granulomatous inflammation is extremely rare; (iii) not associated with germ cell neoplasia in situ; and (iv) rarely metastasize.

Prognosis

- Spermatocytic tumor is a **slow-growing tumor**. It rarely metastasizes.
- Prognosis: Excellent.

Teratoma, Prepubertal-type

Definition: Prepubertal-type teratoma is a germ cell tumor (GCT), usually seen in the prepubertal testis. It is composed of elements resembling somatic tissues derived from one or more of the germinal layers (endoderm, mesoderm, and ectoderm).

Prepubertal-type teratoma are **not associated with germ cell neoplasia in situ (GCNIS), dysgenetic parenchymal changes, scarring, or chromosome 12p amplification**.

Age: Teratomas that develop before puberty (typically before the age of 6 years) are usually **distinct from those that occur in older patients**. Both differ in terms of pathogenesis and clinical behavior. These tumors **do not show cytological atypia and do not recur or metastasize**.

They appear **similar to the benign cystic teratomas of the ovary** rather than with the GCNIS-associated germ cell tumors of the postpubertal testis.

MORPHOLOGY

Gross

The tumors may be solid or may show prominent cystic component filled with keratinous or mucoid material. There may be calcification or bone formation. Grossly identifiable hair may occur, a finding which never occur in postpubertal-type teratomas of the testis.

Microscopy: The tumors have distinct differences from that of postpubertal-type teratomas.

- **Tissues found:** Calcification and hair follicles may be seen; but numerous other ectodermal, mesenchymal, and endodermal tissues also occur. These include glandular and squamous epithelium, meningothelium, fat, cartilage, bone, and muscle
- **Organoid pattern:** Ciliated epithelium, squamous cysts, and smooth muscle tend to be particularly prominent. They are often arranged in an organoid pattern. In this pattern, the smooth muscle encircle the ciliated epithelium-lined cysts and sometimes splitting into distinct layers similar to muscularis mucosa and muscularis propria, with an intervening collection of connective tissue resembling submucosa. Lobular arrangement of glands resembling salivary or pancreatic acinar cells may be found.
- **Lack of atypia:** Virtually all elements lack significant atypia.
- **Absence of GCNIS**: In contrast to the postpubertal-type teratomas, there is no GCNIS.

Clinical Features

- **Painless enlargement** of the testis.
- **Biopsy of a testicular neoplasm is contraindicated** because of risk of tumor spillage.
- Standard treatment of a solid testicular tumor is radical orchiectomy.

Spread of Testicular Tumors

Q. Write short note on spread of testicular tumors.

- **Lymphatic spread:**
  - All types of **malignant testicular tumors spread through lymphatics**.
  - First, spreads to **retroperitoneal para-aortic nodes**.
  - Later, it may spread to mediastinal and supraclavicular nodes.
- **Hematogenous spread:**
  - To the **lungs**, **liver**, **brain**, **and bones**
  - Microscopic appearance of metastases may be different from that of the primary testicular tumor. For example, an embryonal carcinoma of testis may show a teratomatous element in the secondary deposits.

Tumor Markers (Table 21.2)

Q. Write short note on serological markers/tumor markers of testicular tumors.

- Germ cell tumors of the testis may **secrete hormones and enzymes,** which can be detected in blood.
- Tumor markers include **hCG**, **AFP**, and **lactate dehydrogenase**.
- They are **valuable in the diagnosis and management** of testicular neoplasms.
  1. **Lactate dehydrogenase:** The **level** of raised lactate dehydrogenase **correlates with the mass of tumor cells**, and can be **used to assess tumor burden**.
  2. **AFP and hCG: Marked elevation** of these markers found in more than 80% of individuals **with NSGCT**.
     - AFP: produced by yolk sac tumor.
     - hCG levels: Raised in tumor with choriocarcinoma elements. Seminomas with syncytiotrophoblastic giant cells may also cause mild elevation of hCG levels.
- **Importance of tumor markers:**
  - Evaluation of testicular masses.
  - Staging of testicular germ cell tumors.
  - Assessing tumor burden.
  - Monitoring the response to therapy.

Extragonadal site of germ cell tumors:
1. Mediastinum (most common)
2. Retroperitoneum
3. Pineal gland.

Testicular tumor: Any solid testicular mass should be considered neoplastic unless otherwise proved.

Testicular tumor metastasis: Retroperitoneal and para-aortic nodes and not inguinal lymph nodes.

Biopsy of testicular neoplasm is associated with risk of tumor spillage, therefore, radical orchiectomy should be done on presumption of malignancy.

FNAC contraindicated in testicular tumors—it may result in inguinal lymph node metastasis.

Germ cell tumor markers:
- LDH
- hCG
- AFP

Pure choriocarcinoma: Most aggressive testicular tumor.

Yolk sac tumor of testis:
- Raised α-fetoprotein level (AFP).
- Schiller-Duval bodies

Choriocarcinoma: Elevated hCG.

MALE INFERTILITY

Q. Write short note on causes of male infertility.

BOX 21.3: Causes of male infertility.

- **Endocrine disorders:** Gonadotropin deficiency, estrogen excess (e.g., cirrhosis of liver)
- **Testicular causes:** Cryptorchidism, Klinefelter's syndrome, maturation arrest of spermatogenesis (idiopathic), varicocele, irradiation, defective spermatozoa (e.g., immotile cilia)
- **Post-testicular causes:** Blockage of efferent ducts due to congenital cause or secondary to an inflammatory process, impotence (e.g., neurological disorders)

Causes (Box 21.3)

Investigations: These should be performed to determine the cause/precise nature. These include a testicular biopsy to assess the integrity of the seminiferous tubules and the degree of spermatogenesis, hormonal assessment and other relevant investigations.

TABLE 21.2: Tumor markers of testicular germ cell tumors.

Type of tumor	*Tumor marker*
Seminoma	hCG, placental alkaline phosphatase
Teratomas and teratocarcinoma	AFP, hCG
Embryonal carcinoma	CEA
Yolk sac tumor	AFP
Choriocarcinoma	β-hCG

(AFP: α fetoprotein; hCG: human chorionic gonadotropin; CEA: carcinoembryonic antigen).

CHAPTER
22

Female Genital Tract Disorders

CERVIX

NORMAL STRUCTURE

The uterine cervix consists of **ectocervix** which is the part exposed to the vagina and visible on vaginal examination. Ectocervix is lined by mature stratified squamous epithelium and is continuous with the squamous lining of vaginal wall. In the center of ectocervix there is a small opening termed the **external os** that leads to the endocervical canal (or simply termed endocervix). The endocervix is lined by columnar, mucus-secreting epithelium. The endocervical mucosa has clefts and tunnels, commonly referred to as cervical glands and they secrete mucus. The junction of the ectocervix and endocervical canal is a point where the squamous epithelium of ectocervix meet the columnar epithelium of endocervix is referred to as the **squamocolumnar junction**. In this region there is a gradual transition between squamous and columnar epithelia. The position of this squamocolumnar junction is variable and changes with age and hormonal influence. Usually this junction moves upwards into the endocervical canal with time. The columnar/glandular epithelium of endocervix can be replaced by advancing squamous epithelium and this process is called **squamous metaplasia**. The area of the cervix where the columnar epithelium of endocervix abuts the squamous epithelium of ectocervix is termed the "*transformation zon*e" and is significant both clinically and pathologically. It is highly susceptible to infections with human papilloma virus (HPV) which is one of the important etiological agents involved in cancer of cervix.

Immature squamous metaplastic epithelial cells in this transformation zone are most susceptible to infection by HPV, and is the site where cervical precursor lesions and cancers can develop.

Most common lesions of cervix are cervicitis, certain benign tumors, dysplasia, carcinoma in situ and invasive carcinoma.

INFLAMMATIONS

NC: Describe the etiology and morphologic features of cervicitis.

Inflammation of cervix is termed as cervicitis and is extremely common condition of the cervix. It is because of its constant exposure to bacterial flora in the vagina.

- At the onset of menarche, the estrogens are secreted by the ovary. They stimulate maturation of the cervical and vaginal squamous mucosa and intracellular glycogen vacuoles are formed in the squamous cells. As these squamous cells are shed, the glycogen present in these cells provide a substrate for various endogenous vaginal aerobes and anaerobes.
- One of the **dominant microbial species in the normal vagina** is **lactobacilli**. These lactobacilli produce lactic acid, which in turn helps to maintains the vaginal pH below 4.5. This acidic/low pH suppresses the growth of other saprophytic and pathogenic organisms. Also, at this low pH, lactobacilli produce bacteriotoxic hydrogen peroxide (H_2O_2). If the pH becomes alkaline (e.g., due to bleeding, sexual intercourse, antibiotic therapy or vaginal douching), there will be decreased production of H_2O_2 by lactobacilli. This in turn promotes the overgrowth of other microorganisms, and can result in cervicitis or vaginitis.
- Some degree of inflammation is observed in cervix of almost all multiparous women and some nulliparous women and is usually of little clinical significance. The normal intact ectocervical stratified epithelium is usually resistant to infection compared to the endocervical columnar epithelium.

Classification

Depending on the cause:

- *Specific cervicitis:* These are caused by gonococci, *mycoplasmas, mycobacterium tuberculosis*, syphilis,granuloma inguinale, lymphogranuloma venereum, *chlamydia* and chancroid.
- *Nonspecific cervicitis* is more frequent and is generally divided into acute and chronic forms, the latter being quite common.

Depending on the onset as acute or chronic:

Acute cervicitis is usually associated with puerperal infection or gonococcal infection. Other causes include primary chancre and infection with herpes simplex.

Chronic cervicitis:

- Chronic nonspecific cervicitis is quite common and is the common cause of leukorrhea. They are most commonly caused by organisms present in the normal vaginal flora. These include streptococci, enterococci (e.g., *E. coli*) and staphylococci. Other organisms include gonococci, *Trichomonas vaginalis, Candida albicans* and herpes simplex. Predisposing factors include sexual intercourse, trauma of childbirth, instrumentation and excess or deficiency of estrogen.
- Microscopically, it shows subepithelial inflammatory infiltrate composed of lymphocytes, plasma cells, large mononuclear cells and a few neutrophils. In some lymphoid follicles may be formed (*follicular cervicitis)*. The surface epithelium may be either normal, or may show squamous metaplasia.

WHO (2014) CLASSIFICATION OF TUMORS OF UTERINE CERVIX

WHO (2014) classification of tumors of uterine cervix (abridged) is presented in **Box 22.1**.

CERVICAL INTRAEPITHELIAL NEOPLASIA (SQUAMOUS INTRAEPITHELIAL LESIONS)

CC: Describe the epidemiology, pathogenesis, etiology, pathology, screening, diagnosis and progression of carcinoma of the cervix.

Q. Define and write short note on cervical intraepithelial neoplasia (CIN).

Definition: Cervical intraepithelial neoplasia (CIN) is defined as a **spectrum of intraepithelial changes**, which **begins from minimal atypia** and **progresses through** more-marked stages of **intraepithelial abnormalities to invasive squamous cell carcinoma**.

- Age: **CIN** generally occurs **under the age of 40**.

Etiology and Pathogenesis of CIN andCarcinoma of Cervix

Q. Write short essay on etiology and pathogenesis of carcinoma of cervix.

Q. Write sort note on risk factors for carcinoma cervix.

Risk factors: Common risk factors of CIN and carcinoma of cervix include: (1) human papilloma virus, and (2) environmental factors.

Etiology and pathogenesis have common features for both CIN and carcinoma of cervix and are discussed together.

Human Papilloma Virus Infection

Q. Discuss the role of HPV in carcinoma cervix.

Human papilloma virus infection (HPV) is a sexually transmitted DNA virus. Following features are associated with increased exposure to HPV and increased incidence of both CIN and cervical cancer. These features point that HPV is a major risk factor. Increased incidence of CIN and carcinoma cervix is observed in women with:

- **Multiple sexual partners**
- **First intercourse at young age**
- **High parity**
- **A male partner having multiple previous or current sexual partners**
- **Persistent infection** with a high oncogenic risk HPV, e.g., **HPV 16 or HPV 18**

- **Inefficient immune response:** Most HPV infections are transient which are eliminated by the immune response and immunosuppression may result in persistent HPV infection.

Classification of HPV

A. **Low oncogenic risk:**

- Causes **condyloma acuminatum** in vulva, perineal and perianal region.
- **HPV genome** is maintained in a **nonintegrated free episomal** (extrachromosomal) **form**.
- **Virus freely replicates** and **causes the death of the host** cell and it is **known as productive infection**.

B. **High oncogenic risk**:

- There are about 15 types of high oncogenic risk HPVs. Among these types, **HPV 16 and HPV 18 are the most important** in **cervical carcinogenesis**. Others include HPV 31 and 33.
- Can also cause **squamous cell carcinoma** of the **vagina, vulva, penis, anus, tonsil and oropharynx**.
- **Integration into the host DNA:** In cancers, the HPV genome (viral DNA) is integrated into the genome of the host cell, which is important for malignant transformation. **Integration causes:**
  - **Overexpression of the oncoproteins E6 and E7**.
  - **Genomic instability** in the host cell.

Features of Infection by HPV

Even though **HPV is a causative factor** for CIN and cancer of the cervix, **only few infected will develop cancer**. Most women infected with HPV clear the infection by immunological mechanisms.

BOX 22.1: WHO (2014) classification of tumors of uterine cervix (abridged).

Epithelial tumors

- Squamous cell tumors and precursors:
  - Squamous intraepithelial lesions
    - Low-grade squamous intraepithelial lesions (LSIL)
    - High-grade squamous intraepithelial lesions (HSIL)
  - Squamous cell carcinoma:
    - Keratinizing
    - Nonkeratinizing

- Glandular tumors and precursors:
  - Adenocarcinoma in situ
  - Adenocarcinoma
- Neuroendocrine tumors:
  - Low-grade neuroendocrine tumor: Carcinoid tumor
  - High-grade neuroendocrine carcinoma:
    - Small cell neuroendocrine carcinoma (formerly termed small cell carcinoma)
    - Large cell neuroendocrine carcinoma

Mesenchymal tumors

- Benign: Leiomyoma
- Malignant: Leiomyosarcoma

Other tumors

- **Longer the duration of infection**, **higher the risk of CIN** and subsequent **carcinoma**. Infections with high oncogenic risk HPVs last longer than infections with low oncogenic risk HPVs.
- **HPVs infect immature basal cells of the squamous epithelium**, **or immature metaplastic squamous cells** present at the squamocolumnar junction.
- **Infects only damaged surface epithelium** and **not the mature intact superficial squamous cells**. In areas of epithelial breaks or damage, the HPV can reach the immature cells in the basal layer of the epithelium.
- **Replication occurs in the maturing nonproliferating squamous cells which normally are arrested in the G1 phase of the cell cycle** (though the virus infects only the immature squamous cells). However, these mature cells actively progress through the cell cycle when infected with HPV by using the host cell DNA synthesis machinery to replicate its own genome.
- **HPV has to induce DNA synthesis** and must **reactivate the mitotic cycle** in such nonproliferating cells. Viral replication results in a cytopathic effect, **"koilocytic atypia",** consisting of **nuclear atypia** and a **cytoplasmic perinuclear halo**.

Oncogenesis by HPV

High-risk HPV types overexpress **E6 and E7 oncoproteins** that are responsible for the oncogenesis (*refer* pages 226-7 and **Fig. 7.21**).

- **Actions of E7 protein of HPV:** Inactivation of RB and inhibition of cyclin-dependent kinase inhibitors (e.g., p21 and p27). These two actions

increase progression of cell cycle and impair the ability of cells to repair DNA damage.

- **Actions of E6 protein of HPV:** Degradation of the tumor suppressor protein p53 and up-regulates the expression of telomerase → leads to cellular immortalization.
- **Integration of viral DNA into the host cell genome:** In most cancers the effects include (1) increased expression of *E6* and *E7* genes, (2) dysregulation of oncogenes near the sites of viral insertion (e.g., MYC).
- **Extrachromosomal (episomal) form of viral DNA:** It is observed in precursor lesions associated with high-risk HPVs and in condylomata associated with low-risk HPVs.

Environmental Factors

Only HPV infection is not sufficient for carcinogenesis and it acts in association with the environmental factors. The environmental factors include:

- **Presence of co-carcinogens**: Cigarette smoke which contains polycyclic hydrocarbons, acts as a co-carcinogen.
- **Coexisting microbial infections**
- **Dietary deficiencies**
- **Hormonal changes**
- **Use of oral contraceptives**.

Consequences of HPV infection in the cervix are shown in **Figure 22.1**.

Classification of Cervical Precancers (Fig. 22.2)

Q. Write short note on classification of cervical intraepithelial neoplasia.

Terminologies, namely CIN, dysplasia, CIS, and squamous intraepithelial lesion (SIL) are commonly used interchangeably. The classifications presently being used is **Bethesda system** squamous intraepithelial lesion.

Carcinoma In Situ (CIS) System

It is the oldest classification in which **mild dysplasia** is on one end of the spectrum and severe **dysplasia/carcinoma in situ** on the other end.

Cervical Intraepithelial Neoplasia Classification

According to this, mild dysplasia is termed **CIN I**, moderate dysplasia **CIN II**, and severe dysplasia termed as **CIN III**.

Squamous Intraepithelial Lesion

The **Bethesda system** for Reporting Cervical/Vaginal Cytologic Diagnoses, groups these lesions into **low- and high-grade squamous intraepithelial lesions**.

- **Low-grade squamous intraepithelial lesion (LSIL):** CIN I is renamed as low-grade squamous intraepithelial lesion

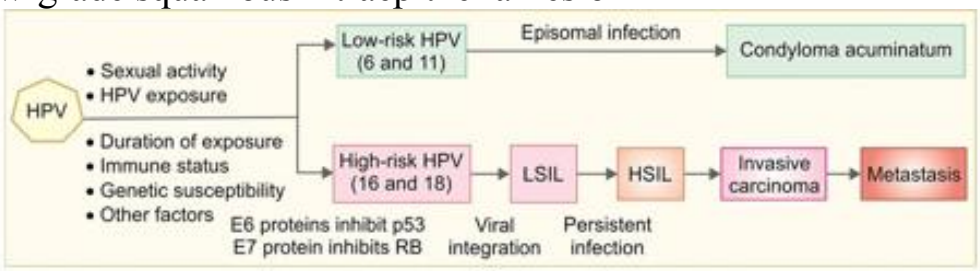


Fig. 22.1: Consequences of HPV infection in the cervix.
(LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion).

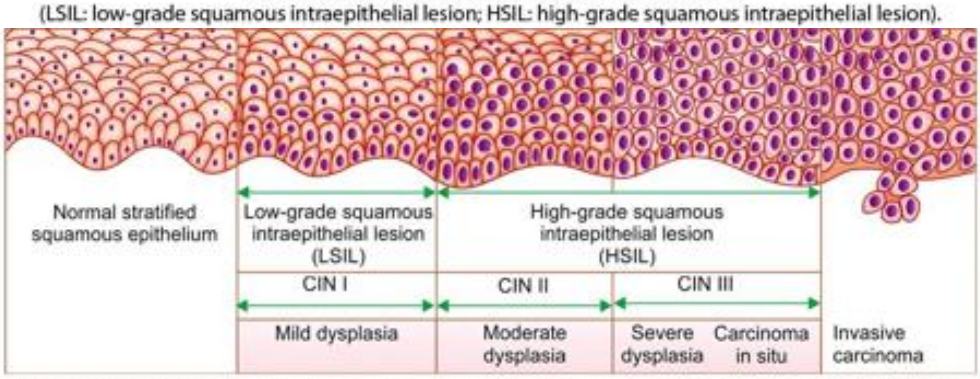


Fig. 22.2: Spectrum of cervical intraepithelial neoplasia with normal stratified squamous epithelium for comparison. LSIL (CIN I/mild dysplasia); HSIL (CIN II and CIN III/severe dysplasia and carcinoma in situ).

  - Associated with productive HPV infection.
  - Most LSILs regress spontaneously.
- **High-grade squamous intraepithelial lesion (HSIL)**: CIN II and CIN III are combined into one category and are known as high-grade squamous intraepithelial lesion.
  - HSIL tend to progress to carcinoma if not treated.

MORPHOLOGY

Q. Write short note on morphology of carcinoma in situ/squamous intraepithelial lesion.

Squamous Intraepithelial Lesion (SIL)

Spectrum of morphologic changes range from **normal**, **low-grade to high-grade SIL (Fig. 22.2)**.

Diagnosis of SIL: It is **based on identification of atypical immature squamous cells** that have following features:

1. **Nuclear atypia:** The characteristics of nuclear atypia are:
   - **Nuclear enlargement**.
   - **Pleomorphic nuclei: Variation of nuclear sizes and shapes**.
   - **Hyperchromasia: Characterized by dark staining of nuclei**.
   - **Coarse chromatin granules**.
2. **Nuclear cytoplasmic ratio: Increased**.
3. **Loss of polarity**.
4. **Cytoplasmic change:** The nuclear atypia mentioned above may be accompanied by perinuclear cytoplasmic halo, which are termed as **koilocytic atypia (Figs. 22.3A to C).** The

koilocyte (from the Greek *koilos*, hollow) is **a superficial or intermediate mature squamous cell** characterized by:

- **Cytoplasm**: Sharply outlined **perinuclear halo (vacuolation)** formed **due to extensive cytoplasmic destruction** by a HPV-encoded protein E5 that is localized to the membranes of the endoplasmic reticulum. **Peripheral cytoplasm** is **dense and shows irregular staining**.
- **Nucleus**: **Enlarged, wrinkled** with an **undulating** (raisin- or prune-like) **nuclear membrane** and **rope-like chromatin** pattern.

Koilocytes are absent in many cases of high-grade dysplasia and all invasive cancers.

Markers of actively dividing cells (e.g., Ki-67) are normally restricted to the actively dividing cells of basal layer of the epithelium. With HPV infection, E6 and E7 proteins of HPV prevent arrest of cell cycle arrest in the upper portion of the epithelium resulting in replication of these cells. Thus, superficial cells express markers, such as Ki-67. Disturbed growth regulation also causes overexpression of p16, a cyclin-dependent kinase inhibitor. Ki-67 and p16 staining show high correlation with HPV infection and are useful for confirming the diagnosis in doubtful cases of SIL.

Grading of SIL

Squamous intraepithelial lesion is graded as LSIL (low) and HSIL (high grade) based on expansion of the immature cell layer from its normal basal location.

- **LSIL:** If the **atypical**, immature squamous **cells** are **confined to the lower one-third of the epithelium**, the lesion is graded as LSIL.
- **HSIL (Figs. 22.3A to F):** If the **atypical,** immature squamous **cells** expand to **involve lower two-thirds of the epithelium**, the lesion is graded as HSIL.

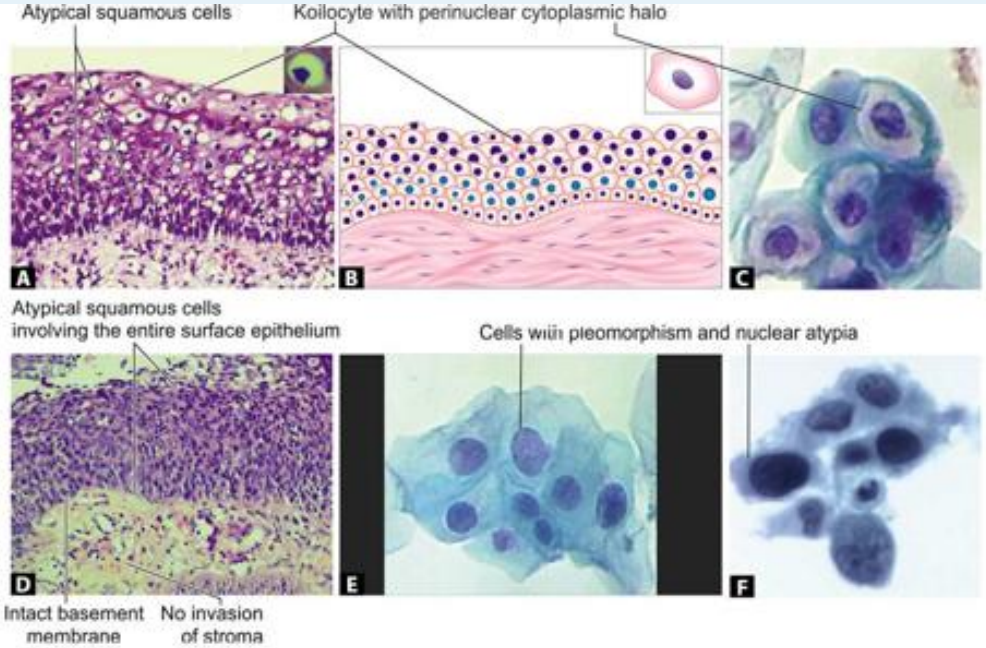
CIN: Spectrum of intraepithelial changes ranging from minimal atypia to marked atypia without invasion of stroma.

Risk factors for CIN and carcinoma cervix related to HPV exposure:
1. Early age at first intercourse
2. Multiple sexual partners
3. High parity
4. High-risk HPV (16 and 18)

HPV types: Low and high oncogenic risk.

CIN and carcinoma cervix: HPV is the major risk factor.

Low oncogenic risk HPV: Virus replicates within the host cell leading to death of infected cell.



Figs. 22.3A to F: (A) Hematoxylin and Eosin (H and E); (B) Diagrammatic LSIL with koilocyte; (C) Cervical cytology showing koilocytes; (D) HSIL (H&E); (E) Low-grade squamous intraepithelial lesion (LSIL); (F) High-grade squamous intraepithelial lesion (HSIL). Cervical cytology with pleomorphism and nuclear atypia.

Condyloma is caused by HPV types: 6 and 11.

High-risk HPV: Types 16 and 18.

HPV: Integration of viral DNA into the host DNA is necessary for malignant transformation of the host cell.

HPV infects:
1. Immature basal cells of squamous epithelium
2. Immature metaplastic squamous cells
3. Damaged surface epithelial cells of squamous epithelium

High-risk HPV: Express E6 and E7 oncoproteins → oncogenesis.

Risk factor for carcinoma of cervix:
- HPV
- Cigarette smoking
- Immunodeficiency

Tumors caused by HPV:
- Squamous intraepithelial lesion (SIL)
- Carcinoma cervix
- Adenocarcinoma or adenosquamous carcinoma
- Neuroendocrine carcinoma

Presently used classification of cervical precancer is Bethesda system. According to this, it is classified as (i) LSIL and (ii) HSIL.

Koilocyte: Superficial or intermediate mature squamous cell with perinuclear halo and enlarged, wrinkled nuclear membrane. It is due to effect of HPV on squamous cells.

Raisin nucleus: (1) Koilocyte, (2) Clear cell RCC.

Koilocytotic atypia:
1. Nuclear atypia
2. Perinuclear vacuolization
3. Caused by HPV
4. Considered as viral “cytopathic” effect

INVASIVE CARCINOMA OF CERVIX

Q. Describe the etiology, pathology and spread of carcinoma of cervix.

- **Precursor lesion**:

- **HSIL** is an immediate precursor of **cervical squamous cell carcinoma.**
- Precursor lesion for adenocarcinoma is called adenocarcinoma in situ.

- **Age**: **Cervical cancer** is usually found between **40 and 60 years** (mean age 54 years) with peak at 45 years.

Etiology and Pathogenesis(Refer pages 226-8 and 738-9)

MORPHOLOGY

Q. Write short note on morphology of carcinoma of cervix.

Gross

Carcinoma of cervix arise from the transformation zone. Three patterns of invasive cervical carcinoma are:

1. **Fungating (exophytic)**: Forms a nodular and **exophytic cauliflower-like** growth protruding into vagina **(Figs. 22.4A and B)**.
2. **Ulcerative lesion**: It appears as **ulcerated, granular, eroded** lesion.
3. **Infiltrative mass**: If the tumor is located within the **endocervical canal**, it may infiltrate the stroma and cause diffuse enlargement and hardening of the cervix (**barrel-shaped cervix**).

Tumors encircling the cervix and penetrating into the underlying stroma produce a **barrel cervix**. This can be identified by direct palpation.

Microscopy

A. **Squamous cell carcinoma:**

- **Most common** (~ 80%) histological subtype of cervical cancer.
- Composed of **solid nests and groups of malignant squamous cells** (either keratinizing or nonkeratinizing) **invading the underlying stroma** of the cervix.
- **Subtypes:** Depending on the cytological features, it can be divided into **two subtypes**:
  1. **Large cell keratinizing:** It consists of nests of keratinized cells, which form concentric whorls known as **keratin or epithelial pearls (Fig. 22.5)**.
  2. **Large cell nonkeratinizing:** This pattern shows nests of large malignant squamous cells with individual **cell keratinization but without any keratin pearls**.

B. **Adenocarcinoma:**
   - **Second most common** tumor type (~ 15%).
   - **Microscopy (Fig. 22.6):**
     - Consists of **malignant endocervical cells forming glandular pattern**.
     - Tumor cells have large, hyperchromatic nuclei and with minimal mucin in the cytoplasm.

C. **Adenosquamous carcinomas** are rare cervical tumors. They are composed of malignant glandular epithelium admixed with malignant squamous epithelium.

D. **Neuroendocrine cervical carcinoma:** It appears similar to small-cell carcinoma of the lung.

Spread of Carcinoma Cervix

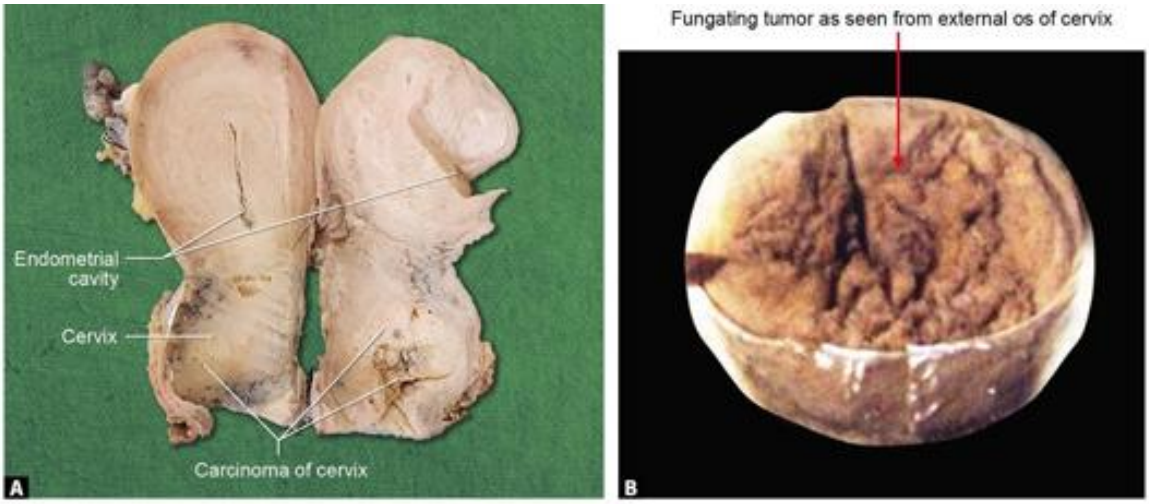
Q. Write short note on spread of carcinoma of cervix.

1. **Direct extension:** Into the surrounding tissues and organs.
   - Paracervical tissues.
   - Urinary bladder and rectal: Their involvement may lead to fistula formation.
   - Ureters: If involved may lead to its obstruction → hydroureter, hydronephrosis, pyelonephritis, and renal failure (uremia).
   - Vagina.
2. **Lymphatics:** Tumor may spread to regional lymph nodes and involve paracervical, hypogastric, and external iliac nodes.
3. **Hematogenous spread:** Liver, lungs and bone marrow.

Clinical Features

- During early stages of cervical cancer, patients most often present with **vaginal bleeding** after intercourse.
- With more advanced tumors, symptoms depend on the route and degree of spread.

Prognosis for invasive carcinomas depends largely on the stage of the carcinoma.



Figs. 22.4A and B: Squamous cell carcinoma of cervix: (A) Longitudinal section of uterus and cervix with a tumor in the cervix; (B) Carcinoma cervix presenting as a fungating growth projecting from cervical os.

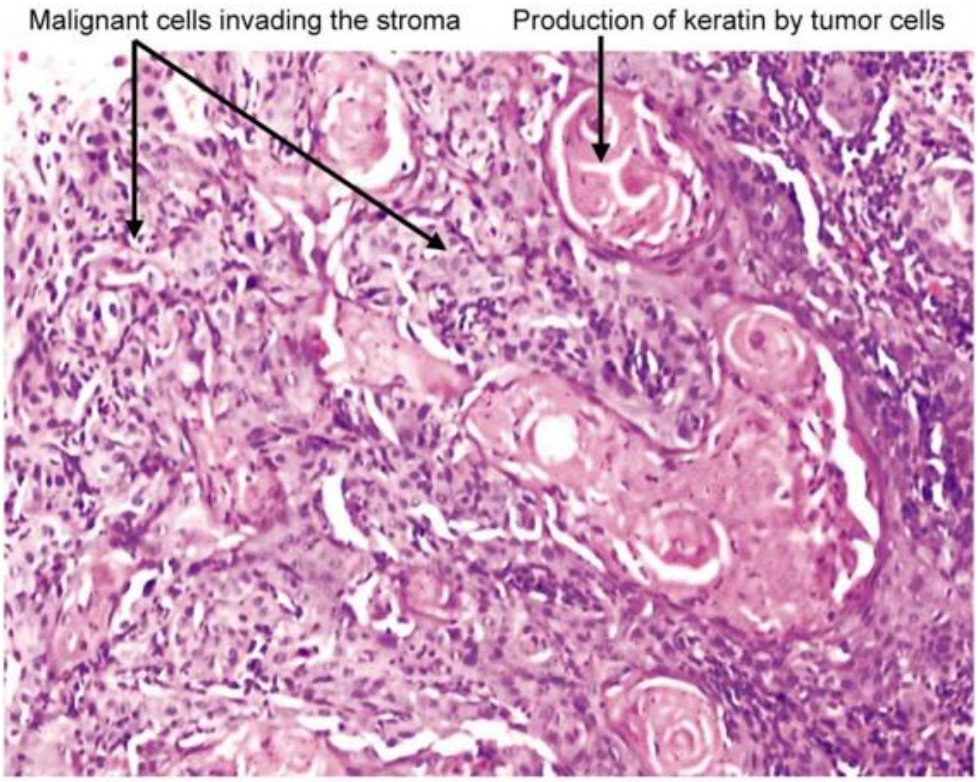


Fig. 22.5: Photomicrograph of invasive keratinizing squamous cell carcinoma of cervix.

Cervical Cancer Screening

Q. Write short note on diagnosis of carcinoma of cervix.

Cytologic Screening by Pap Smear Examination

- Majority of cervical cancers are preceded by a precancerous lesion and **cytologic screening by Pap smear** is the **most reliable screening test for preventing and also detecting noninvasive stage of cervical cancer**.
- **Pap tests** are **cytological preparations of exfoliated cells from the cervix** that are **stained with the Papanicolaou method** (*refer* page 281). Using a spatula **(***refer* **Fig. 10.1)** or brush, the transformation zone of the cervix is circumferentially scraped and the cells are smeared onto a slide. Following fixation and staining, the smears are screened **to identify cytologic abnormalities (Fig. 22.7)**.
- In addition, **HPV DNA testing** may be carried out.

Histologic Diagnosis and Removal of Precancerous Lesions

- If **the Pap test shows abnormal cells**, a **colposcopic examination** of the cervix and vagina is performed to know the extent of the lesion and lesion is biopsied.
- Application of **acetic acid to the cervix highlights abnormal areas**.
- After confirmation by tissue biopsy, women with LSIL can be followed up with repeat smears.
- **HSILs are treated with cervical conization** (excision) and follow-up smears and clinical examinations for life.
- **Surgical removal of invasive cancers with adjunctive therapy** (radiation and chemotherapy).

Cervical Cancer Prevention

- Prophylactic quadrivalent HPV vaccine for HPV types 6, 11, 16, and 18 has been used to reduce the incidence of cervical cancer.
- The vaccine is prepared from noninfectious, DNA-free virus-like particles produced by recombinant technology.
- It produces high levels of serum antibodies in vaccinated individuals.

Q. A-48 year-old woman complains of brownish, foul-smelling vaginal discharge for the last 6 months. She also gives a history of postcoital spotting. On per vaginal and per speculum examination, the cervix showed 3 cm ulceroproliferative growth which bleeds on touch to cervix.

(a) What is the probable diagnosis and give reason? (Ans: Carcinoma cervix)

(b) Discuss the etiopathogenesis of the disease. (refer pages 738-9)

(c) Describe the morphological features of this condition. (refer pages 742)

(d) What screening methods/investigations will help in the diagnosis of this condition? (refer page 743)

Carcinoma cervix: Gross patterns
1. Fungating
2. Ulcerative
3. Infiltrative

Carcinoma cervix: Squamous cell carcinoma is the most common histological subtype.

Carcinoma cervix: Most common cancer in women in India.

Microscopic types of squamous cell carcinoma of cervix:
1. Large cell keratinizing
2. Large cell nonkeratinizing

Cervical Pap smear uses:
- For screening SIL and cervical cancer
- Evaluation of hormonal status

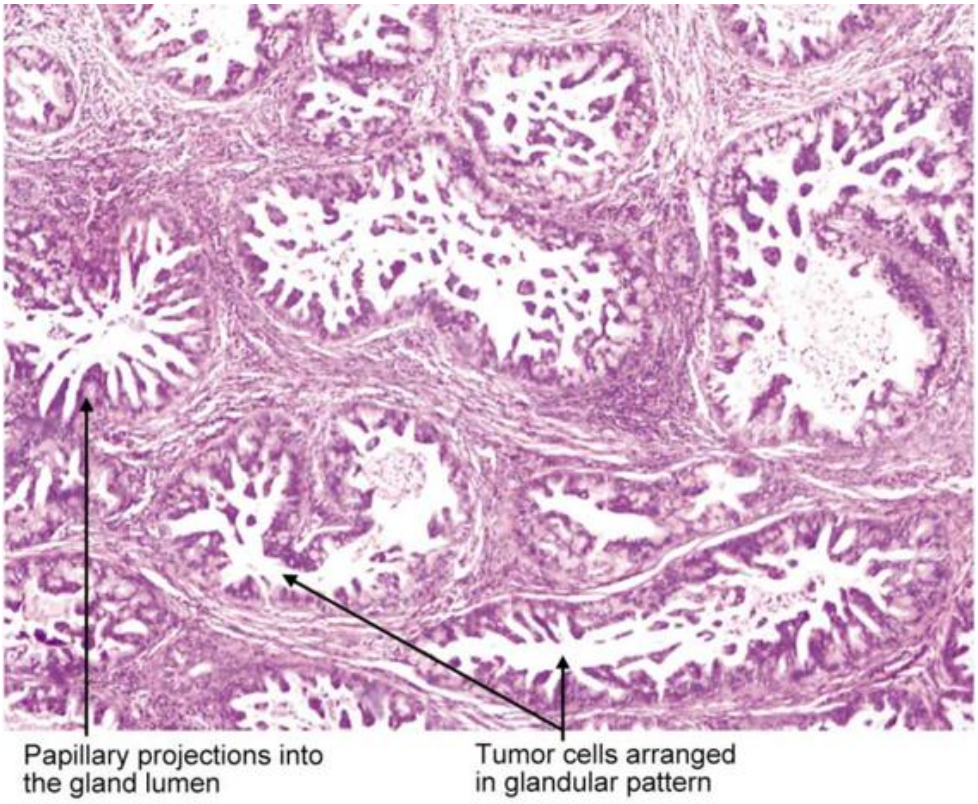
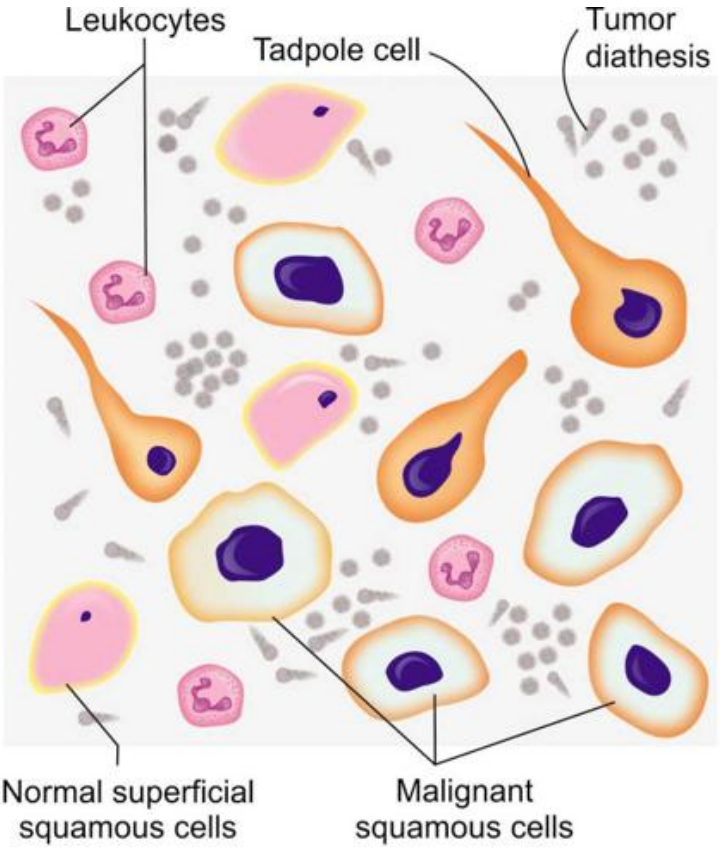


Fig. 22.6: Photomicrograph of adenocarcinoma of cervix.



Cervical cancer: Reduced incidence because of cytologic screening by Pap smear examination.

Pap smear: Highly effective for screening CIN and cervical cancer.

HSIL: Precursor of cervical squamous cell carcinoma.

Fig. 22.7: Cytological appearance of squamous cell carcinoma of cervix (diagrammatic).

UTERUS

MENSTRUAL CYCLE

The endometrium undergoes characteristic morphologic changes during the menstrual cycle due to sex hormones produced in the ovary.

The normal menstrual cycle begins with the shedding of the upper half to two-third of the endometrium during menses.

Proliferative Phase (Figs. 22.8A and B)

- **Endometrial glands:**
  - During the proliferative phase, the endometrial glands are **straight, tubular and evenly distributed**.
  - Glands are **lined by regular, tall, pseudostratified columnar cells**. Mitotic figures are numerous.
- **Endometrial stroma:**
  - Consists of **compactly arranged spindle cells** having scanty cytoplasm but abundant mitotic activity.
  - Spiral arteries are narrow and mostly inconspicuous.

Secretory Phase

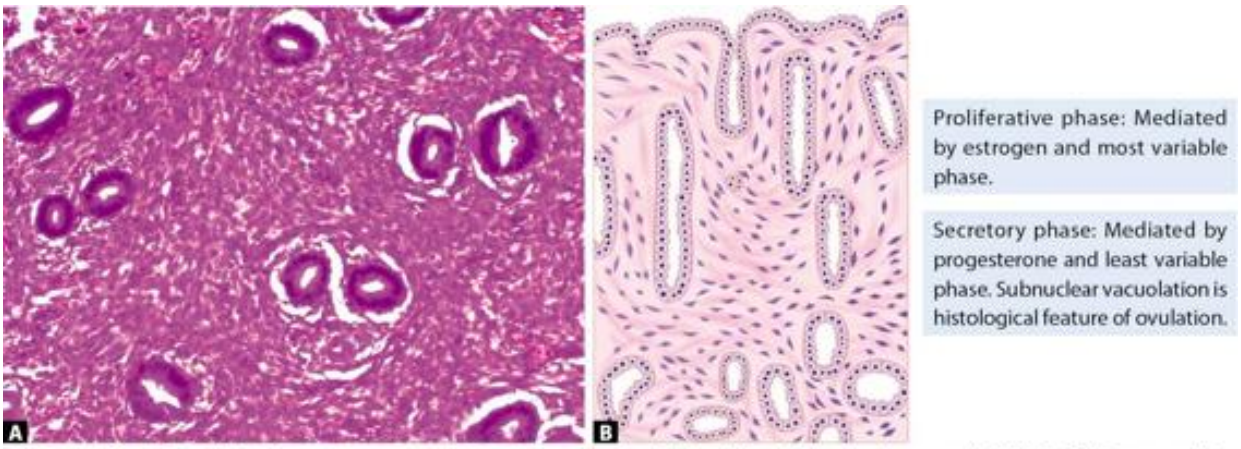
- **Endometrial glands:**
  - **Enlarge, dilate**, and **become more coiled**.
  - The lining cells develop abundant and prominent, glycogen-rich, **subnuclear vacuoles (Fig. 22.9)**.
  - The **secretion increases** and the basal vacuoles progressively push past the nuclei. Later, the **secretions are discharged into the lumen** of the gland → the glands are dilated and tortuous, producing a serrated ("**saw-toothed**") appearance **(Figs. 22.10A and B)** when they are cut in their long axis.
- **Endometrial stroma:**
  - The stromal cell enlarge (hypertrophy) and show **large, round, vesicular nuclei and abundant eosinophilic cytoplasm** and reappearance of stromal mitoses.
  - These cells are the precursors of the decidual cells of pregnancy and are referred to **predecidual change**.

Menstrual Phase

- In the absence of pregnancy, spiral arteries collapse, and the disintegration of the functionalis begins.
- The blood escapes into the stroma, marking the beginning of menstrual shedding. Menses commence on day 28, lasts 3–7 days.

ENDOMETRIOSIS

NC: Describe the etiology, hormonal dependence, features and morphology of endometriosis.



Figs. 22.8A and B: Proliferative phase composed of tubular endometrial glands lined by tall columnar cells (A, H&E; B, diagrammatic).

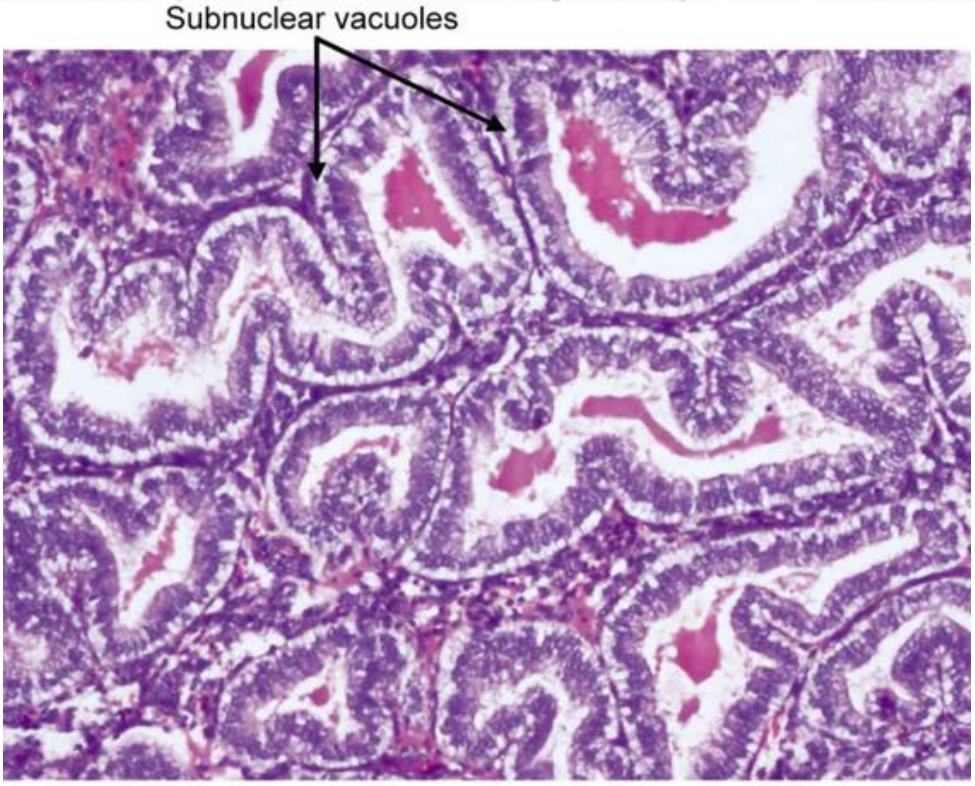


Fig. 22.9: Early secretory phase shows endometrial glands lined by tall columnar cells with subnuclear vacuoles.

Q. Write short note on endometriosis.

Definition: Endometriosis is the **presence of endometrial tissue** (glands and stroma) **outside of the uterus**.

Age: Mainly occurs **during active reproductive life**, mostly in **third and fourth decades**.

Incidence: Affect ~ 5–10% of women.

Pathogenesis

The proposed origin of endometriosis may be from two main sources: (1) from the uterine endometrium and (2) from outside the uterus from cells which has the capacity to give rise to endometrial tissue. Main theories includes:

1. **Regurgitation theory:** According to this, the endometrial tissue by retrograde menstruation passes through the fallopian tubes and are implanted at abnormal/ectopic site. It can explain the distribution of endometriosis within the peritoneal cavity. Retrograde menstruation occurs even in normal women.
2. **Benign metastases theory:** According to this, endometrial tissue can spread from the uterus to distant sites (e.g., bone, lung, and brain) via blood vessels and lymphatic channels.
3. **Metaplastic theory:** According to this theory, endometrium arises directly from metaplasia of celomic epithelium (mesothelium of pelvis or abdomen). During embryonic development, the Müllerian ducts and the endometrium arise from celomic epithelium.
4. **Extrauterine stem/progenitor cell theory:** It is a recent theory according to which the endometrial tissue in ectopic site represents differentiation of stem/progenitor cells from the bone marrow.

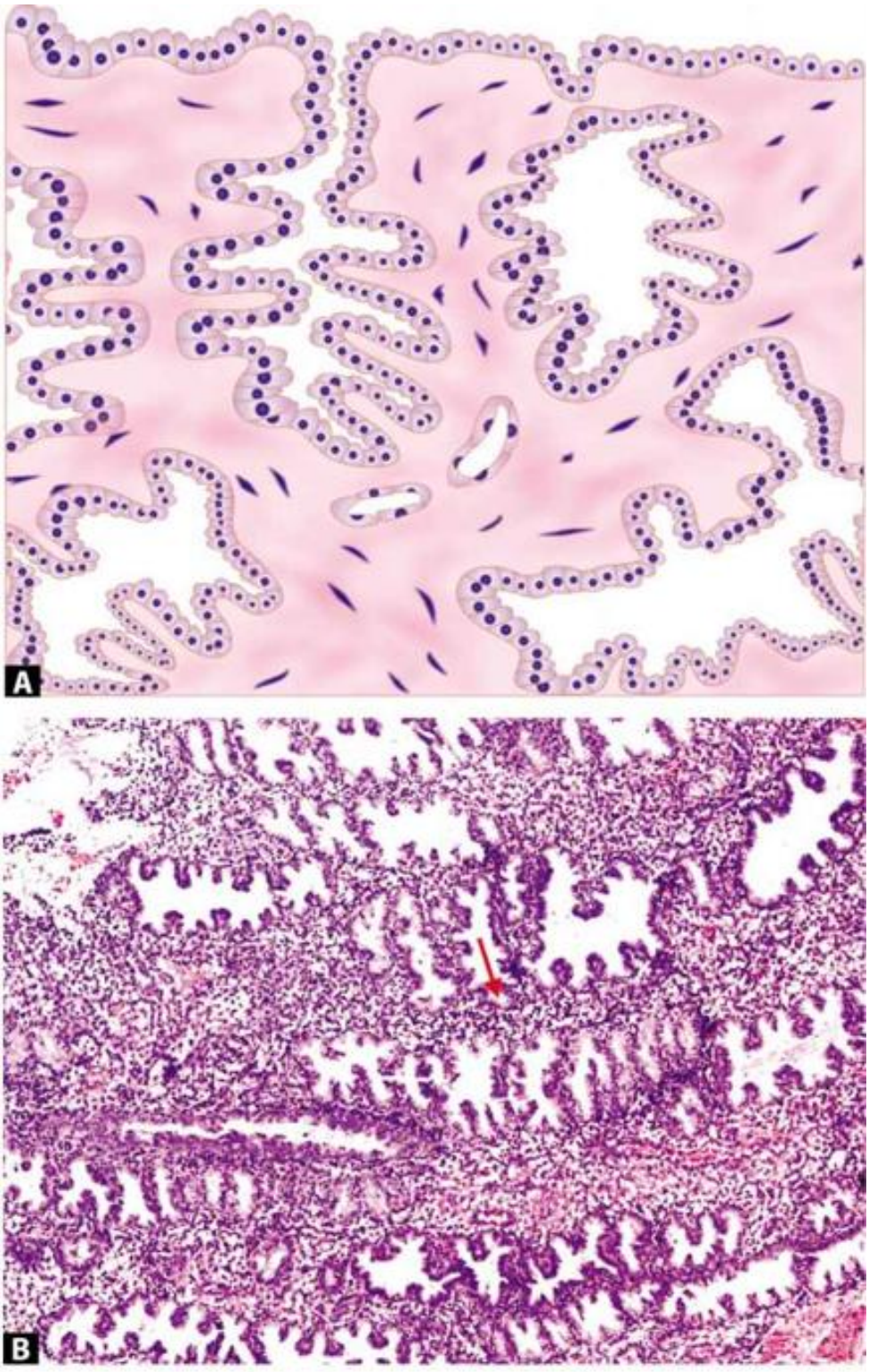
Proposed mechanism of endometriosis: The **endometriotic tissue in endometriosis is not just misplaced but is also abnormal.** Molecular analysis of endometriotic tissue showed some differences when compared to the endometrium in women without endometriosis. They differ from normal in the following:

- **Increased levels of inflammatory mediators,** e.g., prostaglandin E2 (PGE2), IL-1β, TNFα, IL-6 and -8, etc. Probably inflammation results from the recruitment and activation of macrophages by factors synthesized by endometrial stromal cells.
- Stromal cells also secrete aromatase which favors **increased local estrogen production by endometriotic stromal cells.**

The above two factors increase the survival and persistence of the endometriotic tissue in a foreign location (important in the pathogenesis of endometriosis). This is supported by the fact that the use of COX-2

inhibitors (against PGE2) and aromatase inhibitors are found to be beneficial in the treatment of endometriosis.

Association between endometriosis and ovarian endometrioid and clear cell type carcinoma is observed.



Figs. 22.10A and B: Late secretory endometrium saw-tooth appearance of gland and predecidual changes in the stroma: (A) H&E; (B) Diagrammatic.

BOX 22.2: Various sites of endometriosis in descending order of frequency.

1. Ovaries (more than 60%)
2. Uterine ligaments
3. Rectovaginal septum
4. Cul de sac
5. Pelvic peritoneum
6. Large and small bowel and appendix
7. Cervix, vagina, and fallopian tubes
8. Laparotomy, hysterectomy scars

MORPHOLOGY

Sites (Box 22.2).

Gross

- They produce **nodules** with a **red-blue to yellow-brown** appearance at the site of endometriosis.
- Foci of endometriosis **respond to both extrinsic and intrinsic hormonal stimulation** with **periodic bleeding → organization of hemorrhage** causes fibrosis → subsequent **fibrous adhesions** between neighboring structures (e.g., tubes, ovaries and obliterates the pouch of Douglas).
- **Endometriosis of ovary**: Called as chocolate cyst.

Q. Write short note on endometriosis/chocolate cyst of ovary.

- Ovaries may be distorted by numerous **cystic spaces** (1–5 cm in diameter) **filled with dark brown fluid** (due to previous hemorrhage).
- **Repeated hemorrhage** may form **large cysts** up to 15 cm in diameter, which **contain inspissated, chocolate-colored material** → clinically known as **chocolate cysts** or endometriomas.

Microscopy

- Commonly, shows **both endometrial glands and stroma with or without** the presence of **hemosiderin-laden macrophages** at the ectopic site.
- Rarely, may show only endometrial stroma and/or hemosiderin-laden macrophages.

Scar Endometriosis

Q. Write short answer on scar endometriosis.

Rarely, endometriosis can occur outside of the pelvis, and these are called as extrapelvic endometriosis. Endometriosis occurring in scar tissue is rare and is called scar endometriosis. It occurs at incision site, after obstetrical (especially after cesarean section) and gynecological surgeries. The term cesarean scar endometriosis (CSE) is an unusual manifestation of extrapelvic endometriosis. The patient may complain of formation of a mass or lump (size varies) in the surgical scar. There is often pain in the abdominal wall at the incision site at the time of menstruation and is because of the bleeding in the area of endometrial tissue. The bleeding is very irritating to the abdominal organs.

Clinical Features

- Dysmenorrheal (painful menstruation)
- Dyspareunia (pain during intercourse)
- Pelvic pain (due to the intrapelvic bleeding and periuterine adhesions)
- Pain on defecation (rectal wall involvement)
- Dysuria (involvement of serosa of the bladder)
- Intestinal disturbances (when the small intestine is affected)
- Menstrual irregularities

Consequences

- Infertility in ~30–40% of women.
- Malignancy: Uncommon

Endometriosis: Functioning endometrial glands and stroma outside the uterus. Undergoes cyclic bleeding.

Endometriosis of ovary: Most common site → chocolate cysts.

ADENOMYOSIS

NC: Describe the etiology and morphologic features of adenomyosis.

Q. Write short note on adenomyosis of uterus.

Definition: Adenomyosis is defined as the **presence of endometrial tissue within the**

myometrium (uterine wall).

Adenomyosis seen in ~20% of uterine specimens.

Etiology and Pathogenesis

Pathogenesis is **not known**. Adenomyosis is seen in continuation with the endometrium. So, it may develop due to down growth followed by proliferation of endometrial tissue into and between the smooth muscle fascicles of the underlying myometrium.

MORPHOLOGY

Gross

- **Uterus** may show **mild to moderate enlargement**.
- Cut section of the **uterine wall** shows coarse **trabeculations with ill-defined area of hemorrhage**.

Microscopy (Fig. 22.11)

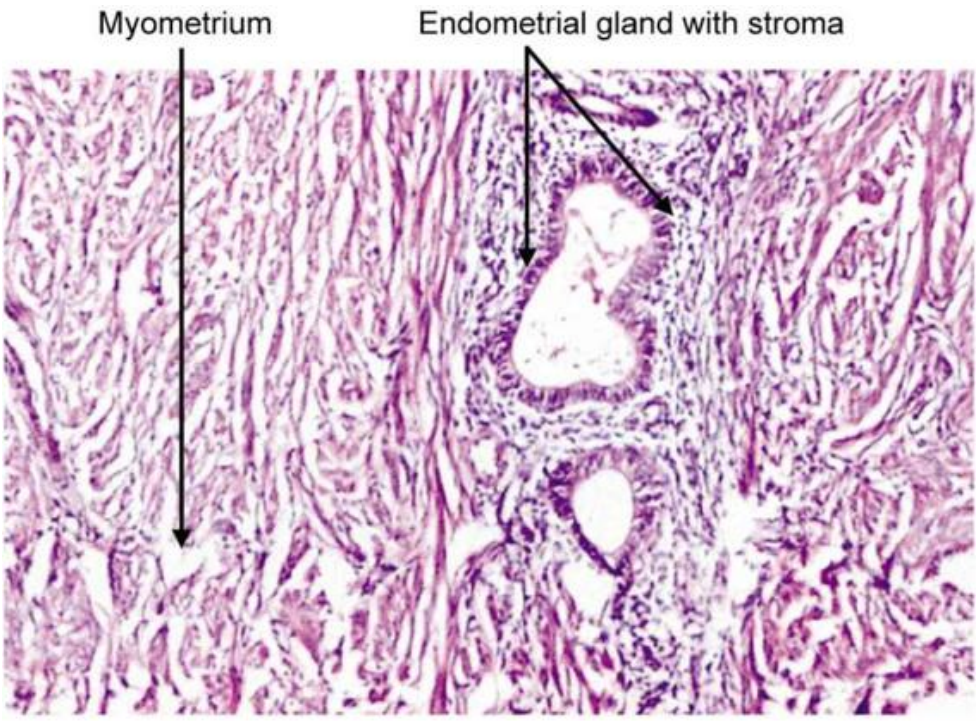
- Shows irregular **nests of Benign endometrial glands and stroma** deep within the **myometrium**.
- The **minimum distance between the basal endometrium and the endometrial tissue** of adenomyosis should be **one low power microscopic field** (i.e., at least 2–3 mm).

Clinical Features

- Metrorrhagia (irregular and heavy menses)
- Colicky dysmenorrhea
- Dyspareunia
- Pelvic pain (during premenstrual period)

ENDOMETRIAL HYPERPLASIA

NC: Describe the etiology, hormonal dependence and morphology of endometrial hyperplasia.



Endometriosis: Extrauterine
- Endometrial glands
- Stroma

Endometriosis triad: (1) Dysmenorrhea; (2) Dyspareunia; (3) Infertility.

Adenomyosis: Endometrial glands and stroma within the myometrium.

Adenomyosis: No cyclic bleeding.

Fig. 22.11: Adenomyosis showing nests of endometrial glands and stroma deep within the myometrium.

Q. Write short note on endometrial hyperplasia/causes/types of endometrial hyperplasia.

Definition: Endometrial hyperplasia is defined as **increased thickness of endometrium** due to an **increased proliferation of the endometrial glands relative to the stroma**. This results in an increased gland-to-stroma ratio when compared with normal proliferative endometrium.

- Endometrial hyperplasia is an important **cause of abnormal uterine bleeding**.

- **Soil for carcinoma**: Endometrial hyperplasia is a soil for endometrial carcinoma and both share specific molecular genetic alterations.

Etiology

Prolonged Estrogen Stimulation

It can lead to endometrial hyperplasia. Increased estrogen may be due to:

- **Increased estrogen production**
  - **Endogenous sources**
    - **Obesity:** It is associated with increased peripheral conversion of androstenedione to estrone by the enzyme aromatase in fat cells.
    - **Polycystic ovarian disease, e.g.,** Stein-Leventhal syndrome.
    - **Functioning granulosa cell tumors** of the ovary.
    - **Excessive cortical function,** e.g., cortical stromal hyperplasia.
  - **Exogenous estrogen**: Prolonged administration of estrogen (e.g., estrogen replacement therapy).
- **Anovulation,** e.g., menopause.

Mutation of Tumor Suppressor Gene PTEN

It is **seen in both endometrial hyperplasias and carcinomas**.

- Loss of PTEN function may **activate cellular pathways** normally activated by estrogen.
- Thus, inactivation of the PTEN and hyperestrogen may act in conjugation both in endometrial hyperplasia and endometrial carcinoma.

Classification

Over the years, the classification of endometrial hyperplasia has undergone number of changes.

Old Classification

It was most widely used and was based on architectural and cytologic features. It consisted of four categories:

1. **Simple hyperplasia without atypia:** Also known as **cystic or mild hyperplasia** and is usually due to persistent estrogen stimulation. Progression to adenocarcinoma is uncommon (~1%). Microscopically, it

shows mild increase in the gland-to-stroma ratio. The glands are of various sizes and irregular shapes with cystic dilatation **(Figs. 22.12A and B)** → results in a **Swiss-cheese** appearance.

2. **Simple hyperplasia with atypia: Uncommon** and its progression to adenocarcinoma is about ~8%. Microscopically **(Fig. 22.13A)**, it appears like simple hyperplasia, but glandular epithelial cells show cytologic atypia.
3. **Complex hyperplasia without atypia:** Only ~3% progress to adenocarcinoma which is lower than that of simple hyperplasia with atypia. Microscopically **(Fig. 22.12C)**, number and size of endometrial glands increased with marked gland crowding (back-to-back with little intervening stroma), branching of glands and abundant mitotic figures. Epithelial cells are cytologically normal.
4. **Complex hyperplasia with atypia (Figs. 22.13B and C):** It **morphologically overlaps** with well-differentiated endometrioid **adenocarcinoma.**

World Health Organization (WHO) 2014 Classification

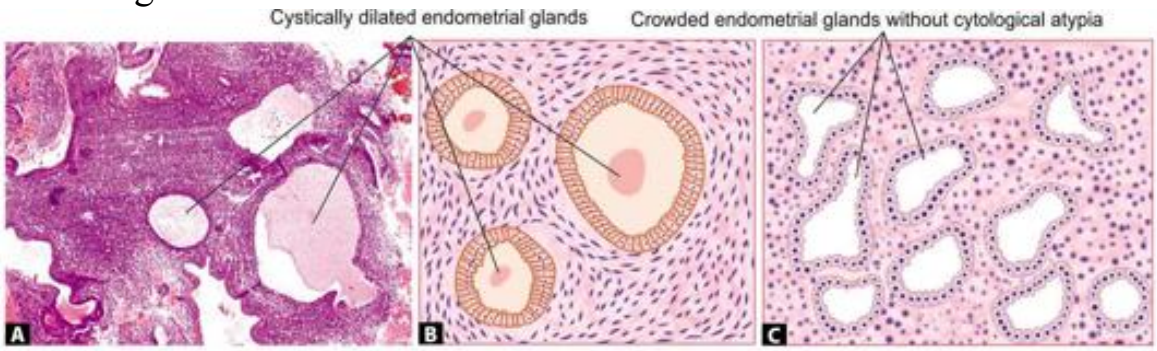
Q. Write a short note on morphological features of endometrial hyperplasia.

The most current classification divides endometrial hyperplasia into two major categories:

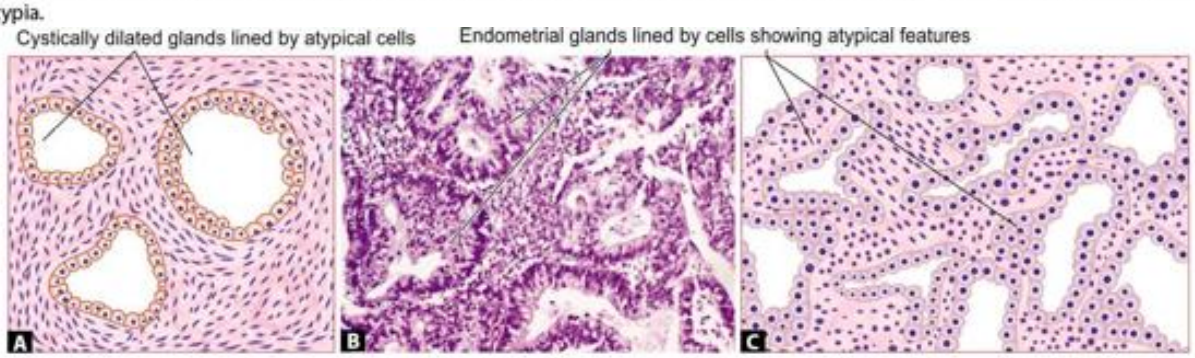
1. Hyperplasia without Atypia/Nonatypical Hyperplasia (Figs. 22.12A to C)

This is also termed hyperplasia without atypia and includes simple hyperplasia without atypia and complex hyperplasia without atypia. Main feature is an increase in the gland-to-stroma ratio. The glands show variation in size and shape and may show dilatation but without significant cytological atypia. There may be focal back-to-back arrangement of glands with some intervening stroma. They are due to persistent estrogen stimulation and

rarely progress to adenocarcinoma (~1–3%). It may undergo cystic atrophy when estrogen is withdrawn.



Figs. 22.12A to C: Endometrial hyperplasia without atypia: (A) Hematoxylin and eosin; (B) (Diagrammatic) Old terminology—simple hyperplasia without atypia showing cystic dilatation of glands; (C) (Diagrammatic) Old terminology—complex hyperplasia without atypia.



Figs. 22.13A to C: Atypical endometrial hyperplasia: (A) (Diagrammatic appearance) Old terminology—simple hyperplasia with atypia; (B) Hematoxylin and eosin; (C) (Diagrammatic) Old terminology—complex hyperplasia with atypia.

2. Atypical Hyperplasia/Endometrioid Intraepithelial Neoplasia (Figs. 22.13A to C)

This includes simple hyperplasia with atypia, complex hyperplasia with atypia and endometrial intraepithelial neoplasia. It consists of complex patterns of proliferating glands with nuclear atypia. The glands are usually crowded, show back-to-back arrangement and often with complex outlines due to branching of glands and loss of polarity. The nuclei are enlarged, pleomorphic and consist of open (vesicular) chromatin and conspicuous nucleoli. These features may overlap with those of well-differentiated endometrioid adenocarcinoma. Differentiating atypical hyperplasia from endometrial cancer may not be possible without hysterectomy.

Endometrial hyperplasia: Increased thickness of endometrium due to increased proliferation of endometrial glands.

Endometrial hyperplasia: Soil for endometrial carcinoma.

Endometrial hyperplasia: Prolonged estrogen stimulation.

Endometrial hyperplasia: Diffuse process involving the entire endometrium.

Mutation of *PTEN* (tumor suppressor) gene: Seen in both hyperplasia and carcinoma of of the endometrium.

WHO (2014) Classification of Tumors of Uterine Corpus (Box 22.3)

Q. Write a short note on classification of tumors of uterus.

BOX 22.3: WHO (2014) classification of tumors of uterine corpus (abridged).

Epithelial tumors and precursors

- **Precursors**
  - Hyperplasia without atypia
  - Atypical hyperplasia/endometrioid intraepithelial neoplasia
- **Endometrial carcinoma**
  - Endometrioid carcinoma (type I)
  - Serous carcinoma (type II)
  - Neuroendocrine tumors
    - Low-grade neuroendocrine tumor: Carcinoid tumor
    - High-grade neuroendocrine carcinoma:
      - Small cell neuroendocrine carcinoma (formerly termed small cell carcinoma)
      - Large cell neuroendocrine carcinoma

Mesenchymal tumors

- Benign: Leiomyoma
- Malignant: Leiomyosarcoma

Other tumors

CARCINOMA OF THE ENDOMETRIUM

CC: Describe the pathogenesis, etiology, pathology, diagnosis, progression and spread of carcinoma of the endometrium.

- Carcinoma of the endometrium is the most common pelvic invasive cancer of the female genital tract.
- **Age group**: Carcinoma of the endometrium is uncommon before 40 years of age, and mainly occur in **postmenopausal women** between **55 and 65 years**.
- Clinically present as **abnormal (postmenopausal) bleeding**.

Molecular Pathogenesis

Q. Risk factors for endometrial carcinoma.

Classification: According to **clinicopathological and molecular features,** carcinoma of the endometrium is classified into two types namely type I (endometrioid) and type II (serous carcinoma) **(Table 22.1)**.

Type I Carcinomas (Endometrioid Cancers)

- **Most common type** (>80%).
- **Majority** of them are **well-differentiated** and mimic proliferative endometrial glands → known as **endometrioid carcinoma**. **Develops from** precursor of **endometrial hyperplasia**.
- Associated with: (1) obesity, (2) diabetes, (3) hypertension, (4) infertility (nulliparous or have anovulatory cycles), and (5) unopposed estrogen stimulation.

Molecular Changes

Endometrial carcinoma develops in a stepwise pattern by acquiring several genetic alterations in tumor suppressor genes and oncogenes. The **hallmark** of type I endometrioid carcinoma is that the **most common mutations increase signaling through the PI3K/AKT pathway.** It is observed that individual tumor may have many mutations that increase PI3K/AKT signaling. This suggests that tumor development and progression is due to successive increase in signal strength. Mutations that increase PI3K/AKT signaling in endometrial carcinomas are:

- Mutations in the *PTEN* tumor suppressor gene (>50%).

- Mutations in *PIK3CA* (oncogene that encodes the catalytic subunit of PI3K and play a role in invasion) (~30%).
- *KRAS* mutations (~25%).
- Loss-of-function mutations in *ARID1A* (a regulator of chromatin structure) (40% low-grade carcinoma).
- Defects involving *DNA mismatch repair genes* and microsatellite instability~35%.
- Loss-of-function mutations in *TP53 (~30%)*

MORPHOLOGY

Gross (Figs. 22.14A to C)

Endometrial carcinoma may be:

- **Diffuse tumor** involving the endometrial surface (infiltrative)
- **Localized polypoid growth or more discrete nodules (exophytic)**.
  Large tumors usually **show areas of hemorrhage and necrosis**.

Microscopy

- Most endometrial carcinomas (~85%) are **endometrioid adenocarcinomas (Figs. 22.15A and B)**.
- **Glandular pattern: Resembles normal endometrial epithelium**
- **Nuclei:** Range from **bland to markedly pleomorphic**
- **Nucleoli: Prominent**.
- **Mitotic figures: Abundant** and may show abnormal mitotic figures in less differentiated tumors.

Grading: It is done according to the glandular differentiation alone.

- **Well-differentiated (grade 1/G1) adenocarcinoma**: It consists of tumor cells forming **easily recognizable glandular** patterns.
- **Moderately-differentiated (grade 2/G2) adenocarcinoma:** It consists of mixture of both **well-formed glands and solid sheets** of malignant cells.

TABLE 22.1: Differentiating features of type I and type II endometrial carcinoma.

Characteristics	*Type I (endometrioid)*	*Type II (serous carcinoma)*
Age	55–65 years	65–75 years

TABLE 22.1: Differentiating features of type I and type II endometrial carcinoma.

Characteristics	*Type I (endometrioid)*	*Type II (serous carcinoma)*
Predisposing conditions	Unopposed estrogen stimulation, obesity, hypertension, infertility, diabetes	Atrophy of endometrium. Thin physique
Precursor lesion	Atypical endometrial hyperplasia	Serous endometrial intraepithelial carcinoma
Molecular features	Mutations or inactivation of *PTEN*, mutations in *PIK3CA, PIK3R1, ARID1A, KRAS, and TP53.* Microsatellite instability	Mutations in *TP53, PIK3CA, FBXW7, PPP2R1A.*Germline *BRCA1/2* mutations
Microscopy	Endometrioid adenocarcinoma ranges from well to poorly differentiated	• Serous • Clear cell • Mixed Müllerian tumor
Spread	Via lymphatics	Intraperitoneal and via lymphatic
Behavior	Indolent	Aggressive

- **Poorly-differentiated (grade 3/G3) adenocarcinoma:** It consists of **solid sheets (more than 50%)** of cells **without recognizable glands**. They show severe degree of nuclear atypia and numerous mitotic figures.

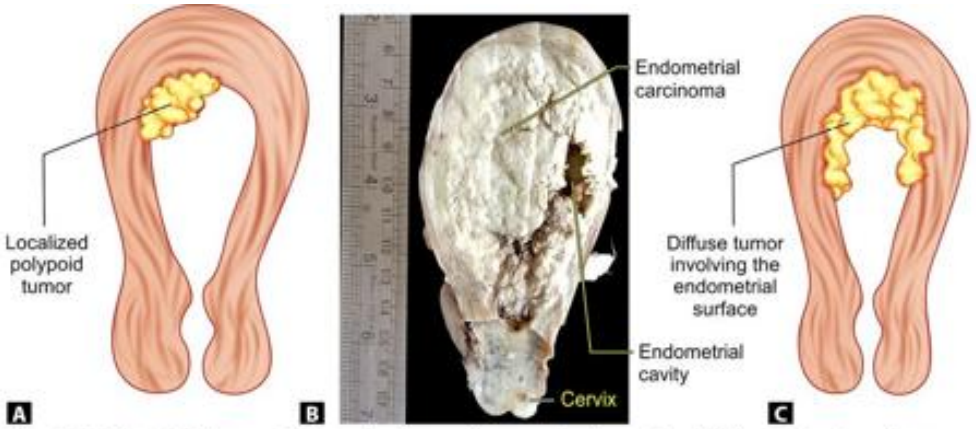
Type II Serous Carcinomas

- **Age:** Usually occur a **decade later than type I carcinoma**.
- **Poorly differentiated** (grade 3) tumors. Constitute ~15% of endometrial carcinoma. Associated with **bad prognosis**. **Usually arise** in the **background of endometrial atrophy**.
- **Serous adenocarcinoma** is the most common subtype and is similar to serous adenocarcinoma of the ovary. It may show papillary projections lined by columnar cells with moderate nuclear atypia similar to ovarian papillary serous carcinoma (**Fig. 22.21C**).
- Less common histological subtypes: Clear cell adenocarcinoma, carcinosarcoma (malignant mixed Müllerian tumor).

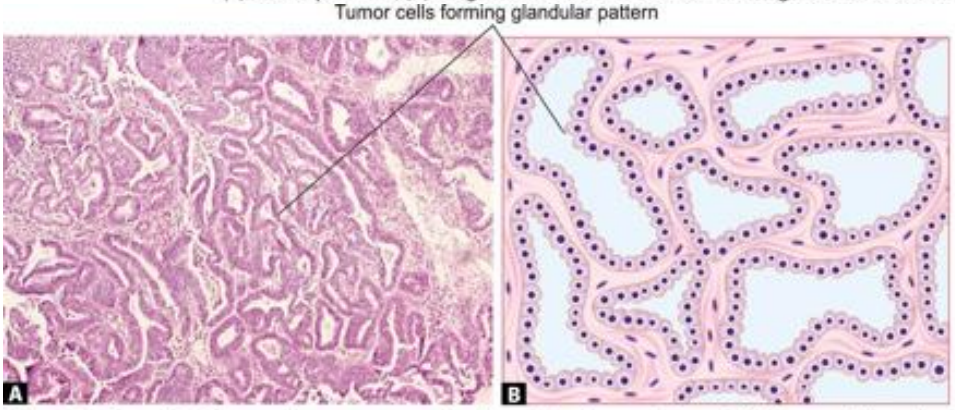
Molecular Changes

Most common mutations include *TP53, PIK3CA, FBXW7 and PPP2R1A* and germline *BRCA1/2* mutations (*refer* **Table 22.2**). Serous carcinoma

probably begins as a surface epithelial neoplasm that extends into adjacent gland structures and later invades endometrial stroma.



Figs. 22.14A to C: Endometrioid (type I) adenocarcinoma of endometrium: (A) Localized polypoid tumor; (B) Gross specimen; (C) Diagrammatic. Diffuse tumor involving the endometrial surface.



Gross appearance of type I endometrial carcinoma:
1. Diffuse
2. Local polypoid.

Figs. 22.15A and B: Microscopic appearance of endometrial adenocarcinoma: (A) Hematoxylin and eosin (H and E); (B) Diagrammatic.

MORPHOLOGY

Serous carcinomas usually arise in small atrophic uteri. They form large bulky mass or deeply invade the myometrium.

Serous Endometrial Intraepithelial Carcinoma (SEIC)

It is the precursor lesion **that arises from a polyp or atrophic endometrium.** The lesion is confined to the endometrium and consists of malignant cells similar to serous carcinoma that arises from the epithelial surfaces.

Invasive Serous Carcinomas

They may have a papillary growth pattern and consists of cells with marked cytological atypia (high nuclear to cytoplasmic ratio, atypical mitotic figures, hyperchromasia, and prominent nucleoli). However, they may have a glandular growth pattern which is differentiated from endometrioid carcinoma by the presence of marked cytological atypia. Irrespective of histological pattern, all these tumors are classified as grade 3. Serous

carcinoma with relatively superficial endometrial involvement shed tumor cells and metastasize extensively to peritoneal surfaces and extrauterine sites by routes (i.e., tubal or lymphatic transmission) other than direct invasion.

Clinical Features of Endometrial Carcinoma

- Age: Mostly seen in postmenopausal women between 55 and 65 years of age.
- Irregular or postmenopausal vaginal bleeding with excessive leukorrhea.
- Diagnosis by histologic examination of tissue obtained by biopsy or curettage.
- Stage determines survival in both types. Serous tumors more frequently produce extrauterine extension and have a worse prognosis than endometrioid carcinomas.

Spread

- **Direct invasion:** The tumor can invade the underlying myometrium and may extend into the periuterine structures.
- **Lymphatic spread:** To the regional lymph nodes. Serous carcinoma spreads to extrauterine (lymphatic or transtubal) site, even when it is confined to the endometrium or its surface epithelium.
- **Hematogenous spread:** To the lungs, liver, bones, and other organs.

MALIGNANT MIXED MÜLLERIAN TUMORS (MMMTs)

Also termed as **carcinosarcomas** are **endometrial adenocarcinomas with a malignant mesenchymal component.** They resemble endometrial carcinoma genetically and have poor prognosis. Microscopically, they usually consist of adenocarcinoma (endometrioid, serous, or clear cell) along with the malignant mesenchymal (sarcomatous) component.

LEIOMYOMAS

CC: Describe the pathogenesis, etiology, pathology, diagnosis, progression and spread of leiomyomas and leiomyosarcomas.

Uterine leiomyomas (commonly called **fibroids**) are **benign smooth muscle neoplasms** and are **most common tumor in females**.

Molecular Changes

Majority of leiomyoma have normal karyotypes, but about 40% may have a simple chromosomal abnormality. These include (i) rearrangements of chromosomes 12q14 and 6p involving the *HMGIC* and *HMGIY* genes, respectively and (ii) mutations in the *MED12* gene.

MORPHOLOGY

Q. Write short note on morphology of leiomyoma (including its sites).

Gross

- Leiomyomas are **sharply circumscribed** (without encapsulation), **discrete, round, firm, gray-white** tumors.
- **Size:** Vary in size from small nodules to massive tumors which fill the pelvis.
- **Number:** May be single or multiple.
- **Cut section:** It has a characteristic **whorled pattern** of smooth muscle bundles and has a **raw (watered) silk appearance**.
- **Red degeneration:** It may be **observed in large tumors** and is characterized grossly by a bulging surface and a **homogeneous dark brown to red appearance on cut section**.

Sites (Figs. 22.16A and B)

- **Most common**:
  - **Within the myometrium** (intramural) of the corpus.
  - Just **beneath the endometrium** (submucosal).
  - **Beneath the serosa** (subserosal) or pedunculated.
- **Rare sites**: Uterine ligaments, lower uterine segment or cervix.

Microscopy (Figs. 22.17A and B)

Q. Write a short answer on microscopy of leiomyoma.

Pattern

- Composed of **interlacing fascicles/whorled bundles** of **smooth muscle cells** (identical to the smooth muscle cells of the uninvolved adjacent myometrium).
- Form circumscribed nodules and have increased cellularity that helps in distinguishing them from the normal myometrium.

Tumor Cells

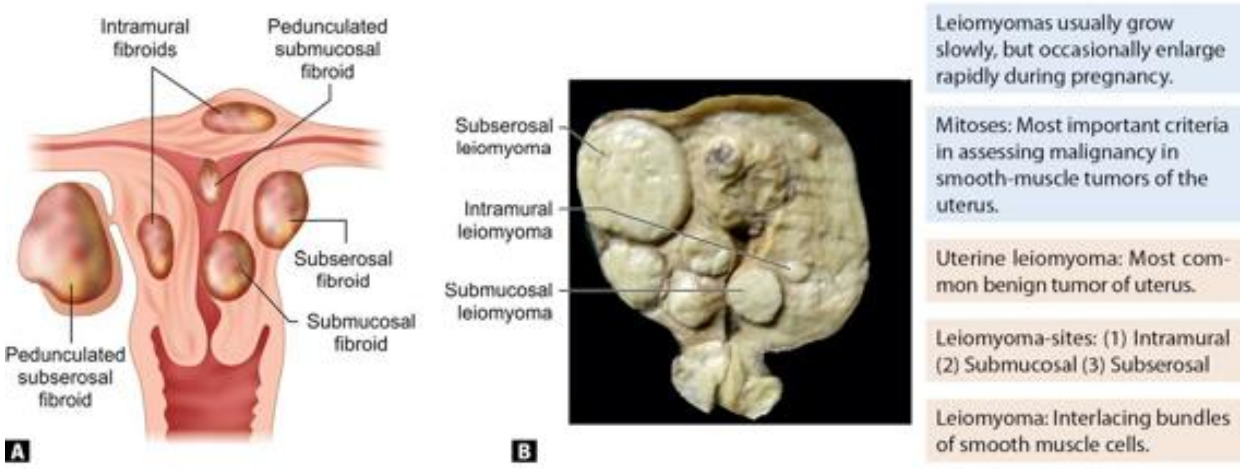
- Individual muscle **tumor cells are uniform in size and shape**.
- **Nuclei:** They are **elongated oval with blunt ends**.
- **Cytoplasm:** It is abundant, eosinophilic with long, slender bipolar cytoplasmic processes.
- **Mitotic figures:** Usually not seen.

Secondary Changes

- **Hyaline change** (degeneration—*refer* **Fig. 2.25**), mucoid or **myxomatous** degeneration, calcification, cystic changes and fatty metamorphosis.
- **Red degeneration:** It shows **extensive coagulative necrosis** and may be **associated with pregnancy** or the use of **contraceptive drugs**.

Clinical Features

- **Age group:** Rare before age 20 and most regress after menopause.



Figs. 22.16A and B: (A) Diagrammatic; (B) Gross specimen, shows different sites of leiomyoma.

- Leiomyomas of the uterus may be asymptomatic.
- Common symptoms:
  - **Abnormal bleeding**
  - **Urinary frequency** (compression of the bladder)
  - **Infertility**

Malignant transformation (leiomyosarcoma) is extremely rare.

Red degeneration in leiomyomas:

- Occurs in large tumors
- Grossly bulging surface
- Cut section—homogeneous dark brown to red
- Extensive coagulative necrosis
- Associated with pregnancy/oral contraceptive

LEIOMYOSARCOMA

Leiomyosarcoma of the uterus is an uncommon malignant smooth muscle tumor. It always arise de novo from the mesenchymal cells of the myometrium. They are always solitary and usually develop in postmenopausal women. This is in contrast to its benign counterpart leiomyomas, which is frequently, are multiple and usually arise in premenopausal women.

MORPHOLOGY

Gross

Leiomyosarcoma are **usually large** and measure about10–15 cm in diameter. They are **soft and show areas of hemorrhage and necrosis**. They have irregular borders and invade into the adjacent myometrium.

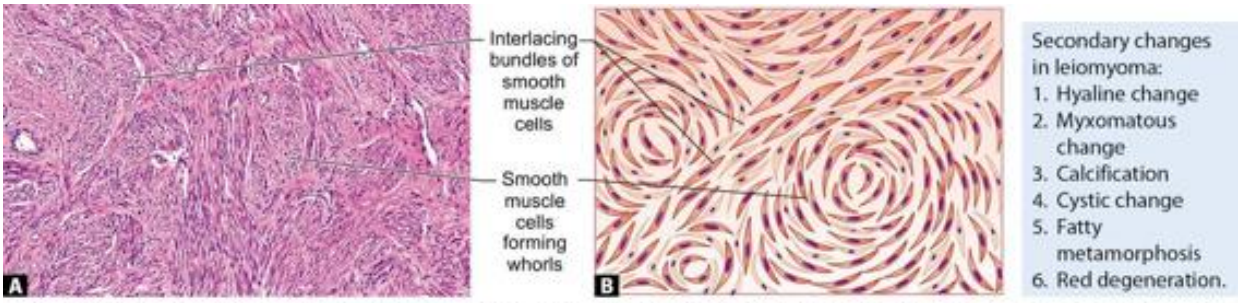
Microscopy

It varies widely, from tumors that closely resemble leiomyoma to wildly anaplastic neoplasms. Well-differentiated tumors may show morphological features of leiomyoma in some areas along with leiomyosarcoma. The diagnostic features include tumor necrosis, cytologic atypia, and mitotic activity. More than 10 mitoses per 10 HPF with or without cellular atypia (if the tumor is more than 5 cm in diameter), or 5–10 mitoses per 10 HPF with cellular atypia and necrosis are considered for the diagnosis of leiomyosarcoma.

Prognosis: Recurrence is common and many tumors metastasize to the lungs.

OVARIES

OVARIAN TUMORS



Figs. 22.17A and B: Microscopic appearance of leiomyoma showing well-differentiated, regular, spindle-shaped smooth muscle cells arranged in interlacing fascicles: (A) Hematoxylin and eosin (H and E); (B) Diagrammatic.

CC: Classify and describe the etiology, pathogenesis, pathology, morphology, clinical course, spread and complications of ovarian tumors.

Incidence: Ovarian cancer constitutes the **third most common female genital tract cancers**, the incidence of which is below only carcinoma of the cervix and the endometrium.

WHO Classification of Ovarian Neoplasms

Q. Classify ovarian tumors.

Classification is **according to the tissue of origin (Box 22.4)**.

- **Primary tumors** may arise from one of three ovarian components:
  - Surface epithelium which is derived from the celomic epithelium.
  - Germ cells which migrate to the ovary from the yolk sac.
  - Sex cord/stroma of the ovary.
- **Secondary or metastatic tumors.**

SURFACE EPITHELIAL TUMORS

Q. Describe the morphological features of surface epithelial tumors.

Surface epithelial tumors are the **most important** and **common primary** neoplasms in the ovary.

Origin

Majority of ovarian tumors arise from the fimbriated end of **the fallopian tube or epithelial cysts in the cortex of the ovary (Fig. 22.18)**. Formerly, they were thought to arise from the celomic epithelium that covers the

surface of the ovary. The epithelium lining the cortical cysts (epithelial cysts of the cortex) in the ovary may be derived from displaced ovarian surface epithelium or the lining of fallopian tube. These can undergo metaplastic or neoplastic transformation and give rise to a number of different epithelial tumors. Benign tumors are usually cystic (cystadenoma) and may be accompanied by stromal component (cystadenofibroma). Malignant tumors also may be cystic (cystadenocarcinoma) or solid (carcinoma).

Classification

Depending on the following features:

A. Histological Types

Three major histological types are:

1. **Serous**
2. **Mucinous**
3. **Endometrioid**.

B. Biological Behavior

It is dependent on the degree of proliferation of the lining epithelium.

1. Benign

Q. Write a short note on classification of surface epithelial tumors of ovary.

Q. Write a short note on classification of sex cord-stromal tumors of ovary.

Q. Write a short note on classification of germ cell tumors of ovary.

BOX 22.4: WHO (2014) classification of ovarian neoplasms (abridged).

A. **PRIMARY TUMORS**
   1. **Surface epithelial tumors**
      - Serous tumors
        – Benign (cystadenoma, cystadenofibroma)

- Borderline (serous borderline tumor)
- Malignant (low- and high-grade serous adenocarcinoma)

- Mucinous tumors, endocervical-like and intestinal type
  - Benign (cystadenoma, adenofibroma)
  - Borderline (mucinous borderline tumor)
  - Malignant (mucinous carcinoma)
- Endometrioid tumors
  - Benign (cystadenoma, adenofibroma)
  - Borderline (endometrioid borderline tumor)
  - Malignant (endometrioid carcinoma)
- Clear cell tumors
  - Benign (cystadenoma, adenofibroma)
  - Borderline (clear cell borderline tumor)
  - Malignant (clear cell carcinoma)
- Brenner tumors
  - Benign Brenner tumor
  - Borderline Brenner tumor/atypical proliferative Brenner tumor
  - Malignant Brenner tumor
- Seromucinous tumors: Benign, borderline and malignant

2. **Germ cell tumors**
   - Mature teratoma: Cystic or solid
   - Immature teratoma
   - Monodermal teratoma and somatic-type tumors arising from dermoid cyst (e.g., struma ovarii, carcinoid)
   - Dysgerminoma
   - Yolk sac tumor (endodermal sinus tumor)
   - Embryonal carcinoma
   - Nongestational choriocarcinoma
   - Mixed germ cell tumors
3. **Sex cord-stromal tumors**
   - Pure stromal tumors
     - Fibroma
     - Cellular fibroma
     - Thecoma
     - Leydig cell tumor
   - Pure sex cord tumors
     - Adult granulosa cell tumor
     - Juvenile granulosa cell tumor
     - Sertoli cell tumor

B. **SECONDARY TUMORS FROM NONOVARIAN PRIMARY**

- **Colonic, appendiceal, gastric, pancreaticobiliary, breast**

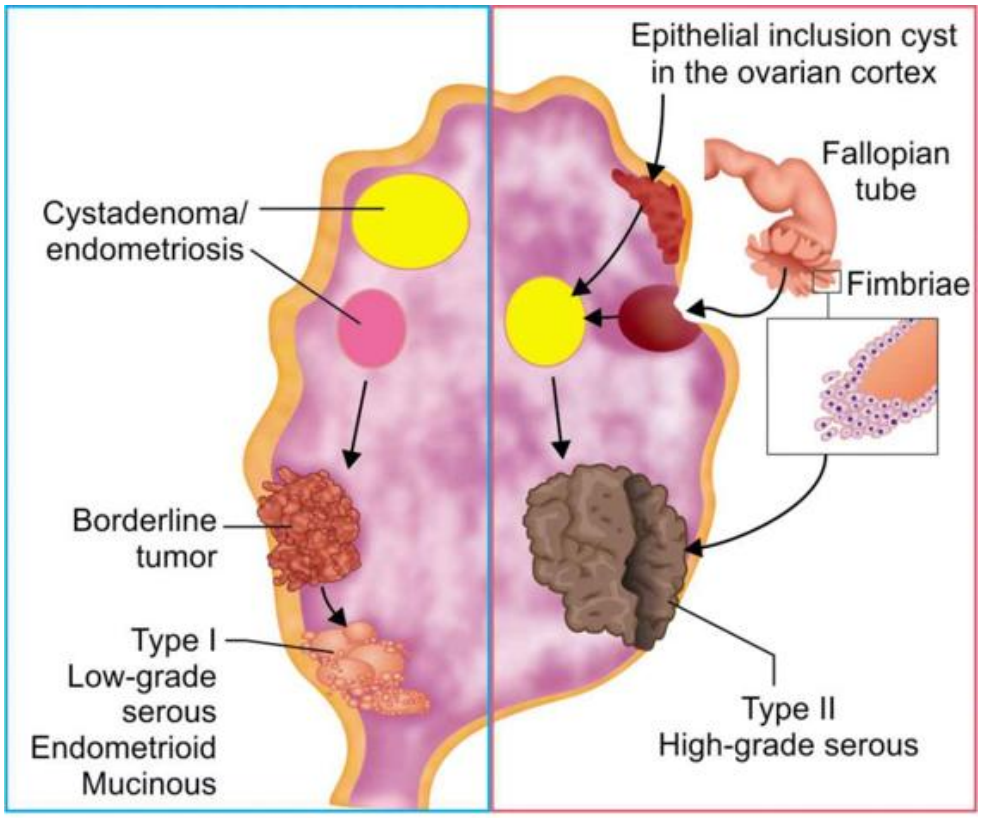


Fig. 22.18: Origin of various ovarian neoplasms. Type I tumors progress from Benign tumors through borderline tumors and may progress to a low-grade carcinoma. Type II tumors arise from inclusion cysts/fallopian tube epithelium via intraepithelial precursors (STIC = serous tubal intraepithelial carcinoma) which are often not identified. These tumors are high-grade and most commonly of serous type.

- Epithelial **proliferation is minimal**.
- **~80% are benign**.
- Mostly seen in young women between the **ages of 20 and 45 years**.

2. Borderline (Also Called Atypical Proliferative)

- Shows **moderate epithelial proliferation**.
- Occur at **slightly older age**.

3. Malignant

- Shows **marked epithelial proliferation** with stromal invasion.
- More common in **older women** between 45 and 65 years.

C. Pattern of Growth and Amount ofFibrous Stroma

The patterns include **cystic, solid** or those that **arise on the surface** of ovary.

1. Benign Tumors

- Cystic areas: Cystadenomas
- Cystic and fibrous areas: Cystadenofibromas
- Predominantly fibrous areas: Adenofibromas
- Surface papillary tumors.

2. Borderline Tumors and Malignant Tumors

They can also have a cystic component, and when malignant they are referred to as cystadenocarcinomas.

Serous Tumors

Q. Write a short essay on serous tumors of ovary.

Incidence: Serous tumors account for about **30% of all ovarian tumors** and about **over 50% of ovarian epithelial tumors**.

About **70% are benign or borderline**. About **30% are malignant**.

Age Group

- **Benign and borderline** tumors: Most common between **20 and 45 years**.
- **Serous carcinomas:** Occur **later** in life.

Molecular Pathogenesis

- **Risk factors** for **benign and borderline** tumors are **unknown**.
- **Risk factors for malignant** serous tumors (serous carcinomas):
  - **Parity**: **Nulliparous** and women with **low parity** have higher risk.
  - **Gonadal dysgenesis** in children is associated with a higher risk.
  - **Genetic factors:** Heritable mutations in tumor suppressor genes ***BRCA1* and *BRCA2*** increase susceptibility to ovarian cancer.
  - **Family history** is a **risk factor**.
  - **Oral contraceptives and sterilization:** During reproductive period **reduces the risk**.

Serous carcinoma: There are two types of serous carcinomas namely (i) low-grade and (ii) high-grade.

- **Low-grade:** They arise from benign or borderline lesions and progress slowly to invasive carcinoma. These tumors are associated with mutations in genes encoding signalling proteins, such as KRAS, a member of the RAS gene family.
- **High-grade:** They develop rapidly and many of them arise in the fimbriated end of the fallopian tube via serous tubal intraepithelial carcinoma (STIC), rather than ovarian celomic epithelium. TP53 mutations are characteristically seen in 95% of cases. Other frequently mutated genes include the tumor suppressors NF1 and RB.

MORPHOLOGY

Q. Describe the morphological features of serous tumors of ovary.

Gross

Benign Serous Cystadenoma (Fig. 22.19)

- **Size: Varies** and may measure from 15 to 30 cm in diameter.
- **Appearance:** It contains one or more thin-walled cysts. The cyst **wall is smooth glistening** without any epithelial thickening.
- **Content:** Lumen filled with **clear watery serous fluid**.
- **Cut section:**
  - Shows **smooth internal surface** and **may show small papillae** projecting into the cavity.
  - Rarely papillae are seen projecting from the outer surface of ovary (surface papilloma).
- **Bilaterality is common** and found in **about 20% of benign** serous cystadenomas.

Borderline Tumors

- **Vary in size, usually >5 cm and typically cystic.**
- Cut section shows **cystic cavities filled** by increased number of **dense and closely packed, cauliflower-like papillary** projections.
- **Bilaterality: 30%**.

Malignant Serous Adenocarcinoma (Fig. 22.20)

- **Usually large**.
- Shows a **mixture of solid and cystic areas** with l**arge solid or papillary areas.** Other features of malignancy includes: Tumor irregularity, and fixation or nodularity of the

capsule.

- Areas of **necrosis and hemorrhage** in the solid area of tumor.
- **Adhesion to adjacent structures** (bowel, uterus and pelvic side wall).
- **Bilateral: ~66%**.

Microscopy

Benign Serous Tumors (Figs. 22.21A and B)

Cysts are lined by nonstratified or stratified cuboidal to columnar epithelial cells (similar to lining epithelium of normal fallopian tube). Cilia are present though sometimes only focally. May also show:

- **Papillae with a fibrovascular core:** They are covered by a single layer of epithelium similar to that of the cyst lining.
- **Psammoma bodies: They are dystrophic calcified tumor cells**.

Borderline Tumors

They are noninvasive tumors with greater epithelial proliferation and cytological atypia than benign but less than low-grade serous carcinoma.

- **Stratification of the epithelium (multilayering):** The epithelial proliferation may produce a delicate, papillae pattern termed as "micropapillary carcinoma." This may be the precursor to low-grade serous carcinoma.

- **Budding or cellular tufting**: These are tiny, irregular tightly packed stroma-free clusters of tumor cells which gets detached and float into the lumen of the cyst.
- **Mild nuclear atypia**.
- **Increased mitotic activity**.
- **Absence of stromal invasion**.
- May also show dense and closely packed **complex papillae** and **psammoma bodies**.

Malignant

Serous carcinoma based on molecular changes is classified as low-grade or high-grade serous carcinomas [Table 22.2 (50–60%)]

Low-grade serous carcinomas

It shows variety of architectural patterns. These include single cells and small nests of irregular shape infiltrating the stroma, and micropapillae (rarely macropapillae).The tumor cells are more uniform with mild to moderate nuclear atypia and limited nuclear pleomorphism.

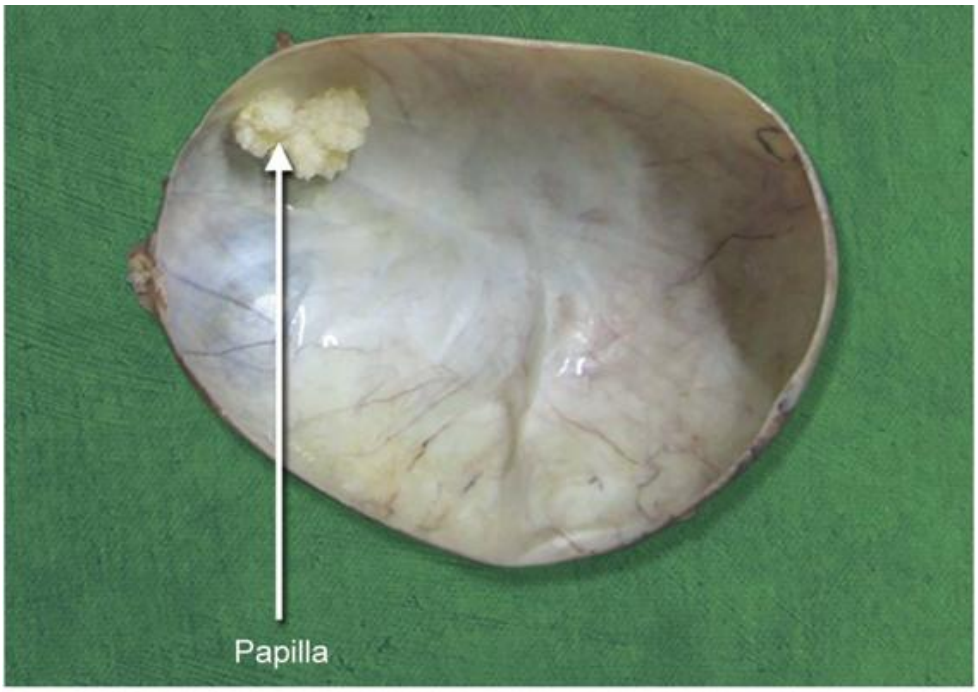


Fig. 22.19: Serous cystadenoma of ovary. Cut section shows uniloculated cyst with a focus of papilla.

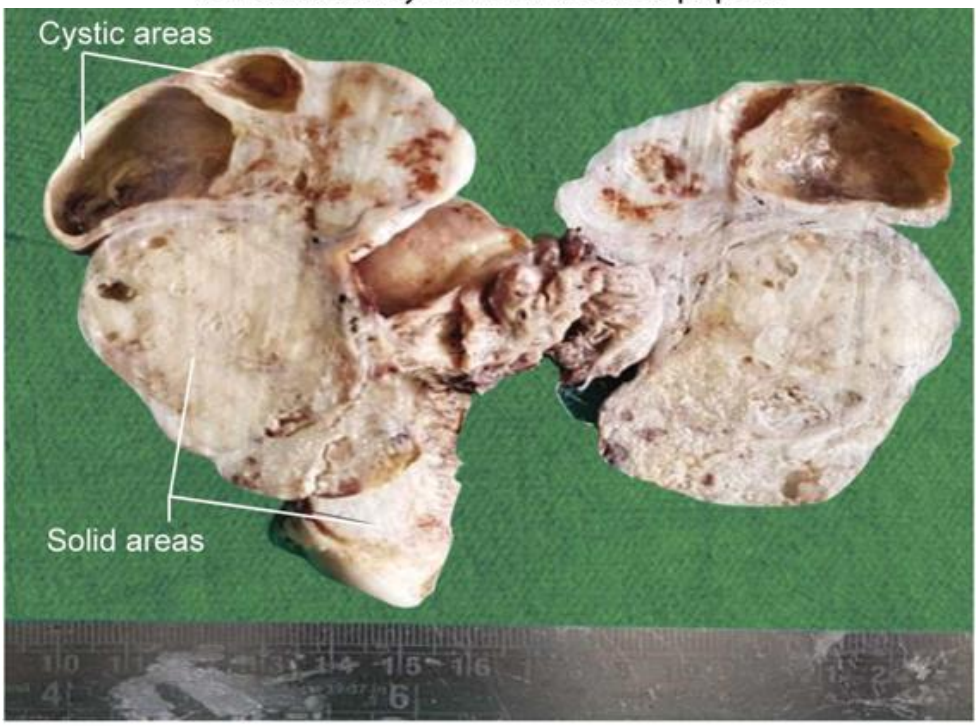
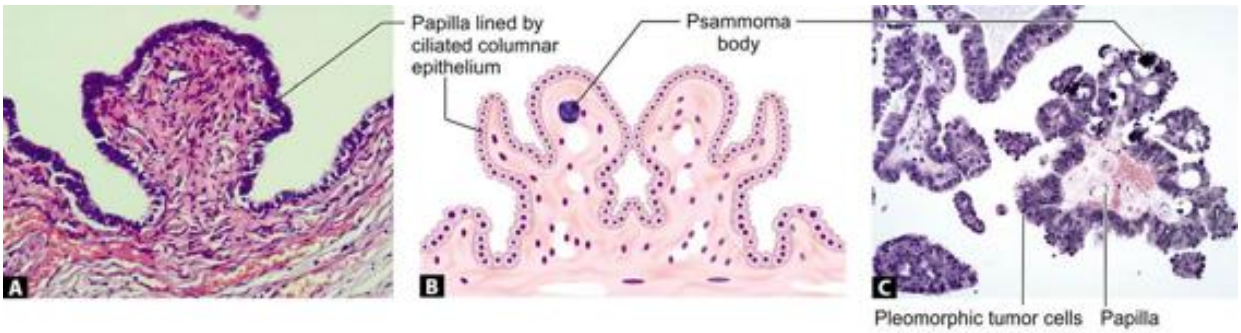


Fig. 22.20: Serous adenocarcinoma of ovary. The tumors shows both solid and cystic areas.



Figs. 22.21A to C: Microscopic appearance of papillary serous cystadenoma of ovary showing cyst wall and papilla lined by single layer of cuboidal epithelium: (A) Hematoxylin and eosin (H and E); (B) (Diagrammatic); (C) Papillary serous adenocarcinoma of ovary showing papillae lined by multilayered columnar epithelium and one core of papilla with psammoma body (H & E).

TABLE 22.2: Molecular basis of classification of serous carcinoma.

Type of carcinoma	***Low-grade (well-differentiated)***	***High-grade (moderately to poorly differentiated)***
Carcinoma arises	In serous borderline tumors	In the fallopian tube fimbriae or from serous inclusion cysts within the ovary
Molecular changes: Mutations		
KRAS or *BRAF* oncogenes (signal transduction)	Present (50-60%)	Absent
TP53 gene mutations (tumor suppressor gene)	Rare	High (almost all)
BRCA1/ BRCA2 mutations (tumor suppressor gene)	Not seen	Seen (50%)

High-grade serous carcinomas

They are distinguished from low-grade carcinoma by the following features:

- More complex growth patterns with solid masses of tumor cells with slit-like spaces.
- Widespread invasion or frank effacement of the underlying stroma **(Fig. 22.21C)**.
- Tumor cells show marked **nuclear atypia, pleomorphism, hyperchromatic nuclei, atypical mitotic figures, and large bizarre form or multinucleation.** These cells in invasive high-grade serous carcinoma may become undifferentiated and the serous features may not be evident on microscopic examination.
- **Psammoma bodies:** These are concentric calcifications observed in serous tumors. However, they are not specific for neoplasia.

Serous tubal intraepithelial carcinomas: The tumor cells appear similar to high-grade serous carcinomas but do not show invasion.

Spread of Ovarian Serous Carcinoma

Local spread: Both low- and high-grade carcinomas may spread to the peritoneal surfaces and omentum and are commonly associated with ascites. Their spread beyond ovary determines the stage of the disease.

- **Umbilical metastasis ("sister Joseph's nodule")** (*refer* page 561 and **Fig. 18.18**).
- Contralateral ovary.
- Abdominal viscera (bowel, liver, spleen)

Lymphatic spread: Para-aortic and pelvic lymph nodes.

Hematogenous spread: Liver and lung.

Mucinous Tumors

Q. Classify ovarian tumors. Describe the gross and microscopy of mucinous tumors of ovary.

Incidence

- Mucinous tumors are **less common than serous tumors**, constitute **~30% of all ovarian neoplasms**.
- About **80% are Benign or borderline** and about **15% are malignant**.
- Primary ovarian mucinous carcinomas are uncommon and account for less than 5% of all ovarian cancers.
- **Age group:** It is seen mainly during **middle adult life** and are rare before puberty and after menopause.

Molecular Pathogenesis

- **Risk factor: Smoking**.
- **Mutations in *KRAS* (oncogene):** This **may occur early** and may be **observed in Benign, borderline** and in **primary ovarian mucinous carcinomas**.

MORPHOLOGY

Q. Write short note on morphology of mucinous tumors of ovary.

General Features

Mucinous tumors **differ from the serous type** in following features:

- **Larger size**

- **Multiloculated** and show hundreds to thousands of small cysts filled with sticky, gelatinous mucinous fluid rich in glycoproteins.
- **Less bilateral:** Only 5% of mucinous cystadenomas and mucinous cystadenocarcinomas are bilateral.
- **Surface involvement: Rare**.

Gross

Benign Mucinous Cystadenoma (Figs. 22.22A and B)

- **Multilocular**, thin-walled cysts with smooth external surface.
- **Cyst content**: **Sticky semi-solid mucinous** material.

Borderline

- Similar to mucinous cystadenoma.

Carcinoma

- Similar to mucinous cystadenoma with additional **solid regions.**
- Lining of tall, columnar epithelial cells with apical mucin that lack cilia.
- Solid areas show **necrosis and hemorrhage**.

Microscopy

Benign Mucinous Cystadenoma (Figs. 22.23A and B)

They are composed of many cysts and glands. These are lined by simple nonstratified tall, nonciliated, columnar mucinous cells with apical mucin and basally situated nuclei. This epithelium resembles gastric foveolar-type or intestinal epithelium. Uncommonly, epithelium may show endocervical type mucinous differentiation.

Borderline

Same criteria as borderline serous tumors, although papillary **projections are less conspicuous**. The cysts are lined by gastrointestinal type of epithelium. The characteristic features are:

- Stratification of the epithelium/multilayering.
- Budding or cellular tufting and/or papillary intraglandular growth (appear similar to tubular adenomas or villous adenomas of the intestine).
- Mild nuclear atypia

- Increased mitotic activity
- Absence of stromal invasion

Mucinous Carcinomas

It may range from well to poorly differentiated carcinomas. Common features are:

- **Epithelial cell stratification** and **atypia:** The atypical epithelium is more than four cells in thickness.
- **Loss of gland architecture**.
- **Necrosis**
- Confluent glandular growth which is a form of "expansile" invasion.
- Infiltration of the serosa is common.

Spread of Mucinous Carcinoma

Q. Write a short note on pseudomyxoma peretonei.

- **Peritoneal** implant and local invasion into neighboring structures like bowel, abdominal wall, and bladder.
- **Pseudomyxoma peritonei:** It is characterized by:
  - **Extensive mucinous ascites**.
  - **Cystic epithelial implants on the peritoneal surfaces**. The abdomen is filled with tenacious, semisolid mucin. Mostly it is caused by metastatic spread of tumors in the gastrointestinal tract, primarily the appendix.
  - Peritoneal adhesions can cause intestinal obstruction.
    - It can also develop due to mucinous tumor of the appendix with secondary ovarian and peritoneal spread.
- **Metastases to distant organs are infrequent**.

Endometrioid Tumors

- Endometrioid tumors **show tubular glands resembling Benign or malignant endometrium**.
- **Benign** endometrioid tumors (endometrioid adenofibromas) and **borderline** endometrioid tumors are rare. But, **endometrioid carcinomas** account for approximately 20% of all ovarian cancers.

Pathogenesis

- About **15– 20%** of cases with **endometrioid carcinoma coexist with endometriosis**.
- About **15–30%** are **accompanied by carcinoma of the endometrium**.

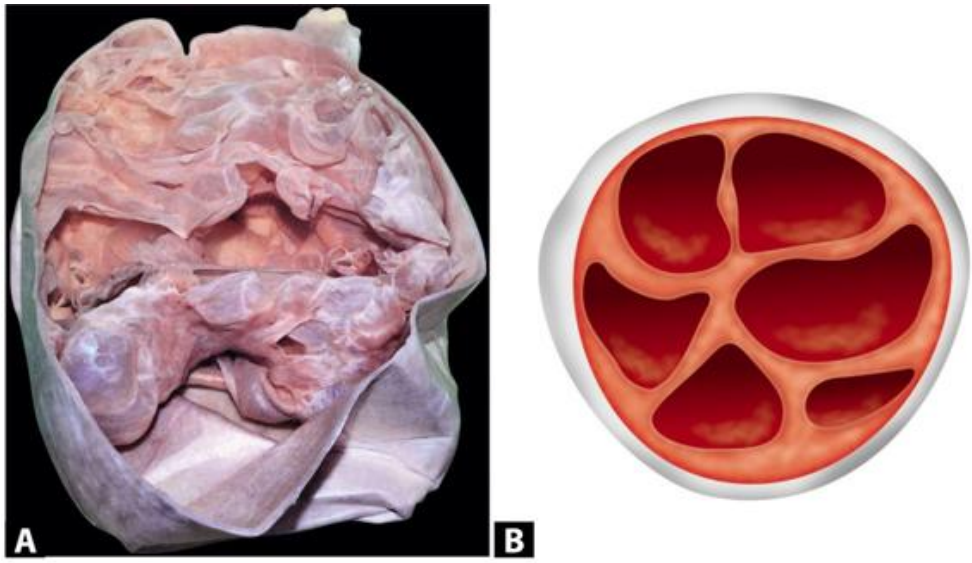
Molecular Changes Similar to Endometrial Endometrioid Carcinoma

Frequent mutations in PTEN, PIK3CA, ARID1A, and KRAS, mismatch DNA repair genes and CTNNB1 (β-catenin). TP53 mutations are common in poorly differentiated tumors.

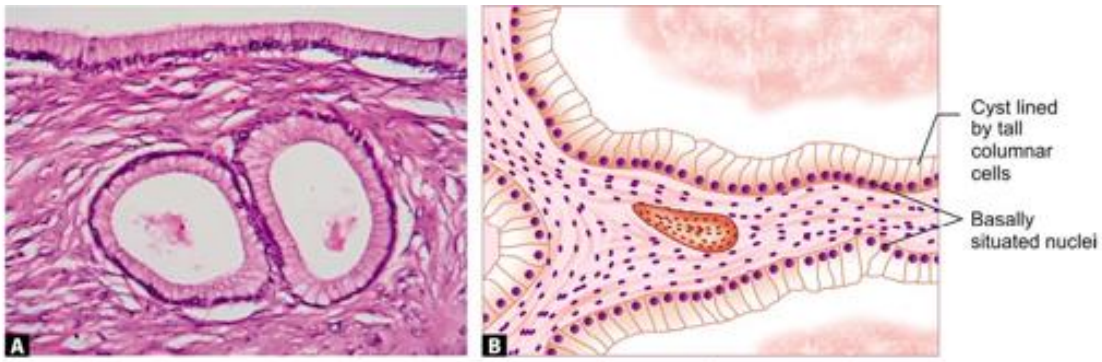
MORPHOLOGY

Gross

- Size varies from 2–30 cm.
- **Most are solid** and exhibit necrotic areas, but some may show combination of solid and cystic areas.



Figs. 22.22A and B: Cut section of mucinous cystadenoma of ovary. (A) Specimen; (B) Diagrammatic.



Figs. 22.23A and B: Microscopic appearance of mucinous cystadenoma of ovary. (A) Photomicrograph; (B) Diagrammatic representation shows cyst lined by uniform, gastrointestinal type of mucinous, columnar cells having basally situated nuclei and apical mucin.

Microscopy

Show **glandular patterns resembling endometrial origin**. They are graded similar to endometrial adenocarcinomas (*refer* page 749).

Brenner Tumor

Q. Write short note on Brenner tumor of ovary.

Brenner tumor consists of nests of bland transitional-type of cells which resemble urothelial cells within a fibromatous stroma. Brenner tumors are **uncommon** tumors and most of them are benign. Borderline (atypical proliferative Brenner tumor) and malignant Brenner tumors have been rarely reported.

MORPHOLOGY

Gross

- May be **predominantly solid or rarely may be cystic**.
- Usually **unilateral** (approximately 90%).
- Vary in size from small lesions >1 cm in diameter to massive tumors up to 20–30 cm.

Microscopy (Fig. 22.24)

- Brenner tumor shows **nests of bland epithelial cells resembling the epithelium of the urinary tract (transitional-type) separated by fibrous stroma,** resembling that of the normal ovary.
- The nuclei of epithelial cells show **nuclear grooves resembling coffee-bean**.

Clinical Presentation of Surface Epithelial Tumors

- Benign tumors are most common and may be **asymptomatic**.
- May be unilateral or bilateral.

- **Most tumors** are **nonfunctional** and produce only mild symptoms. The most common symptoms are:
  - **Lower abdominal pain** and **distention/enlargement**.
  - **Vaginal bleeding**
  - **Due to compression** produced by the tumor, for example:
    - Urinary frequency, dysuria
    - Gastrointestinal tract symptoms
- Most **malignant ovarian tumors** are detected when these spread outside the ovary. Symptoms include:
  - **Progressive weakness, weight loss, and cachexia**.
  - **Massive ascites** due to cancer invasion is common and **ascitic fluid may show exfoliated tumor cells**.
  - **Metastasis**:
    - Regional nodes are often involved.
    - Hematogenous metastasis to liver, lungs, gastrointestinal tract, and elsewhere.
    - Across the midline to the opposite ovary.

Biochemical Markers of Surface Epithelial Tumors

- **CA-125**: It is a high-molecular-weight glycoprotein **present in the serum of about 80% of patients with serous and endometrioid carcinomas**. It is **not a reliable marker** because it can be elevated with nonspecific irritation of the peritoneum (e.g., endometriosis, inflammation).
- **Osteopontin:** It is expressed at **higher levels** in ovarian cancer patients.

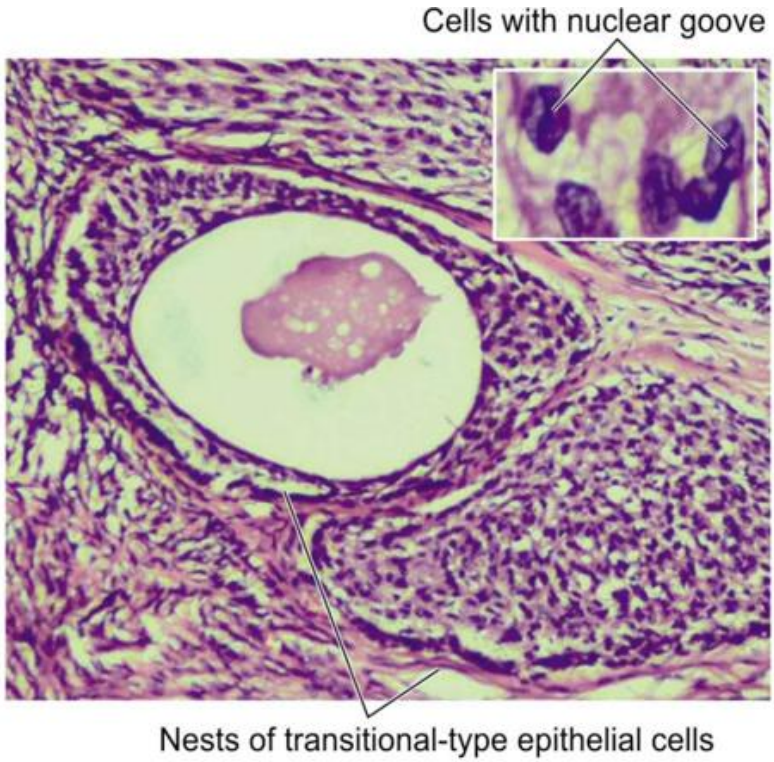


Fig. 22.24: Brenner tumor. Microscopically showing nests of transitional-type cells separated by fibrous stroma. Insert shows tumor cells with nuclear grooves resembling coffee-bean.

Primary ovarian tumors: May arise from surface epithelium, germ cells or sex cord-stromal cells.

Surface epithelial tumors: Most common primary neoplasms in the ovary. Most common malignant ovarian tumors and are seen in women older than 40 years of age.

Surface epithelial tumors:
- Benign
- Borderline
- Malignant subtypes under each type

Serous carcinomas: Most common malignant ovarian tumor and account for 40–50% of all cancers of the ovary.

Serous carcinoma of ovary:
- Large, solid and cystic areas
- Multilayered epithelium
- Invasion

Bilateraliy in serous tumors of ovary:
- Benign ~20%
- Borderline ~30%
- Malignant ~66%

Serous tumors—gross:
- Benign: Unilocular cyst
- Borderline: Predominantly cystic
- Malignant: Solid and cystic

Serous cystadenoma of ovary:
- Unilocular
- Bilateraliy common
- May show papillae

Psammoma bodies:
- Laminated calcified concretions
- Seen in serous tumors (both Benign and malignant)
- Not specific for ovarian tumors

Ovarian cancer: Risk increases as age advances.

Most cases of pseudomyxoma peritonei result from spread of a mucinous tumor located in the appendix (mucocele)

Nuclear grooves resembling coffee-bean:
1. Brenner tumor
2. Granulosa cell tumor

CA-125: Present in the serum of about 80% of patients with serous and endometrioid carcinomas.

GERM CELL TUMORS

Q. Classify ovarian germ cell tumors. Describe germ cell tumors of ovary.

- Germ cell tumors constitute about **20–25% of all ovarian tumors**.
- **In adults**: Most germ cell tumors are **benign** (mature cystic teratoma, dermoid cyst).
- **In children and young adults: Malignant** tumors are the most common.
- They are similar to germ cell tumors in the testis.

Teratomas

Q. Write short note on teratoma of ovary.

Origin

- **All benign ovarian teratomas** have a **karyotype of**v **6,XX**.
- Teratomas **arise from an ovum after the first meiotic division**.
- They **originate from totipotent cells**.
- Teratoma contains **mature or immature cells or tissues representative of more than one germ cell layer** (at least two) and sometimes all three embryonic layers. These **cells or tissues are arranged in a helter-skelter fashion** (disorganized).

Sites

- **Gonadal:** Ovary and testis.
- **Extragonadal:** Rare and arise from **midline embryonic rests,** e.g., mediastinum, retroperitoneum.

Classification

According to WHO (2014) teratomas are classified as follows:

1. **Mature (benign) teratoma:** It consists of all **well-differentiated component** parts derived from two or three germ layers (ectoderm, mesoderm and endoderm). Depending on gross feature, they are further categorized as:
   - **Cystic** (mature cystic teratoma)
   - **Solid** (mature solid teratoma).
2. **Immature (malignant) teratoma:** It consists of **less well-differentiated or immature elements**.
3. **Monodermal or highly specialized presently called as "monodermal teratoma and somatic-type tumors arising from a dermoid cyst."**

Mature (Benign) Teratomas

Q. Write short note on dermoid cyst/benign cystic teratoma of ovary.

- **Most benign ovarian teratomas are cystic** and are better known as **dermoid cysts** (mature cystic teratoma).
- **Age group:** Usually detected in **young women** during the active reproductive years with a peak incidence in the **third decade**.

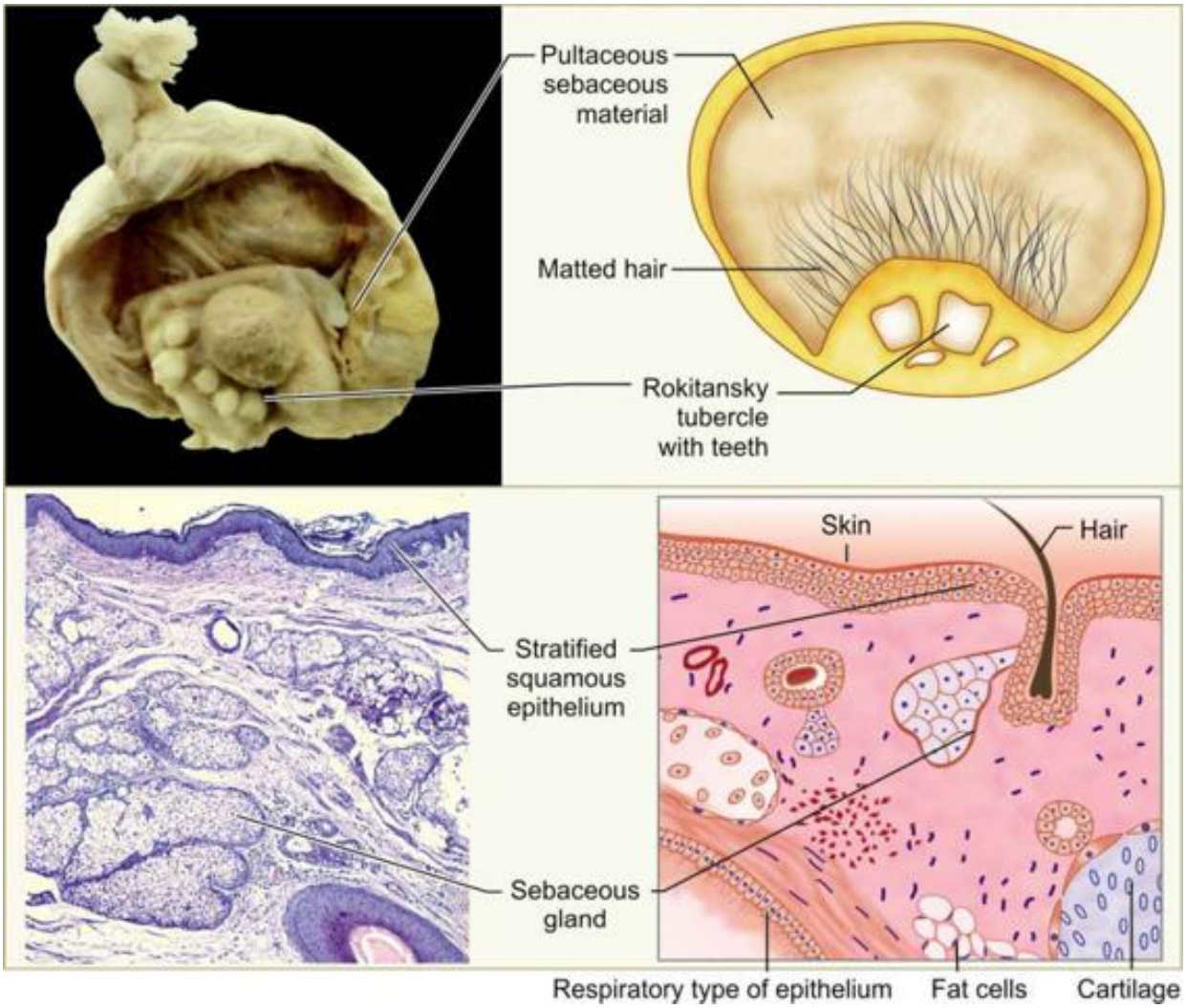
- Clinically they may be discovered incidentally. The karyotype of benign ovarian teratomas is 46,XX.

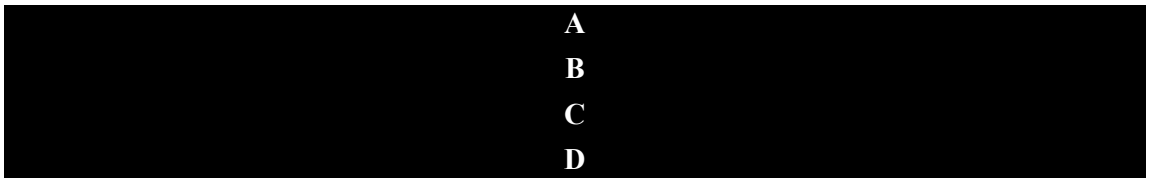
Q. Write a short answer on morphology of benign cystic teratoma.

MORPHOLOGY

Gross (Figs. 22.25A and B)

- Benign teratomas are **bilateral in 10–15%** of cases.
- **Appearance:** It is usually **unilocular, thick-walled cystic tumor** with a smooth, shiny outer surface.





Figs. 22.25A to D: (A and B) Opened mature cystic teratoma (dermoid cyst) of the ovary; (C) Photomicrograph;(D) Diagrammatic appearance of dermoid cyst of ovary.

Cut Section

- Cyst contains yellow or gray, buttery or cheesy **sebaceous material** with variable amount of **hair**.
- **Cyst is lined** by an opaque, gray-white, **wrinkled epidermis.**
- **Tooth** and areas of calcification are common.
- **Rokitansky nodules**/protuberance: These are one or more **foci** of rounded **nodular** structure/s, **covered with hair protruding into the lumen** of the cyst. These are also known as the mammillary body **(tubercle)** and **dermoid nipple**. It shows the greatest variety of tissue types from all three germ cell layers and teeth tend to be located at this site.

Microscopy (Figs. 22.25C and D)

- These tumors **mainly show differentiation along ectodermal line**. The **cyst wall consists of skin** (stratified squamous epithelium) **with skin appendages** (sebaceous glands, hair shafts, and other skin adnexal structures).
- In most cases, **structures from all three germ layers can be identified**, which includes:
  - Ectoderm (e.g., skin, neural tissue, glia)
  - Mesoderm (e.g., smooth muscle, cartilage, bone, fat)
  - Endoderm (e.g., respiratory tract epithelium, gut, thyroid)

Sometimes, teratomas may produce infertility and may undergo torsion (10–15% of cases) which constitutes an acute surgical emergency. Limbic encephalitis may develop as a rare paraneoplastic complication with teratomas containing mature neural tissue. It usually resolves with removal of tumor.

Malignancy in Mature Cystic Teratomas

One of the mature cellular elements may undergo malignant change in about 1% of the dermoids. They tend to occur in older women and include

squamous cell carcinoma, thyroid carcinoma, melanoma, basal cell carcinoma**,** and carcinoid tumor.

Solid Teratoma

Rarely, mature (benign) teratoma can be solid with mature tissues derived from two or three germ layers. On gross examination, these tumors are difficult to differentiate from the malignant, immature teratomas.

Immature Malignant Teratomas

- Immature teratomas are rare tumors **composed of variable amounts of tissues** which **resemble embryonal and immature fetal tissue**.
- **Age:** Mostly found in **prepubertal adolescents** and **young women**. The mean age is 18 years.

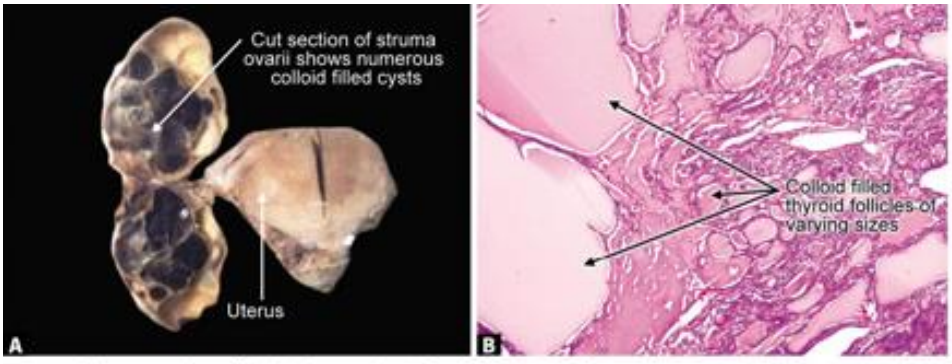
MORPHOLOGY

Gross

Tumors are **bulky with smooth external surface**.

Cut Section

- **Predominantly solid** and have lobulated and **variegated appearance** showing heterogeneous mixture of various tissues.



Germ cell tumors of ovary: Teratoma is the most common Benign germ cell tumor.

Totipotent cells: Have the capacity to differentiate into any of the cell types found in the adult body.

Figs. 22.26A and B: (A) Cut section of struma ovarii involving the ovary; (B) Struma ovarii composed of thyroid follicles containing variable amount of colloid.

- Solid areas may show **grossly recognizable immature bone** and **cartilage**, **hair, sebaceous material**, and calcification.
- Show **areas of necrosis and hemorrhage**.

Microscopy

Varying amounts of **immature tissues** mixed with some mature tissues derived from the two or three germ layers. The immature elements include **immature neuroepithelium (neuroepithelial rosettes and immature glia), cartilage, bone**, muscle, and others.

Grading: It is **based on the amount of immature neuroepithelium**.

Monodermal Teratomas and Somatic-type Tumors Arising from a Dermoid Cyst

Q. Write short note on struma ovarii.

Specialized teratomas are **rare** tumors **composed entirely of one tissue type**. The most common among these rare are struma ovarii and carcinoid.

Struma Ovarii

It is a **cystic lesion** composed entirely or predominantly of **mature thyroid tissue (Figs. 22.26A and B)**. These thyroidal neoplasms may hyperfunction and may lead to hyperthyroidism.

Ovarian Carcinoid

- Probably arise from intestinal epithelium in a teratoma. They may be functional (producing 5-hydroxytryptamine) and result in carcinoid syndrome.
- **Strumal carcinoid:** It is a combination of struma ovarii and carcinoid in the same ovary.

Dysgerminoma

- Dysgerminoma is a **malignant germ cell tumor** and is the **ovarian counterpart of the seminoma** of the **testis**.
- Constitutes ~2% of all ovarian cancers and ~50% of malignant germ cell tumors.
- **Age group:** ~75% occur in the **second and third decades**.
- **Predisposing factors**: **Gonadal dysgenesis** (including pseudohermaphroditism) is one of the risk factors.
- **Functional characteristics**: **Most do not show endocrine function**.
- **Molecular changes:**

- Like seminomas, dysgerminomas **express transcription factors, namely Oct3, Oct4, and Nanog**. These are responsible for pluripotency.
- They also **express the receptor tyrosine kinase *KIT***.

MORPHOLOGY

Q. Write short note on morphology of dysgerminoma.

Gross

- Usually **unilateral** (80–90%)
- **Solid, firm, round to oval**, **encapsulated** tumors.
- **External surface: Smooth, nodular**, **convoluted/bosselated**.
- **Size: Varies** with a mean **diameter 15 cm**.
- **Cut section (Fig. 22.27A): Soft, fleshy** and uniformly **yellow-white to gray-pink (cream colored).**

Microscopy (Figs. 22.27B and C)

Pattern

The tumor cells are arranged in **sheets, groups, cords or nests** separated by **scant fibrous stroma**.

Tumor Cells

Resemble primordial germ cells with following characteristics:

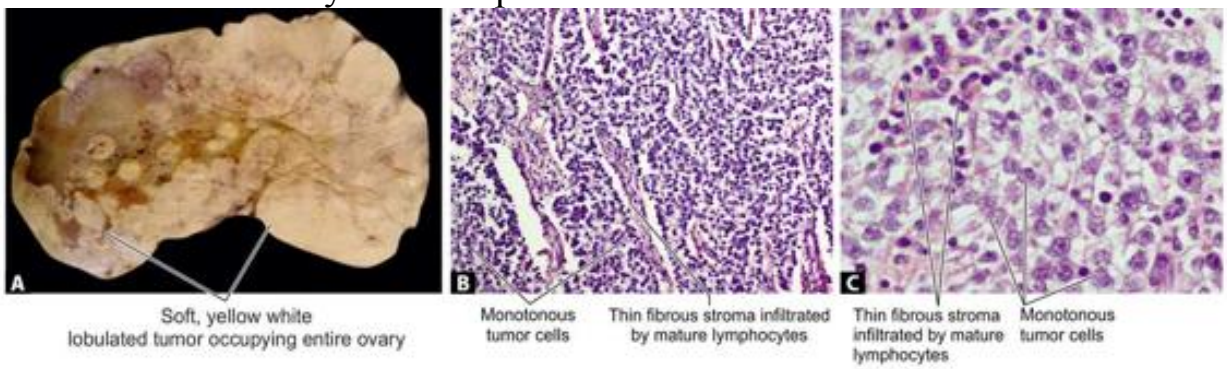
- **Cells**: Individual tumor cells are **monotonous (uniform**).
- **Cytoplasm:** It is **abundant clear to finely granular glycogen-filled** and sometimes fine droplets of fat. The **cell membrane** is **well-defined**.
- **Nuclei:** They are **large, regular** and **centrally placed** with **fine reticular chromatin** and one or more **prominent nucleoli**.
- **Mitotic figures** are usually **numerous**.

Stroma

Similar to seminoma, the fibrous stroma is **infiltrated with mature lymphocytes** (most are of T-cell type) and occasional granulomas.

Spread

- **Local spread:** It can spread into peritoneal cavity and is associated with a decreased survival rate.
- **Lymphatic spread:** Through lymphatics, it spreads commonly to the contralateral ovary and retroperitoneal nodes.



Figs. 22.27A to C: Dysgerminoma: (A) Gross showing a solid tumor, cut section appears fleshy and cream colored; (B) Low magnification and (C) High magnification composed of polyhedral tumor cells with clear cytoplasm reticular nuclei and adjacent scanty fibrous stroma infiltrated by lymphocytes.

- **Blood spread:** Lungs.

Prognosis: All dysgerminomas are **malignant**. Dysgerminoma is treated surgically, and 5-year survival for patients with stage I is almost 100%. The tumor is **highly radiosensitive** and also responsive to chemotherapy.

Endodermal Sinus (Yolk Sac) Tumor

- Endodermal sinus tumor is the **second most common malignant tumor of germ cell** tumor.
- **Origin:** From **differentiation of malignant germ cells along the extraembryonic yolk sac lineage**.
- Tumor is **rich** in **α-fetoprotein and α_1-antitrypsin**.
- **Age: Children or young women**.

MORPHOLOGY

Gross

Usually **unilateral** and **solid**.

Microscopy

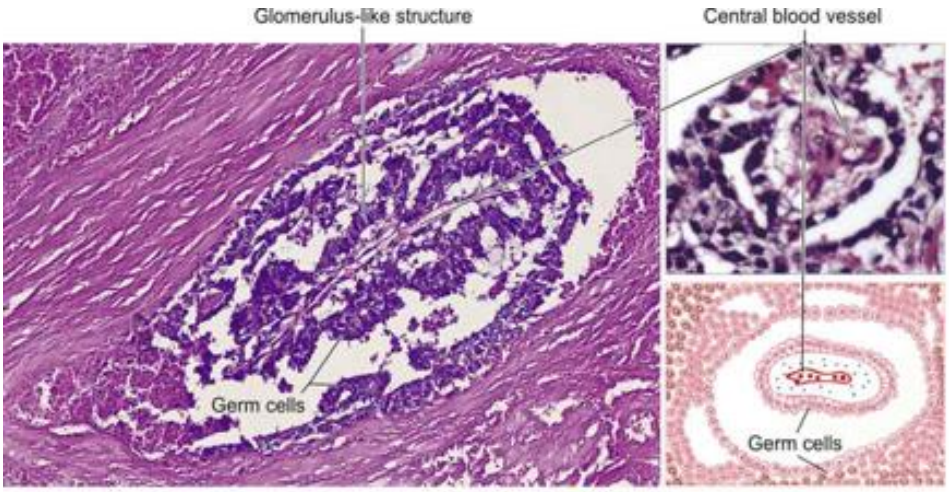
- **Schiller–Duval body:** It is the **characteristic feature.** This is a **glomerulus-like structure composed of a central blood vessel enveloped by germ cells within a space lined by germ cells (Fig. 22.28)**.
- **Hyaline droplets:** All show **prominent intracellular and extracellular PAS positive hyaline droplets.** Some of these **may stain for α-fetoprotein** by immunoperoxidase

techniques.

Tumor Marker

- α-fetoprotein and **α_1-antitrypsin in tumor cells** by immunohistochemistry
- α-fetoprotein in the **serum**

Prognosis: Aggressive and **rapidly growing** tumor.



Teratoma:
1. Gonadal.
   - Ovary
   - Testis
2. Extragonadal

Teratoma: (1) Mature teratoma (cystic or solid), (2) Immature teratoma and (3) Monodermal teratoma and somatic-type tumors arising from dermoid cyst (e.g., struma ovarii, carcinoid).

Fig. 22.28: Endodermal sinus (yolk-sac) tumor showing characteristic glomerulus-like structure known as Schiller–Duval body. It is composed of a central blood vessel enveloped by germ cells within a space lined by germ cells. Inset (upper right—H&E) and lower right (diagrammatic) showing Schiller–Duval bodies.

Nongestational Choriocarcinoma

Choriocarcinoma in females may be:

- **Gestational:** More common of **placental origin.**
- **Nongestational:** Germ cell origin can be confirmed only in the prepubertal girl. This is because after puberty the origin from an ovarian, ectopic pregnancy cannot be ruled out.

Pure choriocarcinomas of ovary are **extremely rare** and most **coexist with other germ cell tumors**.

MORPHOLOGY

Gross: **Solid with large areas of hemorrhage**
Microscopy: Similar to gestational choriocarcinoma (*refer* page 768) and **consists of cytotrophoblast and syncytiotrophoblast without any villi**.

- **Behavior: Aggressive tumors** and generally **metastasize through the bloodstream** to the **lungs, liver, bone**, and other viscera at the time of

diagnosis.

- **Tumor marker:** These tumors **secrete high levels of chorionic gonadotropins (hCG)** which help in diagnosis or detecting recurrences.
- **Prognosis:** In contrast to gestational, these nongestational types generally do not respond to chemotherapy and are **fatal**.

Other Germ Cell Tumors

- **Embryonal carcinoma: Highly malignant** tumor of primitive embryonal elements, similar to that of testes.
- **Mixed germ cell tumors:** They shows various combinations of dysgerminoma, teratoma, endodermal sinus tumor and choriocarcinoma.

Benign teratomas: Bilateral in 10–15% of cases.

Immature teratoma:
- Predominantly solid
- Composed of immature embryonal or fetal tissue admixed with mature tissue.

Monodermal teratomas and somatic-type tumors arising from a dermoid cyst: Rare type of teratoma composed entirely of one tissue type.

Germ cell tumors of ovary: Dysgerminoma is the most common malignant tumor.

Dysgerminoma: Ovarian counterpart of seminoma of testis. Though suffix is 'oma', it is malignant germ cell tumor.
- Sheets of tumor cells separated by scanty fibrous stroma
- Tumor cells are monotonous
- Stroma infiltrated by mature lymphocytes.

Endodermal sinus tumor: Malignant aggressive, rapidly growing germ cell tumor. Can also occur in testis.

Endodermal sinus tumor:
1. Schiller-Duval body. Glomerulus-like structure composed of a central blood vessel enveloped by germ cells within a space lined by germ cells.
2. PAS positive hyaline droplets.

Endodermal sinus tumor: (1) α-fetoprotein; (2) α_1-antitrypsin.

Choriocarcinoma: Consists of cytotrophoblast and syncytiotrophoblast without any villi. Secrets hCG. It may be:
1. Gestational
2. Nongestational.

SEX CORD-STROMAL TUMORS

- Ovarian stroma is derived from the sex cords of the embryonic gonad. So, this group of tumors is known as sex cord-stromal tumors. They are divided into (i) pure sex-cord tumors and (ii) pure stromal tumors.
- **Hormonal activity**: Some of these cells normally secrete estrogens (granulosa and theca cells) or androgens (Leydig cells). The corresponding **tumors may be either feminizing (granulosa-theca cell tumors) or masculinizing (Leydig cell tumors)**.

Pure Sex Cord Tumors

Adult Granulosa Cell Tumor

Q. Write short note on granulosa cell tumor of ovary.

- It is a **low-grade malignant**, sex cord-stromal tumor **composed of granulosa cells often with** a variable number of **fibroblasts and theca cells**. It may be **associated with estrogen secretion**.
- **Incidence:** Account for about 1% of all ovarian tumors.
- **Age group:** About two-thirds occur in **postmenopausal women**.

MORPHOLOGY

Gross (Fig. 22.29A)

- Usually **unilateral**.
- **Size varies** from microscopic foci to large, solid, and cystic encapsulated masses.
- **Cut surface: Hormonally active tumors** have a **yellow color**, due to intracellular lipids (lipid-laden luteinized granulosa cells).

Microscopy

Granulosa Cell Component (Fig. 22.29B)

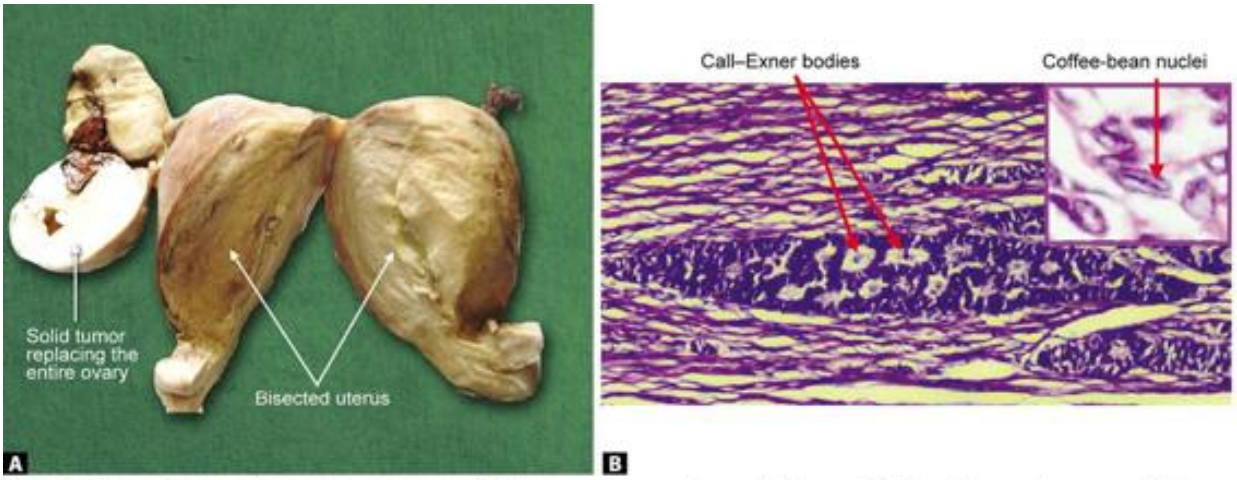
Tumor cells are **small, cuboidal, polygonal cells to spindle-shaped** and commonly have a **cleaved, elongated nucleus (coffee bean appearance—Fig. 22.29B inset).**

- **Growth patterns**
  - **Diffuse** (sarcomatoid) sheets
  - **Insular** (islands of cells in ribbons and in nests)
  - **Trabecular** (anastomotic bands of granulosa cells)
- **Call-Exner bodies (Fig. 22.29B):** These are **small, distinctive, gland-like** (follicular structures/degenerative) **space, filled with an acidophilic material** and appear like an

immature follicles. These characteristic structures when present are **very useful for diagnosis.** They are seen in a minority of tumors.

Stromal Component

Granulosa cell tumors contain a variable amount of fibromatous or thecomatous stroma.
Reticulin stain: It shows reticulin fibers surrounding the nests of tumor cells, except in stromal regions.



Figs. 22.29A and B: Granulosa cell tumor of ovary. (A) Gross appearance shows solid tumor; (B) Photomicrograph composed of tumor cells are arranged in sheets punctuated by small follicle-like structures (Call–Exner bodies). Inset of Figure B shows coffee-bean nucleus of tumor cells.

Clinical Features

Adult granulosa cell tumors occur over a wide age range. Average age is 53 years. Typically they present with **postmenopausal bleeding** in older women and menorrhagia, metrorrhagia, or amenorrhea in younger patients.
Behavior: All granulosa cell tumors are considered as **low-grade malignant tumors** because it may spread locally as well as metastasize.
Biochemical markers: Inhibin is secreted by granulosa cells and **elevated tissue and serum** levels of which are useful for identifying granulosa tumor and monitoring treated patients. Mutations of the *FOXL2* gene is observed in 97% of adult granulosa cell tumors.
Juvenile granulosa cell tumor is a distinctive type of granulosa cell tumor. It occurs mainly in children and young adults.

Pure Stromal Tumors

Fibromas and Thecomas

Q. Write short note on Meigs syndrome.

Tumors arising from ovarian stroma (pure stromal tumors) that are composed of:

- **Only fibroblasts** are called **fibromas** and are hormonally inactive.
- **Plump spindle cells with lipid droplets** are **thecomas**. Pure thecomas are rare, but may be hormonally active.
- Majority of fibromas and thecomas are **benign**.

MORPHOLOGY

Fibroma

Gross (Fig. 22.30)

- **Unilateral** in about 90% of cases.
- Usually **solid, spherical or slightly lobulated, encapsulated, hard, gray-white** masses covered by glistening, intact ovarian serosa.

Microscopy

Consist of **well-differentiated fibroblasts separated by scant collagenous connective tissue**. Focal areas of thecal differentiation may be found.

Clinical Features

Usually present as a **pelvic mass** sometimes accompanied by pain.

Associated Features

- **Meigs syndrome:** It is the combination of **ovarian fibroma, hydrothorax, and ascites**. The mechanism is not known.
- **Ascites** found in about 40% of cases when **tumors are larger than 6 cm** in diameter.
- **Uncommonly**, **hydrothorax** is seen usually only of the **right side**.



Fig. 22.30: Pure fibroma of ovary showing solid, spherical, gray-white apperance on cut section.

- Fibroma may be found in association with basal cell nevus syndrome.

Sex cord-stromal tumors: Produce hormones (estrogen or androgens) and most are benign (but potentially malignant).

Granulosa cell tumor:
- Call–Exner bodies
- Cells with coffee-bean nucleus.

Granulosa cell tumor: Marker is inhibin.

Meigs syndrome: (1) Ovarian fibroma, (2) Hydrothorax usually on right side and (3) Ascites.

METASTATIC TUMORS

Constitute about 3% of ovarian tumors.

Source of Primary Tumor

- **From genital tumors:** Most common metastatic tumors are derived from tumors of the **uterus, fallopian tube**, and **contralateral ovary**.

- **Extragenital tumors:** Most common extra-Müllerian tumors metastatic to the ovary are from **carcinomas of the breast and gastrointestinal tract** (including colon, stomach) biliary tract, and pancreas.
  Gross features, which point to metastatic carcinoma are:
  - **Bilateral** ovarian involvement.
  - **Multinodularity**.

Krukenberg Tumors

Q. Write short note on Krukenberg tumor.

- First described by Krukenberg in 1896. It is a metastatic tumor of ovary.
- **Age**: Usually found between **30 and 60 years.**

Sources of Primary

- **Stomach** is the most common primary site (in 75% of cases).
- **Large intestine.**
- **Breast.**

MORPHOLOGY

Gross (Fig. 22.31A)

- Most are **bilateral**.
- **Moderately enlarged** and **shape of the ovary is maintained**.
- **Capsule** is **intact** and **smooth**.
- Cut section shows a **solid tumor with variegated appearance**.

Microscopy

It shows **nests of mucin-producing, signet-ring** cancer **cells surrounded by ovarian stroma (Fig. 22.31B)**.

Metastatic tumors of ovary: Most common source of metastasis from genital tumors originate from uterus, fallopian tube, and contralateral ovary.

Metastatic tumors of ovary: Most common source of metastasis from extragenital tumors originate from carcinoma of breast and GI tract.

Krukenberg tumor: Microscopy shows nests of mucin-producing, signet-ring cancer cells within a cellular stroma of the ovary.

Krukenberg tumor:
- Metastatic tumor of ovary
- Most common primary is from stomach

GESTATIONAL DISORDERS

GESTATIONAL TROPHOBLASTIC DISEASE

CC: Describe the etiology, pathogenesis, pathology, morphology, clinical course, spread and complications of gestational trophoblastic neoplasms.

Q. Discuss the pathogenesis and morphology of trophoblastic disease.

Gestational trophoblastic disease consists of tumors and tumor-like lesions characterized by proliferation of placental tissue (villous or trophoblastic).



Figs. 22.31A and B: Krukenberg tumor: (A) Gross; (B) Microscopy showing nests of mucin-producing signet-ring cells within a cellular stroma.

BOX 22.5: WHO (2014) classification of gestational trophoblastic disease (abridged).

Molar pregnancies
- Hydatidiform mole
  - Complete
  - Partial
  - Invasive mole

Neoplasms
- Choriocarcinoma
- Placental-site trophoblastic tumor

Non-neoplastic lesions
- Exaggerated placental site

Classification

WHO (2014) classification of gestational trophoblastic disease is enlisted in **Box 22.5**.

Hydatidiform Mole

Q. Write short essay on hydatidiform mole.

Q. Write a short answer on etiopathogenesis of hydatidiform mole.

Definition: Hydatidiform mole is **benign, non-neoplastic, gestational trophoblastic disease of placenta** characterized histologically by **cystic swelling of the chorionic villi**, accompanied by **variable trophoblastic proliferation**. Androgenetic diploidy (diploid paternal-only genome) is the genetic cause in majority of cases.

Significance: Hydatidiform mole is **associated with** an **increased risk of invasive mole or choriocarcinoma**.

Age: Mostly present in the **fourth or fifth month of pregnancy with vaginal bleeding**. Currently, due to routine ultrasound examination during early pregnancy, moles are detected at earlier gestational ages.

Risk Factors

- **Maternal age:** It has two peaks:
  1. Risk is higher in girls **younger than 15 years** of age.
  2. Risk increases progressively **after 40 years**.
- **Ethnic background** and **obstetric history** also **influence the risk** of developing hydatidiform mole.

Types

Q. Write short note on differences between complete and partial mole.

Depending on cytogenetic and histological features, benign, noninvasive moles are divided into **two types**:

1. Complete Hydatidiform Mole(Figs. 22.32A and B)

- Complete mole **results from fertilization** of an **egg that has lost its chromosomes, either by one or two sperms**. Thus, the **characteristic feature is complete absence of maternal chromosomes** (empty ovum) and the **genetic material is completely paternally derived** (from sperm).
- **Most commonly** (~90%), complete moles develop from **fertilization of an empty egg/ovum by one sperm**. The **genetic material/chromosomes of the sperm (23,X not 23,Y) undergoes duplication** a phenomenon called **androgenesis**. Thus, the complete mole formed has a **karyotype 46,XX** diploid pattern, all derived from sperm. It will **never have a karyotype of 46,YY**.
- **Less commonly** (~10%), complete moles are formed by the **fertilization of an empty ovum by two sperm (dispermy).** Depending on genetic material of the two sperms (23,X or 23,Y), the complete mole has either **karyotype 46,XX** (two sperms with 23,X) or 46,XY (one sperm with 23,X and other with 23,Y).

Partial Hydatidiform Mole (Fig. 22.32C)

- Partial moles result from **fertilization of a single ovum with two sperms** (23,X or 23,Y). Thus, in these partial moles the **karyotype is triploid** [(e.g., **69,XXY**—ova (23,X) + sperm (23,X + 23,Y) or **69,XYY**—ova (23,X) + sperm (23,Y + 23,Y)] or even occasionally tetraploid (92,XXXY).
- **Fetal parts are commonly present**.
- In contrast to complete mole, there is **no increased risk for choriocarcinoma**.

MORPHOLOGY

Q. Write short note on morphology of hydatidiform/vesicular mole.

Gross (Fig. 22.33)

Complete hydatidiform mole represents a placenta with **grossly swollen, edematous (hydropic) chorionic villi.**

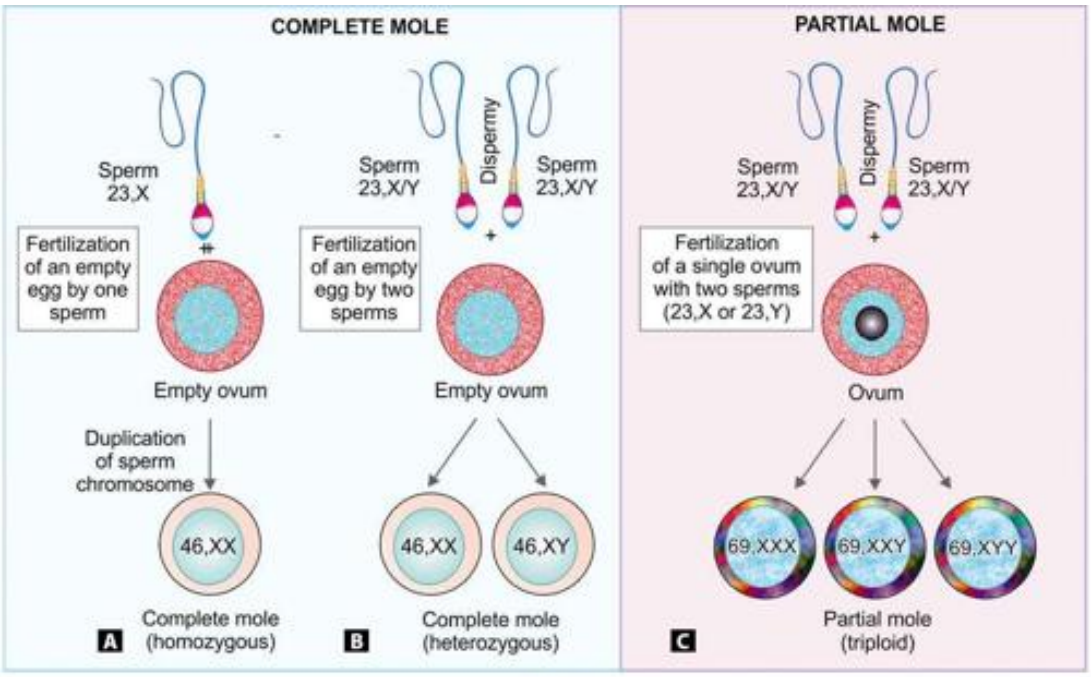
- **Hydropic chorionic villi appear delicate** and form a **friable thin-walled, translucent, cystic mass** which resembles **bunches of grapes (Fig. 22.33)**.

Fetal parts are frequently seen in partial moles.

Microscopy of Complete Mole (Figs. 22.34A and B)

Abnormalities involve all or most of the villi.

- **Characteristics of villi:**
  - Individual chorionic villi are **edematous, enlarged, scalloped in shape**.
  - The **central areas** of the **villi are acellular, devoid of mesenchymal cells, and lack adequately developed vessels.**
  - They show **fluid-filled spaces [cavitation (cisterns/cisternae)]**
- **Trophoblast proliferation:**
  - An **extensive/diffuse trophoblast proliferation (hyperplasia**), which **involves the entire circumference of the villi**.
  - T**rophoblast consists of syncytiotrophoblast, cytotrophoblast** and **intermediate trophoblast**.
  - They may show cellular atypia.
- **Fetal parts are absent**.



Complete mole:
- Diploid
- All chromosomes are paternal
- No embroyonic or fetal tissue

Partial mole:
- Triploid
- Two sets of paternal chromosomes
- Fetal tissue present

Complete mole: The genetic material/chromosomes of the sperm (23,X not 23,Y) undergoes duplication a phenomenon called androgenesis.

Hydatidiform mole: Benign non-neoplastic proliferative disorder of the placenta. Secretes high levels of β-hCG.
Increased risk of invasive mole or choriocarcinoma. It may be complete or partial mole.

Figs. 22.32A to C: Schematic representation of origin of complete and partial hydatidiform moles. (A) More commonly complete moles develop from fertilization of an empty ovum by a single sperm that undergoes duplication of its chromosomes; (B) Less commonly, complete moles may arise from fertilization of an empty ovum by two sperms (dispermy); (C) Partial moles develop from fertilization of single ovum by two sperm and forms partial mole with triploid karyotype.



Fig. 22.33: Complete hydatidiform mole showing marked distention of the uterus by grape-like vesicular chorionic villi.

Microscopy of Partial Mole

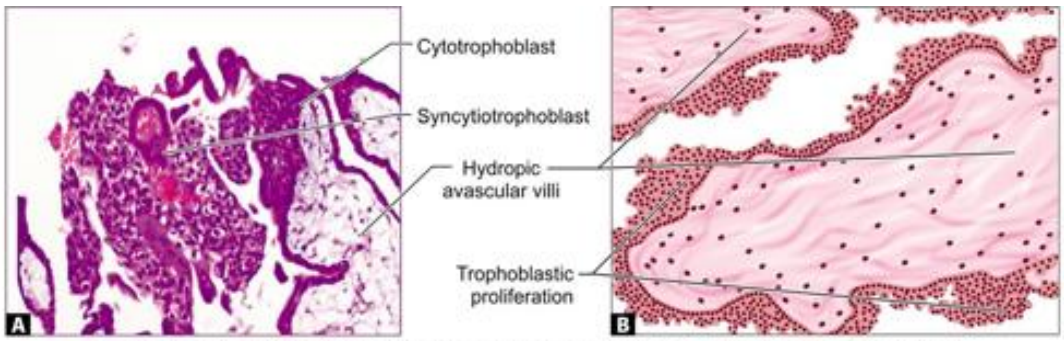
Histological **differentiation of complete mole from partial molar is important**.

- Partial moles have **two populations of chorionic villi**:
  1. **Normal villi**.
  2. **Swollen (**edematous) **villi** due to hydropic swelling and central cavitation.
- The **trophoblastic proliferation is moderate, focal** and **less marked** than that seen in complete mole.
- Chorionic villi show **blood vessels with fetal (nucleated) erythrocytes**.

Clinical Features

Q. Write a short answer on laboratory diagnosis of hydatidiform mole.

- Both partial and early complete moles present with **spontaneous pregnancy loss**.



Figs. 22.34A and B: Complete hydatidiform mole showing marked swollen chorionic villi and circumferential trophoblast proliferation.

- **Human chorionic gonadotropin (β-hCG):**
  - **In complete moles, β-hCG levels are high** compared to normal pregnancy.
  - Serial hormone determination shows increase in the β-hCG levels faster than for the pregnancy.
- **Monitoring serum concentrations of β-hCG is necessary** to determine the early development of invasive moles or gestational choriocarcinoma.
- Majority of moles are treated by thorough curettage.

Invasive Mole

Definition: It is defined as a **hydatidiform mole, complete or partial that penetrates or even perforates the uterine wall**.

- **Microscopy: Invasion of the myometrium by hydropic chorionic villi,** accompanied **by proliferation of both cytotrophoblast and syncytiotrophoblast.**
- **Spread:**
  - **Local spread:** It can spread into parametrial tissue and blood vessels.
  - **Blood spread**: Lungs and brain.
- **Clinical features**: Present as **vaginal bleeding** and **irregular uterine enlargement**.
- **Laboratory findings:**
  - **Persistently elevated** serum **β-hCG**.
  - **Varying degrees of luteinization of the ovaries**.
- **Treatment:** Responds well to **chemotherapy**.

Choriocarcinoma

Definition: Gestational choriocarcinoma is a **rapidly invasive malignant neoplasm of**

trophoblastic cells which **metastasizes widely**. **It responds well to chemotherapy**.

Types

Q. Write short note on choriocarcinoma.

Choriocarcinoma may be subdivided into gestational and nongestational.

- **Gestational:** It is preceded by:
  - **Hydatidiform moles**
  - **Previous abortions**
  - **Normal pregnancies**
  - **Ectopic pregnancies**.
- **Nongestational:**
  - **Very rare**
  - **Develop from germ cells in the ovaries/testis or the mediastinum**.

Q. Describe the gross and microscopy of choriocarcinoma.

MORPHOLOGY

Gross

Size: Choriocarcinoma in the uterus **range from microscopic foci to huge necrotic and hemorrhagic tumors**.

Appearance

- **Soft, fleshy, yellow-white tumor**
- **Secondary changes:**
  - Large pale areas of ischemic necrosis
  - Extensive areas of hemorrhage
  - Foci of cystic softening.

Microscopy (Fig. 22.35A)

Q. Write a short answer on microscopy and laboratory diagnosis of choriocarcinoma.

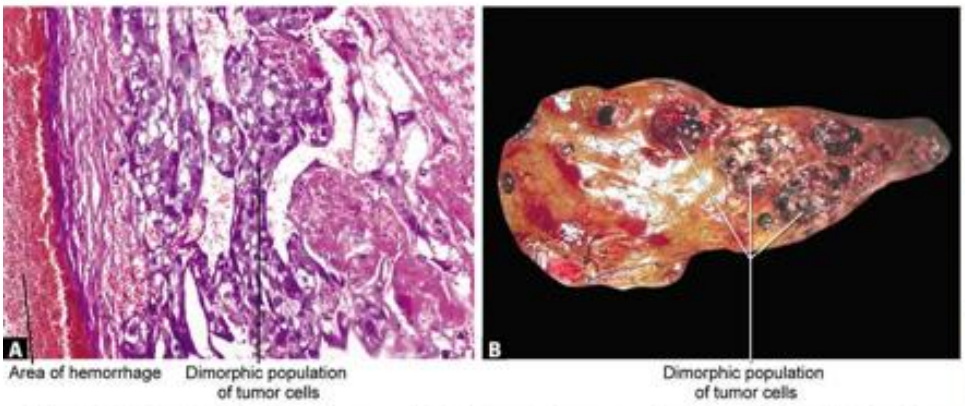
- **Absence of** chorionic **villi**.

- **Trophoblastic cells:** They consist of a **mixed/dimorphic population of syncytiotrophoblasts and cytotrophoblasts**.
- **Mitosis: Abundant** and sometimes abnormal.
- **Secondary changes:** Due to rapid growth, it can undergo:
  - Hemorrhage
  - Ischemic necrosis
  - Secondary inflammation.
- **Invasion:** They can invade into the underlying **myometrium, blood vessels and lymphatics**.

Clinical Features

- Uterine choriocarcinoma usually manifests as **irregular vaginal spotting of a bloody, brown fluid**.
- May follow an apparently normal pregnancy, after a miscarriage, or after curettage.

Laboratory findings: **β-hCG is elevated** more than that found in hydatidiform moles.



Figs. 22.35A and B: (A) Photomicrograph of choriocarcinoma showing dimorphic population of neoplastic cytotrophoblast and syncytiotrophoblast; (B) Liver with multiple secondaries from choriocarcinoma.

Spread

Widespread metastases are characteristic of choriocarcinoma. **Frequent sites** include: **Lungs, brain, bone marrow, liver (Fig. 22.35B)**, **vagina** and other organs.

Treatment of gestational choriocarcinoma: It consists of:

- Evacuation of the contents of the uterus
- Surgery

- Chemotherapy: Results in nearly 100% remission and a high rate of cures.

Placental Site Trophoblastic Tumor

Placental site trophoblastic tumor is **derived from the placental site or intermediate trophoblast**. The morphological and functional features of **intermediate trophoblast** overlap with those of trophoblasts and syncytiotrophoblasts. These are uncommon diploid tumors (often karyotype XX), usually develop a few months after pregnancy. The intermediate trophoblasts **do not produce hCG in large amounts**. Hence, hCG levels are only mildly raised. These tumors produce human placental lactogen.

Prognosis: It has an indolent clinical course, usually with a favorable outcome if the tumor is confined to the endomyometrium. These tumors are not as sensitive to chemotherapy when compared with other trophoblastic tumors. Hence, prognosis is poor when it spreads beyond the uterus.

Ectopic Pregnancy

Q. Write a short note on ectopic pregnancy.

Definition: Ectopic pregnancy/gestation is the **implantation of the fetus** in any site **other than a normal intrauterine location**.

Sites of Ectopic Pregnancy

Q. Write a short answer on sites of ectopic pregnancy.

- Within the **fallopian tubes (Fig. 22.36):** It is the most common site (~90%).
- **Other sites:** Ovary, abdominal cavity, and the intrauterine portion of the fallopian tube (cornual pregnancy).

Predisposing Conditions

- Prior pelvic inflammatory disease resulting in fallopian tube scarring.
- Other causes of peritubal scarring and adhesions, such as appendicitis, endometriosis, and previous surgery.
- Intrauterine contraceptive devices also increase the risk of ectopic pregnancy.

Pathogenesis

- Initially the embryonal sac, surrounded by placental tissue gets **implanted in the lumen of the fallopian tube**.
- With time trophoblastic cells and chorionic villi of the placenta **invade the fallopian tube** wall as they do in the uterus during normal pregnancy.

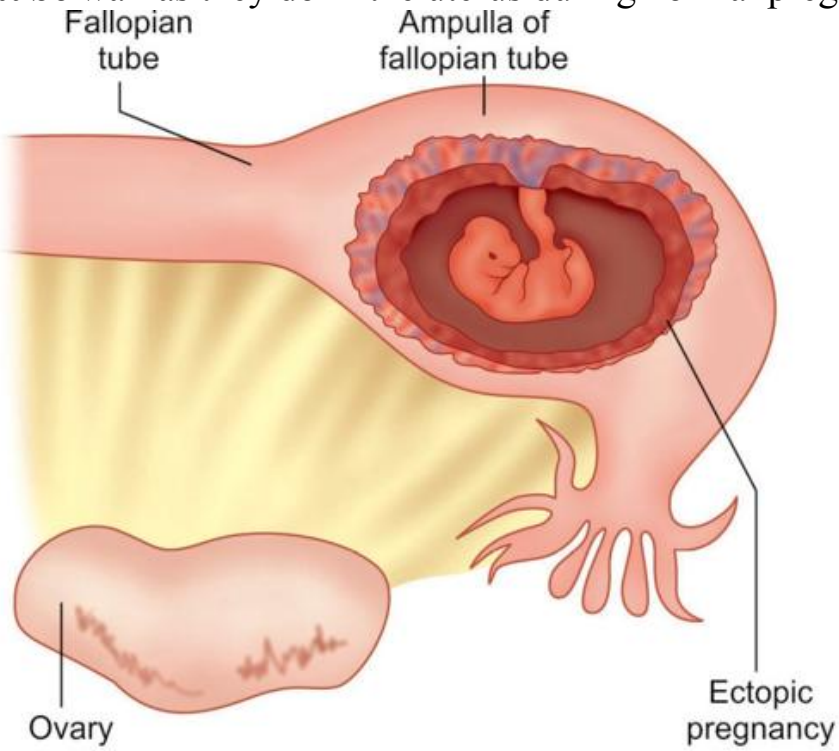


Fig. 22.36: Fallopian tube distended with fetus at the fimbrial end.

- The growth of the gestational sac **distends the fallopian tube** causing thinning and rupture.
- Fallopian tube **rupture usually results in massive intraperitoneal hemorrhage**.

MORPHOLOGY

Tubal pregnancy is the most common **cause of hematosalpinx** (blood-filled fallopian tube). Microscopic examination, usually shows blood clot admixed with chorionic villi.

Clinical Features

The ectopic pregnancy presents as **severe abdominal pain** due to rupture of the tube leading to pelvic hemorrhage and **constitutes a medical**

emergency. It most commonly develops about 6 weeks after a previous normal menstrual period. The patient may rapidly develop **hemorrhagic shock** with signs of an acute abdomen.

Complete hydatidiform mole: Whole placenta is neoplastic without any fetal parts and are diploid. Hydropic avascular villi with trophoblastic proliferation. Only 2% develop choriocarcinoma.

Partial mole: Part of placenta is neoplastic, fetal parts present and triploid (karyotyping shows 69 chromosomes).

Invasive mole:

- Invades or perforates uterine wall
- Shows villi with trophoblastic proliferation

Gestational choriocarcinoma: Highly invasive malignant neoplasm of trophoblastic cells which metastasizes widely. Responsive to chemotherapy and curable.

CHAPTER
23

Breast Disorders

FEMALE BREAST

Breast is made up of two major components: (1) **terminal duct–lobular unit (TDLU)** and (2) **large duct system (Fig. 23.1)**.

- **TDLU:** It **consists of lobule**, together with **its terminal** (intralobular and extralobular) duct. Each **lobule consists of** a variable number of **terminal ductules/acini** embedded within specialized intralobular stroma and is connected to the intralobular terminal duct.
- **Large duct system:** The intralobular ducts emerge from the lobule as extralobular duct and connects with the **subsegmental duct**, which in turn leads to a **segmental duct**. This leads to a **collecting (lactiferous or galactophorous) duct**, which opens onto the surface of the nipple. A fusiform dilation is seen beneath the nipple between the collecting and the segmental duct, known as the **lactiferous sinus**.

Microscopy

- **Lining of ducts and lobules:** Entire ducts and lobules of the breast is lined by a specialized two-cell-type epithelial lining—the **inner epithelium** with secretory and absorptive functions (often simply called epithelium), and the **outer myoepithelial cells** (*refer* **Fig. 23. 5A**).
- **Breast stroma:** It consists of two types namely, **intralobular** and **extralobular**.

BENIGN EPITHELIAL LESIONS

CC: Classify and describe the types, etiology, pathogenesis, hormonal dependency of breast

Q. List benign epithelial lesions of breast.

Classification: According to the subsequent risk of developing breast carcinoma, the benign epithelial lesions of breast can be divided into three groups:

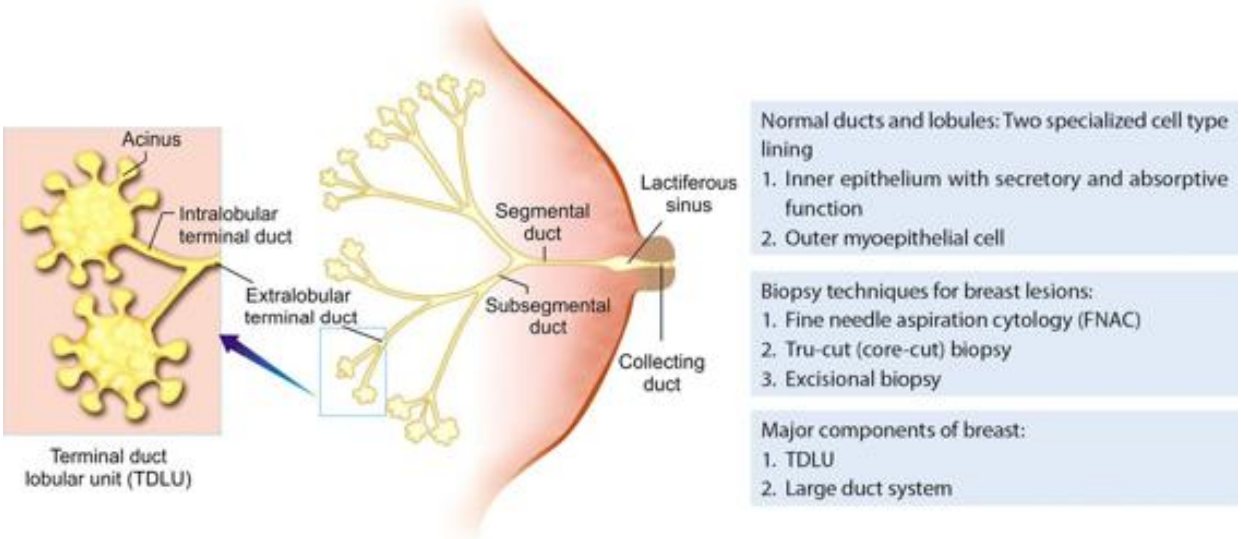


Fig. 23.1: Diagrammatic appearance of breast parenchyma. Terminal duct-lobular unit (TDLU) consists of extralobular terminal duct; intralobular terminal duct and acini.

Nonproliferative Breast Changes (Fibrocystic Changes)

Q. Write short note on fibrocystic disease of breast (fibrocystic changes).

- It is a group that consists of a many **common morphological changes observed in the breast** and is also termed as fibrocystic changes.
- **No increased risk of carcinoma of breast**.

MORPHOLOGY (FIGS. 23.2A TO D)

Q. Write a short note on morphology of nonproliferative breast changes (fibrocystic disease of breast).

Fibrocystic changes **primarily affect the TDLU**. Its characteristic morphological features are:

1. **Cysts:** It can be **microscopic or grossly visible**. Small cysts may coalesce to form larger cysts.
   - **Cysts contain turbid, cloudy yellow and semi-translucent** fluid. Some of these cysts externally appear brown or blue color (**'blue dome cysts' of Bloodgood**).
   - **Microscopically**, cysts are lined either by a **flattened atrophic epithelium** (especially the larger cysts) or by **metaplastic apocrine cells**.
2. **Apocrine metaplasia:** It is a common change, most often seen in **dilated ducts and cystic structures**.
   - Apocrine cells have an **abundant granular, eosinophilic**/acidophilic **cytoplasm** and **round nuclei with prominent nucleolus**. The apical portion of the cytoplasm shows the typical **'apocrine snout'**.
3. **Calcification:** It is less common and line the bottom of a rounded cyst and mammographers use the term "milk of calcium" to describe these calcifications.

4. **Fibrosis:** It is usually seen and its degree varies markedly. Chronic inflammation, fibrosis, hyalinization contribute to the firmness of the breast during palpation.
5. **Adenosis*:*** It is defined as an increase in the number of acini (terminal ductule) per lobule.
   - Acini are often enlarged (blunt-duct adenosis).
   - Acini are lined by columnar cells, which may appear benign or show atypical features. **("flat epithelial atypia")**. Flat epithelial atypia is probably the earliest recognizable precursor of low-grade breast cancers.

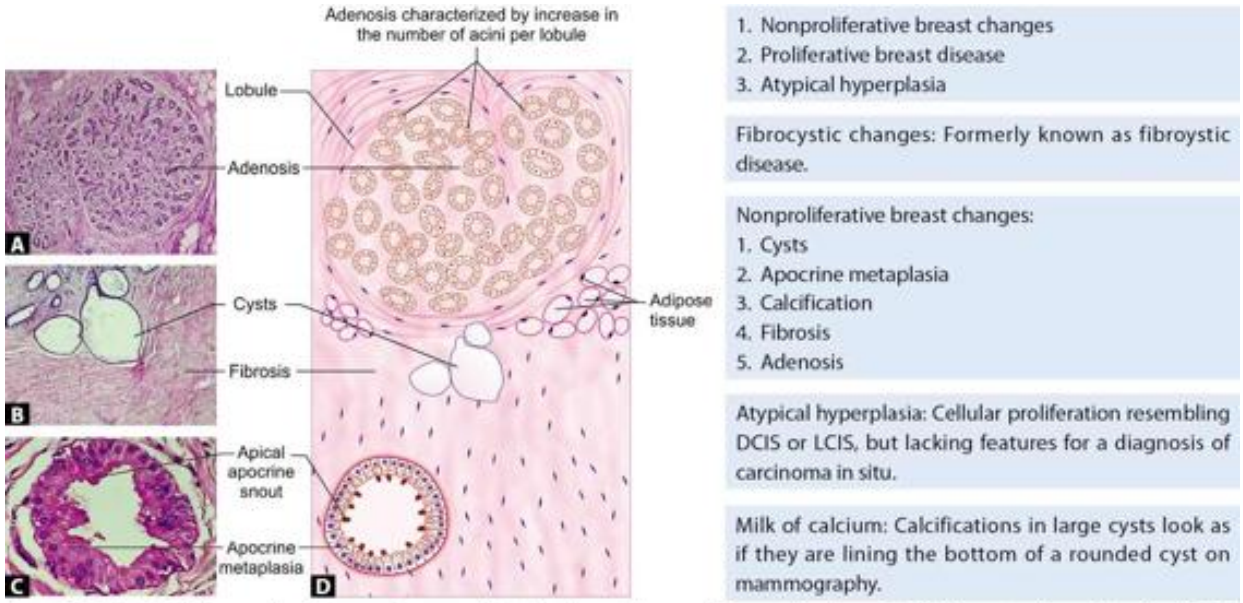
Lactational adenoma: They develop as palpable masses in pregnant or lactating women. They show normal-appearing breast tissue with lactational changes. They represent an exaggerated local response to gestational hormones.

Proliferative Breast Disease without Atypia

Characterized by **proliferation of ductal epithelium and/or stroma without cytologic or architectural features of carcinoma in situ.**

MORPHOLOGY

1. **Epithelial hyperplasia:** Normal breast ducts and lobules are lined by two layers of cells: Inner (luminal) epithelial cells and outer myoepithelial cells.
   - **Epithelial hyperplasia** is defined as the **presence of more than two cell layers**. The additional cells consist of both luminal and myoepithelial cell types. These cells fill and distend ducts and lobules. **Irregular lumens can often be seen at the periphery of the cellular masses.**



Figs. 23.2A to D: Microscopic features of nonproliferative breast change (fibrocystic changes); (A) Hematoxylin and eosin (H & E) showing adenosis; (B) (H and E) Showing fibrosis and cysts; (C) (H and E) Showing cysts with apocrine metaplasia of the lining cells; (D) Diagrammatic appearance showing the above features.

- Epithelial hyperplasia may be mild (when made up of three or four epithelial cells in thickness), moderate to florid (when more pronounced), and atypical.

2. **Sclerosing adenosis:** It is a **form of adenosis**. The **number of acini per lobule is increased** and at least double the number found in uninvolved lobules.
   - The normal lobular arrangement is maintained and is **more cellular centrally than peripherally**.
   - The **acini** are **compressed and distorted in the central portions of the lesion** but dilated at the **periphery**. Myoepithelial cells are usually prominent. On occasion, stromal fibrosis may produce a microscopic **appearance mimicking invasive carcinoma**.
3. **Complex sclerosing lesion:** Its components include sclerosing adenosis, papillomas, and epithelial hyperplasia.
4. **Papillomas:** It consists of **multiple branching central fibrovascular cores lined by luminal and myoepithelial cells**. These **papillae grow within a dilated duct**. Epithelial hyperplasia and apocrine metaplasia are frequently seen.
   - Papillomas are usually **single in large duct** and are found in the lactiferous sinuses of the nipple.
   - Papillomas **in the small duct** are **usually multiple** and located deeper within the ductal system.
   - **Large papillomas** usually **present with nipple discharge**. Torsion on the stalk may produce infarction of the papilloma causing a bloody discharge. Most of the small duct papillomas present as small palpable masses, or detected as densities or calcifications on mammograms.

Proliferative Breast Disease with Atypia

This group includes **atypical ductal hyperplasia** and **atypical lobular hyperplasia**.

MORPHOLOGY

Atypical hyperplasia is a cellular proliferation, which **resembles carcinoma in situ**.

- **Atypical ductal hyperplasia:** It consists of **a monomorphic proliferation** of regularly spaced cells, sometimes with cribriform spaces. It resembles ductal carcinoma in situ (DCIS), but distinguished from it by being limited in extent and **only partially filling ducts**.
- **Atypical lobular hyperplasia:** It is a cellular proliferation identical to those of lobular carcinoma in situ (LCIS), but the **cells do not fill or distend more than 50% of the acini within a lobule**.

Clinical Significance of Benign Epithelial Changes (Box 23.1)

- Nonproliferative changes do not progress to cancer.
- Proliferative disease is associated with a mild increase in risk, while proliferative disease with atypia has a moderate risk of carcinoma.

BOX 23.1: Epithelial pathologic lesion with relative risk of invasive breast cancer.

- Nonproliferative breast changes (fibrocystic changes): 1%
- Proliferative disease without atypia: 1.5–2%
- Proliferative disease with atypia: 4–5%
- Carcinoma in situ: 8–10%

Proliferative breast disease without atypia:
1. Epithelial hyperplasia
2. Sclerosing adenosis
3. Complex sclerosing lesion
4. Papillomas

Epithelial hyperplasia: Ducts and lobules lined by more than two layer of cells.

Proliferative breast disease with atypia: Atypical epithelial hyperplasia.

Atypical hyperplasia:
- Cellular proliferation that resembles carcinoma in situ
- Ductal or lobular

CARCINOMA OF THE BREAST

CC: Classify and describe the epidemiology, pathogenesis, classification, morphology, prognostic factors, hormonal dependency, staging and spread of carcinoma of the breast.

Q. Classify tumors of breast. Discuss the etiopathogenesis and morphology of carcinoma breast.

Q. Discuss the etiopathogenesis and morphology of infiltrating duct carcinoma of breast.

Carcinoma of the breast is the **second most common cancer in females**, first being carcinoma of cervix. Almost all breast carcinomas are adenocarcinomas.

Etiology

Q. Write short note on etiopathogenesis of breast carcinoma.

Risk Factors (Fig. 23.3)

Most important risk factor is gender and breast cancer cases occur in only 1% of male.

1. **Age:** Breast cancer **develops usually after the age of 25**. Its incidence rises as the age advances and peaks at 70 to 80 years and then declining slightly thereafter.
2. **Geographic variations:** They are observed and may be related to following:
   - **Type of diet:** Consumption of **coffee (caffeine) may decrease the risk**.
   - **Reproductive patterns:** These include number and timing of pregnancies.

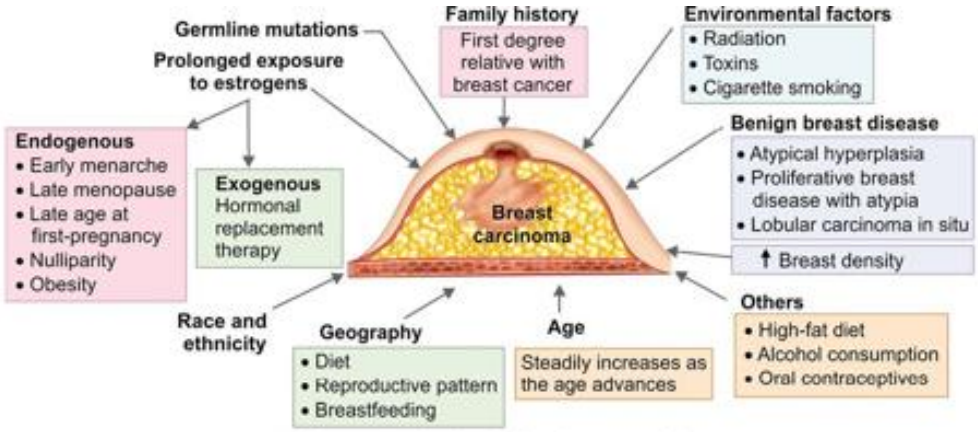


Fig. 23.3: Risk factors involved in the development of breast cancer.

- **Nursing habits/breastfeeding: Longer the women breastfeed**, the **greater the reduction in risk**.
- **Obesity: Physical activity (exercise)** may have a **protective role**.

3. **Race/ethnicity:** The variation in breast cancer risk genes across ethnic groups is in part responsible for racial or ethnic differences. For example, incidence of ***BRCA1*** and ***BRCA2*** mutations occur at different frequencies in different ethnic groups.
4. **Prolonged exposure to estrogens:** It **increases the risk of breast carcinoma.** It may be seen in the following conditions:
   - **Endogenous hormone exposure** occurs with **long duration of reproductive life**:
     - **Early menarche** (<12 years) and **late menopause** (>55 years).
     - **Late age at first-term pregnancy (>35 years) and nulliparity**.
     - **Postmenopausal obese**: **Increased risk** due to the synthesis of estrogens in fat depots.
     - **Carcinoma of the contralateral breast or endometrium:** Both **produces prolonged estrogenic stimulation**.
   - **Exogenous hormone exposure:** It may be due to **postmenopausal hormonal replacement therapy**.

Estrogens stimulate production of growth factors, which promote development of carcinoma.

Reduced estrogen decreases the risk of breast carcinoma.

5. **Germline mutations:** About 5–10% of breast cancers develop in women with germline mutations in tumor suppressor genes (*refer* pathogenesis below).

6. **Family history of first-degree relatives with breast cancer:** First degree relatives include mother, sister or daughter. It is strongly associated with increased risk for breast cancer. The risk is more if the relative had breast cancer at a young age or develop bilateral breast cancer.
7. **Environmental risk factors:**
   - **Radiation exposure:** Radiation to the chest due to cancer therapy, atomic bomb exposure, or nuclear accidents.
   - **Environmental toxins:** Organochlorine pesticides have estrogenic effects.
   - **Cigarette smoking.**
8. **Benign breast disease (Box 23.1): Atypical hyperplasia/proliferative breast disease with atypia/lobular carcinoma in situ.**
9. **Breast density:** Women with very dense breasts (high density) on mammography have a four- to six-fold higher risk of (both ER positive and ER negative) breast cancer compared to women with the lowest density. High breast radiodensity indicates deficient involution of lobules at the end of each menstrual cycle.
10. **Other possible factors:**
    - High-fat diet
    - Moderate or heavy alcohol consumption
    - Oral contraceptives

Pathogenesis (Fig. 23.4)

Breast cancer can be divided into three major subtypes depending on differential expression of hormone receptors and HER2. They arise through distinct pathways that involve the stepwise acquisition of driver mutations in the epithelial cells of the duct/lobular system.

Contributing factors: The contributing factors involved in the development of breast cancer can be categorized as (i) genetic, (ii) hormonal, and (iii) environmental.

Genetic Factors

The mutations in cancer genes that contribute to breast carcinogenesis can be divided into inherited (germline) and acquired mutations.

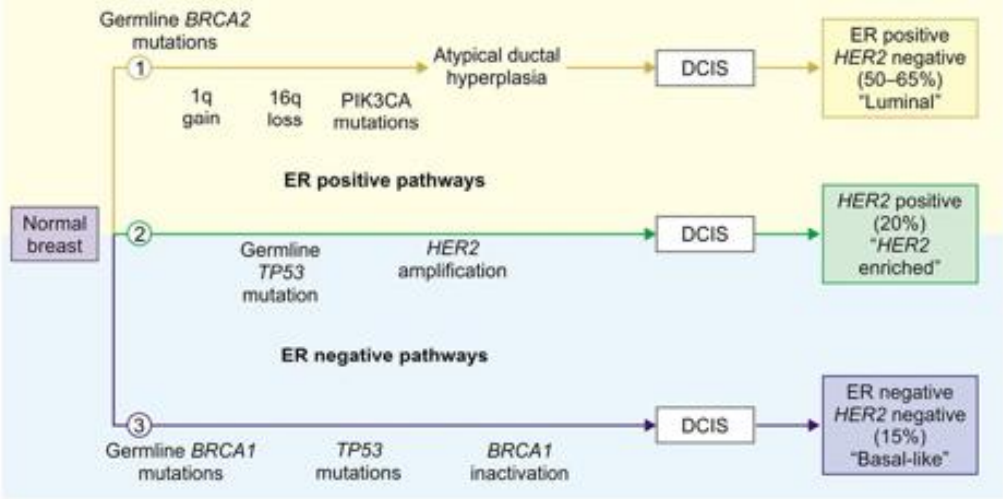


Fig. 23.4: Probable sequence of events in the development of breast carcinoma.
(DCIS; ductal carcinoma in situ: ER; estrogen receptor: PR progesterone receptor).

Familial breast cancer: The major germline mutations in breast cancer affect genes that regulate genomic stability or that are involved in progrowth signaling pathways.

- *BRCA1* and *BRCA2* are classic tumor suppressor genes and are normally expressed in many different cells and tissues. Breast cancer develops only when both alleles are inactivated or defective. *BRCA1* and *BRCA2* encode proteins that are necessary for repair of certain kinds of DNA damage. Germline mutations in these genes increases the risk of breast and serous ovarian cancer. ***BRCA2* mutations are mainly associated with ER-positive tumors, whereas *BRCA1* mutations are associated with triple-negative cancers.**
- **Other mutated genes** associated with familial breast cancer: These include ***TP53*** ("guardian of the genome") and ***PTEN*** (an important negative regulator of the pro-growth PI3K-AKT pathway).

Sporadic breast cancers: The genes mutated in familial breast cancer are often disturbed in sporadic breast cancers.

- Somatic mutations in *BRCA1* and *BRCA2* are rare in sporadic cancers. However, *BRCA1* is inactivated by methylation in up to 50% of triple-negative cancers.
- Somatic mutations in *TP53* are common in breast cancer (especially in triple-negative and HER2-positive tumors). Mutations that activate PI3K-AKT signaling are frequently found in sporadic ER-positive and HER2-positive breast cancers.

Amplification of the *HER2* gene: *HER2* is a receptor tyrosine kinase. It promotes proliferation of cells and opposes apoptosis by stimulating the RAS- and PI3K-AKT signaling pathways. **A common driver mutation in breast cancer is amplification of the *HER2* gene.** Cancers that overexpress *HER2* are pathogenically distinct tumors and highly proliferative. In the past they were consider to be having a poor prognosis. However, the availability of therapeutic agents that specifically target *HER2* receptors markedly improved the prognosis for these patients.

Hormonal Influences

Estrogens stimulate the production of growth factors (e.g., transforming growth factor-α, platelet-derived growth factor, fibroblast growth factor, etc.). These growth factors may promote development of tumor through paracrine and autocrine mechanisms. ER also regulates many other genes in an estrogen dependent fashion, some of which are involved in tumor development or growth. Hormonal influences are involved in the development of cancers from precursor lesions (which typically strongly express ER) to fully malignant and even metastatic carcinomas. The therapeutic use of estrogen antagonists reduce the development of ER-positive cancers in women.

Environmental Factors

The contribution of environmental factors in breast cancer is substantiated by the variable incidence of breast cancer in genetically homogeneous groups and the geographic differences in breast cancer incidence.

Major Pathways of Breast Cancer (Fig. 23.4)

1. **ER-positive, HER2-negative cancers (luminal): They constitute 50–65% of breast cancers.** Most common pathway leads to ER-positive cancers. By gene expression profiling, these cancers are classified as "luminal." This type of cancer arises most commonly in individuals with germline *BRCA2* mutations. They show gains of chromosome 1, losses of chromosome 16, and mutations of *PIK3CA* (the gene encoding PI3K).
2. **HER2-positive cancers (HER2 enriched): They arise through a pathway associated with amplifications of the HER2 gene on**

chromosome 17q. They constitute about 20% (less common) of all breast cancers and may be either ER-positive or ER-negative They are usually associated with germline *TP53* mutations.

3. **ER-negative, HER2-negative cancers (basal-like): They arise via a distinct pathway which is independent of ER-mediated changes in gene expression and HER2 gene amplifications.** They constitute ~15% (least common) of breast cancers but molecularly most distinctive type of breast cancer is negative for ER and HER2 ("triple negative"). These cancers have loss of *BRCA1* and *TP53* function and are genomically unstable. By gene expression profiling, the majority of triple-negative cancers are classified as "basal-like."

Role of stromal cells: Neoplastic epithelial cells are dependent on interactions with stromal cells in the local microenvironment. The stroma consists of fibroblasts, blood vessels, lymphatics, inflammatory cells, and extracellular matrix. The role of stroma is not yet completely known. However, it is observed that cancers develop in the areas with increased mammographic density (due to increased fibrous stroma) which suggests that stroma is both a marker of risk and biologically important for tumorigenesis. Focal changes in the stroma may create a microenvironment required for tumor development and growth. Angiogenesis and tumor-associated inflammation are commonly found with carcinoma even in in situ stage.

Classification of Breast Carcinoma

Q. Write short note on classification of breast carcinoma.

Histological Classification (Box 23.2)

Broad classification depends on a combination of histological pattern and cytological characteristics.

- **Noninvasive carcinoma (carcinoma in situ):** It is characterized by the presence of **malignant epithelial cells within the ducts and lobules** and **has not penetrated the basement membrane**.
- **Invasive carcinoma** ("infiltrating" carcinoma): It is characterized by malignant cells that has **penetrated** through the **basement membrane**

into stroma.

Molecular classification of breast carcinoma is discussed on pages 778-9.

Breast cancer:
- Most common cancer in women in the world
- Most common cancer in urban women in India
- Second most common cause of cancer-related death in women.

Factors which reduce risk of breast carcinoma: Breastfeeding, exercise, healthy body weight.

Carcinoma of breast: Long duration of breastfeeding has a protective effect.

BRCA1 (on chromosome 17q21) and *BRCA2* (on chromosome 13q12.3) are l large genes, and many mutations can occur throughout their coding regions.

More than 95% of malignant tumors of breast are adenocarcinomas.

Origin: All breast carcinomas arise from cells in the terminal duct lobular unit.

Tumors with only *HER2* positivity: High frequency of brain metastasis.

BRCA1 positive woman have 60% increased risk of breast carcinoma.

Upper outer quadrant: Most common site for cancer.

PRECURSOR LESIONS/NONINVASIVE CARCINOMA

Classification

It is subclassified as:

1. **Ductal** carcinoma in situ (DCIS)
2. **Lobular** carcinoma in situ (LCIS).

Carcinoma in situ was originally classified as ductal or lobular based on the resemblance of the involved spaces to normal ducts or lobules (acini).

Presently, these terms are based on differences in tumor cell biology; and "lobular" refers to carcinomas of a specific type, and "ductal" is used more generally for adenocarcinomas that have no other designation. Presently WHO (2012) classifies them as precursor lesions.

BOX 23.2: Classification of common breast tumors.

Epithelial tumors

A. Precursor lesions/Noninvasive carcinoma
   1. Ductal carcinoma in situ (DCIS)
   2. Lobular carcinoma in situ (LCIS)

B. Invasive (infiltrating) breast carcinoma
   1. Invasive carcinoma of no special type (NST), the most common subtype of invasive carcinoma
   2. Invasive lobular carcinoma
   3. Special histologic types of invasive carcinoma
      i. Medullary carcinoma
      ii. Mucinous carcinoma (colloid carcinoma)
      iii. Tubular carcinoma

C. Papillary lesions
   1. Intraductal papilloma
   2. Intraductal papillary carcinoma
   3. Solid papillary carcinoma

Tumors of the nipple

1. Nipple adenoma
2. Paget disease of nipple

Fibroepithelial tumors

1. Fibroadenoma
2. Phyllodes tumor: Low grade and high grade

Clinical pattern

Inflammatory carcinoma

Ductal Carcinoma In Situ(DCIS; Intraductal Carcinoma)

Q. Write short note on ductal carcinoma in situ/intraductal carcinoma.

- DCIS consists of a **malignant cells limited to ducts and lobules** by the basement membrane. The myoepithelial cells are preserved.
- They usually **involve the small and medium-sized ducts**.
- DCIS can **spread throughout ducts and lobules**. Thus, it can produce extensive lesions involving an entire sector of a breast.

MORPHOLOGY

DCIS is divided into two subtypes **depending on the architecture**:

A. DCIS-comedo (high-grade) subtype
B. Non-comedo DCIS
   - Solid
   - Cribriform
   - Papillary
   - Micropapillary

Majority of DCIS show a mixture of above patterns.

A. DCIS-comedo (High-grade) Subtype

- **Gross**: Cut section of the tumor shows **distended duct-like structures** containing **white**, **necrotic material;** similar in appearance to comedones (hence the term comedo).
- **Microscopy (Figs. 23.5A to C):**
  - Ducts contain solid sheets of very **large, pleomorphic epithelial cells** having **pleomorphic, irregular hyperchromatic nuclei**. The **central areas** show **necrosis**.
  - **Central necrotic area** commonly undergoes **dystrophic calcification**.

B. Noncomedo DCIS

- In these tumors, the ducts contain a **monomorphic population of tumor cells** with **nuclear grades ranging from low to high**.
- Both the **tumor cells and nuclei are smaller and more regular** than those of the comedo type.
- **Necrosis is minimal or absent**.
- Architectural patterns of noncomedo DCIS:
  1. **Solid DCIS (Fig. 23.6A):** It shows **tumor cells completely filling the involved spaces.**
  2. **Cribriform DCIS (Fig. 23.6B):** It shows **spaces between the intraductal tumor cells** that are **evenly distributed and regular in shape** (cookie cutter-like).
  3. **Papillary DCIS (Fig. 23.6C):** It shows **papillae with fibrovascular cores** and without the normal myoepithelial cell layer.
  4. **Micropapillary DCIS (Fig. 23.6D):** It shows **bulbous protrusions without a fibrovascular core**.

Prognosis of DCIS: If untreated, women with small, low-grade DCIS may **develop invasive cancer**. Mastectomy for DCIS is curative for over 95% of patients.

Lobular Carcinoma In Situ (LCIS)

- Arises in TDLU
- Tends to be **bilateral**

- More **common** in **young women** and majority occurring before menopause.

Molecular Changes

- LCIS is associated with **mutations of the E-cadherin gene** which results in lack of adhesive molecule E-cadherin.
- Lack of E-cadherin in LCIS result in **rounded tumor cells** due to loss of attachment to adjacent cells.
- **Lack of E-cadherin** expression **confirms the lobular nature** of neoplastic cells.

MORPHOLOGY

- Consists of **loose and noncohesive** (dyscohesive) **cells** having **oval or round, regular nuclei** and **small nucleoli**.
- Tumor cells **smaller** and **more monotonous** than in DCIS.
- Mucin-positive signet-ring cells are commonly present.
- Immunohistochemistry:
  - **Lack of E-cadherin**
  - Almost always **expresses ER and PR**
  - **Does not overexpress HER2/neu**

Carcinoma in situ: DCIS and LCIS

DCIS: Most common malignancy associated with calcifications.

Comedo DCIS: Most likely to result in a palpable abnormality in the breast.

DCIS:
- Low grade: Cribriform, papillary and micropapillary
- High grade: Solid and comedocarcinoma

Bloody nipple discharge: Intraductal papilloma and ductal carcinoma.

E-cadherin: Responsible for cohesion of normal breast epithelial cells, and is expressed by both benign breast epithelium and ductal cancers.

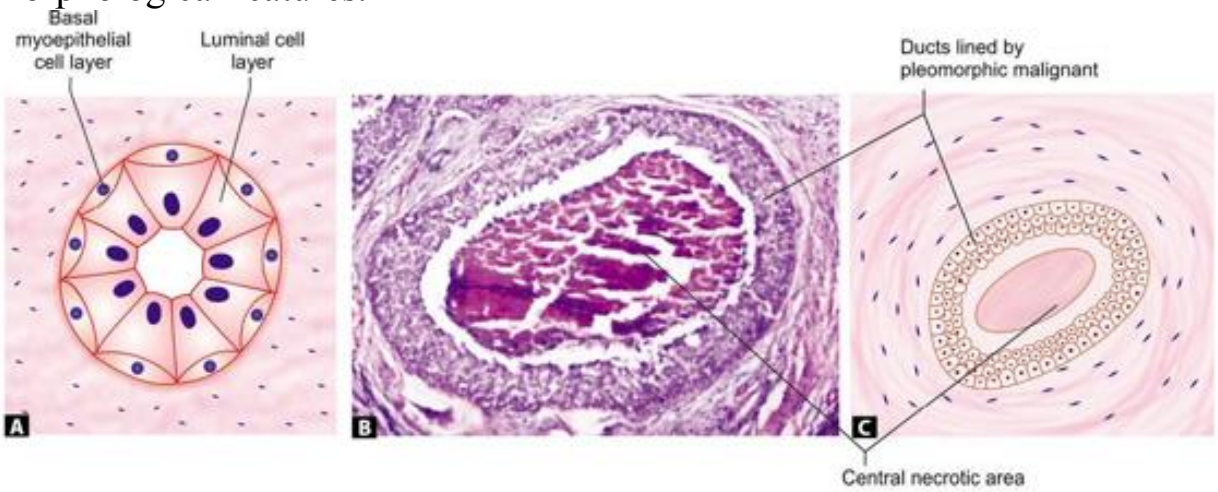
Lobular carcinoma in situ (LCIS): Loss of E-cadherin.

INVASIVE (INFILTRATING) CARCINOMA

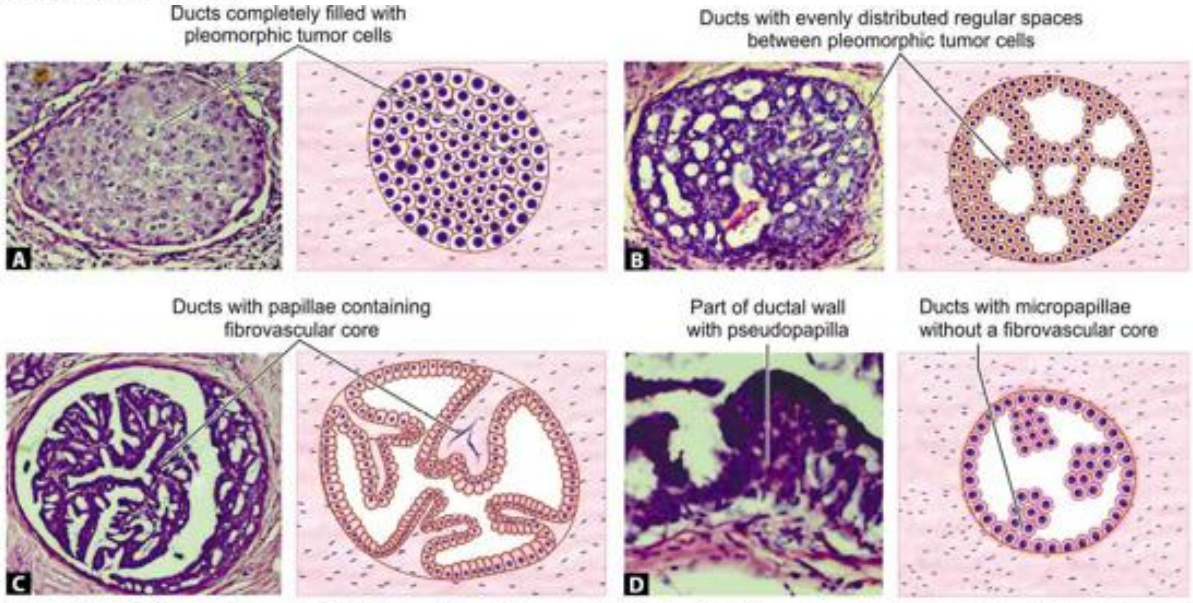
Invasive ductal carcinoma is the **most common histological type** of breast carcinoma.

Classification of Invasive Carcinoma

Invasive carcinomas can be divided depending on the molecular and morphological features.



Figs. 23.5A to C: (A) Normal duct/acinus lined by bilayered epithelium consisting of inner luminal cells and outer myoepithelial cells; (B) (photomicrograph); (C) (diagrammatic) DCIS-comedo subtype showing ducts containing large, pleomorphic epithelial cells and central area of the duct with necrosis.



Figs. 23.6A to D: Photomicrographic (left of each) and diagrammatic (right of each) appearances of noncomedo ductal carcinoma in situ (DCIS): (A) Solid; (B) Cribriform; (C) Papillary; (D) Micropapillary type.

Morphological Classification (Box 23.2)

- **No special type (NST):** Two-thirds are grouped together and called "ductal" or no special type (NST).
- **Special histological subtype:** About one-third of breast cancers are classified into special histologic types.

Molecular Classification

Based on the expression of estrogen receptor (ER) and HER2, invasive breast carcinoma can be divided into three major biologic subgroups **(Table 23.1)**.

1. **ER-positive, HER- negative (luminal):** Constitute **50–65% of cancers** and **is the most common molecular subtype of invasive breast cancer.** It is subdivided into two subgroups based on proliferation rates.
   - **ER-positive, HER2-negative, low proliferation (40–55%): This tumors form the majority of cancers in older women and in men.** Many of them are detected at an early stage. The local recurrence rate is very low and is usually cured by surgery.

TABLE 23.1: Molecular subtypes of invasive breast carcinoma.

Features	*ER-positive, HER2-negative (luminal)*		*HER2-positive (ER-positive or negative)*	*Triple negative (ER, PR, and HER2 negative)*
Percentage	~ 40–55% (low proliferation)	~10% (high proliferation)	~20%	~15%
Special histologic types	Well/moderately differentiated lobular, tubular, mucinous, papillary	Poorly differentiated lobular	Some apocrine, micropapillary	Carcinoma with medullary features
Type of patient	Older women, men	*BRCA2* mutation carriers	Young women, *TP53* mutation carriers (ER positive)	Young women, *BRCA1* mutation carriers
Main grade	Grade 1 and 2		Grade 2 and 3	Grade 3
Similar group defined by mRNA profiling	Luminal A (low grade), luminal B (high grade)		Luminal B (ER positive), HER2-enriched (ER negative)	Basal-like
Common somatic mutations	*PIK3CA* (40%), *TP53* (26%)		*TP53* (75%), *PIK3CA* (40%)	TP53 (85%)
Pattern of metastasis (percentage)	Bone (70%), visceral (25%) or brain (<10%)	Bone (80%), visceral (30%) or brain (10%)	Bone (70%), visceral (45%), and brain (30%)	Bone (40%), visceral (35%), brain (25%)
Complete response to chemotherapy	<10%	~ 10%	15% (ER positive) 30% (ER negative)	~30%
Recurrence	Late (>10 years)	Intermediate	Usually short (<10 years)	Usually short (<8 years)

(ER: estrogen receptor; PR: progesterone receptor; PIK3CA: phosphatidylinositol 3-kinase, catalytic, α-polypeptide)

- **ER-positive, *HER2*-negative, high proliferation (10%):** These tumors are ER-positive, but ER levels may be low and expression of progesterone receptor may be low or absent. It is the most common carcinoma associated with *BRCA2* germline mutations. There is higher expression of genes related to proliferation.

2. ***HER2*-positive (~ 20%):** It is the **second most common molecular subtype of invasive breast cancer.** About 50% of them are ER-positive. When present, ER expression is usually low; progesterone receptor expression is usually absent. They are more common in young women. They are identified by assays of *HER2* protein overexpression or *HER2* gene amplification. They can metastasize early (to viscera and brain) and even when tumor is small in size. About, one- third or more of these patients respond completely to antibodies that bind and block *HER2* activity, and have an excellent prognosis.
3. **ER-negative, *HER2*-negative breast cancers (basal-like/triple negative carcinoma) (~ 15%): They constitute the third major molecular subtype.** They are more common in young premenopausal women. This forms the majority of carcinomas in women with *BRCA1*

mutations. They have high proliferation and rapid growth. Hence, they present as a palpable mass. They share a number of genetic similarities with serous ovarian carcinomas (both types with germline *BRCA1* mutations). These can metastasize when small in size, frequently to viscera and to the brain. However, about 30% respond to chemotherapy and cure may be possible in this chemosensitive subgroup. Recurrences develop within 5 years of treatment. Local recurrence is common, even after mastectomy. Prolonged survival following distant metastasis is rare.

Common Features

- **Invasion:** It is characterized by the presence of tumor cells outside of the duct-lobular units and into the surrounding breast stroma.
- **Desmoplasia:** It is characterized by marked stromal fibrosis.
- **Clinical presentation:**
  - Almost always produces a **palpable mass;** and in over 50% of patients are associated with axillary lymph node metastases.
  - Invasion → **dimpling of the skin** or **fixation** of tumor **to** the underlying **chest wall.**
  - **Retraction of the nipple** may occur when tumor involves the central portion of the breast.
  - **Lymphatic involvement:** Involved lymphatics may block the local area of skin drainage and cause **lymphedema** and thickening of the skin.
- Mammographically, invasive carcinomas most commonly present as a radiodense mass.

Invasive (Infiltrating) Carcinoma, No Special Type (NST); Invasive Ductal Carcinoma

Q. Discuss the pathology of infiltrating duct carcinoma.

Invasive carcinomas of no special type **constitute** about **70 to 80% of carcinomas. Larger carcinomas may infiltrate the pectoralis muscle and be fixed to the chest wall or invade into the dermis and produce dimpling of the skin.** If the tumor is in the central portion of the breast, it

may cause retraction of the nipple. Rarely, breast cancer may first present as metastasis to an axillary node or distant metastasis before cancer is detected in the breast. In these cases, the primary carcinoma may be small, or masked by dense breast tissue, or may not produce a desmoplastic response. This causes difficulty in detecting them by palpation or mammography.

NC (Show How): Describe and identify the morphologic and microscopic features of carcinoma of the breast.
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

MORPHOLOGY

Q. Write short note on morphology of invasive/infiltrating duct carcinoma of breast.

Gross (Figs. 23.7A and B)

- **Firm to hard in consistency** and have an **irregular outline or border**.
- **Cut section** or scraping, they typically produce a characteristic **grating sound** (similar to cutting a water chestnut). The cut surface also **retracts below the surface**.
- **Dense fibrous stroma.**

Microscopy (Figs. 23.8A and B)

Invasive breast carcinoma are graded according to the Nottingham Histologic Score. Carcinomas are scored depending on (1) tubule formation, (2) nuclear pleomorphism, and (3) mitotic rate. The points are added and carcinomas are graded into grade I, II and III.

- **Grade I (well-differentiated carcinomas):** They show tumor cells forming **prominent tubule.** The tumor cells have small round nuclei, and mitotic figures are rare.
- **Grade II (moderately differentiated carcinomas):** Tumor cells may form **tubules**, but they also show **solid clusters or single infiltrating cells**. The tumor cells show a greater degree of nuclear pleomorphism and also have mitotic figures.
- **Grade III (poorly differentiated carcinomas):** They consist of **nests or solid sheets of cells**. The tumor cells also show enlarged irregular nuclei, numerous mitotic figures and areas of tumor necrosis.

ER-positive, HER2-negative carcinoma: It may show different morphological patterns and grade may range from well to poorly differentiated. Mostly, they are well differentiated carcinomas. Mucinous, papillary, cribriform, and lobular patterns may be seen.

HER2-positive carcinoma: Most of them are poorly differentiated carcinomas and they have no specific morphological pattern. About 50% of apocrine carcinomas and 40% of micropapillary carcinomas belong to this group. It is more often associated with DCIS.

ER-negative, *HER2*-negative carcinomas: They are poorly differentiated and have circumscribed pushing borders with a central fibrotic or necrotic center. Tumors with similar appearance with a prominent lymphocytic infiltrate in the stroma (carcinomas with medullary features) also belong to this group.

Special Histologic Types of Invasive Carcinoma

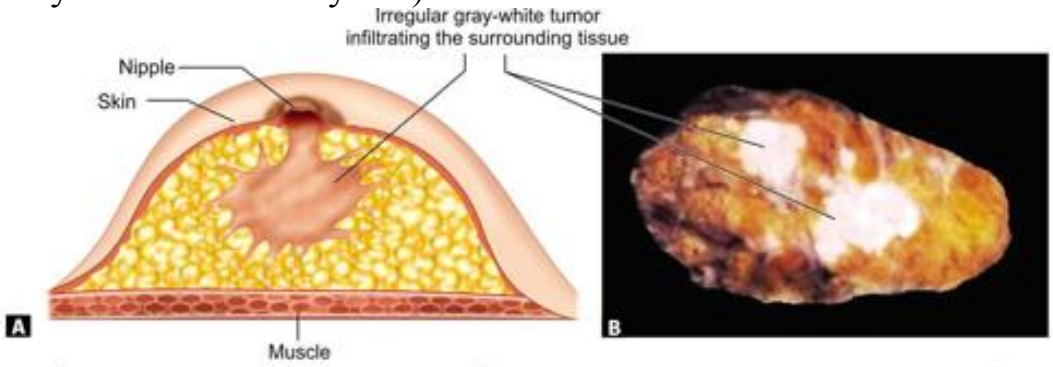
These are invasive carcinoma with distinctive/unique morphological, biologic, genetic and clinical features. Similar to "no special type", of breast cancer, these special tumors can be grouped depending on expression of ER and *HER2*.

Medullary Carcinoma

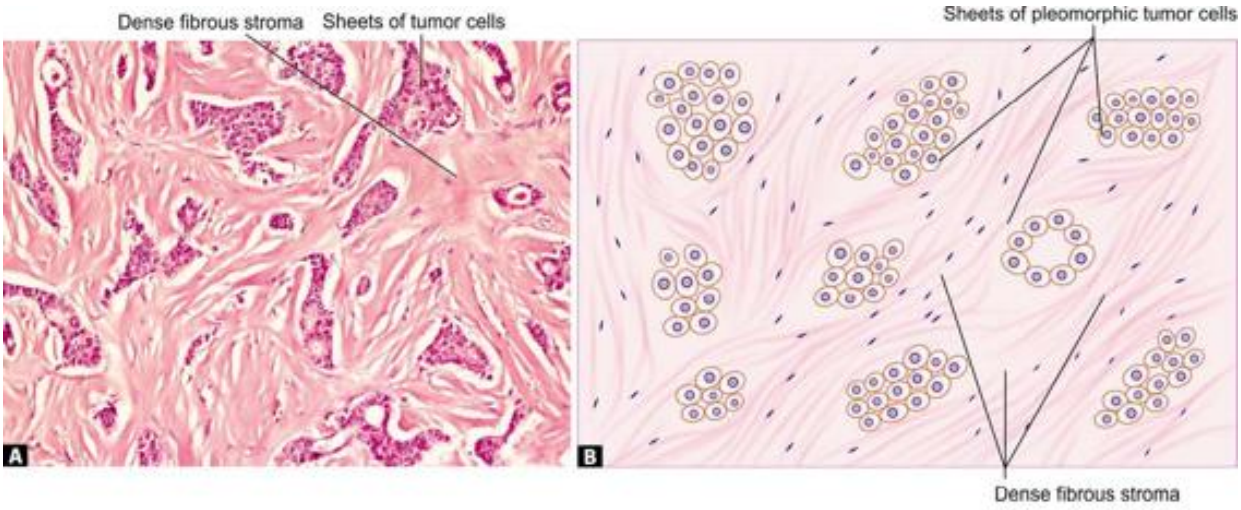
Q. Classify tumors of breast. Discuss lobular and medullary carcinoma of breast.

Q. Write short note on medullary carcinoma of breast.

Carcinoma with medullary features is a special type of triple-negative cancer and form about 5% of all breast cancers. It has no resemblance to medullary carcinoma of thyroid).



Figs. 23.7A and B: Gross appearance of invasive carcinoma of breast. (A) Diagrammatic; (B) Mastectomy specimen with irregular, gray-white tumor.



Figs. 23.8A and B: (A) Hematoxylin and eosin (H & E); and (B) Diagrammatic. Microscopy of invasive carcinoma of breast showing irregular sheets of malignant cells separated by dense fibrous stroma (desmoplasia).

- **Age group:** Most common in the **sixth decade**.
- **Clinical presentation:** Presents as a **well-circumscribed mass** and mimics a benign lesion. It may present as a rapidly growing mass. Lymph node metastases are infrequent.
- Many medullary carcinoma are *BRCA1*-associated carcinomas and in about 67% of cases, hypermethylation of the *BRCA1* promoter, leading to downregulation of *BRCA1* expression is observed. However, germline *BRCA1* mutations is not found in majority of these tumors.

MORPHOLOGY

Q. Write a short note on morphology of medullary carcinoma of breast.

Gross (Fig. 23.9A)

- Medullary carcinoma usually **does not produce desmoplasia**. So, it is more yielding on palpation and cutting than typical breast carcinomas.
- The tumor is **soft, pale gray, fleshy** (medulla is Latin for "marrow").
- **Well circumscribed**.

Microscopy (Fig. 23.9B)

Medullary carcinomas are **poorly differentiated** and its features are:

- **Tumor cells**
  - Arranged in **solid, syncytium-like (cytoplasmic mass containing several nuclei without distinct cell borders) sheets**.

- Tumor cells are **large pleomorphic** with **vesicular, pleomorphic nuclei, and prominent nucleoli.**
- **Mitotic figures** are **frequent.**

- **Stroma: Minimal stroma with** moderate to marked **lymphoplasmacytic infiltrate**.
- **Pushing (noninfiltrative) border**.

The syncytial growth pattern and pushing borders may be due to the **overexpression of adhesion molecules (e.g., E-cadherin)**, which can limit metastatic potential.

Prognosis

- Medullary carcinomas have a **slightly better prognosis** than NST carcinomas probably due to host immune response to tumor antigens (lymphocytes in stroma).
- HER2/neu overexpression is not observed.

Mucinous (Colloid) Carcinoma

- Occur in older women
- Slow growing.

MORPHOLOGY

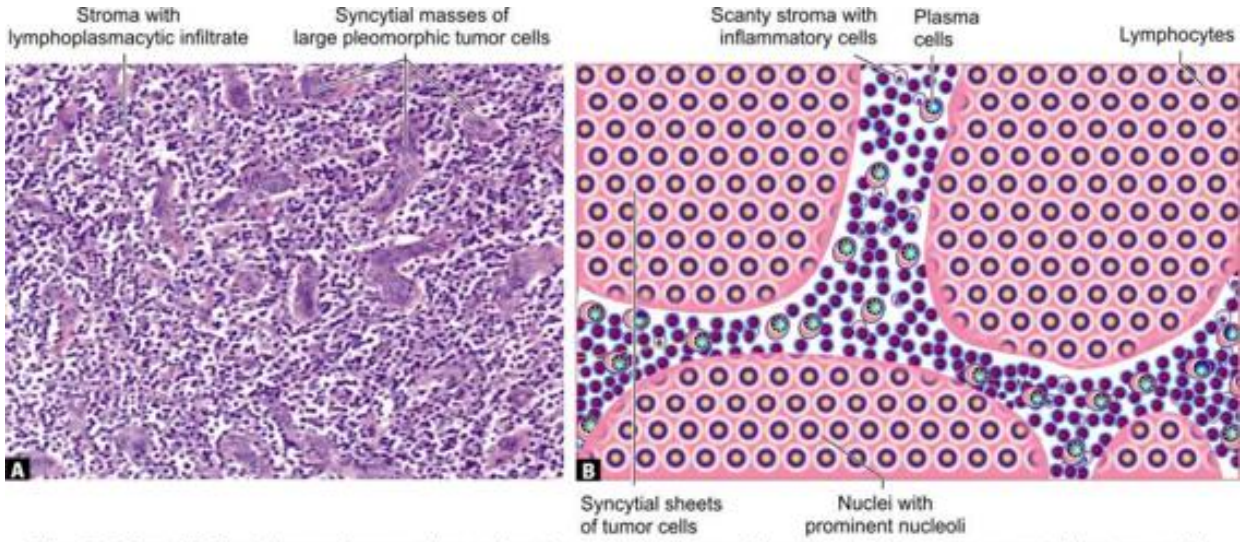
Gross

- Soft or rubbery
- Pale gray-blue gelatinous appearance
- Borders are pushing or circumscribed
- Cut section: Glistening surface and **mucoid consistency**.

Microscopy

- Cuboidal to columnar tumor cells are arranged in clusters and small islands (occasionally forming glands).
- Background shows large amounts of extracellular mucin.

- **Molecular pathology**: They are usually diploid, and ER positive.
- **Lymph node metastases** are **uncommon**.
- **Prognosis**: Better than infiltrating ductal or lobular carcinoma.



Figs. 23.9A and B: Medullary carcinoma of breast showing syncytial masses of large pleomorphic cells separated by stroma with lymphoplasmacytic infiltrate. (A) Photomicrograph; (B) Diagrammatic representation.

Tubular Carcinoma

- Uncommon variant of invasive ductal carcinoma.
- Age group: Late 40s.

MORPHOLOGY

- **Gross:** Smaller than 1 cm in size and irregular.
- **Microscopy:** Very well differentiated and consist of **well-formed, angulated tubules** separated by dense stroma **(Fig. 23.10)**.

- **Metastasis**: Rare.
- **Molecular pathology:** >95% are diploid, **ER positive**, and **HER2/neu negative**.
- **Prognosis:** Excellent.

Invasive Lobular Carcinoma

Q. Write short note on invasive lobular carcinoma of breast.

Most tumors show biallelic loss of expression of *CDH1* (gene encodes E-cadherin). Due to loss of E-cadherin, it has discohesive cells and there is no desmoplastic response.

MORPHOLOGY

Gross

- Present as a palpable mass.
- Greater incidence of **bilaterality**.

Microscopy (Figs. 23.11A and B)

- Shows **loose and non-cohesive (dyscohesive)** infiltrating tumor cells.
- Tumor **cells are smaller and monotonous with oval or round, regular nuclei and small nucleoli**.
- Tumor cells arranged in **single file (Indian file pattern)** or in loose clusters or sheets.
- **Signet-ring cells** containing mucin are common.
- Tubule formation is absent.
- Desmoplasia may be minimal or absent.

Metastasis

Unlike other breast cancers, lobular carcinomas **metastasizes to** the **peritoneum and retroperitoneum**, the **leptomeninges** (carcinoma meningitis), the **gastrointestinal tract**, and the ovaries and uterus.

Clinical Patterns

Inflammatory Carcinoma

MORPHOLOGY

Gross

- Present as **swollen, erythematous** breast due to extensive invasion and obstruction of dermal lymphatics by tumor cells.
- Typically **does not form** a **discrete palpable mass**.
- **Mistaken as an inflammatory condition** and causes delay in diagnosis.
- **Many patients develop metastases at diagnosis and prognosis is poor.**

Carcinoma of breast: Involvement of subdermal lymphatics (permeation and blockage/obstruction) of skin may produce changes in skin that mimic the appearance of an orange skin and termed **peau d' orange**.

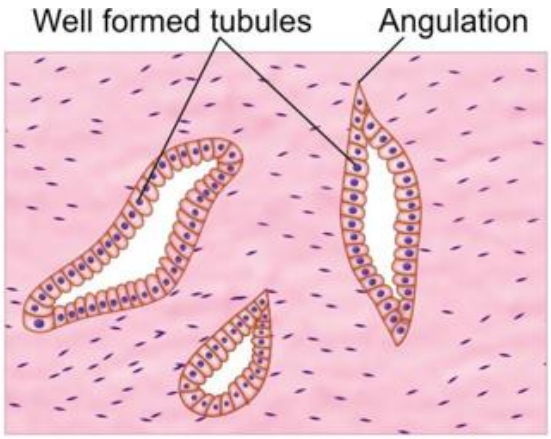
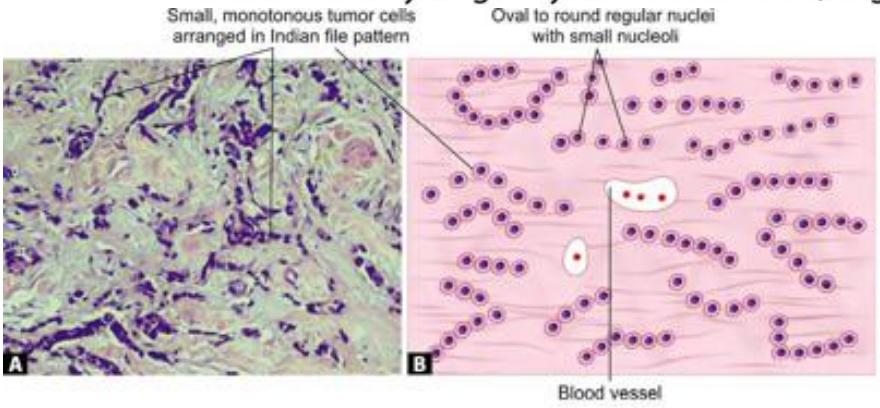


Fig. 23.10: Tubular carcinoma showing well-formed angulated tubules lined by single layer of tumor cells (diagrammatic).



Both lobular carcinoma of breast and signet ring carcinoma of GIT are characterized by the loss of E-cadherin.

Invasive lobular carcinoma:
- Small monotonous tumor cells
- Indian file arrangement
- Cytoplasmic mucoid globules
- Lack of E-cadherin

Figs. 23.11A and B: (A) Photomicrograph and (B) Diagrammatic. Microscopic appearance of lobular carcinoma of breast showing small, monotonous loosely cohesive tumor cells arranged in Indian file pattern.

Invasive carcinoma NST: Firm, irregular and on cut section produce a characteristic grating sound similar to cutting an unripened pear/water chestnut.

Desmoplasia: Marked proliferation of stromal fibroblast in response to invasion by the tumor.

NST: Histological grades
1. Well-differentiated
2. Moderately-differentiated
3. Poorly-differentiated

Medullary carcinoma of breast:
- Well circumscribed
- Syncytial sheets of tumor cells
- Large pleomorphic nuclei
- Minimal stroma infiltrated by lymphocytes and plasma cells

Mucoid carcinoma:
- Elderly
- Slow growing

- Large amount of extracellular mucin
- ER positive
- Lymph node metastasis uncommon
- Better prognosis than NST or lobular carcinoma

Tubular carcinoma of breast:
- Best prognosis
- Distant metastases are rare

Invasive lobular carcinoma:
- Bilateral (more common)
- Multicentric
- Multifocal

Inflammatory carcinoma:
- **Most malignant** type of breast cancer
- Erythematous breast
- Mistaken for inflammatory condition
- Poor prognosis

PAGET DISEASE OF THE NIPPLE

Q. Write short note on Paget disease of breast/nipple.

Paget disease of nipple is a **rare manifestation** (1–4% of cases) **of ductal carcinoma, either in situ or invasive**.

MORPHOLOGY

Q. Write a short note on morphology of Paget disease of breast.

Gross (Fig. 23.12)

- **Skin of the nipple and areola** shows **ulceration** with oozing **resembling eczema.**
- **Underlying ductal carcinoma (in situ or invasive).**

Microscopy (Figs. 23.13A and B)

- **Skin** lesions in the nipple shows **clusters of malignant cells** known as **Paget cells** in the **epidermis** without infiltrating the basement membrane**.** These cells represent extension from underlying ductal carcinoma via the lactiferous sinuses. The tumor cells allow

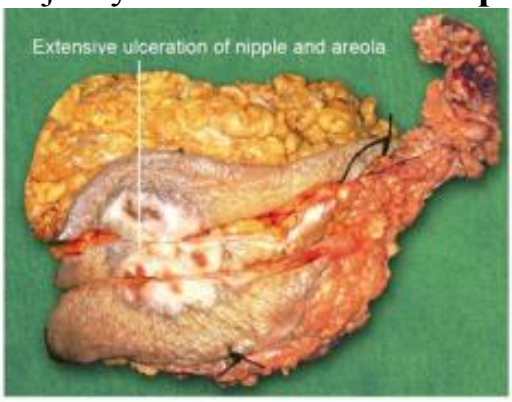
seepage of extracellular fluid onto the nipple surface. The Paget cells can be detected by nipple biopsy or cytologic examination of the exudate.

- **Paget cells** are **large spherical cells** with **clear cytoplasm** and **hyperchromatic nuclei**.

Immunohistochemistry: Underlying carcinoma when present are usually poorly differentiated**, ER negative, and overexpress *HER2*/neu.**

Clinical Presentation

- Presents as a unilateral **erythematous eruption** in the region of nipple and areola with a scale crust.
- **Pruritus** (itching) is common, and the lesion may be **mistaken for eczema**.
- **Palpable mass** in the breast is present in 50–60% of women and shows an **underlying invasive carcinoma**.
- Majority of women **without a palpable mass** have only **DCIS**.



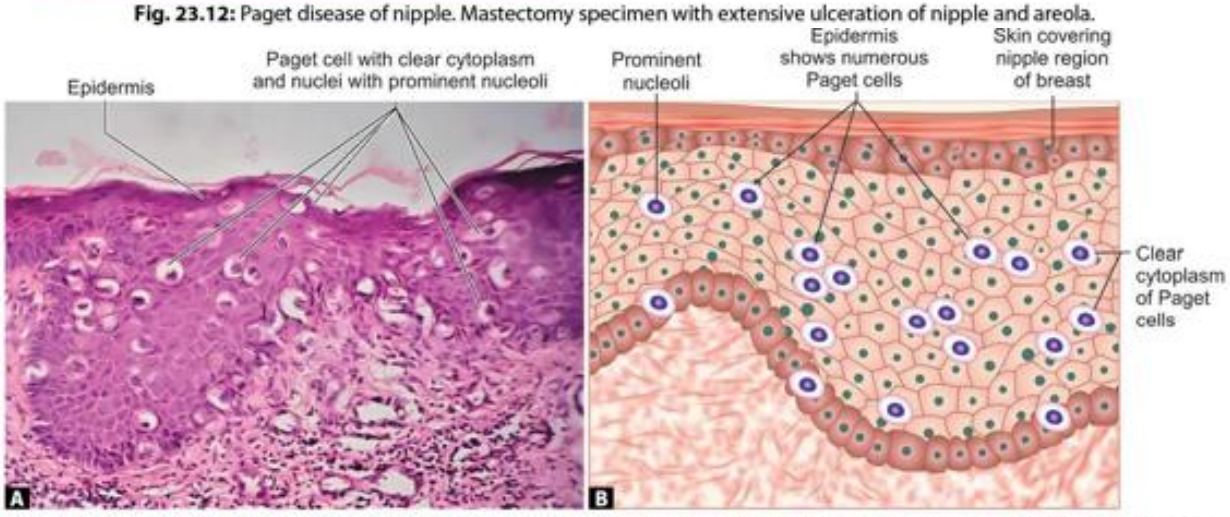
Paget disease of breast: Eczematous lesion of nipple associated with ductal carcinoma (in situ/invasive).

Histologic hallmark of Paget disease of nipple is: Infiltration of the epidermis by malignant (Paget) cells.

Paget disease can also develop in extramammary sites, such as vulva.

Paget disease should not be mistaken for Paget disease of bone.

Fig. 23.12: Paget disease of nipple. Mastectomy specimen with extensive ulceration of nipple and areola.



Figs. 23.13A and B: (A) Photomicrograph and (B) Diagrammatic appearance show microscopic features of Paget disease of breast.

Prognosis: Depends on the features of the underlying ductal carcinoma.

Paget cells: Large spherical with clear cytoplasm and hyperchromatic nuclei. They represent extension from underlying ductal carcinoma via the lactiferous sinuses.

Pattern of lymphatic spread:
- Outer quadrant carcinoma to axillary lymph node
- Inner quadrant carcinoma to internal mammary lymph node

SPREAD OF BREAST CARCINOMA

Q. Write a short answer on spread of breast cancer.

Various routes of spread of breast cancer are mentioned in **Table 23.2**.

TABLE 23.2: Various routes of spread of breast carcinoma.

Direct	*Lymphatics*	*Hematogenous*
• Skin, including nipple and areola • Chest wall	• Axillary lymph node • Internal mammary lymph node • Supraclavicular lymph node	• Lung • Liver • Brain • Bone

Breast carcinoma: Blood spread to lungs and bone.

PROGNOSTIC AND PREDICTIVE FACTORS(TABLE 23.3)

Q. Write short note on prognostic and predictive factors of breast carcinoma.

Prognosis is determined by the the biologic features of the carcinoma (molecular or histologic type) and the extent of cancer spread (stage) at the time of diagnosis. **Two important prognostic factors** are **tumor size and lymph node status**.

TABLE 23.3: Prognostic and predictive factors of breast cancer.

Major factors	*Minor factors*
Lymph node metastases	Histologic subtype
Tumor size	Histological grade

TABLE 23.3: Prognostic and predictive factors of breast cancer.	
Major factors	***Minor factors***
In situ versus invasive carcinoma	Estrogen and progesterone receptors (ER & PR)
Distant metastases	*HER2*/neu
Locally advanced disease	Lymphovascular invasion
Inflammatory carcinoma	Proliferative rate
	DNA content
	Response to neoadjuvant therapy
	Gene expression profiling

Prognostic factors can be divided into two groups:

1. Those related to the extent of carcinoma (stage) and
2. Those related to the underlying biology of the cancer.

Prognostic Factors Related to Extent of Carcinoma

1. **In situ versus invasive carcinoma:** Majority of **adequately treated DICS** are **cured**. About 50% of invasive carcinomas have metastases at the time of diagnosis.
2. **Distant metastases:** Cure is unlikely.
3. **Lymph node metastases**: **Status of the axillary lymph node** is the **most important prognostic factor** in the absence of distant metastases.
   - **Number and level involved** is directly related to the survival rate.
   - Lymphatic vessels in most breast carcinomas drain first to one or two lymph nodes, which are known as **sentinel nodes**. These are **usually examined for metastasis.**
   - Depending on the size of metastatic foci, they can be divided into:
     - **Macrometastases** means metastasis greater than 0.2 cm.
     - **Micrometastases** means metastasis 0.2 cm or less.
   - Till recently, status of lymph node was considered as a major determinant of treatment choice. After the advent of molecular typing of carcinoma, the information obtained from nodal status is becoming less important. In the future, many women need not undergo sampling of nodes.

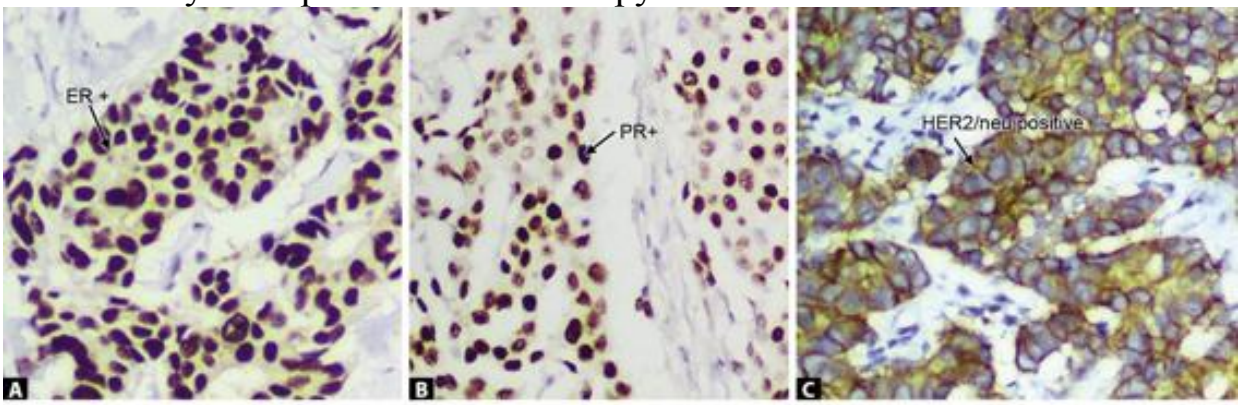
4. **Tumor size:** The risk of metastases to axillary lymph node increases with the size of the primary tumor. However, both are independent prognostic factors. Carcinoma of **less than 1 cm** in size without lymph node metastasis **have a 10-year survival rate of over 90%,** which drops to 77% for cancers more than 2 cm. Size is less important for *HER2*-positive and ER-negative carcinomas. Because they can metastasize even when tumor is quite small.
5. **Locally advanced disease:** Invasion into skin or skeletal muscle has a bad prognosis.
6. **Inflammatory carcinoma:** This is characterized by breast erythema and skin thickening. The edematous skin is tethered to the breast by Cooper ligaments and appears like the surface of an orange peel (peau d'orange). These signs are due to the filling of dermal lymphatics with metastatic carcinoma producing blockage of lymphatic drainage. It has poor prognosis. More than 60% of these cancers are ER-negative and 40–50% overexpress *HER2*.
7. **Lymphovascular invasion:** In about 50% of invasive carcinomas, tumor cells are found within vascular spaces (either lymphatics or small capillaries). This is strongly associated with lymph node metastases. It is a poor prognostic factor in cancers without lymph node metastases and is a risk factor for local recurrence. Severe plugging of the lymphovascular spaces of the dermis with carcinoma cells (inflammatory carcinoma) also has a very poor prognosis.

Prognostic Factors Related to the Underlying Biology of the Cancer

1. **Molecular subtype:** It is determined by immunohistochemistry for the expression of ER and HER2 and proliferation. It is an important prognostic factor.
2. **Special histologic type**: Survival rate for special types of invasive carcinomas (tubular, mucinous, medullary, lobular, and papillary) is greater than NST cancers.
3. **Histological grade: Nottingham histologic score** (also referred to as **Scarff–Bloom–Richardson**) is the **most commonly used grading**

system, which classify invasive carcinomas into three groups (grade 1 to grade 3). These grades are highly correlated with survival. This grading system is **based on: (1) tubule formation, (2) nuclear grade and (3) mitotic rate.**

4. **Proliferative rate:** It can be measured by counting mitotic figures during histological grading or by immunohistochemical detection of proteins that are specifically expressed by actively dividing cells (e.g., cyclins, Ki-67). Proliferative rate is mainly important for ER-positive, *HER2*-negative carcinomas. Most of the ER-negative and/or *HER2*-positive carcinomas have high proliferative rates. Carcinomas with high proliferation rates have a poorer prognosis, but they may respond better to chemotherapy. It is because rapidly growing cancer cells are more sensitive to agents that damage DNA or otherwise interfere with cell division.
5. **Estrogen (ER) and progesterone receptors (PR):**
   - **ER and PR positive (Figs. 23.14A and B)**: These carcinomas **respond to hormonal manipulation**. ER-positive cancers are less likely to respond to chemotherapy.



Figs. 23.14A to C: Immunohistochemistry in breast carcinoma: (A) ER positive; (B) PR positive and (C) HER2/neu positive.

   - **ER and PR negative:** These carcinomas are more likely to respond to chemotherapy.
6. **HER2/neu:** Its **overexpression (Fig. 23.14C)** is associated with **poorer survival**. However, they are predictor of response to agents that target this receptor.
7. **RNA expression profiling:** It is a new method used for subclassifying cancers. For breast cancers, many of the genes that predict prognosis are involved in proliferation. These assays are able to identify patients with

slow-growing, antiestrogen-responsive cancers who can be spared the toxicity of chemotherapy.

Without adequate surgery, the majority of patients die with extensive local disease producing ulceration of the overlying skin. Carcinoma en cuirasse (literally "carcinoma of the breastplate") is a complication that should be prevented, even in patient with distant metastasis.

Q. A 49-year-old woman came to surgical outpatient department for a lump in her left breast noticed 1 month back. On physical examination, a 2.5 cm, firm, irregular mass was palpable in the upper outer quadrant of left breast. There are no overlying skin lesions. Two axillary lymph nodes were palpable. FNAC confirmed the clinical diagnosis and left mastectomy was done.

(a) What is the probable diagnosis? (Ans: Carcinoma of breast)

(b) Discuss the etiopathogenesis of this disease (refer pages 773-5).

(c) Mention the classification of these lesions in breast (refer page 776).

(d) Describe the morphological features of this disease (refer page 780).

Sentinel node: Initial node draining the cancer.

Most common site of metastasis from carcinoma of breast is: Bone (lumbar vertebra>femur> thoracic vertebra> rib> skull).

Triple assessment in breast cancer
1. Clinical examination
2. Radiological examination (mammography)
3. FNAC

Breast cancer diagnosis: FNAC is a very useful, rapid, simple and economical procedure.

STROMAL/FIBROEPITHELIAL TUMORS

Two tumors that arise from intralobular stroma are **fibroadenoma and phyllodes tumor.**

Fibroadenoma

Q. Write short note on fibroadenoma of breast.

- **Most common benign tumor** of the female breast.
- **Age group**: Mostly occur in females between **20 to 30 years.**
- **Origin:** Arise from intralobular stroma.
- **Clinical presentation:** Young women usually present with a **palpable and freely movable mass**.

MORPHOLOGY

Q. Write a short note on morphology of fibroadenoma of breast.

Gross (Fig. 23.15)

- Fibroadenomas can be single or multiple and unilateral or bilateral.
- **Spherical nodules** and are usually **well-circumscribed** and freely movable. The tumor can compress the surrounding breast tissue, but is not fixed. This accounts for its mobility on clinical examination → known as **'breast mouse'**.
- **Cut section**: It appears as **rubbery, glistening, grayish-white** nodules that bulge above the surrounding tissue and often contain **slit-like spaces.**
- **Size:** Varies, usually **1–4 cm** in diameter.

Microscopy (Figs. 23.16A to D)

Q. Write a short answer on microscopy of fibroadenoma of breast.

- Composed of a **mixture of duct-like structures** and **fibrous connective tissue**.
- **Duct-like structures**:
  - Ducts may be either simple and **round or elongated** and **branching**.



Fig. 23.15: Fibroadenoma of breast showing a well-circumscribe tumor and cut surface grayish-white in color.

- Epithelium lining the ducts ranges from the **double layer of epithelium** of normal lobules to varying degrees of hyperplasia.

- **Fibrous connective tissue stroma**:
  - Constitutes most of the tumor
  - Stroma is **delicate, cellular**, often myxoid and resembles normal intralobular stroma.
- **Classification**: According to the microscopic appearance:
  - **Pericanalicular (Figs. 23.16A and B):** In this type, regular round or oval glandular configuration of the glands is maintained. The epithelium forms ducts with patent lumen, because the surrounding stroma proliferates circumferentially around them.
  - **Intracanalicular (Figs. 23.16C and D):** It is a misnomer in which the connective tissue invaginates into the glandular spaces, so that it appears to be within them. The proliferated ducts are compressed and distorted by, fibrous tissue reducing them to form curvilinear slits.

  Usually both patterns co-exist.

- **Hormonal-induced changes:** Similar to normal breast, fibroadenomas can undergo hormonally-induced changes.
  - **Epithelial changes**: During pregnancy, fibroadenomas may grow rapidly in size; the glands may **increase in size** due to lactational changes.
  - **Stromal changes**: In older women (after menopause), the **stroma may become more fibrous** and **densely hyalinized**. It may also calcify and form large, lobulated ("popcorn") calcifications.

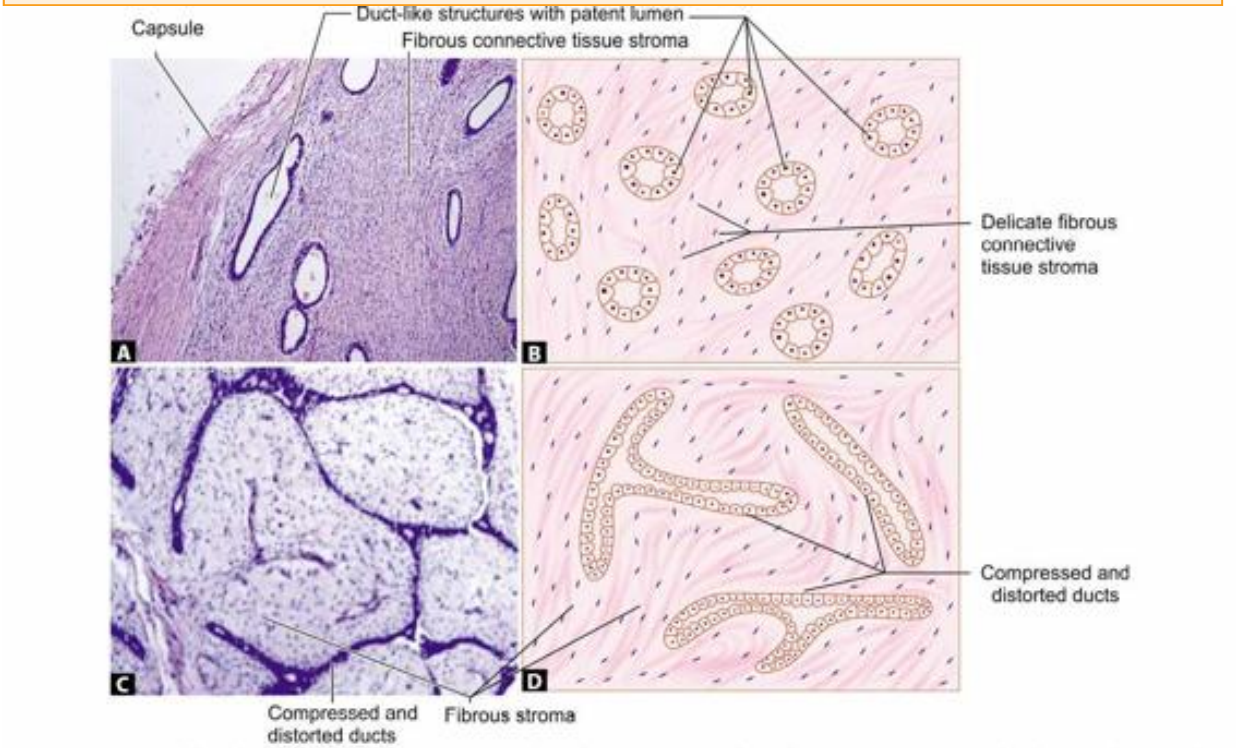
Morphologic Variants of Fibroadenoma

- **Tubular adenoma:** Well-circumscribed tumor composed of closely packed tubules with very scanty stroma.
- **Lactating adenoma:** If an adenoma is composed of acini with secretory activity, it is seen during pregnancy or lactation.
- **Juvenile fibroadenoma:** It is an uncommon variant of fibroadenoma that is larger (over 10 cm), and rapidly growing mass seen in adolescent girls. Microscopically, they are similar to fibroadenoma and does not recur after excision.

Phyllodes Tumor

Q. Write short note on phyllodes tumor.

Definition: Phyllodes tumor is a group of circumscribed **biphasic neoplasm** characterized by a double layered epithelial component arranged in clefts surrounded by a **hypercellular mesenchymal component** typically organized in leaf-like structures.



Figs. 23.16A to D: Fibroadenoma of breast. (A) Hematoxylin and eosin (H & E); and (B) (diagrammatic) Pericanalicular type showing ducts with patent lumen, surrounded by delicate stroma. The border (left) shows sharp demarcation; (C) (H and E); and (D) (diagrammatic) Intracanalicular type composed of slit-like compressed ducts surrounded by fibrous tissue.

- **Origin:** Arises from **intralobular stroma** (like fibroadenomas).
- **Terminology:** Originally known as cystosarcoma phyllodes to imply its malignant behavior. Since, majority of these tumors behaved in a benign fashion, and most are not cystic, the **term phyllodes tumor is preferred**.
- **Age group:** Mostly occur **between 30 and 70 years of age**, with a peak in the fifth decade.
- **Clinical presentation:** Majority are detected as palpable masses.

MORPHOLOGY

Q. Write a short answer on morphology of phyllodes.

Gross

- **Benign phyllodes tumor:** It is **round, sharply circumscribed**.
- **Malignant phyllodes**: It is usually **poorly circumscribed and locally invasive with infiltrative borders**.
- **Cut surface**: Solid, **firm, glistening, gray-white bulging mass.** It shows characteristic whorled pattern with **curved cleft-like spaces** that **resemble the leaf-buds** (**phyllodes** is Greek for "leaflike") Leaf-like appearance is due to the epithelium which covers the nodules of proliferating stroma.
- **Size**: Vary in size with an average size of about **5 cm in diameter**.

Microscopy (Figs. 23.17A and B)

- **Growth pattern:** Typically show **exaggerated intracanalicular growth pattern with leaf-like projections into the dilated lumens**.
- **Two key features: (**1) presence of benign epithelial elements and (2) stromal hypercellularity.
  - **Benign epithelial component:** It consists of **luminal epithelial and myoepithelial cells**. They cover large club-like (bulbous)/leaf-like projections (nodules) of proliferating stroma. In some tumors, these bulbous protrusions push or extend into a cystic space (hence the term cysto).
  - **Stromal hypercellularity:** It is the amount and appearance of the stromal component that determines biological nature of neoplasm.

- **Grading:** Depending on the appearance of the stromal component, phyllodes tumors are divided into (1) low-grade (benign) and (2) high-grade (malignant) phyllodes.
  - **Low-grade (benign) phyllodes:** It resembles fibroadenomas, but the stroma has following additional features:
    - **More cellular** (hypercellular) and resemble fibroblasts.
    - Contain **mitotic figures**.
  - **High-grade (malignant) phyllodes**:
    - **Hypercellular stroma**
    - **Abundant mitotic activity**
    - **Marked pleomorphism of stromal cells** like sarcomas (e.g., malignant fibrous histiocytoma, chondrosarcoma, rhabdomyosarcoma).
    - Majority of high-grade lesions show amplification of EGFR.

Recurrence

- Phyllodes tumors are likely to recur if not excised with wide margins.
- Low-grade tumors may recur locally but rarely metastasize.
- High-grade lesions frequently recur and may also develop hematogenous metastases. Metastatic deposits contain only the stromal component.

Fibroadenoma: Most common breast tumor below 40 years of age.

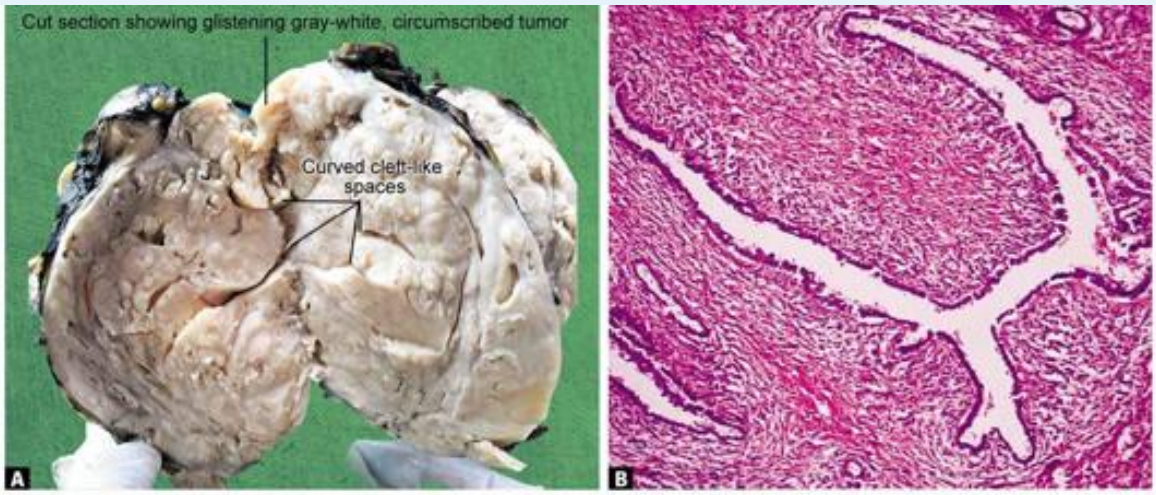
Fibroadenoma: Benign tumor derived from intralobular stroma.

Fibroadenoma: Mixture of duct-like structures separated by delicate fibrous connective tissue.

Fibroadenoma: (1) Intracanalicular or (2) Pericanalicular type

Phyllodes tumor: Grade depends on stromal cellularity.

Phyllodes tumor: Cut surface shows characteristic whorled pattern with curved cleft-like spaces that resemble the leaf-buds.



Figs. 23.17A and B: (A) Gross appearance of phyllodes tumor; (B) Low-grade phyllodes tumor. Compared to a fibroadenoma, there is exaggerated intracanalicular growth pattern, increased stromal cellularity and overgrowth giving rise to the typical leaf-like architecture.

MALE BREAST

Male breast consists of the nipple and a rudimentary duct system without lobule formation.

Gynecomastia

NC: Enumerate and describe the etiology, hormonal dependency and pathogenesis of gynecomastia.

Q. Write short note on gynecomastia.

Definition: Gynecomastia (enlargement of the male breast) is defined as the **enlargement of the male breast** due to **hypertrophy and hyperplasia of both glandular and stromal components**.

Etiology and Pathogenesis

Male breast is subjected to hormonal influences similar to the female breast. Gynecomastia may occur due to an **imbalance between estrogens** (which stimulate breast tissue), **and androgens** (which counteract effects of estrogens).

- Gynecomastia before 25 years of age is usually due to hormonal changes during puberty.
- Gynecomastia during later years (any time during adult life) maybe due to:
  - **Hyperestrinism: Cirrhosis** and hormonally active tumors (Leydig cell tumor of testis, hCG-secreting germ cell tumors, lung carcinoma, or others).
  - Drugs: Examples include, alcohol, marijuana, heroin, antiretroviral therapy, anabolic steroids (used by some athletes and body builders), digitalis, reserpine, phenytoin, and some psychoactive agents.
  - Klinefelter syndrome (XXY karyotype).
  - Idiopathic.

MORPHOLOGY

Gross

Well-circumscribed, oval, disk-shaped mass of elastic consistency.

Microscopy (Fig. 23.18)

- Ducts are lined by multilayered **columnar to cuboidal epithelium** with regular nuclei. The lining shows marked micropapillary epithelial hyperplasia.
- The ducts are surrounded by a **dense collagenous connective tissue stroma**.

Clinical Features

- Unilateral or bilateral.
- Usually centered below the nipple as a button-like subareolar enlargement, an important point in contrast to carcinoma, which tends to be located eccentrically.

Advanced cases, it can simulate the adolescent female breast.

Gynecomastia: Enlargement of the male breast due to hypertrophy and hyperplasia of both glandular and stromal components.



Fig. 23.18: Microscopic appearance of gynecomastia showing ducts are lined by a multilayered cuboidal epithelium surrounded by hyalinized fibrous tissue.

CHAPTER
24

Endocrine Disorders

INTRODUCTION TO ENDOCRINE SYSTEM

The endocrine system consists of various glands located throughout the body **(Box 24.1)**.

BOX 24.1: Various endocrine glands.

- Pituitary gland
- Pineal gland
- Thyroid gland
- Parathyroid gland
- Thymus
- Adrenal gland
- Pancreas
- Ovary/testis

Endocrine glands synthesize and release special chemical messengers called **hormones**. Apart from endocrine glands, numerous hormones are synthesized and released from endocrine cells present in the upper and lower gastrointestinal tract (i.e., incretins). The endocrine system has five general functions.

THYROID GLAND

THYROIDITIS

Thyroiditis is a group of disorders characterized by **inflammation of the thyroid gland (Box 24.2)**.

BOX 24.2: Various types of thyroiditis.

1. Infectious thyroiditis
   - Bacterial including mycobacterial
   - Fungal
2. Hashimoto (chronic lymphocytic) thyroiditis
3. Granulomatous (subacute/de Quervain) thyroiditis
4. Reidel's thyroiditis

Hashimoto Thyroiditis

- Hashimoto (**chronic lymphocytic**) thyroiditis is an **autoimmune disease** → gradual failure of thyroid function.
- **Age:** Peak between **45–65 years** of age.
- **Sex: More common in women** than in men. Female to male ratio 10:1 to 20:1.

Etiology

Q. Describe the pathogenesis of Hashimoto thyroiditis.

Genetic Factor

- Hashimoto thyroiditis has a **strong genetic component** and is supported by the:
  - Concordance of disease in monozygotic twins (40%)
  - Presence of circulating antithyroid antibodies in asymptomatic siblings of Hashimoto patients
  - Association with other autoimmune diseases.
- **Genetic susceptibility:** Autoimmune disease such as Hashimoto and Graves' disease are associated with **polymorphisms** in **genes associated with immune regulation** of T-cell responses such as cytotoxic T lymphocyte-associated antigen-4 *(CTLA4)* gene and *protein tyrosine phosphatase-22 (PTPN22)*.

Pathogenesis (Fig. 24.1)

Hashimoto thyroiditis is **autoimmune disease** caused by a breakdown in self-tolerance to thyroid autoantigens. It is characterized by the presence of

circulating **autoantibodies against thyroglobulin and thyroid peroxidase**.

- **Failure of self-tolerance** to thyroid autoantigens is the initial event. Probably, it is abnormalities of regulatory T-cells (Tregs), or exposure of normally sequestered thyroid antigens.
- **Induction of thyroid autoimmunity:** It is the next event producing circulating autoantibodies against thyroid antigens.
- **Progressive destruction of thyrocytes (thyroid epithelial cells):** It is accompanied by replacement of the thyroid parenchyma by mononuclear cell infiltration and fibrosis.

Mechanism of Thyrocyte Death

Immunologic mechanisms causing death of thyrocytes in the thyroid follicle may be brought out by:

1. **T-cell-mediated cell death: $CD8^+$ cytotoxic T**-cells are main mediators responsible for thyrocyte destruction (type IV hypersensitivity).
2. **Cytokine-mediated cell death: Proliferation of $CD4^+$ T-cells** (T_H1 cell) produces inflammatory **cytokines such as interferon-γ** in the thyroid gland. These cytokines recruit and **activate macrophages** → damages thyrocyte.

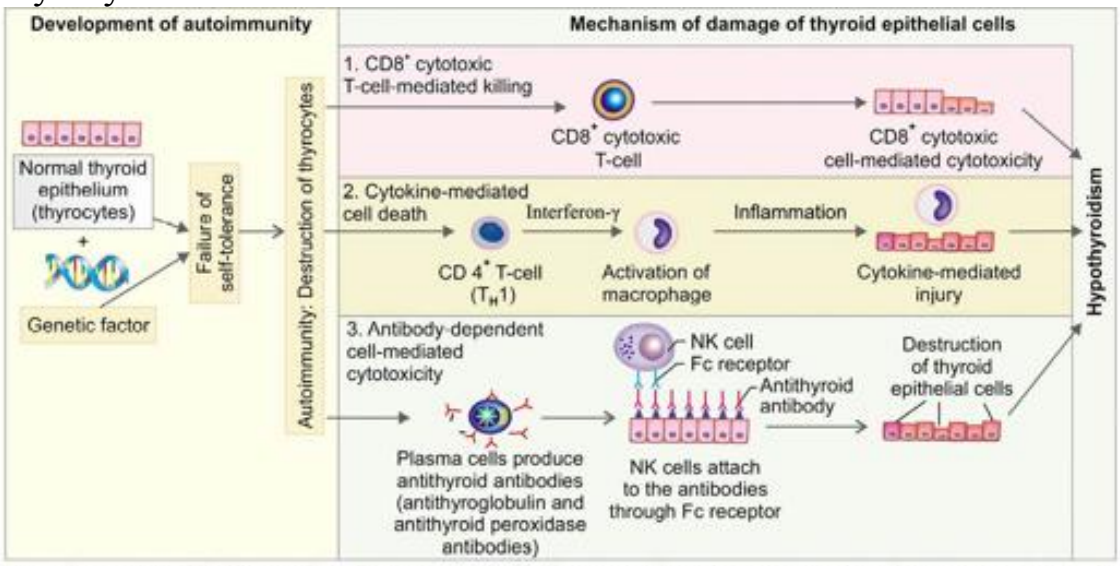


Fig. 24.1: Pathogenesis of Hashimoto thyroiditis. Failure of self-tolerance to thyroid autoantigens, results in progressive autoimmune destruction of thyroid epithelial cells by infiltrating cytotoxic T-cells, through cytokine or by antibody-dependent cell-mediated cytotoxicity.

3. **Antibody-dependent cell-mediated cytotoxicity: Antithyroid antibodies** (antithyroglobulin and antithyroid peroxidase antibodies) **bind to the antigens on the thyrocyte**. **NK cells** bind to these

antibodies through Fc receptor causing **antibody-dependent cell-mediated cytotoxicity** (type II hypersensitivity).

MORPHOLOGY

Q. Describe the morphological changes in Hashimoto thyroiditis.

Gross (Fig. 24.2)

- **Diffuse and symmetric** enlargement of thyroid gland.
- Gland is **firm and nodular**.
- **Capsule** is **intact**, and the thyroid gland is well-demarcated from adjacent structures.
- **Cut surface** is **pale, gray-tan** and shows **accentuation of normal lobulation**.

Microscopy (Figs. 24.3A and B)

Q. Write a answer on Hürthle cell.

1. **Inflammation:**
   - **Dense mononuclear inflammatory infiltrate** consisting of small lymphocytes and plasma cells in the thyroid parenchyma.
   - **Lymphoid follicles** with well-developed **germinal centers**.
2. **Epithelial changes:**
   - **Atrophy of thyroid follicles:** They appear smaller than normal follicles.
   - **Hürthle cell metaplasia: It** is a **metaplastic response** of the follicular epithelium **to injury**. **Hürthle cells (Askanazy/oxyphil cells or oncocytes)** have abundant **eosinophilic, granular cytoplasm** and line some of the follicles. **Ultrastructurally**, they have **prominent mitochondria**.
3. **Fibrosis:** The **interstitial connective tissue** is **increased (fibrosis)** and may cause atrophy of thyroid follicles. In contrast to Reidel thyroiditis, the **fibrosis does not extend beyond the capsule** of the gland.

Fine-needle aspiration cytology (FNAC): It shows Hürthle cells with a heterogeneous population of lymphocytes.

Clinical Course

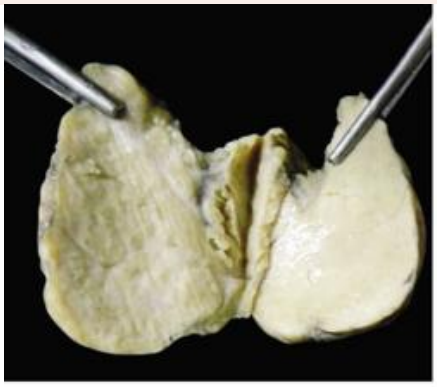
- **Painless enlargement of the thyroid** in middle-aged woman
- **Hypothyroidism** gradually develops
- Early stages may produce transient thyrotoxicosis due to destruction of thyroid follicles, with secondary release of thyroid hormones

(Hashitoxicosis).

Hashimoto (chronic lymphocytic) thyroiditis: Most common cause of hypothyroidism in regions with adequate iodine levels.

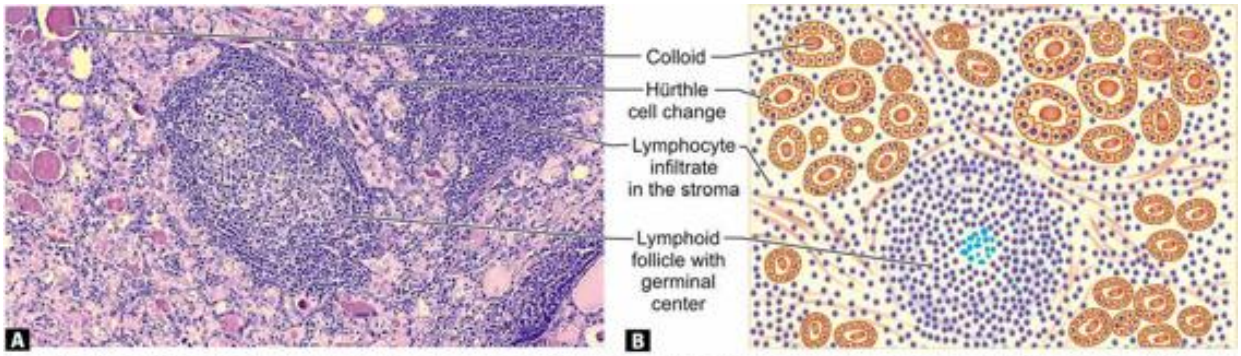
Hashimoto thyroiditis: First reported in 1912 by Hashimoto; goiter and intense lymphocytic infiltration of the thyroid (struma lymphomatosa).

Hashimoto thyroiditis: Autoimmune disease and autoantibodies are against (i) thyroglobulin and (ii) thyroid peroxidase



Risk in Hashimoto thyroiditis:
Development of:
- Other autoimmune diseases
- B-cell non-Hodgkin lymphomas (e.g. MALT lymphomas)
- Papillary carcinomas.

Fig. 24.2: Gross appearance of Hashimoto thyroiditis. Cut section shows enlargement of thyroid, pale gray-tan color and accentuation of normal lobulation.



Figs. 24.3A and B: (A) Photomicrograph; (B) Diagrammatic. Microscopy of Hashimoto thyroiditis shows parenchyma densely infiltrated by lymphocytes with germinal centers. Some of the thyroid follicles lined by deeply eosinophilic Hürthle cells.

Hashimoto thyroiditis—mechanism of damage:
- T-cell mediated cytotoxicity (type IV hypersensitivity)
- Cytokine-mediated cytotoxicity
- Antibody dependent cell-mediated cytotoxicity (type II hypersensitivity)

Hashimoto thyroiditis:
- Destruction of thyroid parenchyma
- Hürthle cell metaplasia
- Lymphoplasmacytic infiltrate

Hürthle cell metaplasia:

- Metaplastic response to injury
- Abundant eosinophilic, granular cytoplasm
- Prominent mitochondria

Hashimoto thyroiditis: Fibrosis does not extend beyond the capsule of the gland.

Subacute (Granulomatous) Thyroiditis

- Subacute (de Quervain) thyroiditis is **less common** than Hashimoto disease
- **Age:** Common between **40 and 50 years** of age
- **Sex: Affects women** more often than men (4:1).

Etiology and Pathogenesis

Subacute thyroiditis is thought to be **initiated by a viral infection**. Points in favor are:

- Majority have an upper respiratory infection just before the onset of thyroiditis
- Seasonal variation with peak occurrence in the summer
- Association with virus infection (e.g., coxsackie virus, mumps, measles, adenovirus).

Pathogenesis

- Pathogenesis is not known
- Probably viral infection exposes a viral or thyroid antigen, which is released secondary to virus-induced host tissue damage
- This antigen stimulates cytotoxic T lymphocytes, which damage thyroid follicular cells
- The immune response is limited and does not progress.

MORPHOLOGY

Gross

- **Unilateral or bilateral enlargement** of the thyroid gland
- **Cut section:** The involved regions are **firm and yellow-white.**

Microscopy

1. **Damaged thyroid follicles** with escape of colloid.
2. **Inflammation:** It is elicited by the escaped colloid seen surrounding the damaged follicles. Consists of aggregates of **lymphocytes, activated macrophages, and plasma cells**.
3. **Granulomatous reaction**: It develops as lesion progresses and shows **multinucleate giant cells surrounding** pools or fragments of **colloid**. Hence, the designation **granulomatous thyroiditis**.
4. **Fibrosis:** It develops at late stages replacing the destroyed area of the gland.

Clinical Course

- **Painful** enlargement of the thyroid.
- **Early phase: Hyperthyroidism** with high serum T_4 and T_3 levels and low serum TSH levels
- **Recovery within 6–8 weeks** and thyroid function returns to normal.

Subacute thyroiditis: Granulomatous reaction against colloid escaped from damaged follicles.

Riedel Thyroiditis

- Less common form of thyroiditis.
- **Etiology: It is unknown**, but the presence of circulating antithyroid antibodies in most patients **suggests** an **autoimmune** etiology.

MORPHOLOGY

- **Gross: Thyroid is stony hard and fixed** which **clinically simulates a thyroid carcinoma**.
- **Microscopy:** Shows **extensive fibrosis** involving the **thyroid and contiguous neck structures**.

Reidel's thyroditis: Fibrous tissue replacement of gland and surrounding tissue.

THYROTOXICOSIS

CC: Describe the etiology, pathogenesis, manifestations, laboratory and imaging features and course of thyrotoxicosis.

Q. Write short essay on thyrotoxicosis.

Definition: Thyrotoxicosis is a **systemic syndrome** (with hypermetabolic state) caused by exposure to **excessive levels of thyroid hormone** (free T_3 and T_4).

Causes of Thyrotoxicosis (Box 24.3)

BOX 24.3: Causes of thyrotoxicosis.

Associated with hyperthyroidism

Primary hyperthyroidism
- Graves' disease (diffuse toxic hyperplasia)
- Toxic multinodular goiter
- Toxic adenoma

Secondary hyperthyroidism
- TSH-secreting pituitary adenoma (rare)

Not associated with hyperthyroidism
- Granulomatous (de Quervain) thyroiditis
- Struma ovarii (ovarian teratoma with ectopic thyroid)
- Factitious thyrotoxicosis (exogenous thyroxine intake)

Thyrotoxicosis Associated with Hyperthyroidism

It is the **clinical consequence due to the excessive circulating thyroid hormone** (**excessive thyroid function**/hyperfunction). It is the **most common cause of thyrotoxicosis**.

Causes of Hyperthyroidism

Excessive synthesis and secretion of thyroid hormone may be primary disorder of thyroid or secondary to other disorders.

- **Primary hyperthyroidism:** It is due to an intrinsic thyroid abnormality.
  - **Abnormal thyroid stimulation:** Diffuse hyperplasia of the thyroid associated with **Graves' disease**.
  - **Intrinsic disease of the thyroid gland**.
    - Toxic multinodular goiter
    - Toxic adenoma of the thyroid.
- **Secondary hyperthyroidism:** It is due to processes arising outside of the thyroid, such as increased TSH-secreting **pituitary adenoma** (rare).

Thyrotoxicosis not Associated with Hyperthyroidism

Excessive release of preformed thyroid hormone from **thyroid** (e.g., in thyroiditis) or an **extrathyroidal source** (e.g., struma ovarii).

Clinical Manifestations of Hyperthyroidism

- Due to the **hypermetabolic state** produced because of excess of thyroid hormone and due to **overactivity of the sympathetic nervous system** (i.e., an increase in the β-adrenergic "tone"). Excessive thyroid hormone results in an **increase in the basal metabolic rate**.
- **Skin: Soft, warm and moist**. **Heat intolerance** and **sweating**.
- **Cardiac manifestations: Increased cardiac output, tachycardia, palpitations, arrhythmias** and **cardiomegaly**.
- **Neuromuscular system: Fine tremor, hyperactivity, nervousness, anxiety, emotional liability,** inability to concentrate, and insomnia.
- **Ocular changes: Lid retraction** → causes a staring appearance. However, **Graves' disease is associated with proptosis** that comprises Graves' ophthalmopathy.
- **Gastrointestinal system: Increased stool frequency**, often with diarrhea.
- **Skeletal system: Osteopenia** and a small increase in fracture rate.
- **Thyroid storm:** It is the **sudden onset of severe hyperthyroidism**. It occurs **most commonly in Graves' disease** and **probably due to sudden elevation in catecholamine levels**.

Diagnosis of Hyperthyroidism

Clinical findings

Laboratory findings:

- **Serum TSH concentration:** It is the **most useful single screening test** for hyperthyroidism, because it is **decreased even at subclinical stage**.
- **Free T_4**: It is **increased**.
- Measurement of **radioactive iodine uptake** by the thyroid gland to determine the cause. For example, diffusely increased uptake in the whole gland is seen in Graves' disease, increased uptake in a solitary nodule in toxic adenoma, or decreased uptake in thyroiditis.

Thyrotoxicosis: Excessive levels of thyroid hormone from any cause.

Hyperthyroidism: Thyrotoxicosis due to excessive synthesis of thyroid hormone. Serum TSH is the most useful screening test.

GRAVES' DISEASE

Graves' disease (also known as Basedow disease) is the **most common cause of hyperthyroidism**.

Triad of Clinical Findings

1. **Hyperthyroidism:** It is due to diffuse hyperplasia of the thyroid.
2. **Infiltrative ophthalmopathy** → results in exophthalmos **(Fig. 24.4)**.
3. Localized, infiltrative **dermopathy** (pretibial myxedema) in few patients.

Age: Peak between **20 and 40 years** of age.
Sex: Females are affected 10 times **more frequently** than males.

Etiology

Graves' disease (hyperthyroidism) **and Hashimoto thyroiditis** (hypothyroidism) are considered as **two extremes of autoimmune thyroid disorders**.

Genetic Factor

- Graves' disease has a **strong genetic component** and is supported by the following observations:
  - Concordance of disease in **monozygotic twins** (60%).
  - **Presence of** circulating **antithyroid antibodies** in asymptomatic siblings of Graves' disease.
  - **Association with other autoimmune diseases**.
- Genetic susceptibility for Graves' disease has been associated with polymorphisms in **genes associated with immune regulation**, e.g., cytotoxic T lymphocyte–associated antigen-4 *(CTLA4)* gene and protein tyrosine phosphatase-22 *(PTPN22)*.

Pathogenesis (Fig. 24.5)

Q. Describe the pathogenesis of Graves' disease.

Graves' disease is an **autoimmune disease** characterized by the **presence of multiple autoantibodies most importantly against** the **TSH receptor**.

- **Failure of self-tolerance** to thyroid autoantigens is the initial event
- **Production of multiple autoantibodies**.

Autoantibodies in Graves' Disease

1. **Thyroid-stimulating immunoglobulin:** It is an immunoglobulin (Ig) G antibody, which binds to the TSH receptor on the plasma membrane of thyrocytes.
   - **Characteristics:** (1) Almost **all patients** show this autoantibody and (2) It is **specific** for Graves' disease.
   - **Action:** They act as agonists and stimulate the TSH receptor and **mimics the action of TSH → increases** the secretion and release of **thyroid hormones** (*refer* **Fig. 6.6**).
2. **Thyroid growth-stimulating immunoglobulin:** It is also directed **against the TSH receptor.**

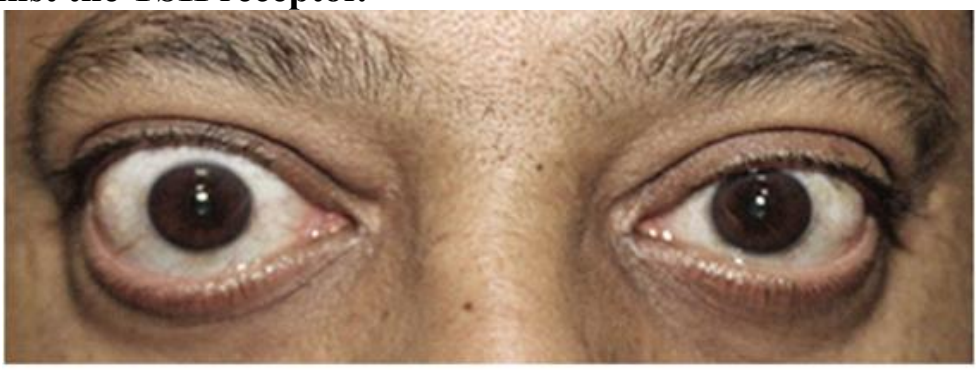


Fig. 24.4: Exophthalmos.

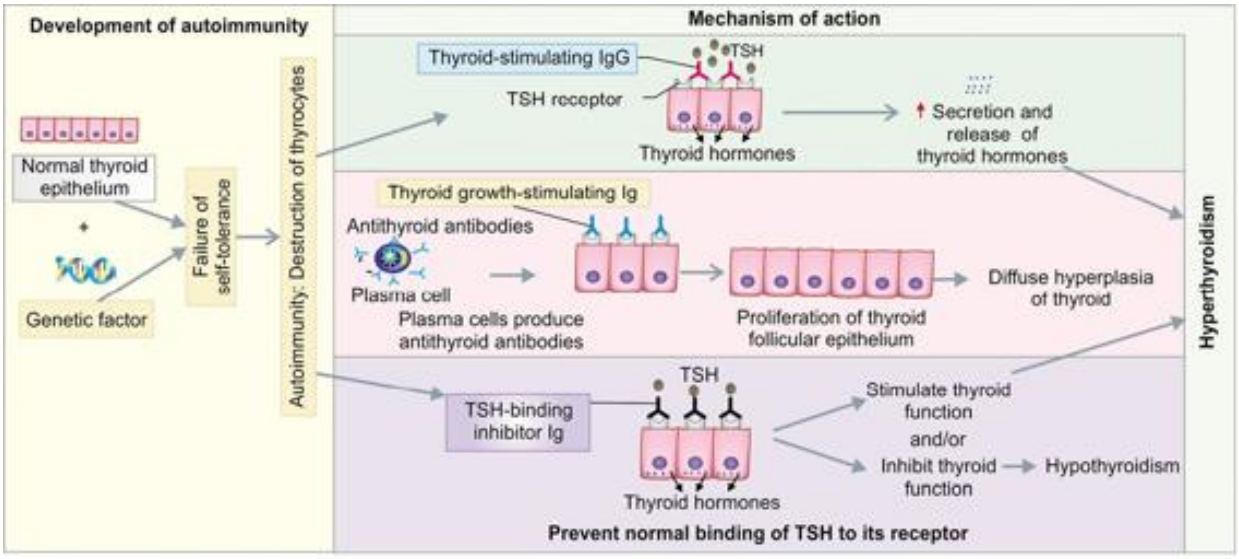
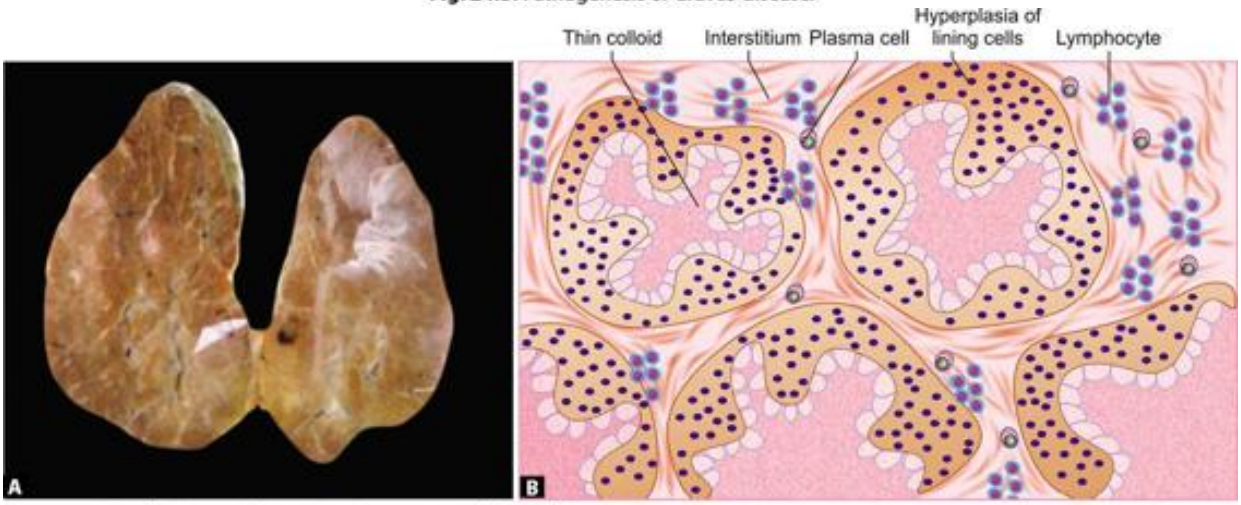


Fig. 24.5: Pathogenesis of Graves' disease.



Figs. 24.6A and B: Graves' disease: (A) Cut section of thyroid showing meaty appearance; (B) Microscopic view (diagrammatic) showing follicles lined by tall, columnar epithelium that are crowed and project into the lumens of the follicles. The follicle contains pale colloid with scalloped appearance of the edges. Stroma shows lymphocytes and plasma cells.

Action: Causes **proliferation of thyroid follicular epithelium → diffuse hyperplasia** of the thyroid gland.

3. **TSH-binding inhibitor immunoglobulin (anti-TSH receptor antibody):** It **prevents normal binding of TSH to its receptor** on thyroid epithelial cells.

   Action: Varies.

   - **Stimulation:** Some forms **mimic** the action of **TSH** → stimulate thyroid epithelial cell causing hyperthyroidism (*refer* pages 151-2 and **Fig. 6.6**).

- **Inhibition:** Some forms may **inhibit** thyroid cell function → hypothyroidism.
- **Coexistence** of **stimulating and inhibiting immunoglobulins:** This action in the same patient → responsible for episodes of hypothyroidism in some patients with Graves' disease.

Infiltrative Ophthalmopathy(Figs. 24.6A and B)

Autoimmunity is responsible for infiltrative ophthalmopathy.

MORPHOLOGY

Q. Describe the morphological changes in Graves' disease.

Gross

- Thyroid gland is **symmetrically enlarged** due to **diffuse hypertrophy and hyperplasia** of thyroid follicular epithelial cells.
- **Weight** of the gland is **increased**.
- **Cut section**: The parenchyma appears **soft and meaty resembling normal muscle (Fig. 24.6A).**

Microscopy (Fig. 24.6B)

A. Changes in Thyroid

- **Thyroid follicles:**
  - **Crowding of epithelial cells:** Epithelial cells lining the thyroid follicles are **tall and more crowded** than normal gland.
  - **Small papillae without fibrovascular cores:** They are formed **due to crowding of epithelial cells** (in contrastthe papillary carcinoma has papillae with fibrovascular core).
  - The **papillae project into the lumen** of the follicles and **encroach on the colloid**.
- **Colloid:** It is **pale with scalloped margins.**
- **Lymphocyte infiltration in the interstitium:** It is seen throughout the gland, along **with mature plasma cells.** These lymphoid aggregates commonly show **germinal centers**.

B. Changes in Extrathyroidal Tissue

- Generalized lymphoid hyperplasia
- **Myocardial hypertrophy** and ischemic changes
- **Ophthalmopathy** (*refer* clinical features)
- **Dermopathy** (*refer* clinical features)

Clinical Features

- **Thyrotoxicosis:** Its degree varies.
- **Unique features:**
  - **Diffuse hyperplasia of the thyroid:** It is seen in all cases and causes enlargement of thyroid.
  - **Ophthalmopathy:** The ophthalmopathy causes **abnormal protrusion of the eyeball (exophthalmos)**. Sympathetic overactivity may produce a characteristic **wide**, **staring gaze and lid lag**. Infiltrative ophthalmopathy is characterized by increase in the volume of the retro-orbital connective tissues and extraocular muscles.
  - **Infiltrative dermopathy** or **pretibial myxedema:** It is present in a minority of patients. This is most commonly seen in the skin overlying the **shins**. It presents as **scaly thickening and induration** of the dermis due to deposition of glycosaminoglycans and infiltration by lymphocytes.

Laboratory Findings in Graves' Disease

- **Elevated free T_4 and T_3 levels**
- **Decreased TSH levels**
- **Radioactive iodine uptake is increased**, and **radioiodine scans show a diffuse uptake of iodine.** This is because of continuous stimulation of the thyroid follicles by thyroid-stimulating immunoglobulins.

Graves' disease: Most common cause of endogenous hyperthyroidism and thyrotoxicosis.

Graves' disease: First reported by Graves as "violent and long continued palpitations in females" associated with enlargement of the thyroid gland.

Triad of Graves' disease:
1. Hyperthyroidism
2. Exophthalmos

3. Pretibial myxedema

Graves' disease: Autoimmune disease caused by autoantibodies most importantly against the TSH receptor. Anti-TSH receptor antibody-type II hypersensitivity reaction.

Autoantibodies in Graves' disease:
1. Thyroid-stimulating Ig
2. Thyroid growth-stimulating Ig
3. TSH-binding inhibitor Ig (anti-TSH receptor antibodies)

Graves' disease: Thyroid is symmetrically enlarged because of diffuse hypertrophy and hyperplasia.

Thyroid storm: Tachyarrhythmias, hyperpyrexia, shock and coma.

Graves' disease has increased risk for other autoimmune diseases:
- Systemic lupus erythematosus
- Pernicious anemia
- Type 1 diabetes
- Addison disease

Radioactive iodine uptake: Evaluates synthetic capacity of thyroid.

TSH levels: Best screening test for thyroid dysfunction.

Increased radioactive iodine uptake: Increased thyroid hormone synthesis; Graves' disease.

Laboratory findings in Graves' disease: Elevated free T_4 and T_3 levels and decreased TSH levels.

HYPOTHYROIDISM

CC: Describe the etiology, pathogenesis, manifestations, laboratory and imaging features and course of hypothyroidism.

Definition: Hypothyroidism is the clinical manifestations due to reduced secretion of thyroid hormone (thyroid hormone deficiency).

Etiology and Pathogenesis

Hypothyroidism is caused by reduced thyroid hormone production either due to structural or functional derangements in the thyroid. Hypothyroidism is a fairly common disorder. It is more common in women

than in men. It can be produced due to a defect anywhere in the hypothalamic-pituitary-thyroid axis.

Types of hypothyroidism (Box 24.4): It may be divided into

1. **Primary hypothyroidism** (>90%): Due to intrinsic abnormality in the thyroid gland itself. There may be enlargement of the thyroid gland (goiter).
2. **Secondary hypothyroidism:** Due to pituitary and hypothalamic disease.

BOX 24.4: Types and causes of hypothyroidism.

Primary

- Autoimmune hypothyroidism: Hashimoto thyroiditis*
- Iatrogenic
  - Post-ablative (removal/excision/destruction): Surgery, radioiodine therapy, or external irradiation
  - Adverse effect of drugs*: Lithium, iodides, amiodarone, p-aminosalicylic acid sulfonamides, and phenylbutazone
- Iodine deficiency*: Common cause in infants and children
- Genetic defects:
  - Congenital biosynthetic defect (dyshormonogenetic goiter) (rare)*
  - Defect in thyroid development (thyroid dysgenesis) (rare)

Secondary (Central)

- Pituitary failure (rare)
- Hypothalamic failure (rare)

*Associated with enlargement of thyroid ("goitrous hypothyroidism").

Cretinism

Definition: It is hypothyroidism that develops in infancy or early childhood.

Etiology

- **Endemic cretinism:** Cretinism formerly was fairly common in areas where there was endemic dietary iodine deficiency [e.g., mountainous areas such as the Himalayas and the Andes (endemic cretinism)]. It is now less frequent due to the widespread supplementation of salt with iodine.
- **Sporadic cretinism:** It is caused due to enzyme defects that interfere with thyroid hormone synthesis.

- **Genetic defects:** Rarely cretinism may be due to genetic defects that interfere with the biosynthesis of thyroid hormone (e.g., dyshormonogenetic goiter).

Clinical Features

In cretinism there is impaired development of the skeletal system and central nervous system. It manifests as **severe mental retardation, short stature, coarse facial features, a protruding tongue, and umbilical hernia**.

Nervous system: Hypothyroidism in pregnancy produces neurologic consequences for the fetus. Normally, maternal hormones namely T3 and T4 cross the placenta. They are important to the development of brain in the fetus. The severity of the **mental impairment** depends on the time at which fetus develop thyroid deficiency in utero.

If there is deficiency of the thyroid hormone in the pregnant women before the development of the fetal thyroid gland, there will be severe mental retardation in the fetus. By contrast, if reduction of thyroid hormones occurs later in pregnancy, after the fetal thyroid has developed, the fetal brain may develop normally.

Myxedema

Definition: It is hypothyroidism that develops in older children and adults.

Clinical Features

Symptoms of hypothyroidism are due to decreased circulating thyroid hormone. In older children with myxedema, the signs and symptoms are intermediate between those of the cretin and those of the adult with hypothyroidism. In the adult, the symptoms of myxedema develop insidiously.

Initial symptoms: Generalized fatigue (tiredness), apathy, and mental sluggishness, which may mimic depression. They are listless, and frequently overweight.

Skin: Cutaneous signs are almost common. Connective tissue matrix substances (glycosaminoglycans and hyaluronic acid) accumulate in the extracellular matrix in the skin and subcutaneous tissue. They bind water and

produce a peculiar form of nonpitting edema called **myxedema**. Myxedematous patients have **broadening and coarsening of facial features** (boggy facies, puffy eyelids), **edema of the hands and feet, enlargement of the tongue, and deepening of the voice** (due to thickening of laryngeal mucous membranes). The skin is cool and pale because of decreased blood flow due to cutaneous vasoconstriction. The skin is also dry, coarse, cold intolerant with decreased sweating. Ecchymoses may develop due to increased capillary fragility. Skin wounds heal slowly.

Nervous system: Hypothyroid adults are **lethargic, show memory loss and slowed mental processes.** Psychiatric symptoms are common and severe agitation termed as **myxedema madness**, may develop. Other symptoms include sensory defects (deafness and night blindness), cerebellar ataxia and slow tendon reflexes.

Heart: Reduced cardiac output produces shortness of breath and decreased exercise capacity. Coronary atherosclerosis is common due to increase in total cholesterol and low-density lipoprotein (LDL) levels.

Gastrointestinal (GI) tract: Decreased sympathetic activity results in constipation.

Laboratory Findings in Hypothyroidism

Diagnosis of hypothyroidism depends on laboratory evaluation because of the nonspecific nature of symptoms.

- **Decreased serum T_3/T_4** in patients with hypothyroidism of any origin
- **Increased serum TSH** is observed **in primary hypothyroidism.** It is the most sensitive screening test. TSH is not increased with hypothyroidism caused by primary hypothalamic or pituitary disease.
- **Hypercholesterolemia** caused by decreased synthesis of LDL receptors [type II hyperlipoproteinemia (HLP)].

DIFFUSE AND MULTINODULAR GOITERS

CC: Enumerate, classify and describe the etiology, pathogenesis, pathology and iodine dependency of thyroid swellings.

Definition: Goiter is defined as **enlargement of thyroid without hyperthyroidism.**

- It is the **most common manifestation of thyroid disease**.
- Two morphological forms of goiter are: (1) diffuse nontoxic goiter and (2) multinodular goiter.

Diffuse Nontoxic (Simple) Goiter

Q. Write a short note on colloid goiter.

- Diffuse nontoxic (simple) goiter is characterized by the **diffuse enlargement of the thyroid gland without any nodularity.**
- Microscopically, it consists of large thyroid follicles distended with colloid → also known as **colloid goiter.**

Etiology

Q. Write short note on goitrogens.

Q. Describe the etiopathogenesis of multinodular goiter.

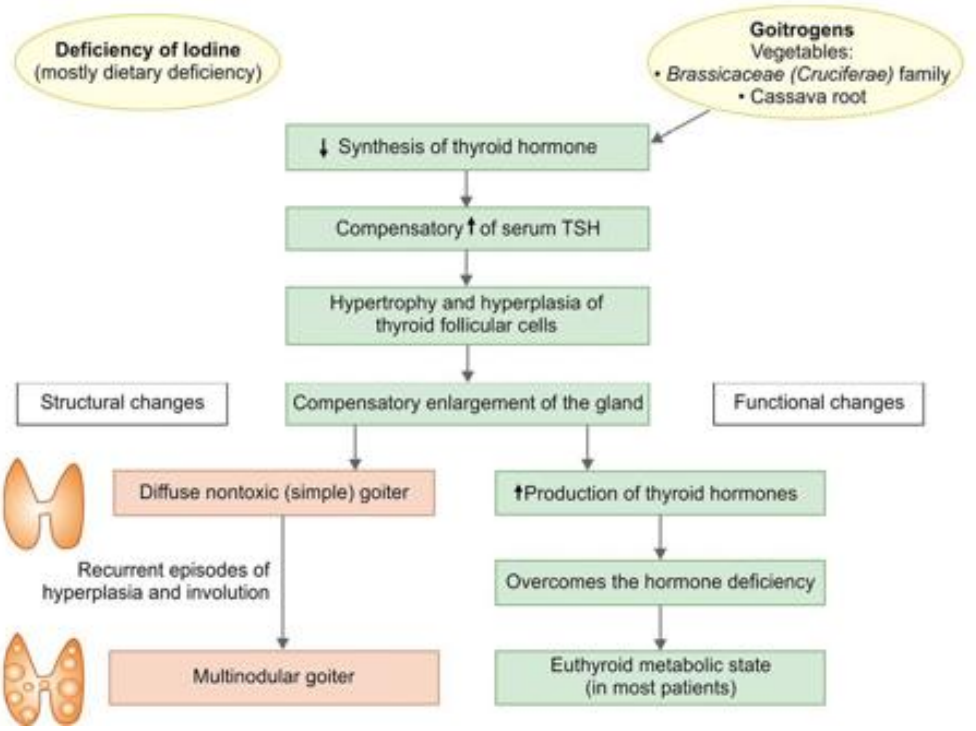
Types: (A) endemic and (B) sporadic.

A. Endemic Goiter

This term is used **when goiters are present** in **more than 10% of the population in a given region.** The causes are:

- **Deficiency of iodine:** This may be due to **low iodine** in the soil, water, and food:

Flowchart 24.1: Sequences of events in the development of goiter.



- Common in mountainous areas (e.g., Himalayas) where there is widespread deficiency of iodine.
- Dietary supplementation of iodine has reduced the frequency and severity of endemic goiter.
- **Consequences of iodine deficiency (Flowchart 24.1): Decreased synthesis of thyroid hormone** → causes compensatory **increase in TSH** → leads to **follicular cell hypertrophy and hyperplasia** and goitrous enlargement.

- **Goitrogens:** These are **substances, ingestion of which interferes with thyroid hormone synthesis**. Goitrogenic substances include vegetables which belong to:
  - ***Brassicaceae (Cruciferae)* family** (e.g., cabbage, cauliflower, Brussels sprouts, and turnips).
  - **Cassava root:** It contains a thiocyanate that inhibits iodide transport within the thyroid. Consumption of this may worsen the concurrent iodine deficiency.

B. Sporadic Goiter

- **Less frequent** than endemic goiter
- **Age:** Puberty or in young adult life

- **Sex: Female preponderance**.
- **Causes:**
  - **Hereditary enzymatic defects** that interfere with thyroid hormone synthesis. Transmitted as autosomal-recessive conditions (e.g., dyshormonogenetic goiter).
  - **Ingestion of substances** that interfere with thyroid hormone synthesis.
  - **Unknown cause**: In most cases of sporadic goiter the cause is not known.

Sequences of events in the development of goiter are shown in **Figure 24.7**.

MORPHOLOGY

Diffuse nontoxic goiter has **two phases**:

1. Hyperplastic Phase

- **Gross:** Thyroid is **moderately, diffusely, and symmetrically enlarged**. The gland rarely exceeds 100–150 g.
- **Microscopy:**
  - **Hyperplasia of lining epithelium** of thyroid follicles. The epithelium consists of crowded columnar cells, which may pile up to form **pseudopapillae** (Sanderson's Polster) and project into the follicular lumen.
  - **Colloid content varies** throughout the gland. Some follicles are distended with colloid, whereas others are small with minimal colloid.

2. Phase of Colloid Involution

- Subsequently, if the dietary content of iodine increases or if the demand for thyroid hormone decreases, the stimulated hyperplastic phase goes into phase of colloid involution.
- **Gross:** Thyroid is **enlarged** and the **cut surface** is usually **brown, glassy, and translucent.**
- **Microscopy:**
  - **Flattened follicular epithelial lining**
  - **Abundant colloid** causes enlargement of follicle **(colloid goiter).**

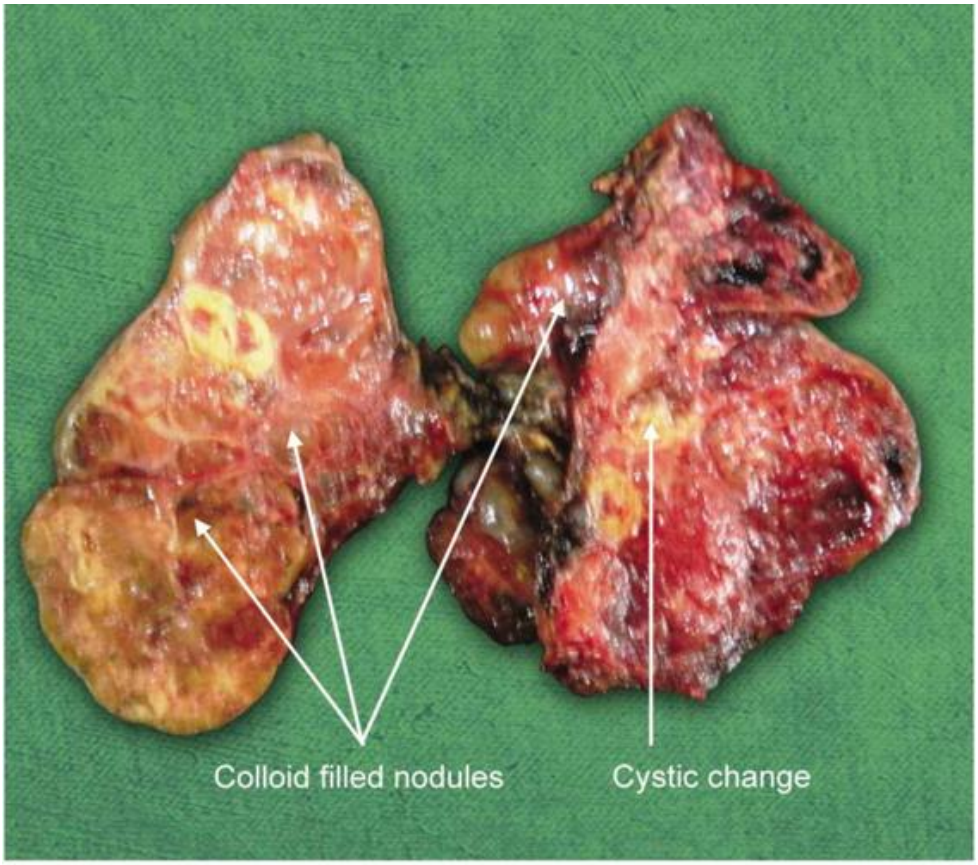


Fig. 24.7: Gross appearance of multinodular goiter showing nodular gland, containing colloid filled nodules, areas of fibrosis and cystic change.

Clinical Course

- **Majority** with simple goiters are **clinically euthyroid**
- **Mass effects** from the enlarged thyroid gland
- In children, dyshormonogenetic goiter due to congenital biosynthetic defect may produce cretinism.

Laboratory Findings

- Serum T_3 and T_4 levels are normal
- **Serum TSH is usually elevated** or at the upper range of normal.

Multinodular Goiter

- In long-standing simple goiters (diffuse and symmetric enlargement), **recurrent episodes of hyperplasia and involution** combine to produce a

more **irregular enlargement of the thyroid** known as multinodular goiters.

- Since, multinodular goiters are derived from simple goiter, they occur in both **sporadic and endemic** forms.

Etiology (*Refer* etiology of diffuse nontoxic goiter, pages 797-8).

Evolution of Multinodular Goiter

Q. Write a short note on stages in evolution of multinodular goiter.

- In long-standing simple goiters, due to **variations among follicular cells** in their **response to external stimuli** (such as trophic hormones), multiple nodules are formed.
- **Uneven follicular hyperplasia**:
  - **Some** cells in a **follicle** contain **clones of proliferating cells**, which may become **autonomous and proliferate** without the external stimulus → **produces new follicles**.
  - **Some follicles** may **accumulate colloid without proliferation** of epithelium → **uneven accumulation of colloid**.
- **Consequences: Produces physical stress** on both follicle and surrounding blood vessels.
  - **Rupture of follicles** may result in **colloid cysts**.
  - **Rupture of vessels** may produce **hemorrhage**s followed by **scarring**, and sometimes **calcifications**. With scarring, **multiple nodules** appear and these nodules show **varying microscopic appearance**.

MORPHOLOGY

Q. Write a short note on morphology of multinodular goiter.

Gross (Fig. 24.7)

- **Multiple nodules:** Thyroid is **asymmetrically enlarged**, and **nodular** due to **multiple nodules of varying sizes**.
- **Pattern of enlargement:** Varies

- **One lobe of thyroid may be more involved** than the other. It may produce pressure on midline structures, such as the trachea and esophagus.
- **One nodule may** become so **prominent** and appear as a **solitary nodule.**
- Goiter may **grow behind the sternum and clavicles** to produce **intrathoracic** or **plunging goiter**.

- **Weight of thyroid:** It is **increased** and varies.
- **Cut section**:
  - Shows **numerous irregular nodules containing variable amounts of colloid**.
  - **When the nodules contain large amounts of colloid**, they appear soft, **glistening, and reddish-brown**, due to gelatinous colloid.
  - **Older lesions** may show areas of **hemorrhage, fibrosis, calcification**, and **cystic change**.

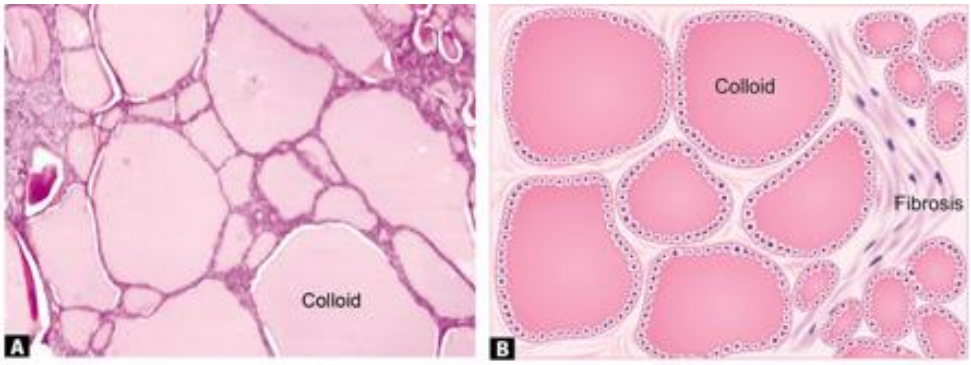
Microscopy (Figs. 24.8A and B)

Multiple **nodules of varying size and shape:**

- **Nodules:** They consist of **follicles of varying sizes distended with colloid** and lined by **flat to cuboidal inactive epithelium**.
- **Colloid cysts:** They may be formed by **fusion (or rupture)** of large **colloid-containing follicles**.
- **Follicular hyperplasia:** Some nodules may show follicular hyperplasia to produce **pseudopapillae** that project into the follicular lumen. These may contain either **minimal or no colloid** and **may be mistaken for follicular adenoma**. In contrast to follicular adenoma, no prominent capsule is seen between the hyperplastic nodules and residual compressed thyroid parenchyma **(Table 24.1)**.

Stroma:

- **Fibrosis and dystrophic calcification**
- **Areas of hemorrhage** and **chronic inflammation** are common. Presence of **hemosiderin deposits and cholesterol granulomas** indicate old hemorrhage.



Figs. 24.8A and B: (A) Photomicrograph; (B) Diagrammatic. Microscopy of a multinodular goiter composed of thyroid follicles of varying sizes distended with variable amount of colloid.

Clinical Course

- **Usually asymptomatic** and present as a **mass in the neck**.
- **Large** multinodular goiter may cause **compression of surrounding structures** and may cause:
  - **Airway obstruction** due to compression of trachea.
  - **Dysphagia** by compressing the esophagus.
  - **Venous congestion of the head and face** (superior vena cava syndrome) due to *c*ompression of large vessels in the neck and upper thorax.
  - **Hoarseness** from recurrent laryngeal nerve compression.
- **Functional status:**
  - **Most patients are euthyroid** and T_4, T_3, and TSH are normal.
  - Some may have subclinical hyperthyroidism with reduced TSH levels.
  - Few may develop hyperthyroidism (toxic multinodular goiter) and this condition is known as **Plummer syndrome**.
- Fine-needle aspiration biopsy is helpful for diagnosis.

Goiter: Enlargement of thyroid gland.

Nontoxic goiter: Absolute or relative deficiency of thyroid hormone. Hyperplasia/hypertrophy → diffuse enlargement of thyroid gland.

Goiter: Endemic or sporadic

Endemic goiter: Goitrogens include vegetables of cruciferae family (cabbage, cauliflower, Brussels sprouts, turnips) and cassava.

Diffuse nontoxic goiter: Two phases
1. Hyperplastic phase
2. Phase of colloid involution

Multinodulr goiter: Multiple nodules of varying sizes. Can produce marked enlargements of thyroid and may be mistaken for neoplasm.

Plunging goiter is: Retrosternal goiter.

Toxic multinodular goiter: One or more nodules become TSH independent.

Exophthalmos and pretibial myxedema are not seen in toxic multinodular goiter.

Plummer syndrome
- Multinodular goiter

- Hyperthyrodism

NEOPLASMS OF THE THYROID

Tumors of the thyroid are usually benign. Usually clinical criteria provide clues to the nature of a thyroid nodule **(Table 24.1)**.

TABLE 24.1: Clinical criteria that generally provide clues to the nature of a thyroid nodule.

More likely to be neoplastic	***More likely to be benign***
• *Solitary nodules* than multiple nodules • *Nodules in younger patients* than are those in older patients • *Nodules in males* than in females • A history of *radiation* treatment to the head and neck region	Functional nodules that take up radioactive iodine in imaging studies *(hot nodules)*

Classification of Thyroid Neoplasms (Box 24.5)

Q. Classify thyroid tumors.

Box 24.5 is shows the classification of thyroid neoplasms (abridged).

Follicular Adenomas

Q. Write short note on follicular adenoma of thyroid.

Adenomas of the thyroid consist of follicular epithelium.

Pathogenesis

Nonfunctional Follicular Adenoma

- **Majority** of adenomas **does not produce thyroid hormones**.

BOX 24.5: Classification of thyroid neoplasms (abridged).

Benign
- Follicular adenoma
- Hyalinizing trabecular adenoma
- Hürthle cell adenoma

Malignant
- Papillary thyroid carcinoma (PTC)

  - Papillary carcinoma.
  - Follicular variant of PTC
  - Encapsulated variant of PTC
  - Papillary microcarcinoma
  - Columnar cell variant of PTC
  - Oncocytic variant of PTC
- Follicular thyroid carcinoma (FTC)
- Hürthle cell carcinoma
- Poorly differentiated carcinoma
- Anaplastic thyroid carcinoma
- Medullary thyroid carcinomas

- **Genetic factors: Less than 20%** of nonfunctioning follicular adenomas patients have any one of the following genetic alterations:
  - **Mutations of *RAS*** (signal transduction protein)
  - **Phosphotidylinositol-3-kinase subunit (*PIK3CA*)**
  - ***PAX8-PPARG*** fusion gene.

All these are genetic alterations also seen in follicular carcinomas.

Functional Follicular Adenoma

- **They produce thyroid hormones (toxic adenomas)** and cause **thyrotoxicosis**.
- **Somatic mutations of TSH receptor signaling pathway:** It has been found in toxic adenomas and in toxic multinodular goiter → leads **secretion of thyroid hormone** by follicular cells independent of TSH stimulation (thyroid autonomy).

MORPHOLOGY

Gross (Fig. 24.9A)

- Follicular adenoma is a **solitary** (single)**, spherical, solid** and **encapsulated** tumor.

- **Size:** It ranges from **1–3 cm in diameter**.
- **Cut surface:**
  - Tumor is **soft and paler** than the surrounding gland
  - **Well-demarcated** and surrounded by a **thin intact,** well-formed **fibrous capsule**
  - Tumor **compresses the adjacent thyroid gland**
  - **Cut surface** of **tumor bulges** when fresh
  - **Color** may range from **gray-white to red-brown**.

- **Secondary changes:** They are common and include **hemorrhage, fibrosis, calcification**, and **cystic change**.

Follicular adenoma should be differentiated from solitary dominant nodule of a multinodular goiter **(Table 24.1)**.

Microscopy (Figs. 24.9B and C)

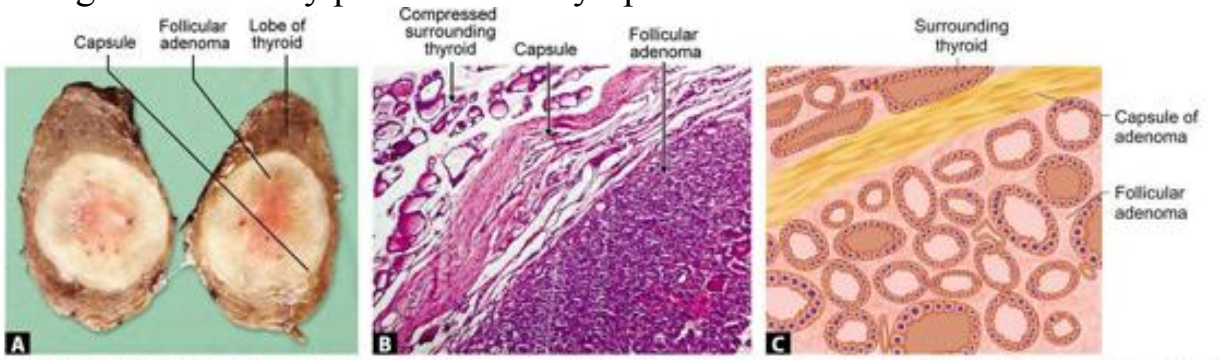
Patterns: Follicular adenomas may show **many histologic patterns**, which **do not have of any significance**. The tumor cells are arranged in follicles, which may **resemble normal thyroid tissue or mimic different stages in the embryonic development of the gland**.

- **Embryonal (trabecular) adenoma:** It consists of follicular cells arranged in a **trabecular pattern** in which poorly formed follicles contain little or no colloid.
- **Fetal** (microfollicular) **adenoma:** It consists of tumor cells which are similar to those of embryonal adenoma, but tend to be arranged in **microfollicles** (small follicles) containing **little colloid**.
- **Simple adenoma:** It shows **mature follicles** with a **normal amount of colloid**.
- **Colloid** (macrofollicular) **adenoma:** It is similar to simple adenoma except that the **follicles are larger** and contain **more abundant colloid**.
- **Hürthle cell adenoma:** It is a solid tumor **composed of oxyphil cells**, small follicles, and scanty colloid.
- **Atypical adenoma:** It is a follicular tumor with **mitoses, excessive cellularity, nuclear atypia** or equivocal capsular invasion.

Differences between nodular goiter from follicular adenoma are shown in **Table 24.2**.

Clinical Features

- Mostly present as a **unilateral painless mass**
- Larger tumors may produce local symptoms.



Figs. 24.9A to C: Follicular adenoma of thyroid. (A) A solitary, well circumscribed, gray white nodule is visible in this gross specimen (Cut section); (B) Photomicrograph; (C) Diagrammatic. Microscopic appearance of part of follicular adenoma of the thyroid showing well-differentiated, uniform thyroid follicles, well-formed capsule and compressed adjacent thyroid follicles.

TABLE 24.2: Comparison of adenomatous goiter nodule and follicular adenoma.

TABLE 24.2: Comparison of adenomatous goiter nodule and follicular adenoma

Characteristics	*Nodule of a nodular goiter*	*Follicular adenoma*
Gross		
Number of nodules	Multiple	Single
Encapsulation	Poor	Good
Microscopy		
Structure of follicles within the nodule	Variable	Uniform
Growth pattern in adjacent gland	Comparable growth pattern in adjacent gland	Distinct architecture inside and outside the capsule
Compression of adjacent gland	No compression	Seen outside the capsule

Investigations

- **Radionuclide scanning:**
  - **Nonfunctioning adenomas: They take up less radioactive iodine** than normal thyroid parenchyma. The nonfunctioning adenomas usually **appear as cold nodules**. About 10% of cold nodules **may be malignant** on microscopic examination.
  - **Functioning follicular adenoma** (toxic adenomas): They appear as **hot nodules**. **Malignancy is rare** in hot nodules.
- **Ultrasonography.**
- **Fine-needle aspiration biopsy.**
- **Histological examination** of surgically resected specimen should be evaluated for capsular integrity, and definitive diagnosis of adenomas.

Prognosis: Excellent and adenomas do not recur or metastasize.

Thyroid neoplasms: Majority present as solitary thyroid nodules, but only ~1% of all thyroid nodules is neoplastic.

Follicular adenoms: Most common benign tumor of thyroid.

Solitary thyroid nodule in female: Majority are benign.

Solitary thyroid nodule in child/male: More likely malignant.

CARCINOMAS

- Carcinomas of the thyroid are **relatively uncommon**.
- **Sex:**
  - **Early and middle adult** years: **Female** predominance.
  - **Childhood and late adult life:** Males and females **equally** affected.
- **Nature: Majority** of thyroid carcinomas are **well-differentiated**.

Major Subtypes

Q. Write a short essay on major subtypes of carcinoma of thyroid and genetic alterations in follicular cell derived neoplasms of thyroid.

1. Papillary carcinoma (>85%)
2. Follicular carcinoma (5–15%)
3. Anaplastic (undifferentiated) carcinoma (<5%)
4. Medullary carcinoma (5%).

Pathogenesis of Thyroid Carcinomas

Origin

- **Follicular cell-derived malignancies:** These include three major types of thyroid cancers namely (1) **follicular**, (2) **papillary** and (3) **anaplastic carcinoma**.
- **Non-follicular cancer:** One type namely **medullary carcinomas** do not arise from the follicular epithelium.

Two factors play major role in thyroid carcinoma: (A) genetic factors and (B) environmental factors.

A. Genetic Factors (Fig. 24.10)

Different genetic changes are involved in the pathogenesis of the four major histologic variants of thyroid cancer.

Follicular Cell-derived Malignancies

General features: Genetic alterations in the three follicular cell-derived malignancies results in **gain-of-function mutations** along components of the **two signaling pathways** namely:

- **Mitogen-activated protein (MAP) kinase pathway**
- **Phosphatidylinositol-3-kinase (PI-3K)/AKT pathway**
  - Normally, these two signaling pathways are transiently activated by binding of soluble growth factor ligands to the extracellular domain of receptor tyrosine kinases. This binding leads to autophosphorylation of the cytoplasmic domain of the receptor, and results in intracellular signal transduction.
  - **Mutation** involving the genes involved in the above two pathways → **leads to** oncogenic **constitutive activation** even in the absence of ligand → promote **carcinogenesis**.

1. Papillary carcinomas: **MAP kinase pathway** activation is a major feature of most papillary carcinomas. The activation can occur by one of the two major mechanisms.

Activation of growth factor receptor: It is the first method of activation of MAP kinase pathway. Two genes encode transmembrane tyrosine kinase growth factor receptors namely: (1) *RET* and (2) *NTRK1* (neurotrophic tyrosine kinase receptor).

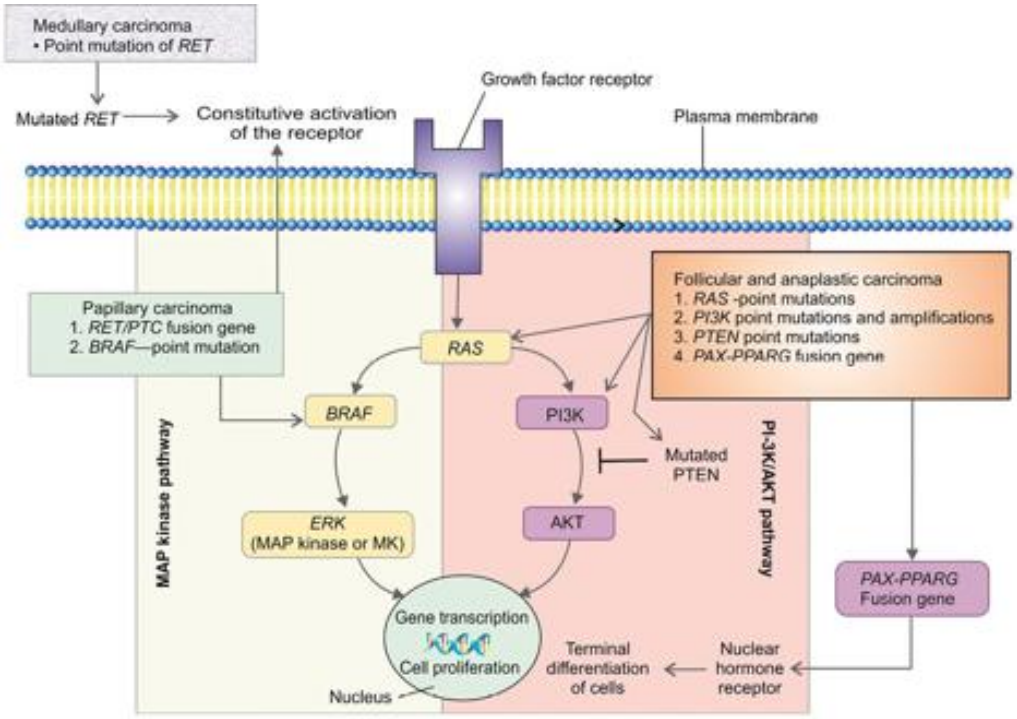


Fig. 24.10: Genetic alterations in follicular cell-derived malignant tumors and medullary carcinoma of the thyroid gland.

- ***RET* gene:** It is **normally not expressed in thyroid follicular cells**. In papillary cancers, chromosomal (**somatic) rearrangements of the *RET* gene** are common. These rearrangements **cause the fusion of the tyrosine kinase domain of RET to various other genes** → creates ***RET/PTC* (*RET*/papillary thyroid carcinoma) fusion oncogenes** → product is RET/PTC fusion protein → constitutive activation of the tyrosine kinase receptor in thyroid follicular cells → activation of the MAP kinase pathway.
  - *RET/PTC*: It is present in about 20–40% of papillary thyroid cancers and frequency is higher in those following radiation exposure.
- **Neurotrophic tyrosine kinase receptor 1 (*NTRK1*) gene**: Its fusion with another gene → resultant fusion proteins → constitutively expressed in thyroid cells → leads to activation of MAP kinase.
  - It observed in 5–10% of papillary thyroid cancers.
- **Mutations in signal transduction genes:** It is the second method of activation of MAP kinase pathway by **activating point mutations in *BRAF***, whose product is an intermediate signaling component in the MAP kinase pathway. *BRAF* is a *RAS* signal transduction protein.
  - **Point mutation of *BRAF* gene:** It is observed in ~30–50% of papillary carcinomas and is **associated with metastatic disease** and **extrathyroidal extension**.

***RET/PTC* rearrangements** and ***BRAF* point mutations** are **not found in follicular adenomas or carcinomas**.

2. Follicular carcinomas:

- **Mutations in PI-3K/AKT cell signaling pathway:** It is observed in ~30–50% of follicular carcinomas. This mutations result in constitutive activation of this oncogenic pathway. The mutations may involve different subset of PI-3K/AKT signaling. These includes:
  - Tumors with gain-of-function point mutations of ***RAS*** (signal transduction protein) and ***PIK3CA***
  - Tumors with amplification of *PIK3CA*
  - ***PTEN*** is a **tumor suppressor gene** and negative regulator of this pathway. Follicular carcinoma may show **mutations of *PTEN*** with **loss-of-function of this tumor suppressor gene**.
- ***PAX8-PPARG* fusion gene:** A unique (2;3)(q13;p25) translocation was found in 30–50% of follicular carcinomas → produces a fusion gene composed of portions of *PAX8 (*gene involved in thyroid development).

3. Anaplastic (undifferentiated) carcinomas: These are highly **aggressive and lethal tumors**. They can arise de novo or more commonly by "dedifferentiation" of a well-differentiated follicular or papillary carcinoma.

- Molecular changes are similar to those found in well-differentiated carcinomas (e.g., *RAS* or *PIK3CA* mutations), but occur at a higher rate.
- **Other genetic changes:** Inactivation of *p53* or mutations of β-catenin, are seen in anaplastic carcinomas and are associated with aggressive behavior.

Non-follicular Cell Derived Malignancies

Medullary thyroid carcinomas:

- **Genes coding growth factor receptors: Familial medullary thyroid carcinomas** occur in multiple endocrine neoplasia type 2 (**MEN-2**) and are associated with **germline *RET* gene mutations**. The ***RET* gene codes for receptor tyrosine kinase** and **oncogenic version** of *RET* gene

(mutated RET) lead to **constitutive activation of the tyrosine kinase receptor and cell proliferation**.

- *RET* mutations are also seen in about 50% of nonfamilial (sporadic) medullary thyroid cancers.
- ***RET/PTC*** observed in papillary carcinomas is **not seen in medullary carcinomas**.

B. Environmental Factors

1. **Ionizing radiation:** It is the major risk factor mainly during the first 2 decades of life.
2. **Deficiency of dietary iodine** and goiter may be associated with a higher frequency of follicular carcinomas.

Papillary Thyroid Carcinoma

Q. Write short note on papillary carcinoma of thyroid.

- The **most common** and constitutes ~85% of primary **thyroid cancer**.
- **Age:** It **can occur at any age**, but **mostly** found between the ages of **25 and 50**.
- Associated with **previous exposure** to **ionizing radiation**.
- Genetic factors (*refer* under pathogenesis of thyroid carcinoma, pages 802-4).

MORPHOLOGY

Q. Write a short note on morphology of papillary carcinoma of thyroid.

Gross

- Papillary carcinomas may be **solitary or multifocal** lesions.
- Tumors may be: (1) **well-circumscribed**, (2) **encapsulated** or (3) **ill-defined** (infiltrate the adjacent parenchyma).
- **Gray white, firm to hard tumor**, which may show areas of fibrosis and calcification. May also be cystic.
- **Cut surface** may show **papillary foci (Fig. 24.11A)**.

Microscopy (Figs. 24.11B and C)

- **Complex branching papillae:** They have a dense central fibrovascular stalk/core.
  - Papillae are covered by a **single to multiple layers of uniform cuboidal to columnar** epithelial **cells**.

- **Nuclear features:** These are **important for diagnosis** of papillary carcinoma, **even in the absence of papillary architecture**.
  - **Ground glass** or **Orphan Annie eye nuclei:** They contain **finely dispersed chromatin**, which gives an **optically clear** or **empty** appearance.
  - **Intranuclear inclusions and intranuclear grooves:** Invaginations of the cytoplasm into the nucleus in cross-sections may give the appearance of eosinophilic **intranuclear inclusions** (pseudoinclusions) or **intranuclear grooves.**
- **Psammoma bodies** (calcospherites): These are **concentrically calcified structures usually** present **within the papillary core**. They are virtually **diagnostic** of papillary carcinoma, and almost **never found in follicular and medullary carcinomas**.
- **Lymphatic spread: Lymphatic invasion** and **spread** to regional cervical lymph nodes are **common**, but **vascular invasion and blood spread are uncommon**.

Histological Variants of Papillary Thyroid Carcinoma

- **Follicular variant:** It is the **most common variant**, in which the **nuclei show characteristic features of papillary carcinoma** but has totally **follicular architecture**.
- **Tall and columnar cell variant:** It is characterized by **tall columnar cells with intensely eosinophilic cytoplasm** lining the papillary structures.
- **Diffuse sclerosing variant:** It shows prominent papillary projections, admixed with nests of squamous metaplasia, **extensive, diffuse fibrosis** with a **prominent lymphocytic infiltrate**.
- **Papillary microcarcinoma:** It is **<1 cm in size**, and usually found as an **incidental finding on surgery**.
- Oncocytic variant.

Clinical Course

Most papillary carcinomas present as **asymptomatic thyroid nodules**, sometimes the presenting symptom may be a mass in a cervical lymph node (due to metastasis).

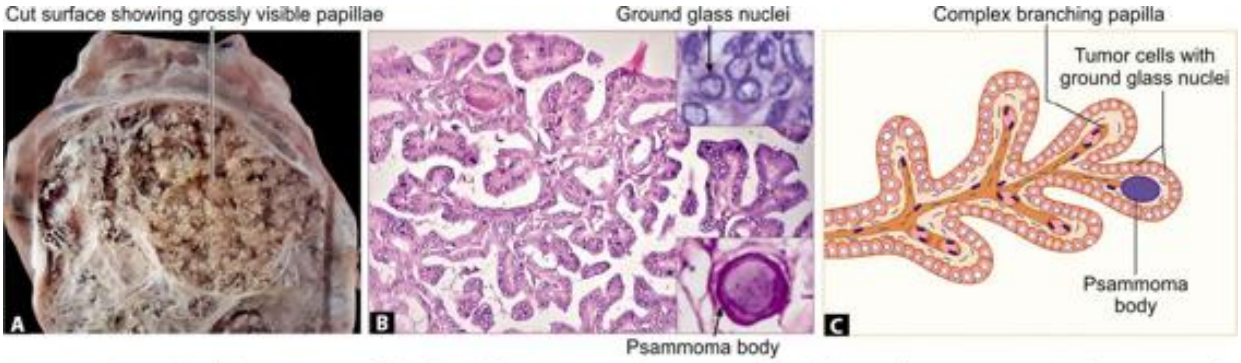
- **Fine-needle aspiration cytology** shows nuclear features characteristic of papillary carcinoma.
- **Prognosis:** Excellent.

Follicular Thyroid Carcinoma

- Follicular carcinomas constitute ~5–15% of primary thyroid cancers.
- Sex: More common in **women** (3:1).
- **Age:** Develop at **an older age than papillary carcinoma** with a peak incidence between **40 and 60 years** of age.

Etiology

More **frequent in regions** where there is **dietary deficiency of iodine**. Genetic factors (*refer* under pathogenesis of thyroid carcinoma, pages 802-4).



Figs. 24.11A to C: Papillary carcinoma of the thyroid: (A) Specimen showing grossly visible papillary structures; (B) Photomicrograph; (C) Diagrammatic. Microscopy shows well-formed, branching papillae lined by cells with characteristic empty-appearing ("Orphan Annie eye") nuclei. Inset in (B): upper right shows Orphan Annie eye nuclei and nuclear groove and right lower shows psammoma body.

MORPHOLOGY

Gross

- Follicular carcinomas are **single nodules** which may be **either well-circumscribed or infiltrate** into the surrounding thyroid parenchyma.
  - Circumscribed tumors may be difficult to distinguish from follicular adenomas on gross examination.
  - Large tumors may penetrate the capsule and infiltrate into the adjacent structures in the neck.
- Cut section of tumor appears **gray to pink** in color.

Microscopy

Follicular Pattern of Tumor Cells

- Tumor consists of **uniform cuboidal to columnar** follicular epithelial **cells**.

- Tumor cells may form **small follicles containing colloid or** may be arranged in **nests or sheets** without forming follicles.
- **Increased mitotic activity**.
- Neither nuclear feature of papillary carcinoma nor psammoma bodies is seen.

Invasion

Depending on the pattern of invasion, follicular carcinoma can be **subdivided into three** variants.

1. **Minimally invasive follicular carcinomas:**
   - Gross: Tumors appear as a **well-defined** and **encapsulated lesion**.
   - Microscopy: **Resemble follicular adenoma** but **shows mitoses, capsular invasion (Fig. 24.12)**.
2. **Encapsulated angioinvasive:** It is an encapsulated tumor which shows **vascular invasion.**
3. **Widely invasive follicular carcinomas:** They **infiltrate the thyroid parenchyma**, its **capsule** and into the surrounding **extrathyroidal soft tissues**.

Metastasis

1. **Lymphatic spread** is **uncommon**.
2. **Hematogenous spread is common to bone, lungs and liver.**

Clinical course: Follicular carcinomas present as **slowly enlarging painless nodules**.

Anaplastic (Undifferentiated) Thyroid Carcinoma

- Anaplastic carcinomas are **undifferentiated tumors** of follicular epithelium.
- Constitute less than 5% of thyroid tumors.
- **Age: Elderly** with a mean age of 65 years.
- Genetic factors (*refer* under pathogenesis of thyroid carcinoma, pages 802-4).

MORPHOLOGY

Gross: Diffusely infiltrative tumor.
Microscopy: Composed of highly anaplastic cells, which includes:

1. **Spindle** cells with a sarcomatous appearance
2. Large, pleomorphic **giant** cells
3. **Mixed** spindle and giant cells

Immunohistochemistry: The tumor cells express epithelial markers like **cytokeratin**, but are usually **negative for thyroglobulin**.

Spread

- **Local spread** into thyroid capsule and adjacent neck structures.
- **Hematogenous spread** to lungs.

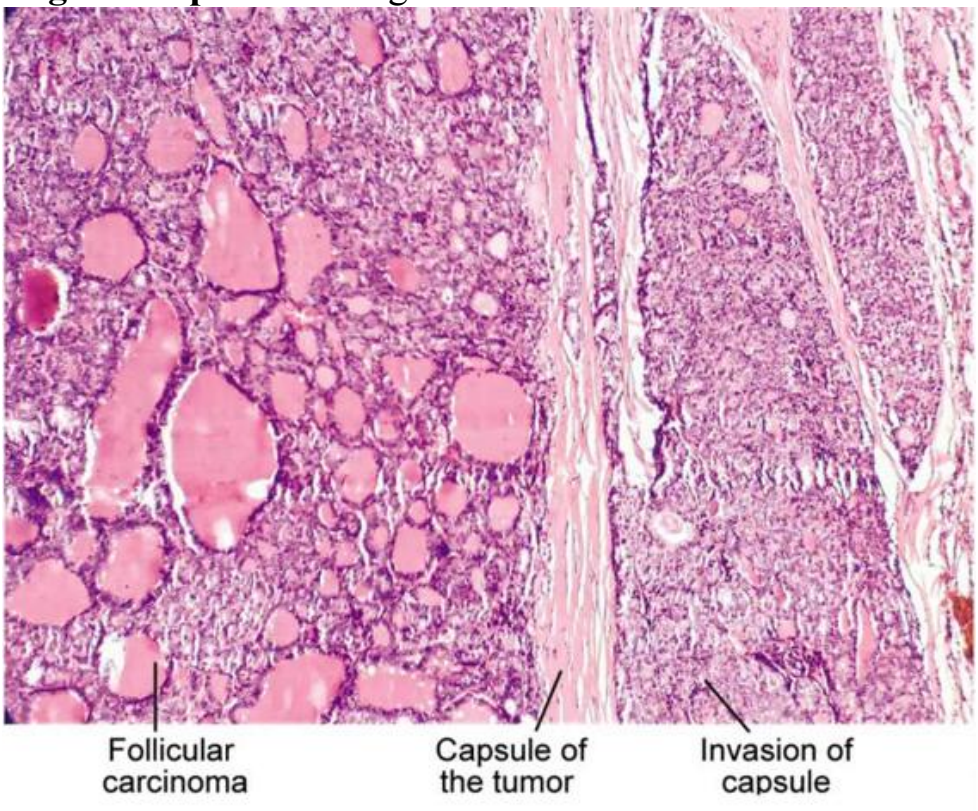


Fig. 24.12: Follicular carcinoma showing capsular invasion.

Clinical Course

- Usually, present as a **rapidly growing bulky neck mass**.
- Symptoms due to **compression and invasion of the neck structures** may cause dyspnea, dysphagia, hoarseness, and cough.

Prognosis: Aggressive tumor with poor prognosis.

Medullary Thyroid Carcinoma

Q. Write short note on medullary carcinoma of thyroid.

- Medullary carcinomas of the thyroid are **neuroendocrine tumors derived from** the **parafollicular cells** or **C-cells** of the thyroid.

- Similar to normal C-cells, they **secrete calcitonin,** measurement of which is **useful in the diagnosis and postoperative follow-up**.
- It constitutes ~5% of thyroid neoplasms.
- Genetic factors (*refer* under pathogenesis of thyroid carcinoma, pages 802-4).

Types

- **Sporadic:** Constitutes about 70% of tumor with a peak incidence in the **40s and 50s**.
- **Familial:** It may occur:
  - **With associated MEN syndrome 2A or 2B** in younger patients.
  - **Without an associated MEN syndrome.**

Point mutations in the *RET* gene is seen in both familial and sporadic medullary carcinomas **(Figs. 24.14A and B)**.

MORPHOLOGY

Gross (Fig. 24.13)

- **Number:**
  - **Solitary nodule** in **sporadic** medullary thyroid carcinomas
  - **Multicentric and bilateral common in familial cases**.
- Tumor is **firm, pale gray, and infiltrative**.
- **Larger lesions** may show areas of **necrosis and hemorrhage** and they **may extend through the capsule of the thyroid**.

Microscopy (Figs. 24.14A and B)

Q. Write a short answer on microscopy of medullary carcinoma of thyroid.

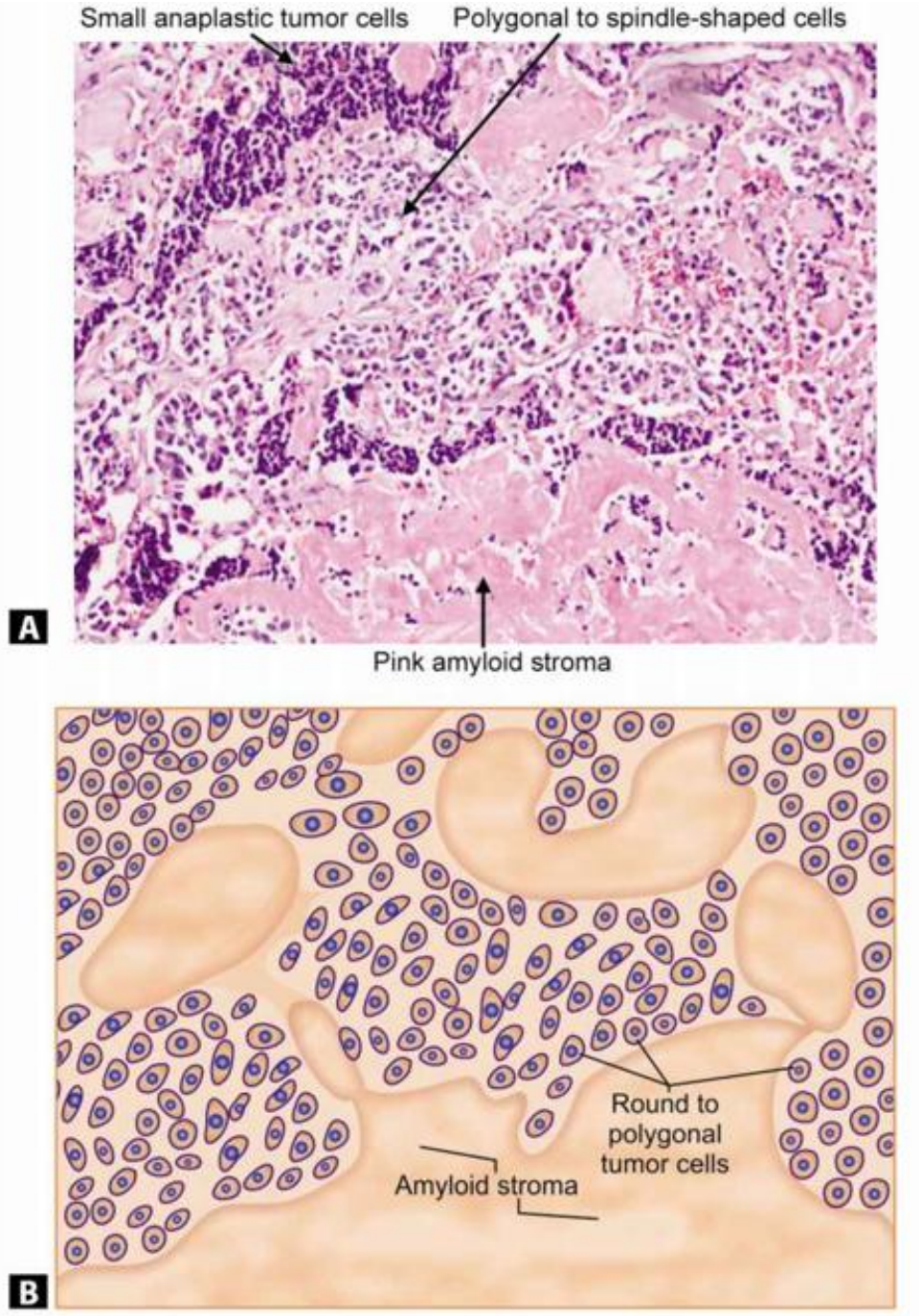
- **Tumor cells:**
  - **Polygonal to spindle-shaped** cells. **Small, more anaplastic cells** may be found **in some** tumors.
  - Cells are arranged as **nests, trabeculae** and even form follicles.
- Acellular **amyloid** deposits **in the stroma (*refer* Fig. 6.26)**: It is found in most of the cases. These are **derived from altered calcitonin polypeptides**.
- **C-cell hyperplasia:** It is seen in the **surrounding thyroid of familial medullary cancers**, which is **not observed in sporadic lesions**. They **represent the precursor lesions** for medullary carcinoma.

Immunohistochemistry: Calcitonin can be demonstrated within the cytoplasm of the tumor cells and in the stromal amyloid by immunohistochemical methods.

Electron microscopy: It shows **membrane-bound electron-dense granules** within the cytoplasm of the tumor cells.



Fig. 24.13: Gross appearance of medullary carcinoma of thyroid.



Figs. 24.14A and B: (A) Photomicrography; (B) Diagrammatic. Medullary carcinoma of thyroid shows nests and trabeculae of round, spindle to polygonal cells separated by abundant homogeneous, extracellular, pink deposits of amyloid.

Clinical Course

1. Sporadic Cases

- Present as a **mass in the neck**
- **Paraneoplastic syndrome**: It may occur in some medullary carcinoma (e.g., diarrhea due to the secretion of VIP, or Cushing syndrome due to ACTH).
- **Tumor markers:** They are useful, mainly for presurgical assessment of tumor load and in calcitonin-negative tumors. These include:
  - Calcitonin
  - Carcinoembryonic antigen (CEA).

2. Familial Cases

- Present with **symptoms localized to the thyroid** or as a component of familial syndromes along with neoplasms in other organs (e.g., adrenal or parathyroid glands).
- Medullary carcinomas arising as a component of MEN-2B are more aggressive and more frequently metastasize than sporadic tumors (MEN-2A or FMTC).

Follicular cell-derived thyroid carcinoma:
- Follicular carcinoma
- Papillary carcinoma
- Anaplastic carcinoma

Thyroid carcinoma: Different genetic changes are involved in its pathogenesis.

Follicular cell-derived thyroid carcinoma: Mutations in two signaling pathways namely:
1. Mitogen-activated protein (MAP) kinase pathway
2. Phosphatidylinositol-3-kinase (PI-3K)/AKT pathway

Papillary carcinoma: Chromosomal rearrangements involving *RET/PTC* fusion gene and *BRAF* mutations.

RET gene codes for growth factor tyrosine kinase receptors.

RET/PTC fusion protein: Causes constitutive activation of the tyrosine kinase growth factor receptor in thyroid follicular cells.

BRAF gene codes for RAS signal transduction protein.

Papillary carcinoma:
- *RET/PTC* fusion gene
- *BRAF* mutation

Follicular carcinoma: Point mutations of:
- *RAS*
- *PI3K*
- *PTEN (tumor suppressor gene)*
- *PAX8/PPARG* fusion gene

Medullary carcinoma: Point mutation of *RET* gene.

Papillary carcinoma: Most common malignant tumor of thyroid.

Papillary carcinoma: Most common endocrine malignancy.

Papillary carcinoma:
- Accounts for 85% of thyroid malignancy in iodine-deficient areas
- Most common thyroid cancer in children and in persons exposed to external radiation
- Most often in women between 30–40 years
- Excellent prognosis

Characteristic nuclear features:
- Ground glass (Orphan Annie) nuclei
- Pseudoinclusions
- Nuclear grooves

Papillary carcinoma: Nuclear features are used as diagnostic criteria even in the absence of papillae.

Papillary carcinoma: Most commonly spreads through lymphatics have excellent prognosis.

Follicular carcinoma: Most common thyroid cancer presenting as a solitary cold nodule.

Follicular thyroid carcinoma is differentiated from follicular adenoma:
- Capsular invasion
- Vascular invasion

Follicular carcinoma: (1) Minimally invasive (2) Encapsulated angioinvasive and (3) Widely invasive.

Follicular neoplasm: Adenoma and carcinoma. Both are composed of well-differentiated follicular epithelial cells. Follicular carcinoma shows capsular and/or vascular invasion.

Follicular carcinoma: Most commonly spreads through hematogenous than through lymphatics.

Pulsating secondaries:
- Follicular carcinoma of thyroid
- Renal cell carcinoma

Anaplastic carcinomas: Undifferentiated tumors of follicular epithelium and are thought to arise by dedifferentiation or more differentiated tumors.

Anaplastic carcinomas:
- Highly aggressive
- Poor prognosis

Medullary carcinoma: Arises from parafollicular C-cells.

Medullary carcinoma:
- Sporadic (~70%)
- Familial (~30%)

Medullary carcinoma-familial:
- Multicentric
- C-cell hyperplasia

Medullary carcinoma of thyroid is associated with mutation in: *RET* gene.

Medullary carcinoma: Amyloid stroma is a characteristic microscopic finding.

Medullary carcinoma: Paraneoplastic syndrome in few
- Diarrhea due to vasoactive intestinal peptide (VIP)
- Cushing syndrome due to adrenocorticotropic hormone (ACTH)

Medullary carcinoma: Tumor markers
- Calcitonin (It is converted to amyloid)
- Carcinoembryonic antigen

PARATHYROID GLANDS

Most individuals have four parathyroid glands. Normally, they are located in posterior thyroid surface in close proximity to the upper and lower poles of each thyroid lobe. They measure 4–6 mm in length and they have a size and color of a grain of saffron-cooked rice.

About ¾ of the cells are **chief and oxyphil cells** and ¼ consists fat cells scattered throughout the parenchyma. Most of the gland consists of **chief cells and their cytoplasmic secretory granules contain parathyroid hormone** (PTH) and PTH related protein. Oxyphil cells appear after puberty

and are found throughout the normal parathyroid either singly or in small clusters. Their cytoplasm is acidophilic due to many mitochondria. They have no secretory granules and do not secrete PTH.

Calcium homeostasis: Parathyroid glands play a key role in the regulators of calcium homeostasis. The activity of the parathyroid glands depends on the blood levels of free (ionized) calcium and magnesium. Ionized Ca, not bound Ca, is functionally active. PTH is stimulated by hypocalcemia and hyperphosphatemia whereas PTH is suppressed by hypercalcemia and hypophosphatemia. Normally, when the free calcium (Ca) level decreases in the blood it stimulate the parathyroid to synthesis and secrete PTH. The **secreted PTH has the following effects (Fig. 24.15)** on its target tissues, the kidneys, the bones and the gastrointestinal tract (GI tract):

- **Increased tubular and intestinal reabsorption of calcium:**
  - Increased reabsorption of calcium by renal tubules.
  - Increased conversion of vitamin D to its active dihydroxy form in the kidneys. This in turn increases absorption of calcium in the gastrointestinal tract
- **Regulation of renal phosphate excretion:** Increased urinary excretion of phosphate lowers serum phosphate levels (since phosphate binds to ionized calcium).
- **Bone resorption:** Increases the number and activity of osteoclasts. Increased osteoclastic activity causes increased bone resorption and thus releases ionized calcium.

The net result of the above activities is an increase in the free calcium level in the blood, which inhibits PTH secretion (acts as a brake on PTH secretion) from chief cells. PTH is degraded in the liver and kidney.

Abnormalities of the parathyroids: These include either hyperfunction or hypofunction. Tumors of the parathyroid glands, unlike thyroid tumors, usually cause of excessive secretion of PTH. Thus, clinical diagnosis is made due to its functional effect rather than mass effects.

HYPERPARATHYROIDISM

NC: Describe the etiology, genetics, pathogenesis, manifestations, laboratory and morphologic features of hyperparathyroidism.

Hyperparathyroidism is a condition caused by elevated PTH.

Classification: Hyperparathyroidism is classified into **primary, secondary, and tertiary** (least common), types.

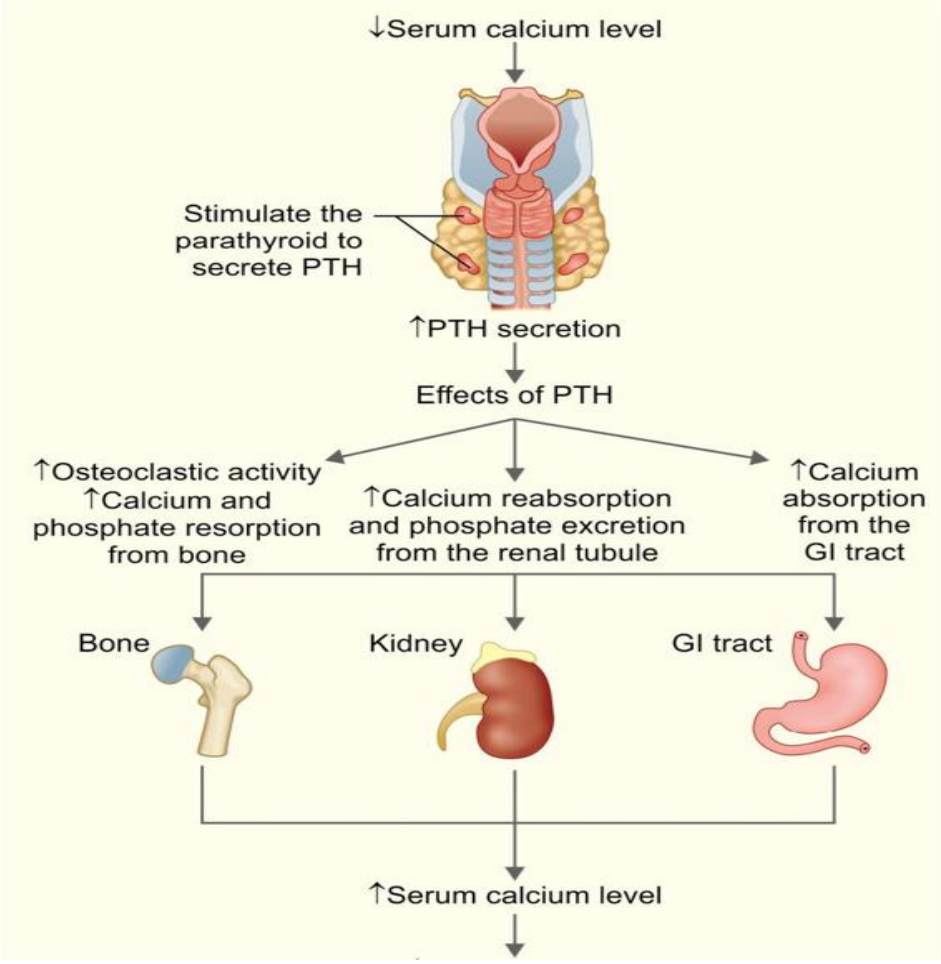


Fig. 24.15: Various effects of PTH (parathyroid hormone).

Primary Hyperparathyroidism

Definition: Primary hyperparathyroidism is a common endocrine disorder characterized by excessive autonomous production and secretion of PTH from the parathyroid glands. **It is an important cause of hypercalcemia.**

Age and gender: Primary hyperparathyroidism is usually seen in adults. It is more common in women than in men (ratio of 4:1).

Etiology: Usually the result from a **parathyroid adenoma** (~85–95%) or **diffuse or nodular primary hyperplasia** of parathyroid tissue (~5–10%) or **parathyroid carcinoma** (~1%).

Genetics and pathogenesis: Abnormalities in two genes are associated with parathyroid tumors:

1. **Cyclin D1 gene rearrangements:** Cyclin D1 is a positive regulator of the cell cycle and *cyclin D1* gene is normally on 11q.
   - **An inversion on chromosome 11 repositions the *cyclin D1* gene from** its normal position on **11q to 11p** adjacent to genomic elements that regulate the *PTH* gene. This drive abnormal expression of cyclin D1 in PTH-producing cells and leads to increased proliferation of these cells. About 10–20% of parathyroid adenomas show this acquired genetic defect of inversion.
   - **Overexpression of cyclin D1:** This is seen in about 40% of parathyroid adenomas.
2. **MEN1 mutations:** About 20–30% of sporadic parathyroid tumors show mutations in both copies of the *MEN1* tumor suppressor gene. Germline mutations of *MEN1* are also found in patients with familial parathyroid adenomas.

MORPHOLOGY

In primary hyperparathyroidism, the morphologic changes are found in the parathyroid glands and also in other organs affected by hypercalcemia. About 75–80% of cases, one of the parathyroid glands shows a solitary adenoma.

Parathyroid Adenoma

- **Gross:** They appear as well-circumscribed, soft, tan nodule or red-brown and solitary mass surrounded by a thin capsule. It measures about 1–3 cm and weigh between 0.5 and 2 g. They are seen in only one gland and the other glands are either normal in size or somewhat shrunken (due to feedback inhibition by raised serum calcium).
- **Microscopy:** They are composed predominantly sheets of neoplastic chief cells in a rich capillary network. A rim of compressed, non-neoplastic, normal parathyroid tissue is usually found outside the capsule and helps to distinguishes adenomas from parathyroid hyperplasias.

Parathyroid Hyperplasia

It may occur sporadically or familial as a component of MEN syndrome.

- **Gross:** Typically, a multiglandular process involving all the four parathyroid glands, but sometimes it may be asymmetrical with sparing of one or two glands. In such cases, enlargement may be grossly apparent in only one or two glands, making it difficult to distinguish between hyperplasia and adenoma.
- **Microscopy:** Most commonly there is chief cell hyperplasia, which may involve the glands in a diffuse or multinodular pattern.

Parathyroid Carcinomas

- **Gross:** They may be circumscribed lesions similar to adenoma or may be clearly invasive neoplasms. They enlarge one parathyroid gland. They appear as gray-white, firm, irregular masses and are usually larger than adenomas (sometimes exceed 10 g in weight).
- **Microscopy:** The tumor cells are usually uniform and resemble normal parathyroid cells. They are arranged in nodular or trabecular patterns. The tumor mass is usually enclosed by a dense, fibrous capsule and occasionally invades capsules or blood vessels. The diagnosis of carcinoma based on cytologic features is not reliable. The only definitive criteria for malignancy are invasion into the surrounding tissues and metastasis. Local recurrence and distant dissemination can occur.

Morphologic Changes in Other Organs

Skeletal changes: Bone shows increased osteoclastic activity. It is characterized by erosion of bone matrix and mobilization of calcium salts, especially in the metaphyses of long tubular bones. Bone resorption is associated with increased osteoblastic activity and the formation of new bone trabeculae.

- **Osteitis fibrosa cystica:** In more severe cases, there may be gross thinning of the cortex and the bone marrow shows increased fibrous tissue accompanied by foci of hemorrhage and cysts. This is termed as generalized osteitis fibrosa cystica (von Recklinghausen disease of bone).

- **Brown tumors of hyperparathyroidism:** Aggregates of osteoclasts, reactive giant cells, and hemorrhagic debris may form masses that may be mistaken for neoplasms and are termed as brown tumors of hyperparathyroidism.
- **Renal changes:** PTH-induced hypercalcemia results in formation of stones in the urinary tract (nephrolithiasis) and calcification of the renal interstitium and tubules (nephrocalcinosis).
- **Metastatic calcification:** It develops due to hypercalcemia and can be found in other sites such as stomach, lungs, myocardium, and blood vessels.

Clinical Features

Primary hyperparathyroidism may be (1) asymptomatic and detected during routine blood chemistry profile, or (2) symptomatic with the classic clinical manifestations of primary hyperparathyroidism.

Asymptomatic primary hyperparathyroidism: Routinely serum calcium levels are assessed in most patients who need blood tests for unrelated conditions. Hence, most patients with primary hyperparathyroidism are diagnosed incidentally when clinically silent hypercalcemia (increase in serum ionized calcium) is found. Primary hyperparathyroidism is the most common cause of asymptomatic hypercalcemia followed by osteolytic metastatic malignancy to bone.

Symptomatic primary hyperparathyroidism: The signs and symptoms of hyperparathyroidism are due to the combined effects of increased PTH secretion and hypercalcemia. These include painful bones, renal stones, abdominal groans (deep inarticulate sound in response to pain), and psychic moans (long, low sound made by a person expressing physical or mental suffering).

- **Bone disease and bone pain:** It is secondary to fractures of bones (pathologic fractures) that are weakened by osteoporosis or osteitis fibrosa cystica.
- **Nephrolithiasis (renal stones):** It causes renal colic and obstructive uropathy. Chronic renal insufficiency and abnormal renal function produces polyuria and secondary polydipsia.
- **Gastrointestinal:** Constipation, nausea, peptic ulcers (hypercalcemia increases serum gastrin, thus stimulating gastric acid secretion), pancreatitis, and gallstones.

- **Central nervous system:** Depression, lethargy, memory defects and seizures.
- **Neuromuscular abnormalities:** Weakness, hypotonia, and fatigue.
- **Cardiac manifestations:** Aortic or mitral valve calcifications (or both).

Laboratory Findings

- Increased serum PTH
- Increased serum calcium
- Decreased serum 1, 25-(OH) 2D (hypovitaminosis D): Hypercalcemia decreases the synthesis of 1-α-hydroxylase in the proximal renal tubule, which decreases the synthesis of 1, 25-(OH) 2D.

Secondary Hyperparathyroidism

Definition: Compensatory parathyroid hypersecretion of the PTH in response to prolonged hypocalcemia, hyperphosphatemia, or decreased serum calcitriol levels.

Etiology and pathogenesis: Secondary hyperparathyroidism is commonly caused by chronic hypocalcemia, most often as a result of chronic renal failure, leading to compensatory hyperplasia of the parathyroids. It can also develop in association with vitamin D deficiency, intestinal malabsorption, Fanconi syndrome and renal tubular acidosis.

Chronic renal insufficiency is associated with decreased phosphate excretion **(Flowchart 24.2)**. This in turn results in hyperphosphatemia. The raised levels of serum phosphate directly depress serum calcium levels. In addition, in diseased kidney, there is loss of renal α1-hydroxylase activity, which is needed for the synthesis of the active form of vitamin D (1, 25(OH) 2D), reduces the intestinal absorption of calcium. All these alterations cause chronic hypocalcemia, which stimulates the parathyroid glands to undergo secondary hyperplasia. Hyperplasia causes increased secretion of PTH.

MORPHOLOGY

Parathyroid glands in secondary hyperparathyroidism: They undergo hyperplastic changes. Microscopically, the hyperplastic glands show increased number of chief cells, or cells with more abundant, clear cytoplasm (water-clear cells).

Skeletal/bone changes and metastatic calcification: Similar to primary hyperparathyroidism (*refer* page 809).

Clinical Features

The clinical features of secondary hyperparathyroidism are usually due to chronic renal failure.

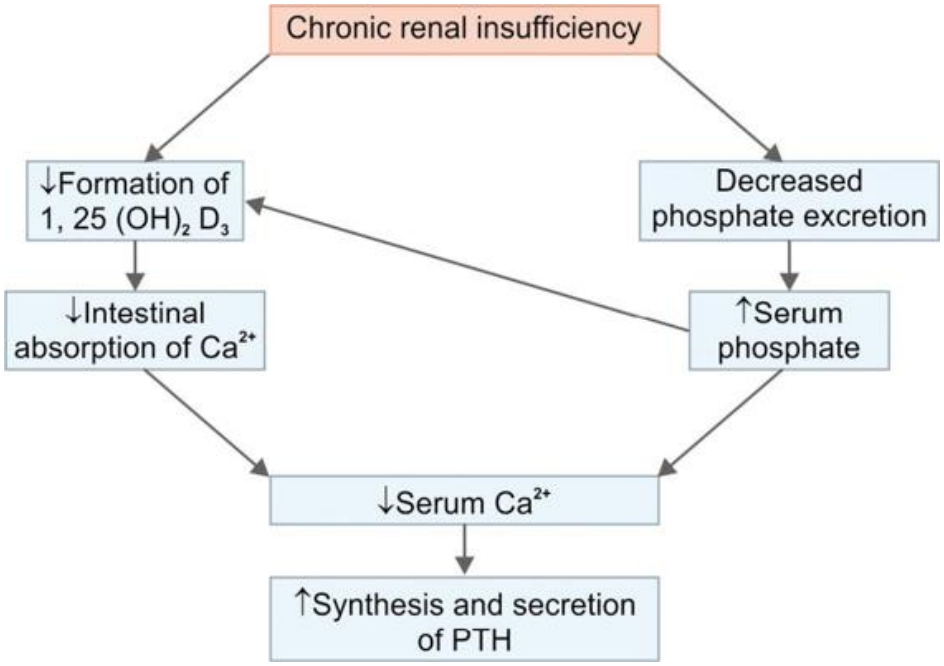
Bone abnormalities (renal osteodystrophy): Excess levels of PTH produce skeletal pain and deformities, osteomalacia and osteitis fibrosis cystica. The osseous manifestations similar to primary hyperparathyroidism (*refer* page 809). They are usually less severe than seen in primary hyperparathyroidism. Rarely, metastatic calcification of blood vessels (secondary to hyperphosphatemia) may produce significant ischemic damage to skin and other organs (process termed to as calciphylaxis).

Laboratory findings: Serum calcium remains near normal. This is due to the compensatory increase in PTH levels which sustains serum calcium.

Tertiary Hyperparathyroidism

Long-standing secondary parathyroid hyperplasia in renal failure may be followed by an autonomous parathyroid hyperplasia with resultant hypercalcemia. In such cases, parathyroid hyperplasia persists and may not regress even after the cause of prolonged hypocalcemia is corrected, for example, after renal transplant. Such patients require parathyroidectomy to control the hyperparathyroidism.

Flowchart 24.2: Mechanism of hypocalcemia and secondary hyperparathyroidism in chronic renal failure produces.



HYPOPARATHYROIDISM

Definition: Hypoparathyroidism is hypofunction of the parathyroid glands leading to hypocalcemia.

Etiology:

- **Surgically induced hypoparathyroidism:** Acquired hypoparathyroidism can occur due to inadvertent removal of parathyroid glands during thyroidectomy or during radical neck dissection (parathyroid glands is mistaken for lymph nodes).
- **Autoimmune hypoparathyroidism:** It may be associated with syndrome known as autoimmune polyendocrine syndrome type 1 (APS1) and is caused by mutations in the autoimmune regulator (AIRE) gene.
- **Autosomal-dominant hypoparathyroidism:** It is due to gain-of-function mutations in the calcium-sensing receptor (CaSR) gene.
- **Familial isolated hypoparathyroidism (FIH);** it is a rare disorder that may be inherited as either autosomal dominant or autosomal recessive.
- **Congenital absence of parathyroid glands.**

Clinical Features

The major clinical manifestations are due to hypocalcemia and depend on its severity and chronicity.

Tetany (hypocalcemia): It is the hallmark of hypocalcemia and is characterized by neuromuscular irritability, resulting from decreased serum calcium levels. The symptoms range from circumoral numbness or paresthesias (tingling) of the distal extremities and carpopedal spasm, to life-threatening laryngospasm and generalized seizures.

Features of chronic hypoparathyroidism:

- Calcification of the basal ganglia: Caused by metastatic calcification. Increased serum phosphates in primary hypoparathyroidism causes calcification of the cerebral basal ganglia.
- Cataracts (opacification of the lens) and dental abnormalities

Laboratory findings in hypoparathyroidism: These include hypocalcemia, hyperphosphatemia, and a decrease in serum PTH.

ADRENAL GLANDS

The adrenal (or suprarenal) glands are paired endocrine organs. They consist of two independent regions namely outer cortex and inner medulla. They differ in structure, function and development.

ADRENAL CORTEX

Adrenal cortex consists of three layers (zones) of distinct cell types namely the (i) **zona glomerulosa** (outermost layer)**, (ii) zona fasciculata** (middle) and (iii) **zona reticularis (innermost layer**). The hormones produced by the adrenal cortex are called steroids and it secrets **three different types of steroids.**

1. **Glucocorticoids (named for their primary effect on glucose metabolism):** Principally, it is cortisol and it opposes the effects of insulin. It is produced primarily in the zona fasciculata, with a small amount from the zona reticularis.

2. **Mineralocorticoids:** Principally, it is aldosterone and is synthesized in the zona glomerulosa. They maintain normal salt and water balance by promoting sodium retention and potassium excretion at the distal renal tubules.
3. **Sex steroids (estrogens and androgens):** They are synthesized mainly in the zona reticularis.

Note: The stimulus for the synthesis and secretion of both cortisol and androgen is ACTH; whereas that for aldosterone is angiotensin II (AII). The activities of these three steroids (hormones) regulate "three S's": sugar, salt, and sex.

Diseases of the adrenal cortex: They can be divided into two major groups namely those associated with (i) **hyperfunction or (ii) hypofunction**.

Adrenocortical Hyperfunction (Hyperadrenalism)

Syndromes of adrenal hyperfunction are caused by overproduction of the one or more of the three major hormones produced by the three layers of the adrenal cortex.

1. **Cushing syndrome:** Due to an excess of cortisol.
2. **Hyperaldosteronism:** Due to excess of mineralocorticoid (aldosterone).
3. **Adrenogenital or virilizing syndromes:** Due to an excess of androgens.

In some of these syndromes, the clinical features may show some similarities because of the shared functions of adrenal steroids.

Hypercortisolism (Cushing Syndrome)

NC: Describe the etiology, pathogenesis, manifestations, laboratory, morphologic features, and complications of Cushing's syndrome.

Definition: The term Cushing syndrome is used to describe the clinical features/abnormalities due to hypercortisolism (excessive production glucocorticoid/cortisol) from any cause.
The term Cushing disease is reserved for pituitary-dependent excessive ACTH secretion by pituitary corticotrope tumors.

Etiopathogenesis

Cushing syndrome is produced by conditions that produce raised glucocorticoid levels.

Causes (Fig. 24.16)

- **Exogenous causes:** Majority of cases are the result of the administration of exogenous glucocorticoids ("iatrogenic" Cushing syndrome). The exogenous steroids may be used for the management diseases such as allergic and autoimmune diseases.
- **Endogenous causes:** The most common endogenous causes are:
  - **ACTH dependent** (~80%)
  - **Primary hypersecretion of ACTH by pituitary diseases (Cushing disease):** About 70% of cases of endogenous hypercortisolism are due to **ACTH-secreting pituitary adenomas/hyperplasia**.
  - **Secretion of ectopic ACTH by nonpituitary neoplasms**: For example, **small cell carcinoma of the lung, bronchial carcinoid** which may secrete ectopic ACTH.
  - **ACTH independent (~20%): Primary adrenocortical neoplasms** (e.g., adrenal adenoma and adrenal cortical carcinoma) are the most common causes. Other rare cause is primary cortical hyperplasia.

MORPHOLOGY

Main lesions of Cushing syndrome are observed in the pituitary and adrenal glands.

Pituitary: Most common changes are due to high levels of endogenous or exogenous glucocorticoids and are called as **Crooke hyaline change.** This is characterized homogeneous and pale appearance of the cytoplasm of ACTH-producing cells in the anterior pituitary.

Adrenals: Depending on the cause of the hypercortisolism the adrenals may show one of the following abnormalities.

- **Cortical atrophy:** Bilateral cortical atrophy is seen in cases due to exogenous glucocorticoids, suppression.
- **Diffuse hyperplasia:** Found in ACTH-dependent Cushing syndrome.
- **Macronodular or micronodular hyperplasia.**
- **Functional adenoma or carcinoma of adrenal cortex (*refer* pages 815-6.)**.

Clinical Course and Complications

Cushing syndrome develops slowly and the clinical features are as follows:

- **Weight gain** caused by hyperinsulinism from **hyperglycemia.** There is also **glucosuria and polydipsia** (secondary diabetes). More characteristic features are due to fat deposition/accumulation in trunk (**truncal obesity), in the face (moon facies)**, and in the posterior neck and upper back (**buffalo hump**).
- **Hypertension**
- **Muscle weakness:** Cortisol breaks down muscles in the extremities (**decreased muscle mass) and produce proximal limb weakness**.
- **Purple abdominal stria (stretch marks): Skin is thin, fragile, and easily bruised**; and cutaneous striae are particularly common in the abdominal area.
- **Osteoporosis:** Hypercortisolism causes increased breakdown and resorption of bone leading to **osteoporosis** and associated with increased susceptibility to fractures.
- Increased risk for a variety of infections.
- Others include several mental disturbances, hirsutism and menstrual abnormalities.

Laboratory findings: The diagnosis of adrenocortical excess depends on laboratory measurements.

- **Serum ACTH level:** It may be help in differentiating between primary (low ACTH level) and secondary (high ACTH level) hypercortisolism.
- **Serum cortisol:** Glucocorticoid levels are increased in all forms of Cushing syndrome.
- **Urinary free cortisol levels:** It is elevated in all forms of hypercortisolism. Usually a 24-hour urine collection is done to exclude diurnal variations in cortisol production.
- **Dexamethasone suppression test:** It helps in differentiation of pituitary causes and ectopic causes of excessive ACTH production. If the ACTH level is increased or normal and the 24-hour urinary free cortisol level is raised, a dexamethasone suppression test may be done.

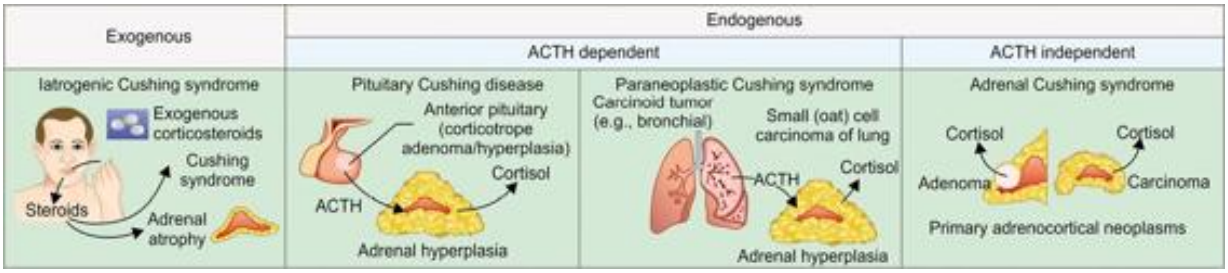


Fig. 24.16: Various causes of Cushing syndrome.
(ACTH: adrenocorticotropic hormone)

The results of the above tests can fall into following three general patterns:

1. **Pituitary Cushing disease/syndrome** (e.g., pituitary adenomas): ACTH levels are elevated show a relative resistance (cannot be suppressed) to feedback inhibition by low dose of cortisol (dexamethasone). Hence, there is no reduction in urinary excretion of cortisol. When a more potent glucocorticoid (dexamethasone) is injected, the pituitary gland responds by reducing (suppressing) ACTH production, which is reflected by suppression of urinary cortisol secretion.
2. **Ectopic ACTH secretion:** Cushing syndrome may be produced due to ectopic production of ACTH by cancer cells (e.g., small cell carcinoma of lung). In such cases, there is elevated level of ACTH. Neither low nor higher doses of dexamethasone (in dexamethasone suppression test) injection in these cases fail to reduce ACTH production. This is because the cancer cells do not respond (completely insensitive) to feedback control. When suppression test is negative, it is necessary to find the ectopic cancer source.
3. **Cushing syndrome due to adrenal cortical tumors:** They have very low levels of ACTH because of feedback inhibition of the pituitary. Similar to ectopic ACTH secretion, both low-dose and high-dose dexamethasone fail to suppress cortisol excretion.

Hyperaldosteronism

Definition: Hyperaldosteronism is a group of closely related conditions characterized by chronic excessive secretion of aldosterone. Aldosterone is not under pituitary control.

Causes: Hyperaldosteronism may be primary (due to adrenal cause), or it may be secondary to an extra-adrenal cause.

Primary Hyperaldosteronism

It is characterized by autonomous excessive production of aldosterone. This in turn suppresses the renin-angiotensin system and decreased plasma renin activity. **Raised blood pressure is the most common manifestation of primary hyperaldosteronism.** The causes of primary hyperaldosteronism are as follows:

- **Bilateral idiopathic hyperaldosteronism (IHA):** It is characterized by nodular hyperplasia of the both adrenal glands and is the most common (about 60%) cause of primary hyperaldosteronism. Its exact pathogenesis is not known and few patients show germline mutations in the *KCNJ5* gene, which encodes a potassium channel protein that is expressed in the adrenal gland. Usually it occurs in older individuals and have less severe hypertension than those with adrenal neoplasms.
- **Adrenocortical neoplasm:** This may be an aldosterone-producing adrenocortical adenoma (cause about 35%) or, rarely, an adrenocortical carcinoma. Primary hyperaldosteronism caused by a solitary aldosterone-secreting adenoma is called as Conn syndrome. This occurs mostly in adult middle life and the female to male ratio is 2:1. Somatic mutations of *KCNJ5* may be also seen in a subset of patients with aldosterone secreting adenomas.
- **Glucocorticoid-remediable hyperaldosteronism:** It is an uncommon cause of **familial hyperaldosteronism**: It is very rare and is due to a genetic defect that leads to overactivity of the *aldosterone synthase gene, CYP11B2.*

Secondary Hyperaldosteronism

In secondary hyperaldosteronism, there is release of aldosterone in response to activation of the renin-angiotensin system. There are increased levels of plasma renin due to an extra-adrenal cause such as:

- **Decreased renal perfusion:** Renal artery stenosis
- **Arterial hypovolemia and edema:** Congestive heart failure, cirrhosis, nephrotic syndrome
- **Pregnancy:** Estrogen-induced increase in plasma renin substrate

Clinical Features

- **Hypertension**: It is the common symptom and the long-term hyperaldosteronism-induced hypertension can lead to cardiovascular complications (e.g., left ventricular hypertrophy, reduced diastolic volumes and myocardial infarction) and an increased incidence stroke.
- **Hypokalemia**: Potassium moves from blood into renal tubule and lost through kidneys. This causes hypokalemia (decreased potassium in blood). Hypokalemia can produce neuromuscular manifestations such as weakness, paresthesias, visual disturbances, and occasionally frank tetany.

Adrenogenital Syndromes

Adrenal cortex can secrete excess androgens due to either of the following:

- Adrenocortical neoplasms (usually *virilizing* carcinomas).
- Congenital adrenal hyperplasia (CAH): It consists of a group of autosomal recessive disorders characterized by defects in steroid biosynthesis, usually cortisol. Reduced secretion of cortisol causes a compensatory increase in ACTH secretion, which in turn stimulates androgen production.
- Androgens have virilizing effects. These effects include masculinization in females (ambiguous genitalia, oligomenorrhea, hirsutism), precocious puberty in males.

Adrenocortical Insufficiency/Hypofunction (Hypoadrenalism)

NC: Describe the etiology, pathogenesis, manifestations, laboratory, morphologic features, complications of adrenal insufficiency.

Definition: Lack of or reduced production of glucocorticoids or mineralocorticoids by the adrenal cortex.

Etiology and Pathogenesis

Adrenocortical insufficiency (hypofunction or hyposecretion of adrenocortical hormones can be due to disease of the adrenal cortex (primary

adrenocortical insufficiency, Addison disease) or due to the inadequate secretion of ACTH from the anterior pituitary gland (secondary adrenal insufficiency). Though hypofunction can affect all the adrenocortical hormones, the most severe clinical manifestations of adrenocortical insufficiency are because of low/inadequate levels of circulating cortisol.

Etiology: Causes of are listed in **Box 24.6.**

BOX 24.6: Causes of adrenocortical insufficiency or hypofunction.

- **Primary adrenocortical insufficiency (hypoadrenalism):** It is due to adrenal disease and can be acute or chronic.
  - **Acute:** Characterized by acute adrenal crisis
    - Sudden or abrupt withdrawal of corticosteroids in a patient with adrenal atrophy due to long-term corticosteroid therapy and is the most common cause of acute adrenal insufficiency
    - Waterhouse–Friderichsen syndrome (acute hemorrhagic necrosis), anticoagulation therapy (massive hemorrhage into adrenals)
    - Stress of infection or surgery in patient with chronic adrenal insufficiency
  - **Chronic (Addison disease)**
    - Autoimmune adrenalitis
    - Infections: Tuberculosis, acquired immunodeficiency syndrome, fungal infections *(Histoplasma capsulatum and Coccidioides immitis)*
    - Amyloidosis, sarcoidosis, hemochromatosis
    - Metastatic carcinoma to the adrenals: Carcinomas of the lung and breast
- **Secondary adrenocortical insufficiency (hypothalamic-pituitary dysfunction):** Decreased stimulation of the adrenals due to a deficiency of ACTH (secondary hypoadrenalism).
  - Hypothalamic pituitary disease: Destruction of the pituitary with loss of pituitary function by —
    - Inflammation (sarcoidosis, tuberculosis, pyogens, fungi) and infections due to opportunistic pathogens associated with the human immunodeficiency virus
    - Pituitary tumors, craniopharyngioma. metastatic tumors to pituitary, pituitary infarction, trauma, surgery and radiation therapy
  - Hypothalamic pituitary suppression
    - Long-term steroid administration

Primary Adrenocortical Insufficiency

Acute Adrenocortical Insufficiency

Definition: A sudden lack of production of glucocorticoids or mineralocorticoids by the adrenal cortex is termed acute adrenocortical insufficiency or adrenal crisis. There is sudden loss of adrenal

cortical function.

Causes of acute adrenocortical insufficiency are listed in **Box 24.6**.

Waterhouse–Friderichsen syndrome: It is catastrophic syndrome characterized by acute, adrenal crisis due to bilateral, hemorrhagic infarction of the adrenal cortex. It is usually secondary to meningococcus (*Neisseria meningitidis*) or *Pseudomonas septicemia*. Waterhouse-Friderichsen syndrome is more common in children.

Clinical feature: Adrenal crisis are usually manifest with hypotension and shock. Nonspecific symptoms include: fatigue, weakness, and gastrointestinal disturbances which may progress to coma.

Chronic Adrenocortical Insufficiency:Addison Disease

Definition: Chronic adrenal insufficiency (Addison disease) is characterized by decreased production of glucocorticoids and mineralocorticoids, with a corresponding increase in ACTH.

Etiology: Addison disease is an uncommon disorder resulting from progressive destruction of the adrenal cortex. Causes of chronic adrenocortical insufficiency are listed in **Box 24.6**. More than 90% of cases are due to one of four disorders namely: autoimmune adrenalitis, tuberculosis, the acquired immune deficiency syndrome (AIDS), or metastatic cancer.

Clinical features and complications

Usually, the clinical manifestations may not appear until at least 90% of the adrenal cortex has been compromised.

- **Nonspecific symptoms:** These include—progressive weakness, tiredness, fatigue, and anorexia.
- **Gastrointestinal disturbances:** These include—anorexia, nausea, vomiting, weight loss, and diarrhea.
- **Diffuse hyperpigmentation of skin and mucosal surfaces:** In primary adrenal disease, increased plasma levels of ACTH hormone stimulate melanocytes. This causes hyperpigmentation of the skin and mucosal surfaces. In contrast, hyperpigmentation does not occur in secondary

adrenocortical insufficiency because there is low level of ACTH and melanotropic hormone levels are not increased.

- **Electrolyte disturbances and hypotension:** Decreased mineralocorticoid (aldosterone) in primary adrenal insufficiency results in potassium retention and sodium loss. This leads to hyperkalemia, hyponatremia, volume depletion, and hypotension (blood pressures around 80/50 mm Hg). In secondary hypoadrenalism, there is deficient cortisol and androgen output but normal or near-normal aldosterone synthesis.
- **Hypoglycemia:** It may be occasionally observed in infants and children and is due to glucocorticoid deficiency and impaired gluconeogenesis.

Laboratory findings in addison disease

- Increased plasma ACTH
- Short and prolonged ACTH stimulation test: There is no increase in serum cortisol in both tests
- Electrolyte findings: These include hyponatremia and hyperkalemia
- Anemia
- Increased serum blood urea nitrogen (BUN) and creatinine (azotemia) caused by volume loss fluid and electrolytes in urine.
- Fasting hypoglycemia: This is because cortisol is a gluconeogenic hormone and there is loss of gluconeogenesis.

Other investigations: Abdominal computerized tomography (CT) or magnetic resonance imaging (MRI) to determine the size of the adrenal glands. In autoimmune adrenalitis, the adrenal glands are smaller; in tuberculosis glands are large and calcified.

Secondary Adrenocortical Insufficiency

Secondary adrenal insufficiency reflects lack of ACTH. Thus, any disorder of the hypothalamus and pituitary that reduces the output of ACTH can cause secondary adrenal insufficiency.

Causes of secondary adrenocortical insufficiency: Destruction of the pituitary and consequent panhypopituitarism result in secondary adrenal insufficiency and its causes are listed in **Box 24.6**. ACTH deficiency may occur alone or sometimes as one component of panhypopituitarism (deficiencies of multiple pituitary hormones).

Differentiation of Primary and Secondary Adrenocortical Insufficiency

- **Response to exogenous ACTH:**
  - **No response to ACTH: In primary adrenocortical insufficiency**, there is destruction of the adrenal cortex. This prevents increased plasma levels of cortisol response when an exogenous ACTH is administered.
  - **Response to ACTH:** In contrast, **in secondary adrenocortical insufficiency**, there is low serum ACTH and there will be rise in plasma cortisol levels (due to secretion of glucocorticoids by adrenal) in response to exogenous ACTH administration.
- **Hyperpigmentation** (no raised levels of melanocyte-stimulating hormone) and **electrolyte abnormalities** (hyponatremia and hyperkalemia) of primary Addison disease are typically **absent in secondary adrenal insufficiency** because these processes are not regulated by ACTH.

MORPHOLOGY

The appearance of the adrenal glands in adrenocortical insufficiency depends on the underlying cause.

Secondary hypoadrenalism: It is due to hypothalamic or pituitary disease and the appearance of the adrenals depend on the severity of ACTH deficiency. The adrenal glands are small and flattened (moderate to marked reduction in size). A thin rim of atrophic yellow cortex surrounds a central, unaffected/intact medulla.

Microscopically shows atrophy of cortical cells with loss of cytoplasmic lipid (mainly in the zona fasciculata and zona reticularis).

Primary autoimmune adrenalitis: It shows irregular shrinkage of adrenal glands. Microscopically, the adrenal cortex shows only scattered residual cortical cells in a collapsed network of connective tissue. A variable amount of lymphocyte infiltrate is seen in the cortex and this may extend into the subjacent normal adrenal medulla.

Tuberculosis or fungal diseases: The architecture of adrenal gland may be effaced by a granulomatous inflammatory reaction (similar to that observed in other sites of infection).

Metastatic carcinoma: The adrenals are enlarged, and their normal architecture is distorted by the infiltrating neoplasm.

ADRENAL NEOPLASMS

Adrenal neoplasms may arise either from adrenal cortex or adrenal medulla and they may be functional or non-functional.

Adrenocortical Neoplasms

NC: Describe the etiology, pathogenesis, manifestations, laboratory and morphologic features of adrenal neoplasms.

Adrenocortical neoplasms include: Adenomas and carcinomas. Most cortical neoplasms are sporadic. They may be functional (producing steroid hormones) or non-functional. Functional adrenocortical neoplasms produce steroid hormones. To determine whether a cortical neoplasm is functional or not, it is necessary to clinically evaluate the patient and measure the hormone or its metabolites in the laboratory.

Adenoma

Adenomas and carcinomas are about equally common in adults.

Etiopathogenesis: Adenomas are frequently seen as part of syndromes such as MEN1 (multiple endocrine neoplasia 1), Carney complex and McCune Albright syndrome.

MORPHOLOGY

Most of adrenocortical adenomas are non-functional and usually are discovered as incidental ("adrenal incidentaloma") findings at the time of autopsy or during abdominal imaging for an unrelated cause.

Gross

A typical adenoma is small measuring about 1–4 cm, encapsulated, firm, yellow and slightly lobulated. They usually weigh 10–50 g. Cut surface are usually yellow to yellow brown, due to the presence of lipid within the neoplastic cells.

Microscopy

It is composed of clear, lipid-laden (fasciculate type) tumor cells arranged in sheets or nests. These tumor cells appear similar to the cells of normal adrenal cortex. As with other endocrine tumors, pleomorphism may be seen in benign tumors and is not a reliable marker of malignancy.

Clinical Features

Functional adenomas commonly produce hormones, the most common being cortisol and aldosterone. Thus, they are associated with hyperaldosteronism and Cushing's syndrome.

Adrenocortical Carcinomas

They are rare and may occur at any age and are predominant in children.

Etiopathogenesis: Two rare inherited causes of adrenal cortical carcinomas are Li–Fraumeni syndrome and Beckwith–Wiedemann syndrome.

MORPHOLOGY

Gross

They are large, invasive lesions and may be more than 20 cm in diameter and efface the native adrenal gland. Cut surfaces are variegated pink, brown or yellow and poorly demarcated lesions. They sually show areas of necrosis, hemorrhage and cystic change.

Microscopy

They are composed of well-differentiated cells similar to that of cortical adenomas or bizarre, pleomorphic cells, monstrous giant cells, which may be difficult to distinguish from those of an undifferentiated carcinoma metastatic to the adrenal gland. It is to be kept in mind that carcinomas metastatic to the adrenal cortex are more frequent than a primary adrenocortical carcinoma. Adrenal carcinomas have a strong tendency to invade the adrenal vein, vena cava, and lymphatics. It can metastasize to regional and periaortic nodes and through blood to the lungs and other viscera. Bone metastases are not common.

Clinical Features

Functional adrenal cortical carcinomas secrete glucocorticoids and androgens and are associated with virilization.

Laboratory Findings in Adrenocortical Neoplasm

- Blood and urine tests: To measure the amount of adrenal hormones (if it is functional tumor that secrets hormones)
- Biopsy
- Radiological: CT or CAT scan and MRI

ADRENAL MEDULLA

The adrenal medulla is entirely surrounded by the adrenal cortex. It is embryologically, functionally, and structurally distinct from the adrenal cortex. It consists of neuroendocrine cells, or **chromaffin cells derived from the neural crest** (neuroendocrine) and their supporting (sustentacular) cells. The chromaffin cells are so named because **catecholamines in their cytoplasmic granules bind chromium (chromium affinity) salts.** These granules darken and develop a brown-black color after exposure to potassium dichromate (causes oxidation). The adrenal medulla is the major source of catecholamines (epinephrine, norepinephrine).
The most important diseases of the adrenal medulla are neoplasms. These include both neuronal neoplasms (including neuroblastomas and more mature ganglion cell tumors) and neoplasms composed of chromaffin cells (pheochromocytomas).

TUMORS OF THE ADRENAL MEDULLA

NC: Describe the etiology, pathogenesis, manifestations, laboratory and morphologic features of adrenal neoplasms.

Pheochromocytoma

Q. Write short note on pheochromocytoma.

- Pheochromocytomas are **neoplasms composed of chromaffin cells**.
- The **tumor cells synthesize and release catecholamines** and some may produce peptide hormones.
- These tumors are the rare cause of surgically correctable **hypertension**.

Rule of 10s

- About 10% of pheochromocytomas are **extra-adrenal**.
  - They occur in organs of Zuckerkandl and the carotid body.
  - Extra-adrenal pheochromocytomas are called as **paragangliomas.**
- About 10% of sporadic adrenal pheochromocytomas are **bilateral**. In pheochromocytomas associated with familial syndromes up to 50% may be bilateral.

- About 10% of adrenal pheochromocytomas **metastasize** and are **malignant**.
  - Malignancy is more common in extra-adrenal paragangliomas, and tumors developing due to germline mutations.
- About 10% of adrenal pheochromocytomas are **not associated with hypertension**.

Etiology

About 25% of individuals with pheochromocytomas and paragangliomas harbor a germline mutation in one of six known genes *RET, NF1, VHL* and three succinate dehydrogenase complex subunit genes, i.e., SDHB, SDHC, and SDHD.

MORPHOLOGY

Gross (Fig. 24.17)

- **Size: Varies** and may range from small, circumscribed lesions to large hemorrhagic masses.
- **Weight:** Average **100 g**, but may be range from 1–4,000 g.
- Larger tumors are **well-demarcated** and **may** produce a **lobular pattern.** Part of the adrenal gland can be seen over the surface of the tumor.
- **Cut surface**:
  - Small tumors have **yellow tan.**
  - Large tumors show areas of **hemorrhage, necrosis, and cystic changes and typically efface the adrenal gland.**
- **Chromaffin reaction:** When the **fresh tumor tissue is incubated in potassium dichromate solution**; the **tumor turns dark brown in color (Fig. 24.17)** due to oxidation of stored catecholamines. This is termed positive **chromaffin reaction**.

Microscopy (Fig. 24.18)

- **Zellballen pattern:** Tumor consists of **polygonal to spindle-shaped chromaffin cells or chief cells**, clustered with the sustentacular cells **into small nests or alveoli (Zellballen)** separated by a **rich vascular network (Fig. 24.18)**.
- **Cytoplasm:** It has a **fine granular appearance due to** the presence of granules containing **catecholamines**. It is best **demonstrated with silver stains**.
- **Nuclei:** They are **round to oval**, with a stippled **"salt and pepper"** chromatin that is characteristic of neuroendocrine tumors.

Electron microscopy: It shows **membrane-bound, electron-dense secretory granules**.
Immunohistochemistry: Neuroendocrine markers (chromogranin and synaptophysin) are positive in the chief cells**.** The peripheral sustentacular cells stain with antibodies against

S-100, a calcium-binding protein expressed by a variety of mesenchymal cell types.

Criteria for Malignancy

- None of the histologic feature can reliably predict clinical behavior.
- **Histologic features associated with an aggressive behavior and increased risk of metastasis** include:
  - **Numbers of mitoses**
  - **Confluent tumor necrosis**
  - **Spindle cell morphology**
- **Capsular and vascular invasion may be found in benign lesions.**

Spread

- **Definitive diagnosis of malignancy in pheochromocytomas is made only when they develop metastases**.
- The tumor may metastasize to regional lymph nodes as well as more distant sites, including liver, lung, and bone.

Clinical Course

Hypertension in 90% of patients.

- **Paroxysmal episodes:** It is characterized by abrupt, precipitous elevation in blood pressure, associated with tachycardia, palpitations, headache, sweating, tremor, and a sense of apprehension.
- **Isolated paroxysmal episodes:** Less common.
- **Chronic**, sustained elevation in blood pressure punctuated by paroxysms.

The elevations of blood pressure are induced by the sudden release of catecholamines. This may precipitate congestive heart failure, pulmonary edema, myocardial infarction, ventricular fibrillation, and cerebrovascular accidents.

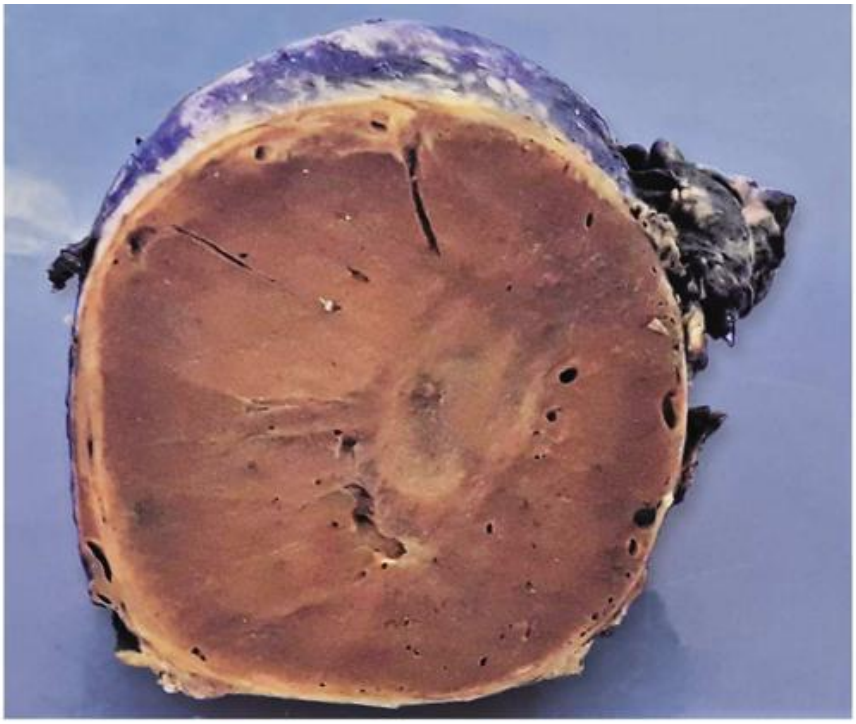


Fig. 24.17: Cut section of pheochromocytomas showing dark brown color after incubation in potassium dichromate solution.

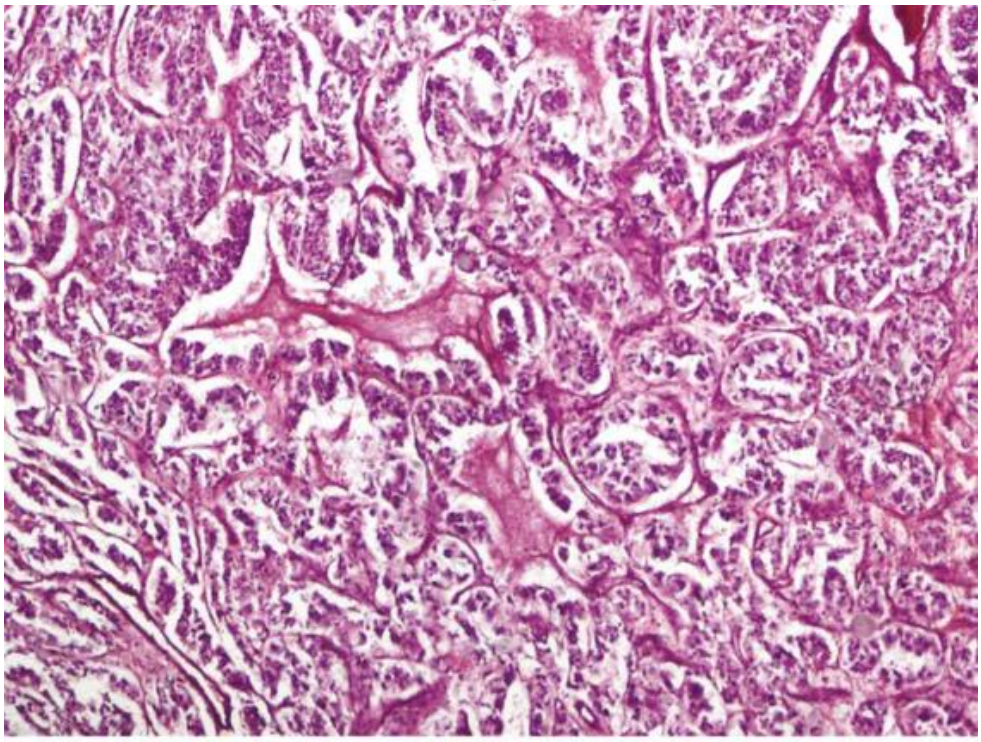


Fig. 24.18: Pheochromocytoma composed of characteristic nests of cells (Zellballen) with abundant cytoplasm.

Complications

Cardiac complications are called **catecholamine cardiomyopathy**, or catecholamine-induced myocardial instability and ventricular arrhythmias.

Laboratory Diagnosis

Demonstration of **increased urinary excretion of free catecholamines and their metabolites**, such as vanillylmandelic acid and metanephrines.

Pheochromocytoma: Chromaffin cells that synthesize and release catecholamines.

Pheochromocytoma: Majority is benign, unilateral and occur in adrenal medulla. Extra-adrenal pheochromocytomas are called as paragangliomas.

Zellballen pattern: Feature of the carotid tumor which is a prototype of parasympathetic paraganglioma.

Definitive diagnosis of malignancy in pheochromocytomas: Metastases.

- Most common cancer of childhood: Leukemia (30%)>Brain tumor (22%).
- Most common solid tumor of childhood: Brain tumor.
- Most common soft tissue tumor in infants and children: Rhabdomyosarcoma.

Neuroblastoma

Q. Write short note on neuroblastoma.

- The most **important neuroblastic tumor.** Neuroblastic tumors are group of tumors of the sympathetic ganglia and adrenal medulla that are derived from primordial neural crest cells.
- **Age: Most common extracranial childhood solid tumor**. It is most frequently diagnosed during infancy. It is the second most common solid malignancy of childhood after brain tumors.
- **Sporadic and familial types**:
  - Mostly occur sporadically, but 1–2% is familial.
  - **Germline mutations in** the **anaplastic lymphoma kinase *(ALK)*** gene are observed in familial neuroblastoma.
  - Somatic gain-of-function *ALK* mutations are also found in a few sporadic neuroblastomas.

- Tumors having *ALK* mutations respond to drugs that target their activity.

- Amplification of *N-MYC* (*MYC* family of proto-oncogenes) is found in about 30% of neuroblastomas (*refer* **Fig. 7.15** and pages 205-6).

MORPHOLOGY

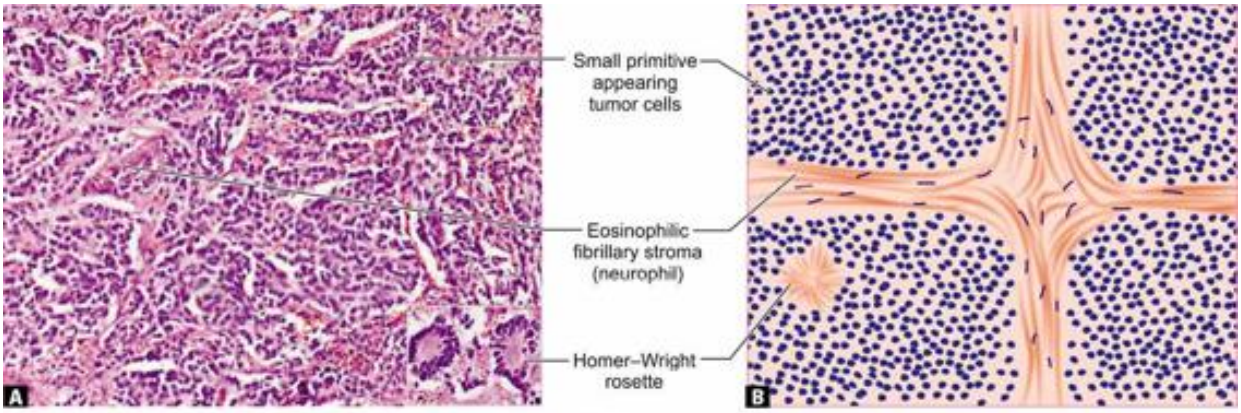
Gross

- **Site:** About 40% of neuroblastomas occur in the **adrenal medulla**.
  - **Other sites:** It may develop along the sympathetic chain.
    - **Paravertebral region of the abdomen** (25%).
    - **Posterior mediastinum** (15%).
    - Pelvis, the neck, and brain (**cerebral neuroblastomas**).
- **Size: Vary** from minute nodules (as in situ lesions) to large tumors weighing 1 kg.
- **Nature: Majority** are **silent** and **regress spontaneously**.
- May be **sharply demarcated** by a fibrous pseudocapsule **or infiltrate** the surrounding structures (kidneys, renal vein, and vena cava, and aorta).
- **Cut section: Soft, brain-like tissue and gray-tan**. **Large tumors** may show areas of **necrosis, cystic change** and **hemorrhage**.

Microscopy (Figs. 24.19A and B)

Q. Write a short answer on microscopic features of neuroblastoma.

- **Tumor cells:** They are arranged in solid sheets. The tumor cells appear as:
  - **Small, primitive tumor cells containing dark nuclei**
  - **Scant cytoplasm with poorly defined cell borders.**
- Mitotic activity, karyorrhexis (breakdown of nuclear material), and pleomorphism may be prominent.
- These tumors may be **difficult to differentiate morphologically from** other **small round blue cell tumors.**
- **Background:** Shows a faintly **eosinophilic fibrillary material** (**neuropil**), which represents the neuritic processes of the primitive neuroblasts.
- **Homer–Wright pseudorosettes:** May be seen which consist of tumor cells concentrically arranged about a central space filled with neuropil. Because of the absence of an actual central lumen they are called pseudorosettes.



Figs. 24.19A and B: (A) Photomicrograph; and (B) Diagrammatic. Neuroblastoma consists of small primitive appearing cells with scant cytoplasm embedded in a finely fibrillar matrix. Inset of A shows two Homer–Wright rosettes.

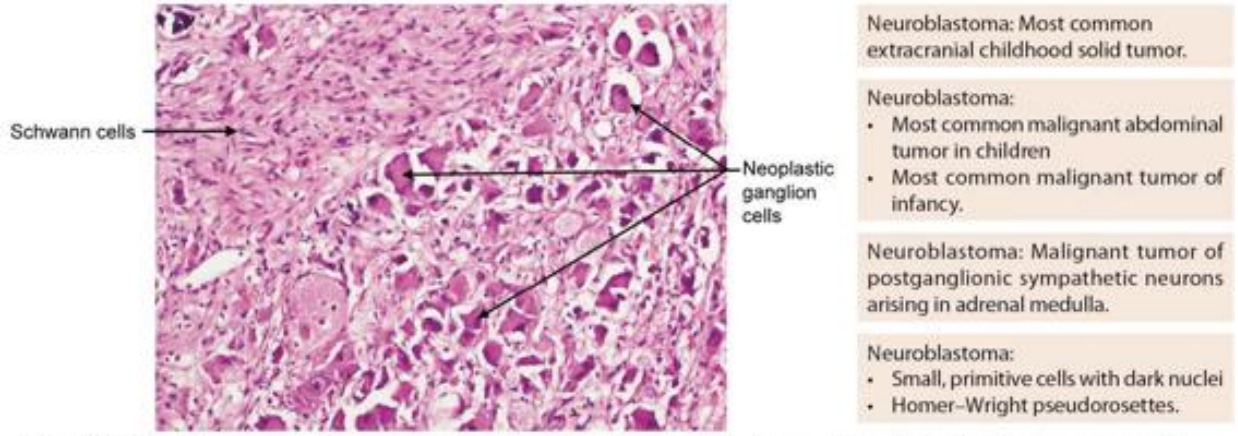


Fig. 24.20: Ganglioneuroma with numerous large cells with vesicular nuclei and abundant eosinophilic cytoplasm, representing neoplastic ganglion cells (right half). Spindle-shaped Schwann cells are present in the background stroma.

Immunohistochemistry: Neuron-specific enolase positive.

Electron microscopy: It shows **small, membrane-bound, catecholamine-containing secretory granules in the cytoplasm**.

Maturation: Neuroblastomas have several unique features in their natural history. Some neoplasms may show **spontaneous or induced** (by therapy) **maturation**. These differentiated lesions include ganglioneuroblastoma and ganglioneuroma. Maturation is **characterized by the presence of Schwannian stroma** composed of organized fascicles of neuritic processes, mature Schwann cells, and fibroblasts. This type of stroma is required for the designation of ganglioneuroblastoma and ganglioneuroma. The presence of ganglion cells is not a criteria for maturation.

- **Ganglioneuroblastoma:** It consists of **primitive neuroblasts** that may be **admixed with ganglion cells** in various stages of maturation. The

ganglion cells appear as large cells with abundant cytoplasm having large vesicular nuclei and a prominent nucleolus.

- **Ganglioneuroma (Fig. 24.20):** It is a **more mature tumor than ganglioneuroblastoma**. It contains many **more large cells resembling mature ganglion cells** with few if any residual neuroblasts. Maturation of neuroblasts into ganglion cells is usually accompanied by the appearance of **Schwann cells**. The presence of **Schwannian stroma is associated with** a **favorable outcome**.

Spread of Tumor

- Local infiltration
- Lymph node spread
- Bloodspread: Liver, lungs, bone marrow, and bones.

Clinical Course

- Children below 2 years of age: Usually present as **large abdominal masses, fever, and weight loss**.
- Older children: Symptoms develop due to metastases such as bone pain, respiratory symptoms, or gastrointestinal complaints.
- Ganglioneuromas may present either as asymptomatic mass or symptoms related to compression.

Laboratory Finding

- **Majority** (~90%) of neuroblastomas, **secrete catecholamines** (similar to pheochromocytomas) → raised blood levels of catecholamines (hypertension is less frequent).
- **Raised urine levels** of the **metabolites vanillylmandelic acid** (VMA) and **homovanillic acid** (HVA).

Course: It is extremely variable.

Prognostic Factors

Many factors influence prognosis and the most important being the stage of the tumor and the age of the patient.

- **Staging of neuroblastomas:** It is importance in establishing a prognosis.
- **Morphology:** It is an **independent prognostic factor**. Accordingly, tumors are divided into favorable and unfavorable histologic subtypes.

- **Age and stage:** Children **younger than 18 months of age** have **excellent prognosis** regardless of the stage of the neoplasm.
- **Amplification of the *N-MYC* oncogene → high-risk category**, irrespective of age, stage, or histology.
- **Ploidy of the tumor cells:** It is of **prognostic value in children younger than 2 years** and loses its prognostic significance in older children.

Neuroblastoma: Child with large abdominal mass.

Spontaneous regression of tumor is seen in: Neuroblastoma.

Neuroblastoma: Raised urine levels of the metabolites of catecholamines
- Vanillylmandelic acid (VMA)
- Homovanillic acid (HVA)

Catecholamines are increased in:
- Neuroblastoma
- Pheochromocytoma

Amplification of the N-MYC oncogene: High-risk neuroblastoma.

PITUITARY

Tumors of pituitary may be benign or malignant.

PITUITARY ADENOMAS

Q. Write short note on pituitary adenoma.

The most common cause of hyperpituitarism is a functioning (hormone producing) adenoma of anterior pituitary. Less commonly due to hyperplasia and carcinomas of the anterior pituitary, secretion of hormones by few extrapituitary tumors and few disorders of hypothalamus.

Classification

Pituitary adenomas may be functional or non-functional.

1. **Functional tumors:** They are classified depending on the hormone(s) produced by the tumor cells (detected by immunohistochemical stains on tissue sections). The hormones lead to endocrine abnormalities. They predominantly produce a single hormone. Some may secrete two different hormones (e.g., growth hormone and prolactin) and rarely they may produce many hormones.
   - **Lactotroph adenomas**: **Prolactin-secreting lactotroph adenomas are the most common type (about 30%).** Hyperprolactinemia clinically causes amenorrhea, galactorrhea, loss of libido, and infertility.
   - **Somatotroph adenomas: Growth hormone–secreting somatotroph adenomas are the second most common type** of functioning pituitary adenoma. They **produce gigantism in children or acromegaly in adults.**
   - **Corticotroph adenomas**: **Excess production of ACTH leads to adrenal hypersecretion of cortisol and the development of hypercortisolism (Cushing syndrome).**
2. **Non-functional tumors:**
   - **Mass effects:** They do not produce any hormone but produce mass effects.
   - **Silent tumors:** The hormone production can be demonstrated at the tissue level only and are without clinical manifestations of hormone excess.

Pathogenesis

Many genetic abnormalities have been identified. These include:

- **G-protein mutations:** G-proteins play a major role in signal transduction, transmitting signals from cell surface receptors to intracellular effectors, which in turn generate second messengers.
- **Genetic predisposition:** About 5% of pituitary adenomas have genetic predisposition and 4 genes have been identified as a cause of familial pituitary adenomas. These include *MEN1*, *CDKN1B*, *PRKAR1A*, and *AIP*. These genes regulate transcription and the cell cycle.
- **Molecular abnormalities:** The *aggressive tumors are associated with aberrations in cell cycle checkpoint genes* (e.g., overexpression of cyclin

D1), mutations of *TP53,* and epigenetic silencing of the retinoblastoma gene *(RB)*.

MORPHOLOGY

Gross

Usually these tumors are well-circumscribed and soft. Small tumors may be confined to the sella turcica, whereas larger tumors may compress the optic chiasm and adjacent structures. About 30% of tumors are nonencapsulated and infiltrate adjacent bone and dura. There may be foci of hemorrhage and/or necrosis in larger adenomas. Depending on the size they are classified as:

- **Microadenomas:** Size less than 1 cm in diameter
- **Macroadenomas:** Size more than 1 cm in diameter. Non-functioning adenomas usually come to clinical attention at a later stage and are, therefore, likely to be macroadenomas. They may encroach upon and destroy adjacent anterior pituitary parenchyma and may produce hypopituitarism.

Microscopy

Both functional and non-functioning pituitary adenomas usually are composed of a single cell type. The tumor consists of relatively uniform, polygonal cells arranged in sheets, cords, or papillae. The cytoplasm may be acidophilic, basophilic, or chromophobic, depending on the type and amount of secretory product within the cell. The nuclei of the tumor cells may be uniform or pleomorphic and mitotic figures are very few. The connective tissue or reticulin between the tumor cells is minimal. The functional status of the adenoma cannot be predicted by the microscopic appearance.

MULTIPLE ENDOCRINE NEOPLASIA SYNDROMES

Q. Write short note on MEN (multiple endocrine neoplasia).

Definition: MEN syndromes are a group of inherited diseases caused due to proliferative lesions (hyperplasias, adenomas, and carcinomas) of multiple endocrine organs.

Characteristic Features

- **Age:** These tumors develop at a younger age compared to sporadic cancers.
- **Multiple:** They occur in multiple endocrine organs, either synchronously (at the same time) or metachronously (at different times).
- **Multifocal:** Even in one organ involved, the tumors are often multifocal.
- **Clinical features:** They are usually preceded by an asymptomatic stage of endocrine hyperplasia of cell of origin of the tumor (e.g., in MEN-2 there is C cell hyperplasia in the thyroid parenchyma adjacent to medullary thyroid carcinomas).
- **Nature of tumor**: Usually, these tumors are more aggressive and recur compared to sporadic endocrine tumors.

There are two main types of MEN namely MEN type 1 and MEN type 2.

Multiple Endocrine Neoplasia Type 1

MEN-1 syndrome is caused due to germline mutations in the *MEN1* tumor suppressor gene. MEN1 gene encodes a protein called menin that is a component of several different transcription factor complexes. Loss of menin function causes deregulation of the corresponding binding partners and leads to endocrine neoplasia.

Organs Involved

3 Ps: Most commonly involves (1) parathyroid, (2) pancreas, and (3) pituitary.

1. **Parathyroid: Primary hyperparathyroidism** is the most common feature of MEN-1 (80–95%) and is the presenting feature in most patients. Usually it is detected by 40 to 50 years of age. **Parathyroid shows hyperplasia and adenomas**.
2. **Pancreas: Endocrine tumors of the pancreas** are the leading cause of death in patients with MEN-1. These tumors usually are aggressive and present with metastatic disease. Pancreatic endocrine tumors often are functional (i.e., secrete hormones). Zollinger-Ellison syndrome

associated with gastrinomas, and hypoglycemia, associated with insulinomas, is common endocrine manifestations.

3. **Pituitary:** The most frequent pituitary tumor in patients with MEN-1 syndrome is a **prolactin-secreting macroadenoma**. In some cases, acromegaly develops in association with somatotropin-secreting tumors.

Multiple Endocrine Neoplasia Type 2

MEN-2 syndrome consists of two distinct groups/types of disorders (type 2A and 2B) characterized by the presence of activating (i.e., gain-of-function) **mutations of the *RET*** proto-oncogene at chromosomal locus 10q11.2. MEN-2 is inherited in an **autosomal dominant disorder**. All persons with germline RET mutations should undergo prophylactic thyroidectomy to prevent the inevitable development of medullary carcinomas.

Multiple Endocrine Neoplasia Type 2A

Commonly affected organs in MEN-2A are:

- **Medullary carcinoma of the thyroid:** It is found in almost all untreated cases. It usually manifest during the first 2 decades of life. The tumors are usually multifocal and associated with foci of C cell hyperplasia in the adjacent thyroid.
  - **Familial medullary thyroid cancer:** It may be seen in a variant of MEN-2, without the other features of MEN-2A. They usually occur at an older age and follow a more indolent course.
- **Pheochromocytomas of adrenal medulla:** Observed in 50% of the patients and no more than 10% of these are malignant.
- **Hyperplasia of parathyroid gland:** Found in about 10–20% of patients and they manifest with features of primary hyperparathyroidism.

Multiple Endocrine Neoplasia Type 2B

In MEN type 2B, there is a single amino acid change in RET, which is distinct from the mutations observed in MEN-2A.

Features

- **Medullary thyroid carcinomas**: These patients have medullary thyroid carcinomas, that are usually multifocal and more aggressive than in

MEN-2A.

- **Pheochromocytomas**.
- **No primary hyperparathyroidism in MEN-2B**.
- **Extraendocrine manifestations:** These are characteristic of MEN-2B. These include ganglioneuromas of mucosa (e.g., gastrointestinal tract, lips, tongue) and a **marfanoid habitus** (long bones appear similar to that in Marfan syndrome).

DIABETES MELLITUS

INTRODUCTION

CC: Classify and describe the epidemiology, etiology, pathogenesis, pathology, clinical laboratory features, complications and progression of diabetes mellitus.

Definition: Diabetes mellitus is a **group of metabolic disorders having features of hyperglycemia**.

The prevalence of diabetes is increasing sharply in the developing countries because of more sedentary lifestyles. India and China have the largest prevalence of diabetics.

Glucose Levels and its Significance (Table 24.3)

Normally, the **blood glucose levels** are maintained in a very narrow range of **70–120 mg/dL**. Glycemia can be classified into three categories: euglycemia (normal), pre-diabetes (impaired) and diabetes **(Table 24.3).** The various definitions according to the American Diabetes Association (ADD) are as follows:

TABLE 24.3: WHO criteria levels of blood glucose in normal, prediabetes and diabetes.

	Euglycemic	***Prediabetes***	***Diabetes***
Fasting glucose level	<**100** mg/dL	>**100** mg/dL but <**126** mg/dL	>**126** mg/dL on more than one occasion
OGTT	<**140** mg/dL	>**140** mg/dL but <**200** mg/dL	>**200** mg/dL

1. **Euglycemic**: Individuals are considered to be euglycemic, when:
   - **Fasting glucose** level is **less than 100 mg/dL**, or
   - Glucose level **less than 140 mg/dL** following an **OGTT**.
2. **Impaired glucose tolerance (prediabetes):** It is defined as condition in which there is **impaired glucose tolerance**, but elevated **blood sugar does not reach the criterion accepted for an outright diagnosis of diabetes**. The criteria for diagnosis are:
   - A fasting plasma glucose between 100 and 125 mg/dL ("impaired fasting glucose")
   - 2-hour plasma glucose between 140 and 199 mg/dL following a 75-g glucose OGTT, and/or
   - A glycated/glycosylated hemoglobin (Hb_{AIC}) level between 5.7% and 6.4%.

Risks in prediabetes: (1) **Progression to frank diabetes** over time and (2) **cardiovascular disease.**

3. **Diabetes:** WHO criteria for the diagnosis of diabetes mellitus and criteria for diagnosis of diabetes (American Diabetes Association 2016) are listed in **Boxes 24.3 and 24.4** respectively.

Classification

Q. Classify diabetes mellitus.

Diabetes is classified according to etiopathogenesis into different groups **(Box 24.7)** but **majority of cases fall into one of two** broad classes namely: **type 1 and type 2**.

Normal fasting blood glucose level: 70–120 mg/dL.

Prediabetes: Impaired glucose tolerance, but elevated blood sugar does not reach the criterion accepted for diagnosis of diabetes.

TYPE 1 DIABETES

- Type 1 diabetes (T1D) Accounts for ~**5–10%** of all cases.
- **Age:** Most common in **childhood** (**younger than 20 years** of age). Since, it can develop at any age, the term "juvenile diabetes" should be avoided.

Causes

- **Autoimmune disease** characterized by:
  - **Pancreatic β-cell destruction**
  - **Absolute deficiency of insulin**.
- **Idiopathic:** It is a rare form in which there is no evidence for autoimmunity.

Pathogenesis of Type 1 Diabetes Mellitus (Flowchart 24.3)

Q. Write short note on pathogenesis of type 1 diabetes mellitus.

Involves interplay of both genetic susceptibility and environmental factors.

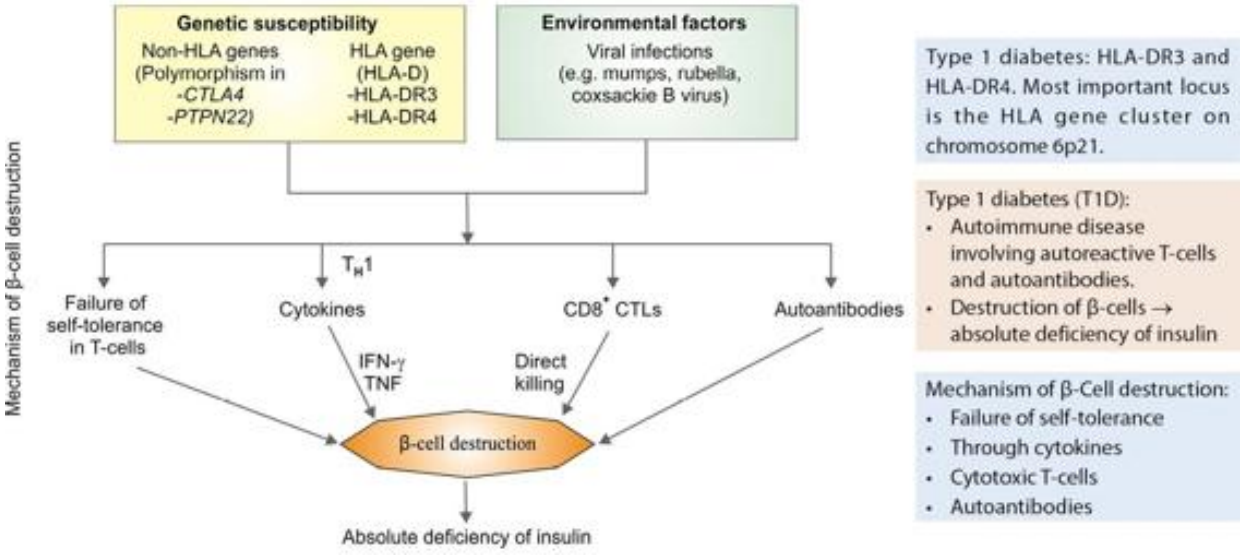
- **Genetic susceptibility:**
  - Incidence of type 1 diabetes is greater in twins of affected individuals than in the general population, and greater in monozygotic than in dizygotic twins.
  - **HLA genes:** About 95% of patients with type 1 diabetes have either human leukocyte antigen **(HLA)-DR3 or HLA-DR4, or both**, compared with the general population.
  - **Non-HLA genes:** It includes polymorphism in
    - Gene coding insulin.
    - *CTLA4* and *PTPN22* genes (involved in immune tolerance) are associated with excessive T-cell activation.
    - CD25.
- **Environmental factors:**
  - **Viral infections:** They **may trigger islet cell destruction** and associations have been found between type 1 diabetes and infection with ***mumps, rubella, coxsackie B, or cytomegalovirus***. **Three different mechanisms** explain the role of viruses in the induction of autoimmunity.
    1. **Release of hidden or sequestered antigens:** Viral infections cause islet injury and inflammation, thereby release the sequestered β-cell antigens and **activates autoreactive T-cells**.

2. **Molecular mimicry: Viruses** can **produce proteins that mimic β-cell antigens**. The **immune response** produced against the viral protein may **cross-reacts with the self-tissue (β-cell antigens)**.

BOX 24.7: Classification of diabetes mellitus.

1. **Type 1 diabetes**: Immune-mediated (type 1A) or idiopathic (type 1B). Due to beta cell destruction, usually leading to absolute insulin deficiency.
2. **Type 2 diabetes:** Combination of insulin resistance and beta cell dysfunction.
3. **Genetic defects of β-cell development or function:** Maturity-onset diabetes of the young (MODY), neonatal diabetes.
4. **Genetic defects in insulin action:** Insulin receptor mutations.
5. **Exocrine pancreatic defects**: Chronic pancreatitis, hemochromatosis, pancreatectomy, cystic fibrosis.
6. **Endocrinopathies**: Acromegaly, Cushing syndrome, hyperthyroidism, pheochromocytoma.
7. **Infections:** Cytomegalovirus, coxsackie B virus, congenital rubella.
8. **Drugs:** Glucocorticoids, thyroid hormone, β-Adrenergic agonists.
9. **Genetic syndromes associated with diabetes:** Down syndrome, Klinefelter syndrome, Turner syndrome.
10. **Gestational diabetes mellitus:** Diabetes associated with pregnancy.
11. **Latent autoimmune diabetes in adults** (LADA).

Flowchart 24.3: Pathogenesis of type 1 diabetes mellitus.



3. **Sharing of antigen epitopes:** First viral infections by a **predisposing virus**, during early in life might persist in the β-cells. Subsequent **re-infection** with a related virus known as **precipitating virus**, that shares antigenic epitopes may leads to an immune response against the β-cells.

Mechanisms of β-Cell Destruction

The autoimmune damage starts many years before the disease becomes clinically evident. **Hyperglycemia and ketosis occur after more than 90% of the β-cells have been destroyed**. Various mechanisms include:

- **Failure of self-tolerance in T-cells:** It is the **basic abnormality** in type 1 diabetes.
  Cause: Failure of self-tolerance may be due to combination of:
  - Defective clonal deletion of self-reactive T-cells in the thymus.
  - Defects in the functions of regulatory T-cells.
  - Resistance of effector T-cells to suppression by regulatory cells.
- **Through cytokines:** T_H1 cells may destroy β-cells by secreting cytokines, including **IFN-γ** and **TNF**.
- **Directly by cytotoxic T-cells**: The $CD8^+$ CTLs may directly kill β-cells.
- **Autoantibodies against b-cell antigens** (e.g., islet antigens b-cell enzymes): They are found in 70–80% of patients with type 1 diabetes, and in asymptomatic family members. It is not known whether autoantibodies cause injury or are result of islet injury.

Self-tolerance: Absence of immune response to an individual's own antigens. It is responsible for our ability to live in harmony with our cells and tissues.

Type 1 DM: Autoantibodies include anti-insulin, anti-GAD, anti-ICA512.

TYPE 2 DIABETES

Q. Discuss the pathogenesis of type 2 diabetes mellitus. Mention its major long-term complications.

- Accounts for ~**90–95% of diabetic patients**.
- **Majority** of patients are **overweight**.
- Though formerly known as "adult-onset," it is now found in children and adolescents.

Pathogenesis of Type 2 Diabetes Mellitus (Flowchart 24.4)

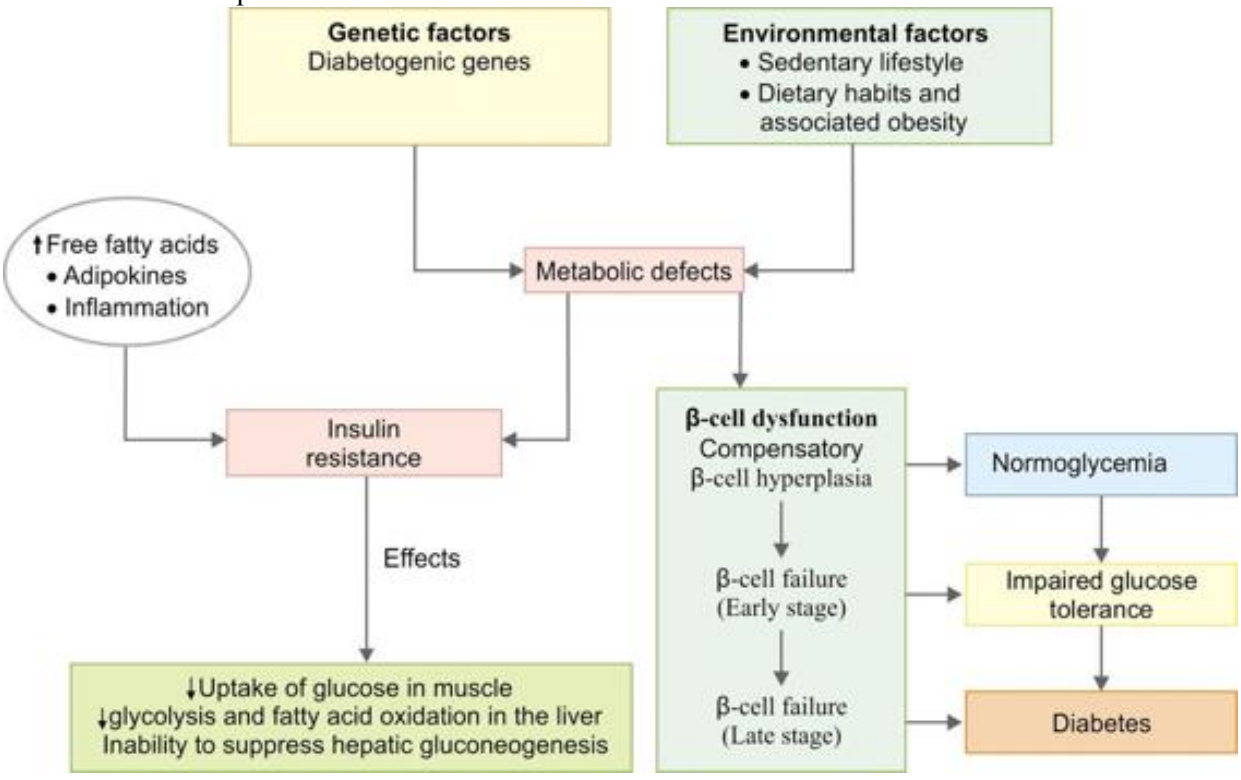
Type 2 diabetes is a **multifactorial disease**.

- **Environmental factors** play a role and includes:
  - **Sedentary lifestyle.**
  - **Dietary habits and associated obesity particularly central or visceral obesity.**
- **Genetic factors:**
  - Type 2 diabetes has a **concordance rate of 35–60% in monozygotic twins compared with 17–30% in dizygotic twins**.
  - **Lifetime risk** for type 2 diabetes in an offspring is more than double **if both parents** are **affected**.
  - **Diabetogenic genes:** They have been found.
  - There is **no evidence of an autoimmune basis**.

Metabolic Defects in Type 2 Diabetes

Two important metabolic defects are:

Flowchart 24.4: Pathogenesis of type 2 diabetes. Insulin resistance associated with obesity is induced by free fatty acids, adipokines, and chronic inflammation in adipose tissue. Insulin resistance causes β-cells of pancreas to undergo compensatory hyperplasia and the resulting hypersecretion of insulin maintains normoglycemia. However, at some point, β-cell compensation is followed by β-cell failure, and diabetes develops.



Type 2 diabetes (T2D): Multifactorial disease caused by insulin resistance and dysfunction of β-cells → relative deficiency of insulin.

Metabolic defects in type 2 diabetes:
- Insulin resistance
- β-cell dysfunction.

Type 2 diabetes (T2D): Obesity has an important role in the development of insulin resistance.

Insulin Resistance

Definition: Insulin resistance is **the decrease/failure of target (peripheral) tissues to insulin action. Main factors** in the development of insulin resistance is **obesity**.

- **Obesity and insulin resistance:** Obesity is associated with type 2 diabetes and the **visceral obesity is found in more than 80% of patients**.
  - **Amount of fat:** Insulin resistance is found even in simple obesity without hyperglycemia. The **risk for diabetes increases as the body mass index** (a measure of body fat content) **increases**.
  - **Distribution of body fat:** It has an effect on insulin sensitivity. **Central obesity** (abdominal fat) is **more associated with insulin resistance than are peripheral** (gluteal/subcutaneous) fat depots.
- **Causes of insulin resistance in obesity:** It is induced by (i) nonesterified free fatty acids, (ii) adipokines, (iii) chronic inflammation in adipose tissue, and (iv) activation of PPAR γ.
  - **Excess of free fatty acids (FFAs):** There is an **inverse correlation between fasting plasma FFAs and insulin sensitivity**.
    - Obese individuals have excess circulating FFAs → gets deposited as triglycerides in muscle and liver tissues → results in markedly increased level of intracellular triglycerides.
    - Central adipose tissue is more "lipolytic" than peripheral sites, and central obesity is associated with insulin resistance.
    - Excess intracellular FFAs → increases the fatty acid oxidation pathways → leads to accumulation of cytoplasmic toxic intermediates such as diacylglycerol (DAG) and ceramide →

reduce the insulin signaling, thereby increases gluconeogenesis (insulin normally inhibits hepatic gluconeogenesis).

- **Adipokines: Adipose tissue** acts as a functional endocrine organ. It **secrets variety of proteins** into the systemic circulation, which are **termed adipokines** (or adipose cytokines). Adipokines can divided into:
  - Prohyperglycemic adipokines, for example, resisting, retinol binding protein 4 (RBP4).
  - Antihyperglycemic adipokines: Leptin and adiponectin improve insulin sensitivity. In obesity, adiponectin levels are reduced, which contributes to insulin resistance.
- **Inflammation:** Proinflammatory cytokines are secreted as a response to excess FFAs and glucose. They cause both insulin resistance and β-cell dysfunction. Excess FFAs within macrophages and β-cells activate the inflammasome (a multiprotein cytoplasmic complex) and secret cytokine interleukin IL-1β. IL-1β secrets additional proinflammatory cytokines from macrophages, islet cells, and other cells and release into the circulation. IL-1 and other cytokines act on the major sites of insulin action and produce insulin resistance. Thus, excess FFAs can directly disturb insulin signaling within peripheral tissues and also indirectly through the secretion of proinflammatory cytokines.

β-Cell Dysfunction

In type 2 diabetes, β-cell dysfunction manifests as **inadequate insulin secretion by the pancreatic β-cells (relative insulin deficiency)** in association with insulin resistance and hyperglycemia. β-cell dysfunction is multifactorial in origin.

- **Obesity and β-cell dysfunction:**
  - **Compensatory β-cell hyperplasia: Pancreatic β-cells initially respond** to long-term demands of peripheral insulin resistance **by undergoing compensatory hyperplasia leading to increased insulin secretion (hypersecretion)**. Thus, the insulin secretion is initially higher for each level of glucose than in controls. This hyperinsulinemic state can compensate for peripheral resistance and maintain normal blood glucose for years.

- **β-cell failure (early stage):** However, at some point, β-cells exhaust their capacity to adapt. β-cell compensation **cannot maintain normal blood sugar level**. This stage, the patient develop impaired glucose tolerance.
- **β-cell failure (late stage):** The early stage of β-cell failure is followed by decreased insulin secretion, hyperglycemia and frank diabetes develops.

- **Molecular mechanisms of β-cell dysfunction:**
  - **Excess FFAs and decreased insulin signaling** (lipotoxicity): It may be responsible for both insulin resistance and β-cell failure.
  - **Chronic hyperglycemia ("glucotoxicity").**
  - An abnormal "incretin effect," with reduced secretion of hormones that promote insulin release (e.g., GIP and GLP-1).
  - **Direct toxicity by amyloid deposits:** In long-standing type 2 diabetes, amyloid deposits in islets is seen in more than 90% of cases. The islet **amyloid protein may be directly cytotoxic to islets** causing β-cell dysfunction.

Type 2 diabetes: Insulin resistance develops before hyperglycemia and is usually associated with compensatory hyperfunction of β-cell and hyperinsulinemia.

Insulin resistance: Failure of target tissues to respond normally to insulin.

Central obesity is associated with type 2 diabetes.

Monogenic diabetes: Mutation of PPARG.

Type 2 diabetes (T2D): Obesity causes insulin resistance by:
- Increasing FFAs
- Reducing antihyperglycemic adipokines
- Secreting proinflammatory cytokines which increase cell stress.

β-cell dysfunction in DM: Initially compensatory β-cell hyperplasia followed by β-cell failure.

PATHOGENESIS OF THE COMPLICATIONS OF DIABETES

Q. Write short note on pathogenesis of complications in diabetes mellitus.

Pathogenesis of complications of diabetes is multifactorial and includes:

- Hyperglycemia (glucotoxicity) is the main mediator
- Insulin resistance (described already)
- Obesity (described already).

Hyperglycemia

- **Control of blood sugar level** (glycemic control) **can reduce the long-term complications** of diabetes.
- **Glycemic control is assessed by** estimation of **glycosylated hemoglobins (Hb_{A1C})**. Hb_{A1C} is formed by addition of glucose to hemoglobin in red cells. Hb_{A1C} should be maintained below 7% in diabetic patients and its **measurement is helpful in knowing the glycemic control over the lifespan of a red cell** (120 days).

Organ Damage by Hyperglycemia

- The chronic hyperglycemia and the metabolic disorders cause secondary **damage in multiple organ** systems.
- Common organs damaged are **kidneys** (end-stage renal disease)**, eyes** (adult-onset blindness)**, nerves,** and **blood vessels (**gangrene of lower extremity).

Effects of Hyperglycemia

Harmful effects of persistent hyperglycemia on peripheral tissues can be brought out by **three distinct metabolic pathways.**

Formation of Advanced Glycation End Products (AGEs) (Fig. 24.21)

In diabetics, glucose binds to proteins nonenzymatically and **is termed nonenzymatic glycosylation**.

- **Initial products:** The initial products of nonenzymatic glycosylation are chemically known as Schiff bases **are labile and can dissociate rapidly.**
- **Later products:** The initial labile products form → **stable advanced glycation end products** (AGEs). **The rate of AGE formation is markedly increased in the presence of hyperglycemia**.

- **Effects of AGEs:** It may be brought out by binding of AGEs to its specific receptor (RAGE-receptor for AGE) or by its direct action.
  - **Through RAGE: AGEs binds to RAGE on inflammatory cells (macrophages and T-cells), endothelial cells, and vascular smooth muscle cells**. **In the vascular compartment**, AGE-RAGE has following actions:

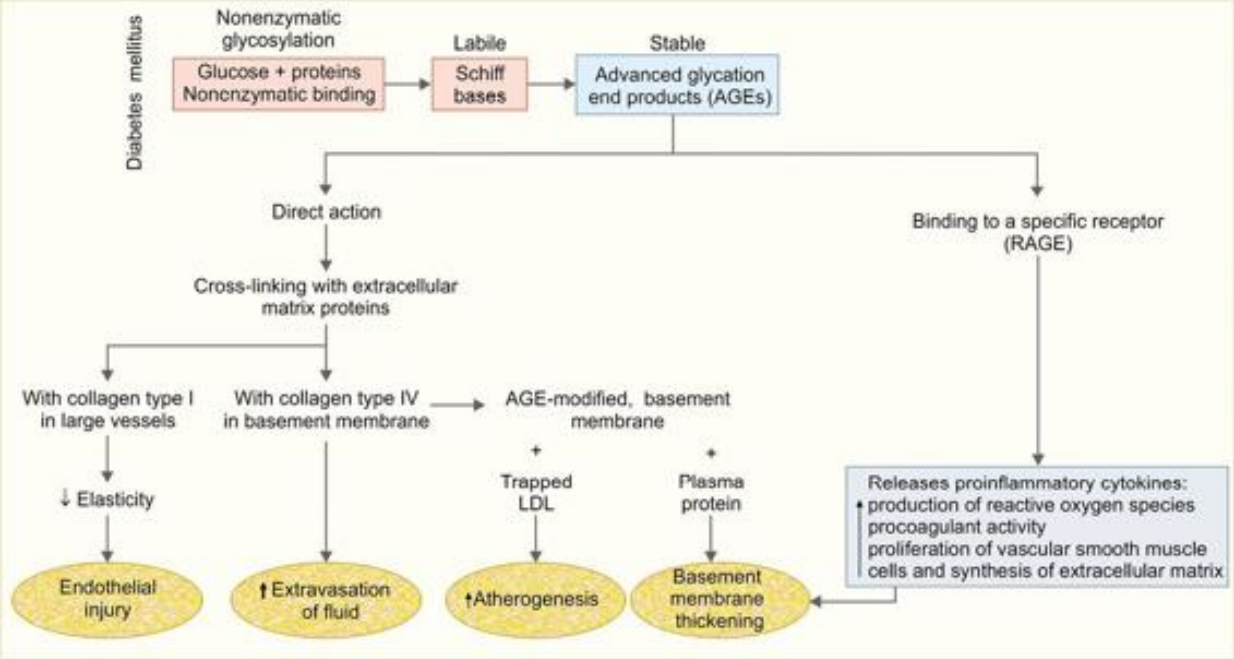


Fig. 24.21: Pathogenetic mechanism of injury advanced glycation end products (AGEs) in diabetes mellitus. (RAGE: receptor for AGE; LDL: low-density lipoproteins).

    - Releases proinflammatory cytokines and **growth factors** from intimal macrophages.
    - Production of reactive oxygen species in endothelial cells.
    - **Increases procoagulant activity** on endothelial cells and macrophages.
    - **Increases proliferation of vascular smooth muscle cells** and synthesis of extracellular matrix.
  - **Direct effect of AGE:** AGEs can **directly cross-link extracellular matrix proteins** and its consequences are:
    - Resist proteolytic digestion → results in decreased removal and increased deposition of protein → **trapping of proteins in the glycosylated collagen of the basement membrane → basement membrane thickening**.
    - **Trapping of LDL in the intima → accelerating atherogenesis**.

The effects of cross-linking of AGEs in the vasculature are presented in **Table 24.4**.

Activation of Protein Kinase C (PKC)

In patients with hyperglycemia, **intracellular hyperglycemia stimulates synthesis of diacylglycerol (DAG) from glycolytic intermediates**. DAG **activates intracellular protein kinase C**.

Consequences of PKC activation: It produces:

- **Proangiogenic molecules** such as **vascular endothelial growth factor (VEGF)** → causes **neovascularization** characteristic of **diabetic retinopathy**.
- **Profibrogenic factors** (e.g., **TGF-β**) → results in **increased production of extracellular matrix and basement membrane material**.
- Plasminogen activator inhibitor (PAI-1) → **reduces fibrinolysis** and **favors vascular occlusion** (by forming atherosclerotic plaques or thrombus).

Disturbances in Polyol Pathways

- **Some tissues** (e.g., nerves, lenses, kidneys, blood vessels) **do not require insulin for glucose transport**.
- **Persistent hyperglycemia increases** the **intracellular glucose in these tissues → excess intracellular glucose is metabolized by the enzyme aldose reductase → to sorbitol (polyol)** → to fructose.
- This **reaction uses NADPH** (the reduced form of nicotinamide dinucleotide phosphate) as a cofactor.
- NADPH is also necessary for the regeneration of reduced glutathione (GSH). GSH protects against injury by free radicals.

TABLE 24.4: Effects of cross-linking of AGEs in the vasculature.

Cross-linking with	*Effects*
Collagen type I in large vessels	Decreases elasticity and predispose them to tear and endothelial injury
Collagen type IV in basement membrane	Decreases endothelial cell adhesion and increases extravasation of fluid

- Reduced NADPH → decrease in GSH → increases cells **susceptibility to oxidative stress**.

MORPHOLOGY

Pancreas

Lesions of pancreas are **not diagnostic** and are more common with type 1 than with type 2 diabetes. The morphological changes include:

- **Reduced number and size of islets:** It is seen in type 1 diabetes which is mild in type 2 diabetes.
- **Infiltration of islets (insulitis):** Mainly by T lymphocytes predominantly in type 1 diabetes.
- **Amyloid deposition within islets:** It is observed in and around capillaries and between cells in type 2 diabetes. In advanced stages, the islets may be virtually obliterated and may show fibrosis.
- **Increase in the number and size of islets: It may be seen in nondiabetic newborns of diabetic mothers** as a hyperplastic response to the maternal hyperglycemia.

Blood Vessels

- **Hyaline arteriolosclerosis:** It can be found in hypertension, elderly nondiabetics without hypertension and more severe degree in diabetics also.
- In diabetics, it is **related to the duration of diabetes and the level of blood pressure**.
- It is characterized by **amorphous, hyaline thickening of the wall of the arterioles**, which may **narrow the lumen** of the vessel **(***refer* **Figs. 16.7A and B)**.
- **Diabetic microangiopathy:** It is characterized by **diffuse thickening of basement membranes,** which is **mostly seen in the capillaries of the skin, skeletal muscle, retina, renal glomeruli, and renal medulla**.
- Though **capillaries show basement membrane thickening, they are leakier to plasma proteins than normal.**
- The microangiopathy results in diabetic nephropathy, retinopathy, and some forms of neuropathy.

Renal changes in diabetes mellitus are discussed in pages 682-3.

Clinical Features of Diabetes (Fig. 24.22)

Type 1 Diabetes Mellitus

- **Age:** Type 1 diabetes can occur at any age.
- **Classical classic triad of diabetes**: It consists of **polyuria, polydipsia, polyphagia**, and **in severe cases ketoacidosis**, are due to metabolic derangements.

- **Insulin requirement:** In the **initial 1 or 2 years**, the exogenous insulin required may be **minimal** because of endogenous insulin secretion → **later**, its requirement suddenly **increases**.

Consequences of Insulin Deficiency

Insulin is an anabolic hormone and its deficiency → results in a catabolic state, which affects glucose metabolism, fat and protein metabolism.

- **Carbohydrate metabolism:**
  - **Diminished transport of glucose** into **muscle cells** and **adipose tissue**.
  - **Reduction of stored glycogen in liver and muscle** due to **glycogenolysis** → this **further aggravates the hyperglycemia**.
  - When **hyperglycemia exceeds the renal threshold** level → develops **glycosuria** → leads to osmotic diuresis → **increased quantity of urine known as polyuria,** with **loss of water and electrolytes**.
  - Water loss through urine + hyperosmolarity (due to hyperglycemia) → causes **depletion of intracellular water** → **stimulates** the osmoreceptors of the **thirst centers** of the brain → results in increased thirst known as **polydipsia**.
- **Protein and fat metabolism:**
  - Insulin deficiency → causes **catabolism of proteins and fats** → produces a **negative energy balance** → leads to **increased appetite** (**polyphagia**).
  - In spite of increased appetite, catabolic effects insulin results in paradoxical **loss of weight and muscle weakness**.
- **Diabetic ketoacidosis:**
  - Serious complication of diabetes.
  - More **common and marked in type 1 diabetes**, but may also occur in type 2 diabetes.
  - **Mechanism:**
    - **Diuresis and dehydration: Marked insulin deficiency** + associated **epinephrine** release → stimulates the **secretion of glucagon** → leads to **decreased peripheral utilization of glucose** + **increased gluconeogenesis** → causes **severe hyperglycemia** (the blood glucose levels of 500–700 mg/dL) → osmotic **diuresis and dehydration** (characteristic features of ketoacidosis).
    - **Activation of the ketogenic machinery**:

- Insulin deficiency → **stimulates lipoprotein lipase** → which **breaks down fat stores → increases the free fatty acids levels**.
- **Free fatty acids** are **esterified to fatty acyl coenzyme A in the liver**.
- In the mitochondria of liver cells, **fatty acyl coenzyme A molecules are oxidized** to **ketone bodies (acetoacetic acid and β-hydroxybutyric acid)**.
- When the **rate of production of ketone bodies exceeds the rate of utilization** by peripheral tissues, it results in **ketonemia** and **ketonuria**.

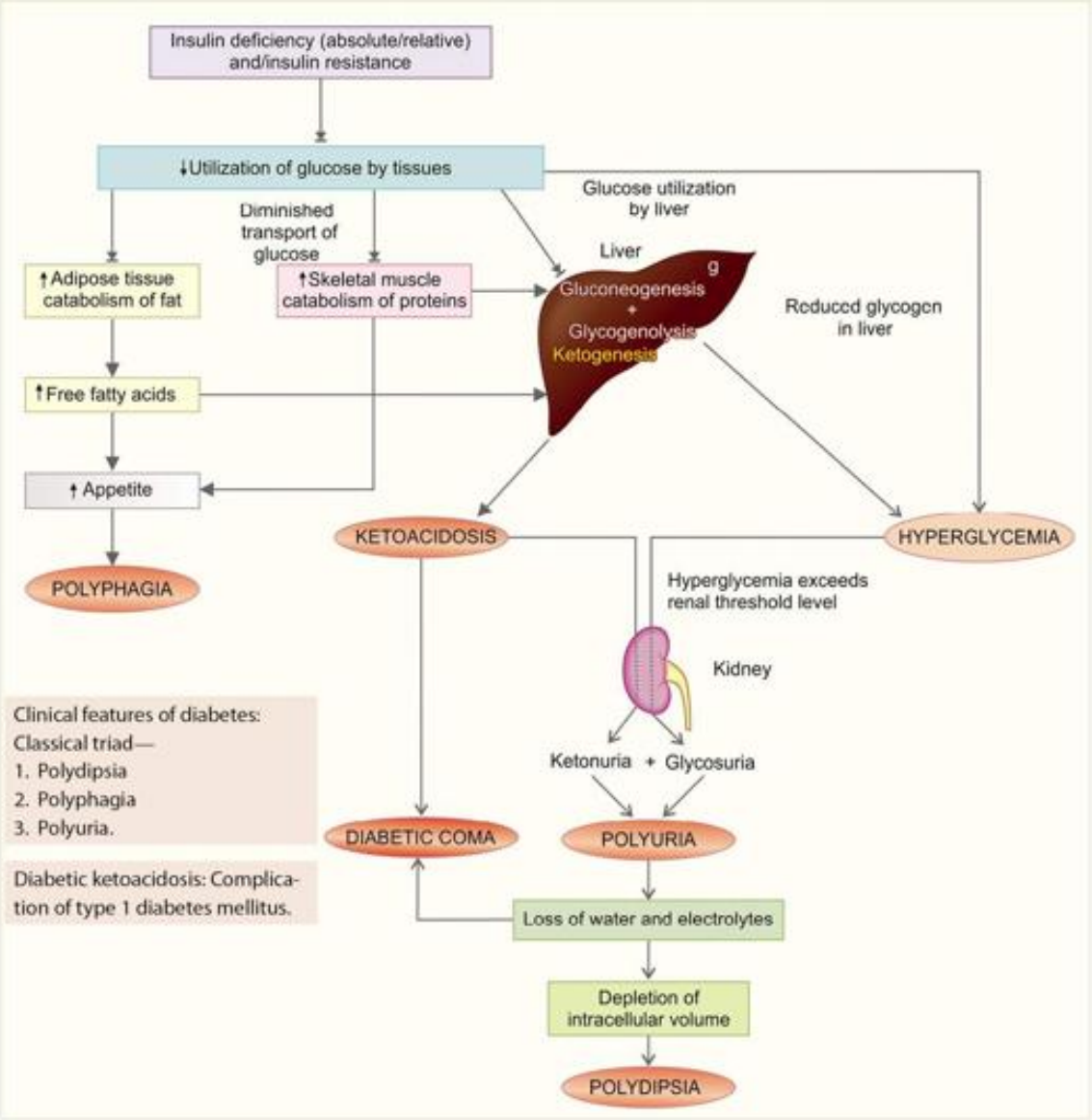


Fig. 24.22: Sequence of metabolic derangements underlying the symptoms and signs of uncontrolled hyperglycemia in diabetes mellitus.

- If the excretion of ketone bodies in the urinary is compromised by dehydration, it results in **systemic metabolic ketoacidosis**.

Type 2 Diabetes Mellitus

- **Age:** Usually occurs in older **above the age of 40 years** and frequently in **obese individuals**. Due to increase in obesity and sedentary lifestyle, it is now detected also in children and adolescents.
- **Presentation: Polyuria, polydipsia, unexplained weakness or weight loss. Ketoacidosis is infrequent** and presentation is usually mild.

- In **asymptomatic individuals**, the **diagnosis is made after routine blood or urine testing**.

Differences between type 1 and type 2 diabetes mellitus are listed in **Table 24.5**.

Complications (Fig. 24.23)

Q. Write short note on complications of type 1 diabetes mellitus.

Refer pages 825-6 for pathogenesis of complication.

- Complications are similar in both type 1 and type 2 diabetes.
- **Long-term complications of diabetes** usually develop about 15–20 years after the onset of hyperglycemia. They are responsible for the majority of the morbidity and mortality.

Q. List the differences between type 1 and type 2 diabetes mellitus.

TABLE 24.5: Differences between type 1 and type 2 diabetes mellitus.

	Type 1 diabetes mellitus	*Type 2 diabetes mellitus*
Clinical		
Age of onset	Childhood and adolescence	Adult, also seen in childhood and adolescence
Weight	Normal or present with weight loss	Majority are obese
Insulin levels	Progressive decrease	Increased (early); normal or moderate decrease (late)
Circulating islet autoantibodies	Detected (anti-insulin, anti-GAD, anti-ICA512)	Not found
Diabetic ketoacidosis	Develops in absence of insulin therapy	Nonketotic hyperosmolar coma
Genetics		
Human leukocyte antigen (HLA) association	Major linkage to MHC (major histocompatibility complex) class I and II genes HLA-DR3 and/-HLA-DR4	No HLA association
Non-HLA genes	Polymorphisms in *CTLA4* and *PTPN22*	Diabetogenic and obesity-related genes
Etiopathogenesis		
Mode of development	Autoimmune	Multifactorial
Mechanism	Breakdown in self-tolerance to islet autoantigens	Insulin resistance in peripheral tissues, β-cell dysfunction
Morphology		
Insulitis (inflammation of islets)	Inflammatory infiltrate of T-cells and macrophages in islets	No insulitis; amyloid deposition in islets
β-cell depletion	Moderate to severe	Mild

Diabetic Macrovascular Disease

The lesions of large- and medium-sized muscular arteries are the **most common causes of mortality** in long-standing diabetes. These include:

- **Atherosclerosis:** Diabetes is one of the major modifiable risk factor for atherosclerosis and other cardiovascular morbidities. The **atherosclerosis is more severe and occurs at earlier age**. Diabetics have **increased levels of** plasminogen activator inhibitor **(PAI-1),** which **inhibits fibrinolysis and favors development of atherosclerotic plaques**. **Renal arteries** also develop **severe atherosclerosis**.
- **Myocardial infarction:** It is due to atherosclerosis of the coronary arteries and is the **most common cause of death in diabetics.** Diabetics have greater risk of coronary artery disease and cardiovascular complications than nondiabetics. Risk for cardiovascular disease is more even in prediabetics.
- **Gangrene of the lower extremities:** It results from advanced vascular disease and is more common in diabetics.
- **Renal vascular insufficiency**.
- **Cerebrovascular accidents** (stroke).

Microvascular Disease (Microangiopathy)

It involves small vessels and is characterized by capillary dysfunction in target organs and is mainly observed in kidneys (nephropathy), retina (diabetic retinopathy) and peripheral nerves (neuropathy).

- **Diabetic nephropathy** (*refer* pages 682-3): About **30–40% of all diabetics** develop nephropathy and is **leading cause of end-stage renal disease**. The different stages in diabetic nephropathy are:
  - **Microalbuminuria:** It is the **earliest manifestation** of diabetic nephropathy in which the urine has low amounts of albumin (>30 mg/day, but <300 mg/day). Microalbuminuria is also a marker for increased cardiovascular morbidity and mortality in either type 1 or type 2 diabetics.
  - **Nephropathy with macroalbuminuria:** Without specific interventions, diabetics will develop **overt nephropathy with macroalbuminuria** (>300 mg of urinary albumin per day), usually associated with hypertension.
  - **End-stage renal disease:** The overt nephropathy may progress to end-stage renal disease.
- **Diabetic retinopathy (Fig. 24.26):** The ocular involvement in diabetes may be in the form of (i) retinopathy, (ii) cataract formation, or (ii)

glaucoma. Retinopathy is the most common and it develops in **~60-80% of diabetics, about 15–20 years after diagnosis.** Retinopathy may in be seen in two forms: nonproliferative retinopathy and proliferative retinopathy. The basic lesion of retinopathy is **neovascularization caused by hypoxia-induced overexpression of VEGF in the retina**.

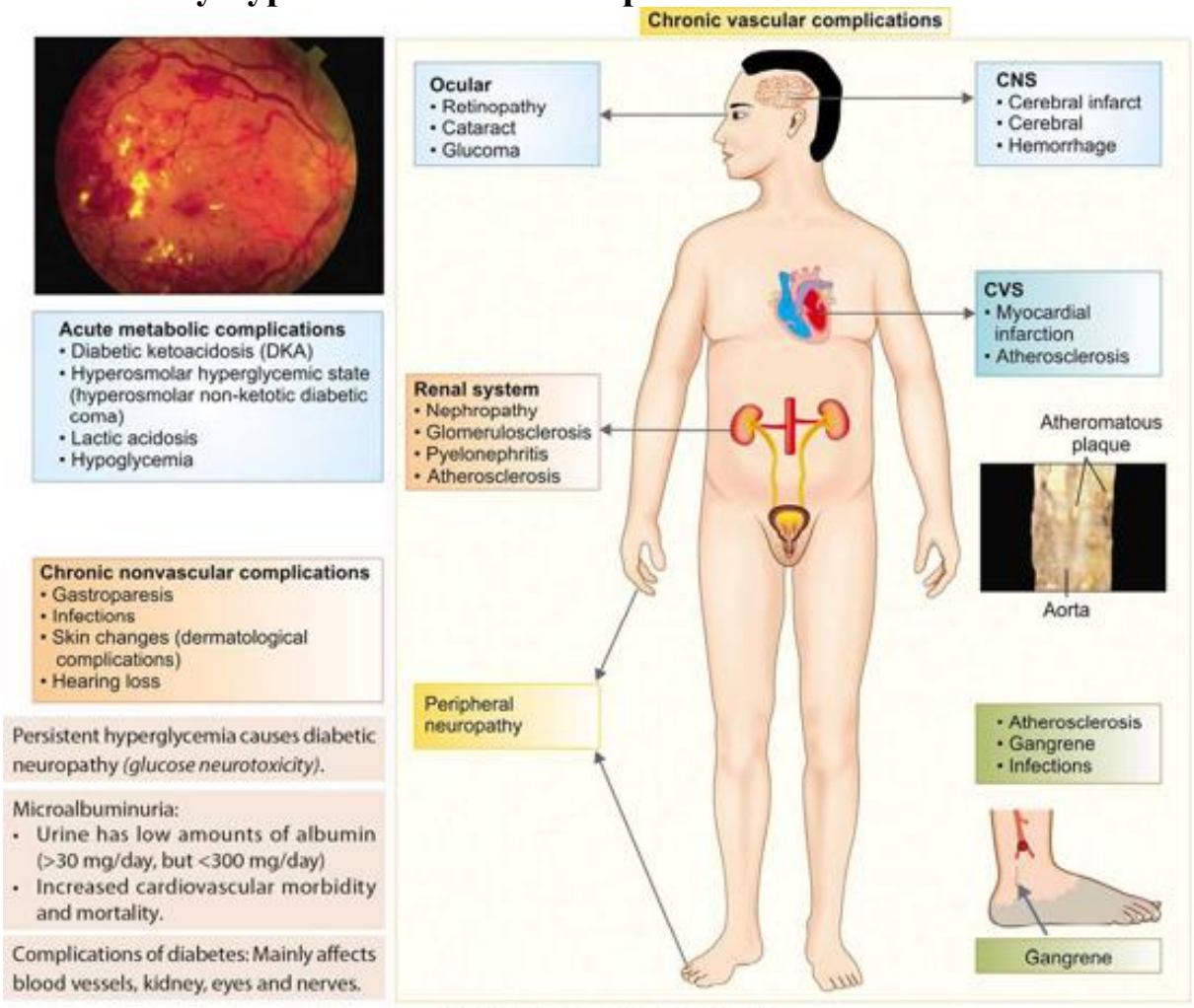


Fig. 24.23: Complications of diabetes.
(CNS: central nervous system; CVS: cardiovascular system).

- **Nonproliferative retinopathy:** The lesions include intraretinal or preretinal hemorrhages, retinal exudates, microaneurysms (appear as small red dots under the ophthalmoscope), venous dilations, edema, and the thickening of the retinal capillaries (microangiopathy). The retinal exudates can be "soft" (microinfarcts) or "hard" (deposits of plasma proteins and lipids).
- **Proliferative retinopathy:** It is a process of neovascularization and fibrosis. If it involves the macula, it can produce blindness. Vitreous

hemorrhages due to the rupture of newly formed capillaries and their subsequent organization can lead to retinal detachment.

- **Diabetic neuropathy:** It may involve the **central and peripheral nervous systems**.
  - **Distal symmetric polyneuropathy:** It affects **both motor and sensory function** of the lower extremities later, it may involve the upper extremities leading to **"glove and stocking" pattern of polyneuropathy**.
  - **Autonomic neuropathy**: **Produces disturbances in bowel and bladder function** and sometimes sexual impotence.
  - **Diabetic mononeuropathy:** It may manifest as sudden footdrop, wristdrop, or isolated cranial nerve palsies.

Diabetic Dyslipidemia

It is a condition in which there are **increased blood levels of triglycerides and LDL and decreased levels of the high-density lipoprotein** (protective). This develops due to insulin resistance, which causes increased production of atherogenic lipoproteins by liver and decreased uptake of circulating lipids in peripheral tissues.

Increased Susceptibility to Infections

Infections of the skin, tuberculosis, pneumonia, and pyelonephritis are common in diabetes. The increased susceptibility may be due to **defective neutrophil functions** (chemotaxis, adherence to the endothelium, phagocytosis, and microbicidal activity), and **impaired cytokine production by macrophages**.

Hyaline arteriosclerosis is discussed in page 447.

Glycosylated hemoglobins (Hb_{A1C}): Marker of glycemic control.

Diabetes mellitus: Good glycemic control prevents complications.

AGEs formation is markedly increased in the presence of hyperglycemia.

DM-effects of AGEs on vessel:
- Through RAGE
  - Endothelial injury
  - Increased ECM
- Direct effect

- Increased atherogenesis
- Basement membrane thickening.

DM-effects of hyperglycemia:
- Formation of AGEs
- Activation of PKC
- Disturbances in polyol pathways.

DM-consequences of PKC activation:
- Neovascularization by VEGF
- Basement membrane thickening by TGF-β
- Reduced fibrinolysis and vascular occlusion by PAI-1

DM-consequence of disturbance in polyol pathway: Increases susceptibility of cells to oxidative stress.

Pancreas in DM: Lesions are not diagnostic.
- Reduce number and size of islets
- Insulitis
- Amyloid deposition in islets

Hyaline arteriosclerosis:
- Diabetes mellitus
- Hypertension
- Elderly nondiabetic without hypertension

Diabetic microangiopathy: Diffuse thickening of capillaries mostly seen in skin, skeletal muscles, retina and kidney.

Most abundant glycoprotein present in basement membrane is: Laminin.

DM: Increased susceptibility to infections due to defective neutrophil functions.

LABORATORY DIAGNOSIS OF DIABETES

Q. Write short essay on laboratory diagnosis of diabetes mellitus.

Hyperglycemia: It is the fundamental basis for the diagnosis of diabetes mellitus.

In symptomatic cases: Diabetes can be confirmed by finding glucosuria and a random plasma glucose level above 200 mg/dL. The severity of clinical

symptoms of polyuria, polyphagia and polydipsia depends on the degree of hyperglycemia.

In asymptomatic cases: Most of these patients have persistently elevated fasting plasma glucose level. However, in asymptomatic patients (suspected to be suffering from diabetes mellitus) with normal fasting glucose level in the plasma need oral glucose tolerance test (GTT).

WHO criteria for the diagnosis of diabetes mellitus is mentioned in **Box 24.8**.

The American Diabetes Association (2016) has recommended definite diagnostic criteria for early diagnosis of diabetes mellitus **(Box 24.9)**.

Investigations for the Diagnosis of Diabetes Mellitus

1. Urine Examination

Urine examination is economical and convenient test for the diagnosis of diabetes mellitus. However, urine alone may show false-positives and false-negative results. It can be used as screening test. Urine is examined for the presence of glucose and ketones.

Glucosuria

- **Benedict's qualitative test** (*refer* page 397): It detects any reducing substance in the urine and is not specific for glucose.

BOX 24.8: WHO criteria for the diagnosis of diabetes mellitus.

- Fasting plasma glucose >126 mg/dL (7.0 mmol/L)
- Random plasma glucose >200 mg/dL (11.1 mmol/L) with classical signs and symptoms.
- One abnormal laboratory value is diagnostic in symptomatic individuals.
  - Two values are needed in asymptomatic individuals
  - The glucose tolerance test is only required for borderline cases and for diagnosis of gestational diabetes.
- HbA1c >6.5 (48 mmol/mol)

BOX 24.9: Criteria for diagnosis of diabetes (American Diabetes Association, 2016).

Typical symptoms of DM (polyuria, polydipsia, polyuria, weight loss) + random plasma glucose >**200 mg/dL** (symptoms of diabetes + random whole blood glucose >175 mg/dL)

OR

Fasting plasma glucose **≥126 mg/dL** (fasting whole blood glucose ≥110 mg/dL)

OR

2-hour plasma glucose **≥200 mg/dL** during an oral glucose tolerance test (OGTT) (whole blood ≥ 175 mg/dL during an oral 75 g glucose tolerance test).

OR

Glycated hemoglobin (HbA1c) **≥6.5%**

- **Dipstick method** (*refer* page 397)**:** This method is more sensitive and specific for glucose.

Disadvantages: Apart from diabetes mellitus, *glucosuria* may be found in other conditions (*refer* **Box 14.8**).

Ketonuria

Tests for ketone bodies in the urine are needed for assessing the severity of diabetes and not for diagnosis of diabetes. However, if both glucose and ketone bodies are present, diagnosis of diabetes is almost certain. Rothera's test (nitroprusside reaction) and dipstick methods can be used for detection of ketonuria (*refer* page 398).

2. Single Plasma Glucose Estimation

Estimation of plasma glucose is absolutely necessary for diagnosis of diabetes mellitus. A fasting plasma glucose level above 126 mg/dL is indicative of diabetes. In other cases, oral GTT is performed.

3. Population Screening by Fasting Plasma Glucose Test for Type 2 DM

Estimation of fasting plasma glucose is a screening test used for type 2DM. It is advisable that all individuals above 45 years of age must undergo screening fasting glucose test every 3-years, and relatively earlier if the person is overweight.

4. Oral Glucose Tolerance Test (OGTT)

Indication: It is not recommended for routine diagnosis of either type 1 or type 2 DM. This is because fasting plasma glucose identifies the abnormal

glucose metabolism.

- In patients where the fasting plasma glucose is in the range of impaired fasting glucose (100–125 mg/dL)
- Diagnosis of gestational diabetes mellitus
- Uncertainty about the diagnosis of diabetes

Preparation of the patient: Patient should be taking an unrestricted carbohydrate diet for at least 3 days or more prior to the test. Because carbohydrate-restricted diet reduces glucose tolerance, patient should be with normal physical activity.

- OGTT is performed in the morning after patient has fasted overnight (at least 8 hours).
- The patient should rest for at least half an hour before starting the test, and refrain from smoking, tea/coffee or exercise during the test.

Test: A fasting venous sample of blood is drawn to estimate the glucose level.

- Patients are given 75 g of anhydrous glucose dissolved in 250–300 mL of water orally over 5 minutes. Dose in children is 1.75 g of glucose/kg body weight up to a maximum of 75 g. Time of starting glucose is taken as 0 hour.
- A single venous sample of blood is drawn 2 hours after the glucose administration and glucose level is estimated (previously, blood samples were collected at 1/2 hour, 1 hour, 1 hour, 2 hours).

5. Postprandial Plasma Glucose

Determination of plasma glucose level 2 hours after food has no standardized role in the diagnosis of diabetes mellitus. However, it is useful in patients on treatment.

6. Glycosylated Hemoglobin (HbA1c)

Blood glucose level in diabetics varies with the dietary intake of the previous day of estimating blood glucose. Long-term assessment (for the last 90–120 days) of degree of glycemic control is assessed by measurement of glycosylated hemoglobin (HbA1c). It is a minor hemoglobin component present in normal individuals. This test does not need any dietary preparation or fasting. Advantages of HbA1c assay are (i) it has a direct relation between

poor control and development of complications and (ii) it is also a good measure of prediction of microvascular complications. Reference range is 3.8 to 6.3% and target for therapy is <7%.

7. Glycosylated Plasma Proteins (Fructosamine)

It may also be measured as an index of diabetic control. It measures glycation of all serum proteins and the major component being **glycosylated albumin**. Since albumin accounts for most of the protein in blood, the measurement of fructosamine, for practical purposes, measures glycated albumin. As albumin has a turnover of about 2 weeks, fructosamine reflects **glycemia over the preceding 2–3 weeks** (shorter period). It is **useful in diabetic patients with anemia or hemoglobinopathy and in pregnancy** (when hemoglobin turnover is changeable).

8. Other Investigations

These are sometimes performed in specific conditions in diabetics and for research purposes. These include:

- **Other GTTs**
  - **Extended GTT:** In this, the oral GTT is extended to 3–4 hours for appearance of symptoms of hyperglycemia. It is a useful in cases of reactive hypoglycemia of early diabetes.
  - **Intravenous GTT:** It is test performed in individuals suffering from intestinal malabsorption or those who have undergone gastrectomy.
  - **Cortisone-primed GTT:** It is provocative test and is a useful in potential diabetics.
- **Insulin assay:** Deficiency of plasma insulin levels is crucial for type 1 DM but is not essential for making the diagnosis of DM. It can be estimated by radioimmunoassay and ELISA technique.
- **Proinsulin assay:** Insulin is synthesized first as a precursor molecule namely proinsulin. Normally, it is <20% of total insulin and can be estimated by immunoassay.
- **C-peptide assay:** The A and B chains in insulin are held together by a peptide called C-peptide. This C-peptide is released into the circulation during conversion of proinsulin to insulin in equimolar quantities to insulin. Its levels correlate with insulin level in blood except in islet cell

tumors and in obesity. This test is more sensitive than insulin assay because its levels are not affected by treatment with insulin.

- **Islet autoantibodies:** Markers of cell-mediated autoimmune destruction of islet β cells can be used as a marker for type 1 diabetes mellitus**. These include (i) islet cell antibodies (ICAs), (ii) autoantibodies to insulin (IAAs), (iii) autoantibodies to g**lutamic acid decarboxylase (GAD65) and (iv) autoantibodies to tyrosine phosphatases IA-2α and IA-2β.
- **Screening for diabetes-associated complications:** Apart from making the diagnosis of DM based on the defined criteria, screening tests can be performed for DM-associated complications (e.g., microalbuminuria, dyslipidemia, thyroid dysfunction, etc.).

CHAPTER 25

Bones, Joints, and Soft Tissue Tumors

INTRODUCTION

The skeletal system consists of the bones, their associated cartilages, and the joints. Bone is a specialized connective tissue which has structural, protective, metabolic and hematopoietic functions (produces blood cells).

Different Regions in the Bone (Fig. 25.1)

- **Epiphysis:** It is the area of the bone which extends from the base of the articular surface (the site of close approximation of two bones) to the region of the growth plate.
- **Metaphysis:** It is the region which extends from the region of the growth plate to area where the diameter of the bone becomes significantly narrow (becomes funnel-shaped).
- **Diaphysis (shaft):** It is the zone which extends from base of one metaphysis to the base of the opposing metaphysis.

Cells of the Bone Tissue

- **Osteoblasts** produce osteoid and are thus bone forming cells. Osteoid is unmineralized organic bone matrix.
- **Osteocytes:** When osteoblast is enveloped by bone matrix it becomes osteocyte. With time osteocytes lose the capacity for matrix synthesis.
- **Osteoclasts:** These are mature multinucleated cells (on an average 6–12 nuclei) that are responsible for bone resorption (removal by absorption).

HEALING OF A FRACTURE

Q. Discuss the healing of fractured bone (or long bone) and its complications.

A fracture is a broken bone. It can range from a thin crack to a complete break in the bone.

Phases of Fracture Healing (Figs. 25.2A to E)

Bone can repair by reactivating processes that normally takes place during embryogenesis. There are three major phases of fracture healing.

1. Inflammatory Phase

Fracture and Hemorrhage

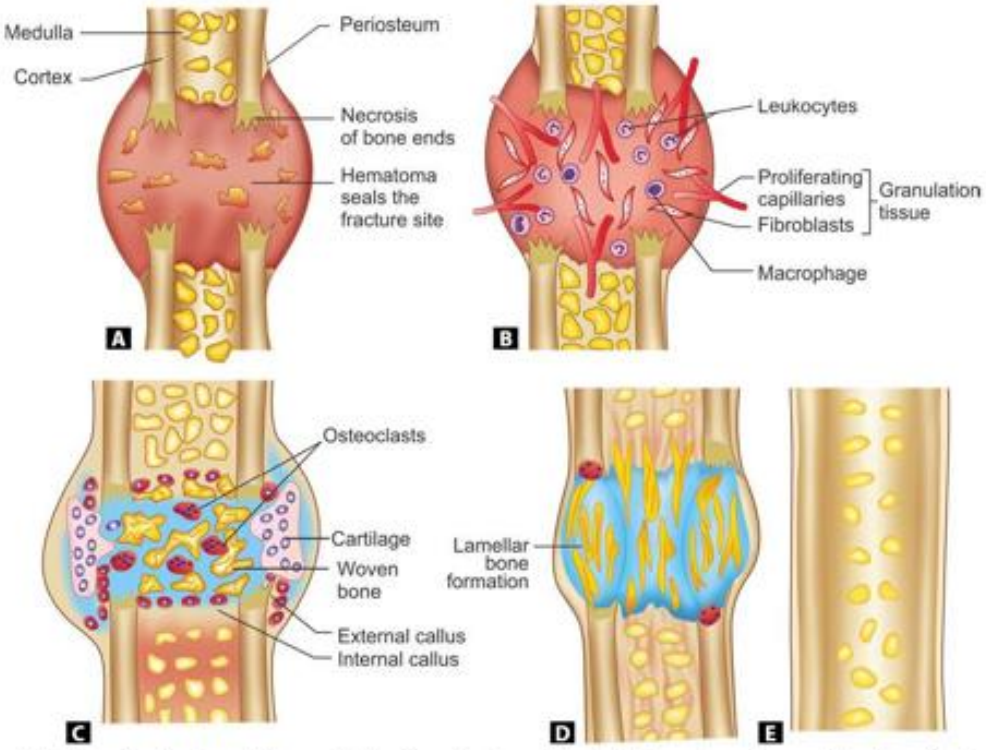
- Soon **after fracture**, **blood vessels** (in the periosteum, cortex and medullary cavity) **rupture** which leads to extensive hemorrhage (hematoma) at the fracture site and surrounding tissue.
- **Necrosis of bone** also occurs at the fracture site.

Inflammatory Cells

- **Fibrin meshwork** in the clotted blood **helps**
  - To seal the fracture site
  - Influx of inflammatory cells (neutrophils and macrophages) to the area
  - Ingrowth of fibroblasts and new capillary vessels (neovascularization) to the site, producing granulation tissue between the fracture fragments.
- **Activation of osteoprogenitor cells**: The **inflammatory cells and platelets release cytokines** (PDGF, TGF-β, FGF and interleukins) → **activate the osteoprogenitor cells** in:
  - Periosteum
  - Medullary cavity
  - Surrounding soft tissues.



Fig. 25.1: Different regions of bone.



Figs. 25.2A to E: Healing of a fracture: (A) Immediately after a fracture, blood clot/hematoma forms at the site of fracture; (B) During the inflammatory phase of fracture healing, the inflammatory cells (neutrophils and macrophages) migrate to the area of fracture and neovascularization develops; (C) The reparative phase of fracture healing is characterized by the formation of a callus near the fracture site; (D) In the remodeling phase, the reactive bone (lamellar or woven) develops; (E) Healing is complete and bone attains its original contour.

Formation of Granulation Tissue

It consists of proliferating capillaries and fibroblasts and are formed at the site of fractures. Simultaneously, degranulated platelets and migrating inflammatory cells release PDGF, TGF-β, FGF, and other factors. They activate osteoprogenitor cells in the periosteum, medullary cavity, and surrounding soft tissues.

Soft-tissue Callus or Procallus Formation

- **Osteoprogenitor cells → activate both osteoblastic and osteoclastic activities** at the fracture site.
- **Osteoblasts** derived from activated osteoprogenitor cells **migrate into the granulation tissue** and **differentiate into osteoid synthesizing units**. They **deposit** large quantities of **osteoid collagen in a haphazard pattern** producing **woven bone** (unmineralized bone is called osteoid).

- **Granulation tissue containing** (mineralized or unmineralized) **bone or cartilage** is termed a **callus.**
- At this stage, **callus** is predominantly **uncalcified** and is called **soft-tissue callus or procallus**, which **provides a type of temporary connection between the ends of the fractured bones**. However, procallus does not have any structural rigidity for any weight-bearing. The callus depending on its site and appearance can be divided into external and internal callus.
  - **External callus:** It is formed from the osteoprogenitor cells of periosteum and surrounding soft tissue and is found on the surface of the bone. It bridges the fracture site outside the bone and continues to grow inwards toward the fracture site. In this region, the osteoprogenitor cells may also differentiate into chondroblasts, which form fibrocartilage and hyaline cartilage around the fracture site.
  - **Internal callus:** It is derived from osteoprogenitor cells of medullary cavity and grows outward towards the fracture site. This bridges the fracture in the region of medullary cavity but in contrast to external callus does not contain cartilage.
- The **repair tissue** attains **maximal thickness at the end of the second or third week** and consists of hyaline cartilage and woven bone.

2. Reparative Phase

Lamellar Bone Formation

As the healing advances, the hyaline cartilage and woven bone of the original fracture **callus are replaced by lamellar bone.** This is stronger and consists of **parallel collagen fibers**.

Endochondral Ossification

The replacement process is known as **endochondral ossification** with respect to the hyaline cartilage and **bony substitution** with respect to the woven bone.

Bony Callus

At this stage, the **callus is mineralized** (calcified) and is known as **bony (osseous) callus**. As the mineralization proceeds, the stiffness and strength

of the callus increases. By the second or third week, controlled weight-bearing can be tolerated.

3. Remodeling Phase

- Several weeks after a callus has sealed the bone ends, the remodeling phase begins.
- **Resorption of excess portions of bony callus**: During healing, **excess of bony callus is formed around the fracture site**. As the callus is subjected to weight-bearing forces, the portions of bony callus that are not physically stressed by this weight are **slowly resorbed by osteoclasts**. Thus, the osteoclasts act to remodel bone and decrease the size of callus.
- The remodeling phase **substitutes the trabecular bone with compact bone**.
- Remodeling phase continues till the original bone shape (contour), outline and strength of the fractured bone is re-established.

The **whole process of healing** of a bone fracture usually takes **about 6–8 weeks**.

Major Causes of Delayed Fracture Healing(Table 25.1)

TABLE 25.1: Major causes of delayed fracture healing.

Local factors	*General factors*
Excessive movement of fractured bone during healing process	Old age
Infection of the fractured site	Poor general health
Severe local soft-tissue injury	Drugs, e.g., corticosteroids
Wide separation of fracture ends	
Extensive necrosis of the fractured bone	
Poor or impaired blood supply (e.g., tibia, head of femur)	

Complications of Healing

Q. Mention the complications of healing of fracture bone.

- **Delayed union and nonunion** of fracture.
- **Pseudoarthrosis:** In case of nonunion, too much movement along the fracture gap can cause cystic degeneration in the callus, creating a false joint or pseudoarthrosis.
- **Large callus with deformity**.

Fracture healing: Three phases
1. Inflammatory
2. Reparative
3. Remodeling

Inflammatory phase
- Fracture and inflammatory cells
- Granulation tissue formation

Callus: Granulation tissue containing (mineralized or unmineralized) bone or cartilage.

Fracture healing: First forms woven bone followed by lamellar bone.

Reparative phase
- Callus formation
- Lamellar bone deposition

Fracture healing: Mineralized callus is called bony/osseous callus.

Rate of newly synthesized osteoid mineralization is best estimated by: Tetracycline labeling.

Remodeling phase: Remodeling to original bone contour.

Complications of fracture healing:
- Delayed union/nonunion
- Pseudoarthrosis
- Large callus with deformity

INFECTIONS—OSTEOMYELITIS

CC: Classify and describe the etiology, pathogenesis, manifestations, radiologic and

morphologic features and complications of osteomyelitis.

Q. Write short essay on osteomyelitis.

Definition: Osteomyelitis is defined as inflammation of the bone and marrow.

Classification

- **Primary:** Where bone is the primary solitary focus of disease.
- **Secondary:** Develops as a complication of any systemic infection.

Any infection (bacteria, viruses, parasites, fungi) may cause osteomyelitis, but infections by certain pyogenic bacteria and mycobacteria are the most common.

Pyogenic Osteomyelitis

Q. Write a short essay on pyogenic osteomyelitis.

Etiology

Q. Write short note on etiology of osteomyelitis.

It is **usually caused by bacteria**.

- Most common pathogens are *Staphylococcus* **species** (*aureus* in 80–90% of the cases).
- **Other organisms**:
  - *Escherichia coli*, *Pseudomonas*, *Klebsiella, Neisseria gonorrhoeae, Haemophilus influenzae* and *Salmonella* species.
  - *Escherichia coli* in patients with genitourinary tract infections or intravenous drug abusers.
  - **Neonatal period**: *Haemophilus influenzae* and group B streptococci.
  - **Patients with sickle cell disease**: *Salmonella* infection.
- **Mixed bacterial infections**: It is due to direct spread or surgery or open fractures.
- In about 50%, no organisms can be isolated.

Portal of Entry of Organism

Causative organisms may reach the bone through the **bloodstream, directly** or extend **from a contiguous site**.

1. Hematogenous Spread

- Source of organisms may be a **focus of infection anywhere in the body** (e.g., skin pustule or infected teeth and gums, intestinal mucosa).
- **Minor injuries** to the mucosa (vigorous chewing of hard foods, brushing of teeth), or minor infections of the skin, **release these organisms** into the blood → causing temporary **bacteremia → reach the bone**.
- In **children** (5–15 years) and **drug addicts** (infected needles), it develops in the long bones.

2. Direct Implantation

Organisms may enter into bone by penetrating wounds, open fractures, or surgical procedures (staphylococci, streptococci, anaerobic organisms).

3. Spread from Adjacent (Contiguous) Site

For example, infections of the feet may spread into the bone in diabetics.

Location of infection: It **varies** with age.

- **Children** and with hematogenous spread: **Metaphysis of long bones** (knee, ankle, and hip).
- N**eonate: Metaphysis, epiphysis, or both**.
- **Adult**: **Epiphysis and subchondral regions**.

Pathogenesis and Morphology (Figs. 25.3A to E)

Q. Write short note on pathogenesis and morphology of pyogenic osteomyelitis.

The sequence of events and morphological features in osteomyelitis are described together.

- **Transient bacteremia**: Mild injury or trauma can initiate bacteremia by organisms (e.g., *Staphylococcus aureus*).
- **Infection reaches metaphysis of long bone:** Because in the metaphysis, capillaries form loop → which slows the blood flow → provides time for bacteria to penetrate blood vessel walls and establish infective foci within the marrow.
- **Inflammatory reaction**: Once in bone, the bacteria grow and induce an **acute inflammatory reaction with exudates**.
- **Necrosis of bone: Exudate increases the pressure** on the adjacent vessels and further **decreases the blood supply** → produces **bone necrosis**. The necrotic areas coalesce and allow **further bacterial proliferation**.
- **Formation of sequestrum**

Q. Write a short answer on sequestrum.

- Bacterial **infections and pus spreads into cortex** and collect beneath the periosteum → may **lift the periosteum → reduces the blood supply** to the affected region → results in **segmental necrosis of the bone** due to both suppuration and ischemia.
- The fragment of **dead necrotic piece of bone**, which is embedded in the pus, is known as a **sequestrum**.

- **Draining sinus**:
  - The **pus penetrates the periosteum** and leads to a **soft-tissue abscess** → may penetrate the skin → form a **draining sinus**. **Hole formed in the bone** during the formation of a draining sinus is known as **cloaca**.
  - The sinus tract may become epithelialized and may remain open, continually draining pus, necrotic bone, and bacteria.

Q. Write a short answer on involucrum.

Involucrum: After first week, chronic inflammatory cells become more numerous and the cytokines released stimulates osteoclastic bone resorption and **deposition of reactive bone in the periphery**. Reactive new bone forms a sheath around the necrotic (segment of devitalized infected bone) sequestrum. This **reactive new bone formed is known as involucrum**.

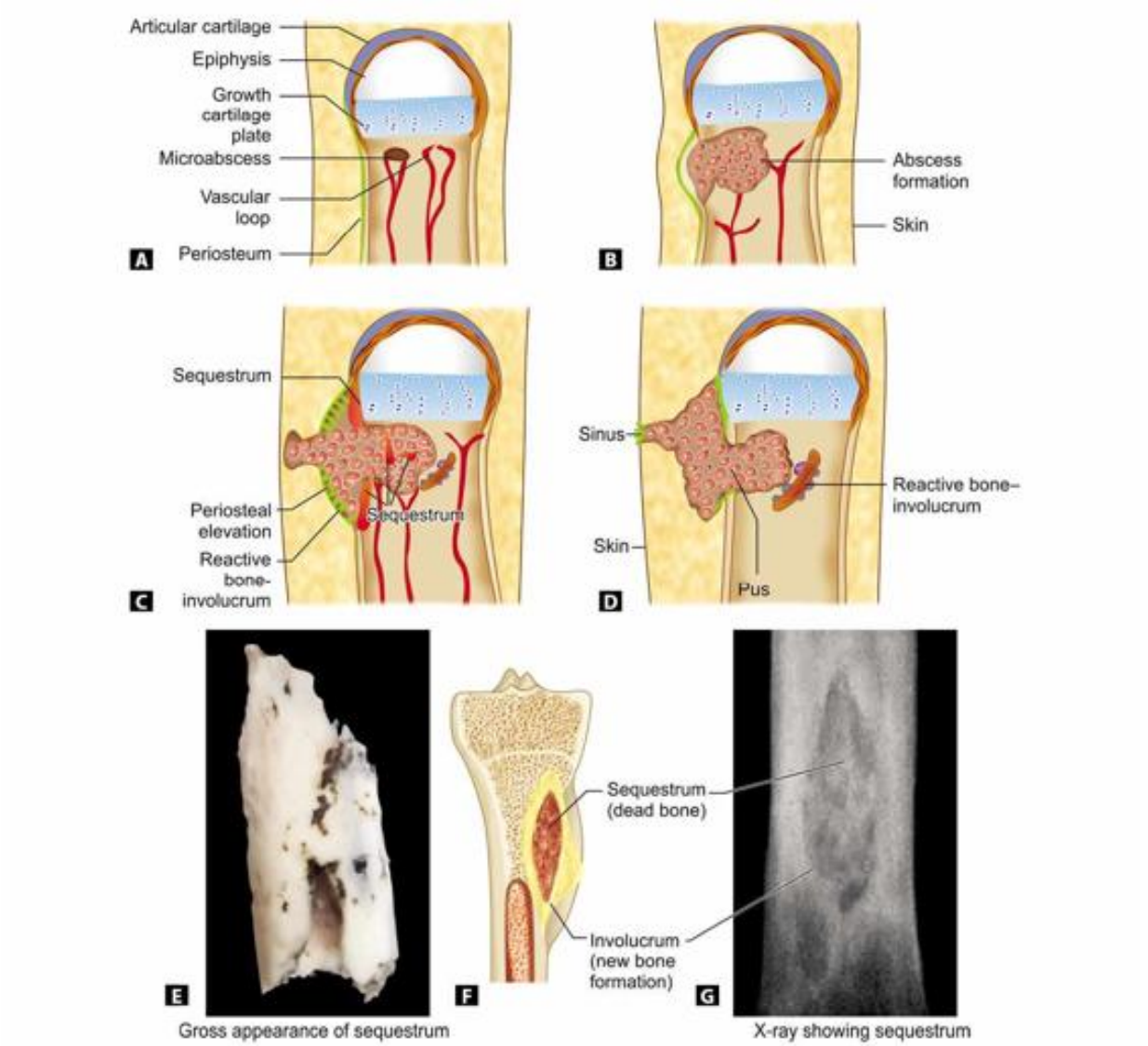
MORPHOLOGY

Q. Write a short essay on morphology of pyogenic osteomyelitis.

The morphologic features **depend on the stage** (acute, subacute, or chronic) and location of the infection.

Gross

Sequestrum appears as a fragment of bone with ragged brownish-to-black colored margins **(Fig. 25.3E)**.



Figs. 25.3A to G: Pathogenesis of hematogenous osteomyelitis: (A) A small, septic microabscess is formed at the capillary loop; (B) Expansion of the septic focus stimulates resorption of adjacent bony trabeculae. The abscess expands and stimulates reactive bone formation by the periosteum; (C) The abscess, which continues to expand through the cortex into the subperiosteal tissue; (D) The extension of this process into the skin produces a draining sinus; (E) The necrotic bone is called a sequestrum; (F) The viable bone surrounding a sequestrum is termed the involucrum; (G) X-ray showing sequestrum.

Microscopy

- In the **initial stages**, only the **neutrophils** are seen. As the diseases become chronic, neutrophils admixed with chronic inflammatory cells (lymphocytes and plasma cells) are seen.
- Sequestrum does not show osteocytes and have empty lacunae.

Morphologic Variants of Osteomyelitis

- **Brodie abscess:** It is a distinctive form of **subacute pyogenic osteomyelitis**.
  - It appears as a **small, solitary, intraosseous abscess localized to the metaphysis** and is surrounded by reactive bone.
  - It may be **due to inadequate treatment of infection by less virulent organisms**.
- **Sclerosing osteomyelitis of Garré**: It is characterized by **extensive new bone formation**, which obscures the underlying structure of the bone and **typically develops in the jaw**.

Complications

Q. Write short note on complications of pyogenic osteomyelitis.

- **Septicemia**: From infection in the bone, organisms may disseminate through the bloodstream and cause septicemia.
- **Acute suppurative arthritis**: Infection may spread through the articular surface into a joint → producing suppurative arthritis → may lead to destruction of the articular cartilage and permanent disability. It is more common in infants.
- **Pathologic fractures**.
- **Squamous cell carcinoma:** It may arise from the epithelialized sinus tract, rarely sarcoma of bone may develop.
- **Secondary amyloidosis**.
- **Chronic osteomyelitis:** It may develop due to delay in diagnosis, extensive bone necrosis, and inadequate therapy.

Clinical Course

- Present with malaise**, fever, chills, leukocytosis**, and **throbbing pain over the affected region**.
- **Diagnosis**
  - **Radiography:** Lytic focus of bone destruction surrounded by a zone of sclerosis.
  - **Blood cultures** are positive.
  - **Biopsy** and bone cultures.

Types of osteomyelitis
- Pyogenic
- Tuberculous
- Others: Chronic nonspecific osteomyelitis

Pyogenic osteomyelitis: *Staphylococcus aureus* is the most common pathogen.

Pyogenic osteomyelitis: In sickle cell anemia due to *Salmonella*.

Pyogenic osteomyelitis:
1. Hematogenous
2. Direct
3. Spread from adjacent site

Osteomyelitis:
- Usually due to hematogenous spread
- Metaphysis is the most common site involved

Sequestrum: Fragment of dead/devitalized necrotic piece of bone embedded in the pus.

Involucrum: Reactive new bone around the sequestrum.

Brodie abscess: Small, solitary, intraosseous abscess localized to the metaphysis surrounded by reactive bone

Complications of pyogenic osteomyelitis:
1. Septicemia
2. Acute suppurative arthritis
3. Pathologic fractures
4. Squamous cell carcinoma
5. Amyloidosis
6. Chronic osteomyelitis

Tuberculous Osteomyelitis

Q. Write short note on tuberculous osteomyelitis.

Tuberculous osteomyelitis is **usually solitary** but in patients with acquired immunodeficiency syndrome, it is frequently multifocal. It tends to be **more destructive and resistant to control** than pyogenic osteomyelitis.

- **Age**: Usually **adolescents or young adults** in developing countries.
- **Source of infection: Pulmonary or extrapulmonary** tuberculosis.
- **Predisposing factors:** Diabetes, elderly, immune compromised states and general debility.
- **Route of infection:**
  - **Blood borne**: Usually blood-borne infection, which is from a focus of **active pulmonary or extrapulmonary disease**.
  - **Direct extension**: From
    - Lung into a rib.
    - Tracheobronchial nodes into adjacent vertebrae.

Sites

Q. Write a short answer on Pott's spine.

- **Spine** (thoracic and lumbar vertebrae) commonly known as **Pott disease (tuberculous spondylitis).** The **infection breaks through intervertebral disks** to involve multiple vertebrae and **extends down into the soft tissues** forming abscesses (**cold abscess—psoas abscess**).
- **Knees and hips**.

Microscopy

It shows dead bone surrounded by **tuberculous granuloma**. The granuloma consists of central area of caseous necrosis surrounded by epithelioid cells and Langhans giant cells, which in turn is surrounded by lymphocytes **(Fig. 16.17)**. The epithelioid cells have a pale-pink granular cytoplasm with indistinct cell boundaries, often appearing to merge into one another. The nucleus is oval or elongate.

Clinical Course

- Low-grade fever with evening rise of temperature
- Pain on motion, localized tenderness
- Weight loss

Complications

- **Spine:**
  - **Destruction of vertebrae**: Causes severe **scoliosis or kyphosis a**nd neurologic deficits due to spinal cord and nerve compression.
  - **Psoas abscess:** Infection from spine may rupture into the soft tissue anteriorly and pus and necrotic debris may drain along the spinal ligaments and form a **cold abscess, i.e., an abscess lacking acute inflammation.** P**soas abscess** is the condition in which infection from lower lumbar vertebrae dissects along the pelvis, and appears as a draining sinus of the skin in the inguinal region. It may be the first manifestation of tuberculous spondylitis.
- **Tuberculous arthritis**
- **Sinus tract formation**
- **Amyloidosis.**

Pott disease: Tuberculosis of spine (thoracic and lumbar vertebrae).

Psoas abscess: May be the first manifestation of tuberculous spondylitis.

PAGET DISEASE OF BONE (OSTEITIS DEFORMANS)

NC: Classify and describe the etiology, pathogenesis, manifestations, radiologic and morphologic features and complications of Paget disease of the bone.

Q. Write short note on Paget disease of bone.

Definition: Paget disease is a unique skeletal disease or **condition characterized by increased, but disordered and structurally unsound, bone**.

Pathogenesis

Genetic and environmental factors are responsible for Paget disease.

- **Genetic factors:** About 50% are familial cases and 10% of sporadic cases show mutations in the *SQSTM1* gene. These mutations increase the activity of NF-κB, which increases osteoclast activity.
- **Environmental factors:** Infection of osteoclast precursors with viruses (e.g., measles or other RNA viruses) alters vitamin D sensitivity and IL-6

secretion. Both of them can lead to increased bone resorption.

MORPHOLOGY

Paget disease shows histologic variation throughout its course of disease and from site to site.

Phase: It can be divided into three sequential phases namely

1. **Initial osteolytic stage:** It is characterized by numerous large osteoclasts and resorption pits. The osteoclasts may have 100 or more nuclei. The findings are not specific.
2. **Mixed osteoclastic–osteoblastic stage: In this phase, there is both osteoclastic–osteoblastic activity.** Osteoclasts persist but many of the bone surfaces are also lined by prominent osteoblasts. It progress to predominance of osteoblastic activity and evolves into the next final phase.
3. **Final burned-out quiescent osteosclerotic stage: The hallmark is a mosaic pattern of lamellar bone during the sclerotic phase.** The unusually **prominent cement lines**, which join haphazardly oriented units of lamellar bone produces a **jigsaw puzzle–like appearance.** During the sclerotic phase, the bone is thickened but without structural stability. Thus, the bone is susceptible to deformation and fracture.

Clinical Course

- **Bones involved:** Paget disease may involve one bone (*monostotic)* in about 15% and multiple bones (*polyostotic)* in about 85% of cases. The axial skeleton or proximal femur is affected in up to 80% of cases. Most cases are asymptomatic.
- **Age:** Usually begins in late adulthood. Usually detected as an incidental radiographic finding.

Symptoms

- **Pain:** Commonly complains of pain localized to the involved bone. Pain may be due to microfractures or by bone overgrowth which may compresses spinal and cranial nerve roots.
- **Enlargement of the craniofacial skeleton:** It may produce *leontiasis ossea* (lion face). The cranium becomes heavy and it becomes difficult to hold the head erect. The weakened Pagetic bone may cause invagination of the skull base *(platybasia)* and compression of the posterior fossa.
- **Leg bones:** Weight-bearing produces anterior bowing of the femurs and tibiae and may distort the femoral heads. It may result in severe

secondary osteoarthritis. *Chalk stick–type fractures* may develop in the long bones of the lower extremities.

- **Compression fractures of the spine:** It may produce injury to spinal cord and kyphosis.

Diagnosis

- **Radiological findings:** Diagnosis is usually done by the radiographic findings.
- **Serum:** Raised serum alkaline phosphatase levels but normal serum calcium and phosphorus.

Complications

Most of the patients have mild symptoms which are suppressed by treatment with calcitonin and bisphosphonates. However, **variety of tumor and tumor-like conditions may develop in Pagetic bone. Secondary osteosarcoma may develop** in less than 1% of cases and about 5–10% in patients with severe polyostotic disease.

BONE TUMORS

CC: Classify and describe the etiology, pathogenesis, manifestations, radiologic and morphologic features and complications and metastases of bone tumors.

Q. Classify bone tumors. Describe the etiology, pathogenesis, types, gross and microscopy of osteosarcoma.

Q. Classify bone tumors. Describe the morphological and radiological features of giant cell tumor of bone.

Classification of Bone Tumors (Table 25.2)

Q. Enlist bone forming and chondrogenic/cartilaginous tumors.

TABLE 25.2: Major primary tumors of bone.

Histologic type	*Benign*	*Malignant*
Osteogenic/bone forming	• Osteoma • Osteoid osteoma • Osteoblastoma	Osteosarcoma (primary/ secondary)
Chondrogenic/ Cartilaginous	• Osteochondroma • Chondroma • Chondroblastoma	• Chondrosarcoma • Dedifferentiated chondrosarcoma
Hematopoietic		• Myeloma • Malignant lymphoma
Unknown origin	• Giant-cell tumor (primitive osteoblast precursors) • Unicameral cyst • Aneurysmal bone cyst	Ewing sarcoma
Neuroectodermal		Chordoma

Osteoid Osteoma and Osteoblastoma

Q. Write a short note on osteoid osteoma.

Osteoid osteoma and osteoblastoma are benign bone producing tumors. Both microscopically have similar features but differ clinically and radiographically.

Osteoid Osteomas

- **Gender and age:** Most common in young men.
- **Site:** About 50% of them arise from the cortex of femur or tibia.
- **Size:** They are usually **<2 cm in diameter** and have a thick rim of reactive cortical bone.

- **Clinical features:** Though small, they present with severe nocturnal pain probably due to prostaglandin E2 produced by osteoblasts. This **pain is relieved by aspirin** and other nonsteroidal anti-inflammatory drugs. Treatment is by radiofrequency ablation.

Osteoblastoma

It is **> 2 cm** and usually involves the posterior components of the vertebrae (laminae and pedicles). The **pain does not respond to aspirin.** Treatment is by either curettage or excision.

MORPHOLOGY

Gross

Both osteoid osteoma and osteoblastoma appear as well circumscribed, round-to-oval mass of gritty tan tissue with hemorrhage.

Microscopy

Composed of randomly interconnecting delicate trabeculae of woven bone rimmed by a single layer of osteoblasts. The neoplastic bone is surrounded by loose connective tissue stroma containing many dilated and congested capillaries.

Osteochondroma

- Osteochondroma (exostosis) is the **most common benign cartilage-capped tumor**, which attaches to the underlying bone by a stalk.
- **Age** group: **Late adolescence** and **early adulthood**.
- **Sex**: **Men are affected three times more** often than women.

MORPHOLOGY (FIGS. 25.4A AND B)

Site and location: Arise from the **metaphysis near the growth plate** of **long tubular bones**, especially about the knee.

Osteochondromas are sessile or mushroom shaped, and range in size from 1 to 20 cm. The **cap is composed of benign hyaline cartilage and underlying lamellar bone.**

Clinical Features

Slow-growing masses, detected as an incidental finding.

Bone tumors: Classified according to their normal tissue counterpart.

Osteochondroma: Most common benign cartilage-capped bone tumor.

Osteosarcoma

Q. Write short note on osteosarcoma.

- Osteosarcoma (osteogenic sarcoma—OS) is the **most common (20%) primary malignant bone tumor**, excluding of myeloma and lymphoma.

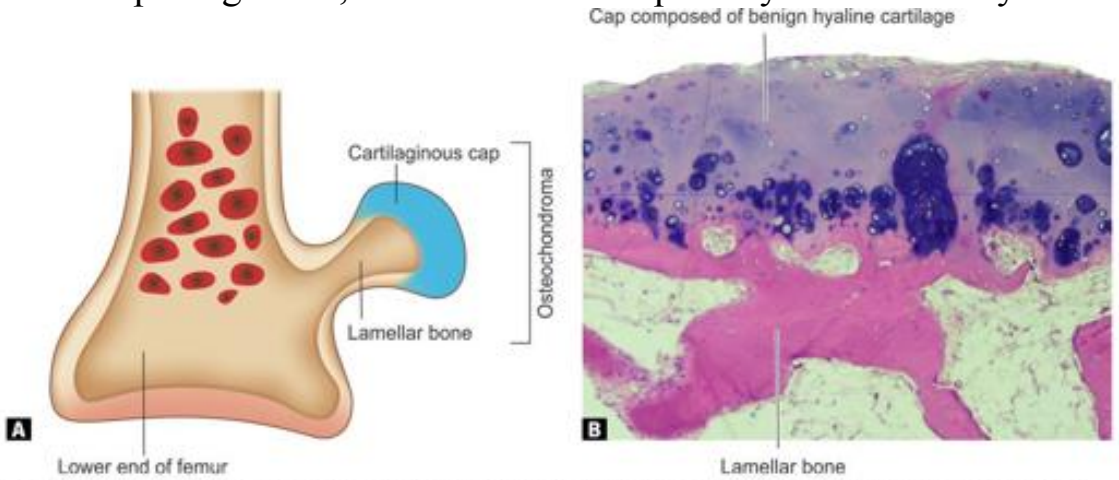
Definition: Osteosarcoma is a **highly malignant bone tumor** characterized by formation of **bone matrix or osteoid** (unmineralized bone) **by malignant tumor cells**.

- **Age group:** OS has a **bimodal age distribution**.
  - Three-fourth occur in between **10 and 20 years of age**.
  - **Secondary** OS (following **Paget disease, bone infarcts, irradiation**, etc.) develop in **elderly after 40 years of age**.
- **Sex:** Affects boys more commonly than girls (2:1).

Q. Write a short note on pathogenesis of osteosarcoma.

Pathogenesis

Based on the pathogenesis, OS is divided into primary and secondary.



Figs. 25.4A and B: (A) Osteochondroma appears as a mushroom-shaped tumor covered by cartilage; (B) Microscopy of osteochondroma showing cap composed of benign hyaline cartilage and underlying lamellar bone.

Primary Osteosarcomas

In this type, the underlying bone is unremarkable. About 70% of OS have acquired genetic abnormalities, such as **ploidy changes** and **chromosomal aberrations**.

- **Mutations in tumor suppressor gene:**
  - ***RB*, the retinoblastoma gene** (a cell cycle regulator) mutations is associated with 1000-fold increased risk of OS and its mutation is found in about two-thirds of patients.
  - **Patients with Li-Fraumeni syndrome (germline *TP53* mutations)** have a greater incidence of OS.
- **Inactivation of *CDKN2A*,** which codes p16 (a negative regulator of cyclin-dependent kinases) and p14 (it augments the function of tumor suppressor gene *p53*). have also been found in OS.
- **MDM2 and CDK4:** They are cell cycle regulators that inhibit functions of *p53* and RB, respectively. They are overexpressed in many low-grade OS.

Peak incidence of osteosarcomas is during the time of the adolescent growth spurt. It occur mostly in the growth plate region of the bones with the fastest growth. The increased proliferation at these sites may predispose to osteosarcoma.

Secondary Osteosarcoma

Osteosarcomas in older persons almost always develop in association with pre-existing bone disorders. These include:

- **Paget disease of bone**.
- **Radiation exposure:**
  - Following therapeutic radiation for tumor, such as lymphoma.
  - Radium watch dial painters, who wetted their brushes by licking them, developed OS many years later.
- **Chemotherapy:** Children treated with **alkylating agents** (other malignancies) have an increased risk.
- **Pre-existing benign bone lesions.** For example, **fibrous dysplasia, osteomyelitis**, and bone marrow infarcts. Trauma may call attention to an existing OS rather than causing the tumor.

Classification

Q. Write short note on morphology and radiological appearance of osteosarcoma.

It can be classified in different ways:

1. **Anatomic site of origin**
   - **Conventional** (classical, medullary, intramedullary, textbook)
   - **Intracortical**
   - **Surface OS**
     - Parosteal (juxtacortical): They arise on the surface of the cortex.
     - Periosteal: They arise on the surface of periosteum.
2. **Degree of differentiation:**
   - Well differentiated
   - Moderately differentiated
   - Poorly differentiated.
3. **Number of tumors**
   - **Solitary**
   - **Multicentric**
     - Synchronous
     - Metachronous.
4. **Pathogenesis** (described above)
   - **Primary**
   - **Secondary** to pre-existing disorders.
5. **Histologic features** (described below).

MORPHOLOGY

Most Common Subtype

- Primary
- Solitary
- Conventional/Intramedullary
- Poorly differentiated.

Location: Usually arise in the **metaphyseal region of the long bones** of the extremities.

Sites: It usually arises near the knee or shoulder. The common site is:

- Lower femur
- Upper tibia, or fibula
- Proximal humerus.

Gross (Figs. 25.5A to C)

- Gross appearance **varies depending on the proportions of bone, cartilage, stroma and blood vessels**.
- **Size:** Usually **big bulky tumors**.
- **Cut surface**: **Gray-white** in color, **gritty**, shows **areas of hemorrhage and cystic degeneration**.

Microscopy (Figs. 25.5A to C)

Q. Write a short note on histological types of osteosarcoma.

Malignant Tumor Cells

- **Tumor cells vary in size and shape**
- **Nucleus:** Usually show **large hyperchromatic** nuclei and **often shows mitotic figures**
- **Bizarre tumor giant cells** are common.

Matrix Component

Production of **osteoid** (unmineralized/noncalcified) **or bone** (calcified osteoid) **by malignant tumor cells** is the single **diagnostic feature** of conventional OS.

- **Osteoid:** It appears as a **dense, uniform, eosinophilic glassy intercellular material**.
- **Neoplastic bone:** It usually has a coarse, lace-like architecture.

Histological Subtypes

Most tumors contain a **mixture of cells with varying amounts of matrices** mentioned below. **Depending on the one predominant matrix**, conventional OS is **divided into**:

- **Osteoblastic**: **Large amount of osteoid and bony trabeculae**.
- **Chondroblastic: Abundant malignant cartilage**.
- **Fibroblastic/fibrohistiocytic:** They appear **similar to malignant fibrous histiocytoma**.
- **Telangiectatic: Large cavernous dilated vascular channels** and it is **more aggressive**.
- **Small cell:** The tumor **cells are small in size, uniform** and simulate the appearance of Ewing's sarcoma and malignant lymphoma.
- **Giant cell:** This subtype contains **numerous giant cells**.

Vascular invasion and necrotic area may be conspicuous.

Immunohistochemistry: The malignant tumor cells **stain prominently for alkaline phosphatase** and **osteonectin**.

Surface Osteosarcoma

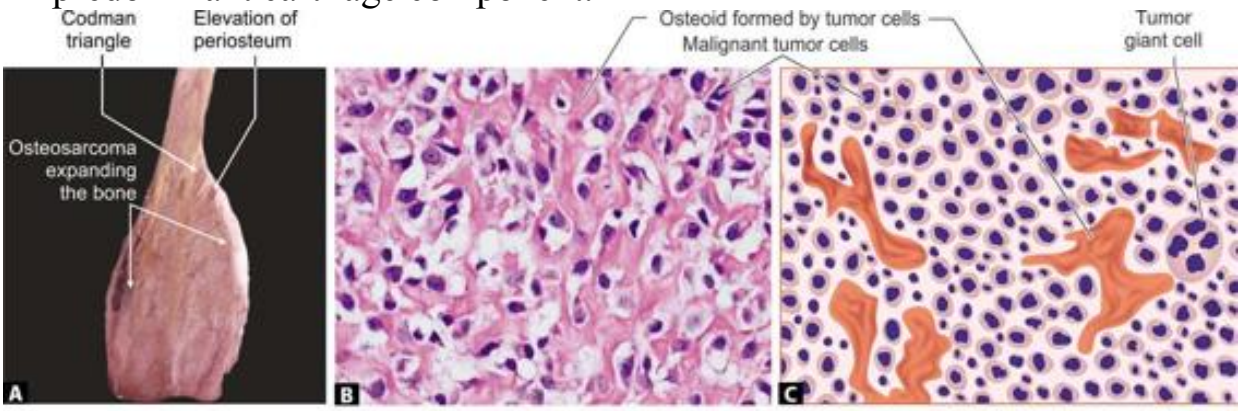
There are two variants:

Juxtacortical (Parosteal) OS

- A variant of **low-grade OS**.
- Usually **arises on the surface of the bone** and **grows along the external surface** of the bone, **both linearly and longitudinally**, without involving the medullary cavity.
- **Prognosis: Excellent.**

Periosteal OS

- A rare variant of OS.
- **Occurs on the periosteal surface** of the bone, **between the cortex and periosteum**.
- Microscopically, it is **high-grade osteosarcoma**, and characteristically has predominant cartilage component.



Figs. 25.5A to C: (A) Gross appearance of osteosarcoma of the lower part of distal femur showing a tan-white tumor, filling most of the medullary cavity of the metaphysis and proximal diaphysis. The tumor has infiltrated through the cortex, lifted the periosteum and this region on X-ray appears as Codman triangle; (B) Photomicrograph; (C) Diagrammatic of microscopy of osteosarcoma composed of pleomorphic tumor cells separated by osteoid.

Clinical Course

- Usually present as **painful, progressively enlarging masses** around the knee or other involved site.

- The involved area is **swollen and tender** and the function of adjacent joint becomes reduced.
- **Serum alkaline phosphatase is increased**.

Radiological Appearance

OS shows a **large destructive, mixed lytic** (bone destruction) and **blastic mass** (neoplastic bone formation) with infiltrative margins.

Codman Triangle

Q. Write short note on radiological features/Codman triangle.

The tumor frequently **infiltrates the cortex and lifts the periosteum**, and produces a **space between the cortex and lifted ends of periosteum**. Radiologically, this space appears as **triangular shadow** and is known as **Codman triangle (characteristic but not diagnostic** of this tumor).

The **space** mainly contains **reactive new bone**, **which is arranged perpendicular to the bone surface**, but it may also contain malignant tumor.

Sunburst Appearance

When the malignant **tumor extends into soft tissue, parallel lines of mineral deposition** in the **periosteal region** gives an appearance of rays of sun → called as "sunburst" appearance **(Fig. 25.6)**.

Spread

Local Spread

- **Invasion of the adjacent cortex** and destroys the nearby cortex.
- **Elevation or perforation of the periosteum** by tumor tissue.
- **Spread along the** medullary (**marrow**) **cavity**.
- **Extension into the epiphysis**:

- Articular end of the bone is generally not involved in the initial stage.
- Later, it may infiltrate the epiphyseal plate and may even involve the joint space.

- **Extension into the soft tissues** and may involve skin.

Blood Spread

It is an aggressive tumor, which may spread through the bloodstream to the **lungs**. Less commonly, it may metastasize to other bones, the pleura, brain, and the heart.

Q. An 18-year-old male presented to orthopedic department with a complaint of pain and progressive swelling around his right knee for the past 3 months. Physical finding showed a diffuse hard swelling with local pain over the area of the distal right femur. An X-ray of the right knee showed an ill-defined mass involving the metaphyseal region of the distal right femur with elevation of the adjacent periosteum. A bone biopsy specimen was obtained and it confirmed the provisional clinical diagnosis.

(a) What is the probable diagnosis? (Ans: Osteosarcoma lower end of femur).

(b) Describe the morphological appearance of the lesion (refer pages 843-44).

(c) What are the salient radiological appearances of this disease? (refer above).

Osteosarcoma: Most common primary malignant bone tumor.

Osteitis deformans (Paget disease of bone):

- Mosaic pattern of lamellar bone histology
- Increased risk of OS

Osteosarcoma: Histological subtypes
1. Osteoblastic
2. Chondroblastic
3. Fibroblastic/Fibrohistiocytic
4. Telangiectatic
5. Small cell
6. Giant cell

Osteosarcoma: Stain positive for
- Alkaline phosphatase
- Osteonectin

Osteosarcoma: Codman triangle and sunburst appearance on X-ray.

Codman triangle: Characteristic but not diagnostic of osteosarcoma.

Osteosarcoma: Single diagnostic feature is production of osteoid or bone by malignant tumor cells.

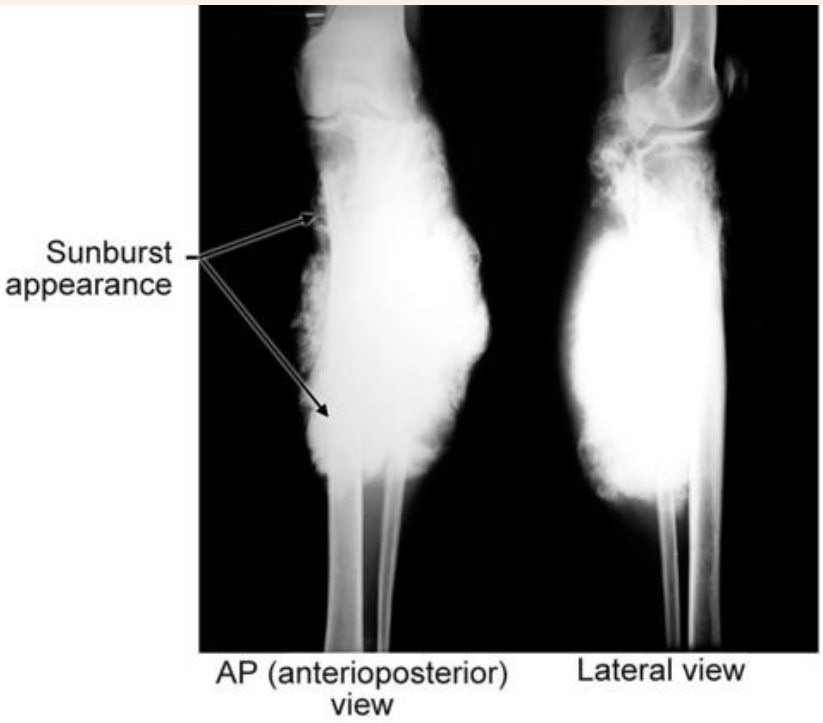


Fig. 25.6: X-ray of the knee joint. Osteosarcoma of proximal tibia extending into the soft tissue with parallel lines of mineral deposition giving rise to sunburst appearance.

Giant-cell Tumor

Q. Write short note on giant-cell tumor/osteoclastoma.

Definition: Giant-cell tumor (GCT synonym osteoclastoma) is characterized by **multinucleated osteoclast-type giant cells randomly and uniformly distributed in a background of mononuclear cells**.

- Giant-cell tumor is a **relatively uncommon, locally aggressive** and **potentially malignant neoplasm**.
- **Age**: Usually seen between **20 and 40 years**. In elderly, it may be secondary to irradiation.
- **Sex**: Slight predilection for women.
- **Cell of origin**: Primitive stromal cells.

Pathogenesis

The neoplastic cells of giant cell tumor are primitive osteoblast precursors and form only a minority of the tumor cells. The major cells of the tumor are non-neoplastic osteoclasts and their precursors. The neoplastic cells express high levels of RANKL. They promote the proliferation of osteoclast precursors and their differentiation into mature osteoclasts are through RANK expressed by these cells.

MORPHOLOGY

Q. Write a short essay on gross and microscopy of giant cell tumor.

Site

Giant-cell tumors involve **epiphyses but may extend into the metaphysis** of a **long bone**.

Bones Involved

Any bone can be involved, majority arise in:

- Knee area: Distal femur and proximal tibia.
- Lower end of radius, Humerus and fibula.

Gross (Fig. 25.7A)

Large tumor, clearly circumscribed and frequently undergo cystic change.

Cut surface

- **Soft and red-brown** without bone or calcification
- **Numerous hemorrhagic areas** give an **appearance of a sponge** full of blood.

Microscopy (Figs. 25.7B and C)

Two types of cells

1. Mononuclear (Stromal) Cells

They are of two types.

i. **Neoplastic mononuclear cells:** They are **uniform oval and plump** with **large nuclei, prominent nucleoli** and **scanty cytoplasm**. Tumor mostly consists of these neoplastic cells. Mitotic activity is common in these cells but not found in the giant cells. **Diagnosis of malignancy depends upon the morphology of the mononuclear cells rather than that of the multinucleated giant cells**. The nuclei of these cells and the osteoclasts are similar. Thus, it is difficult to identify the neoplastic osteoblast precursors on routine histology. The tumor cells do not synthesize bone or cartilage.

ii. **Non-neoplastic:** These are mononuclear cells derived from macrophage–monocyte.

2. Multinucleated (Osteoclastic) Giant Cells

- The **background** shows **numerous large osteoclast-type giant cells** throughout the richly vascularized stroma.
- These **cells have 100 or more nuclei**, which resemble those of the mononuclear cells.

Area of cystic degeneration, necrosis and mitotic figures may be seen.

Secondary features: These include areas of **hemorrhage, necrosis**, hemosiderin deposition, and reactive bone formation.

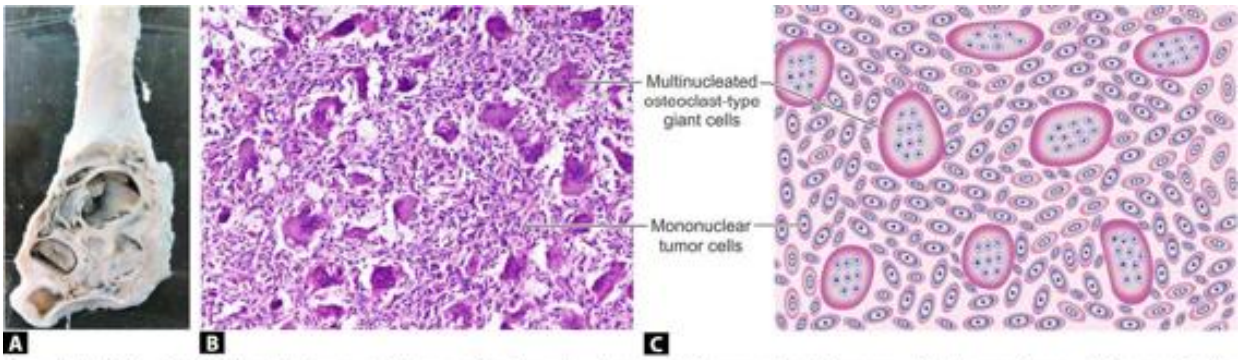
Radiological Appearance

Q. Write a short answer on radiological features of giant cell tumor.

- It produces a **lytic lesion** and **grows slowly, expands the bone** and **destroys the overlying cortex**.
- The tumor produces a bulging **soft tissue mass surrounded by a thin, shell of reactive bone** due to periosteal reaction. This on radiology has multiloculated or **soap-bubble** appearance **(Fig. 25.8)**.

Clinical Course

- Commonly seen near knee joints.
- Pain (usually in the joint adjacent to the tumor) and arthritis-like symptoms.
- Microfractures and pathologic fractures may develop due to thinning of the cortex.



Figs. 25.7A to C: Giant-cell tumor of bone: (A) Grossly shows a circumscribed tumor with hemorrhage; Microscopically; (B) Photomicrograph; (C) Diagrammatic shows numerous multinucleated giant cells. The background shows mononuclear stromal cells.



Fig. 25.8: X-ray of the knee joint. Giant cell tumor of distal femur showing soap-bubble appearance.

Biologic Behavior

- Majority of GCTs behave in a **benign fashion**, but **locally aggressive tumors may locally recur**.
- However, **all GCTs** must be **considered as potentially malignant**, because they may metastasize.

Spread

- **Local spread**: Tumor is usually restricted within the involved bone surrounded by periosteum.

- Aggressive tumors may penetrate the cortex and the periosteum, and may involve the joint capsule and the synovial membrane.

- **Hematogenous spread:** Common site is lung.

Q. A 30-year-old female complains of pain in the region of her left knee for the past one month. On physical examination, there is tenderness to palpation of the distal left thigh. The area is firm, but there is no erythema or warmth. An X-ray showed a five centimeter mass involving the distal femoral epiphyseal region with a "soap bubble" appearance. Biopsy confirmed the clinical diagnosis.

(a) What is the probable diagnosis? (Ans: Giant cell tumor of the lower end of femur)

(b) Describe the morphological appearance of the lesion (refer page 845).

(c) What are the salient radiological appearances of this disease? (refer page 845).

Giant-cell tumor:
- Neoplastic mononuclear cells
- Reactive multinucleated osteoclast type of giant cells

Q. Write a short answer on giant cell lesions of bone and synovium.

Giant cell lesions of bone and synovium:
- Giant cell tumor
- Brown tumor (hyperparathyroidism)
- Giant cell reparative granuloma

- Chondroblastoma
- Tenosynovial giant cell tumor (previously known as pigmented villonodular synovitis).

Giant-cell tumor: Soap-bubble appearance on X-ray examination.

GCTs must be considered as potentially malignant.

Chondrosarcoma

Q. Write short note on chondrosarcoma.

Chondrosarcomas are **malignant bone tumors** characterized by the **production of neoplastic cartilage.**

- **Age group**: Usually around **40 years or older** (fourth to sixth decades).
- **Sex**: **Males are twice frequently** affected than females (2:1).

Pathogenesis

Chondrosarcomas are genetically heterogeneous. Few of them produce reproducible abnormalities. Chondrosarcomas arising in multiple osteochondroma syndrome show mutations in the *EXT* genes. Sporadic chondrosarcomas may show *IDH1* and *IDH2* mutations, mutation of the collagen *COL2A1* gene and silencing of the *CDKN2A* tumor suppressor gene by DNA methylation.

Classification

- **According to anatomic site**:
  - **Central (intramedullary) tumors** (about 90%)
  - **Peripheral (juxtacortical and surface)**: Less common and arise outside the bone.
- **Microscopic appearance:**
  - Conventional (hyaline and/or myxoid)
  - Clear cell
  - Dedifferentiated
  - Mesenchymal
- **Presence or absence of pre-existing lesion**
  - **Primary chondrosarcoma:** No known pre-existing lesion.
  - **Secondary chondrosarcoma:** Developing in a pre-existing lesion
    - Enchondroma
    - Solitary osteochondroma
    - Hereditary multiple osteochondromas

MORPHOLOGY

Q. Write a short note on morphology of chondrosarcoma.

Central conventional chondrosarcoma: It consists of malignant hyaline and myxoid cartilage.

Location

Chondrosarcomas commonly arise in the **central medullary cavity** of:

- **Pelvic bones**
- **Shoulder**
- Ribs
- Long bones

Gross (Fig. 25.9A)

Large bulky tumors, and consists of **nodules of gray-white/light-blue with translucent glistening areas (represents cartilage)**.
Cut section:

- **Mucoid/myxoid feel** and the matrix may ooze from the cut surface.
- **Spotty calcifications** are common and appear as white gritty areas.
- **Central area of necrosis** may produce cystic spaces.
- Adjacent cortex may show thickening or erosion.

Microscopy

It consists of **malignant cartilage cells** in **various stages of maturity**. It is graded from 1 to 3, depending on the degree of cellularity, cytological atypia and mitotic activity.

Low-grade or Grade 1 (Fig. 25.9B)

- Mild hypercellularity.
  - **Cells:**
    - **Chondrocytes show plump vesicular nuclei with small nucleoli**.
    - **Binucleate cells** are **few** in number.
- **Mitotic figures** are **rare**.
- **Matrix:** Portions of the matrix frequently mineralize, and the cartilage may undergo endochondral ossification.

Grade 2

Moderate cellularity and more cellular than grade 1. Chondrocytes show more **pronounced nuclear changes**.

Grade 3

They are uncommon and show **marked hypercellularity.**

- Chondrocytes show **extreme pleomorphism**
- **Bizarre tumor giant cells**
- **Mitoses frequent**

Variants

- **Dedifferentiated chondrosarcoma** is a low-grade chondrosarcoma with a second, high-grade component which do not have cartilage.
- **Clear cell chondrosarcoma** composed of large, malignant chondrocytes with abundant clear cytoplasm, numerous osteoclast-type giant cells, and reactive bone formation within the tumor.
- **Mesenchymal chondrosarcoma** consists of islands of well-differentiated hyaline cartilage surrounded by sheets of small round cells.

Radiological Appearance

Shows a tumor in the **medullary cavity** with **poorly defined borders**. The shaft is thickened and tumor perforates the cortex. The calcified matrix appears as foci of **flocculent densities** with **ring-like ossifications**.

Clinical Features

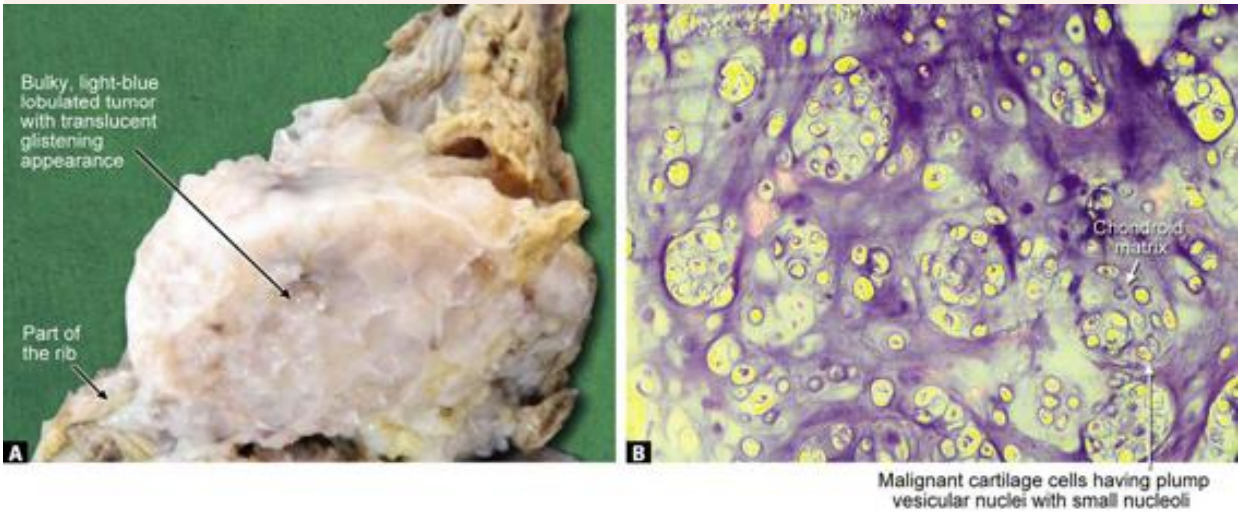
Painful, progressively enlarging masses. Biologic behavior of the tumor depends on the histological grade.

Spread

- **Local spread**: Locally aggressive tumor. It may **infiltrate the marrow space** and may grow with broad pushing fronts into the **surrounding soft tissue**.
- **Hematogenous spread**: Lungs and skeleton.

Chondrosarcoma: Malignant mesenchymal tumor forming cartilage.

Chondrosarcoma: Popcorn calcification, flocculent densities with ring-like ossifications.



Figs. 25.9A and B: (A) Chondrosarcoma of rib; (B) Microscopic appearance of well-differentiated chondrosarcoma.

Ewing Sarcoma/Primitive Neuroectodermal Tumor

Q. Write short note on Ewing sarcoma.

Definition: Ewing sarcoma family of tumors is **primary malignant small round-cell tumors of bone and soft tissue**.

- **Uncommon** tumors and constitute ~ 6–10% of primary malignant bone tumors.
- **Age:** Mostly seen between **10 and 15 years** of age, and about 80% are seen in patients younger than 20 years. In children, it is second most common group of bone sarcomas, first being osteosarcoma.
- **Sex**: More frequent in **boys** than girls (2:1).

Categories

It consists of **two tumors that differ only in their degree of neural differentiation**. This distinction has no clinical significance.

1. **Ewing sarcoma** (EWS): **Undifferentiated** tumors.
2. **Primitive neuroectodermal tumor** (PNET): With **neural differentiation**.

Etiology and Pathogenesis

Origin: The cell of origin is not known. Probably, it arises from **multipotent mesenchymal stem cell** and primitive neuroectodermal cells.

Chromosomal abnormalities.

- **Balanced (11; 22) (q24; q12) translocation:** It produces **fusion of *EWSR1* gene on chromosome 22** to the ***FLI1* gene on chromosome 11** in about 85% of tumors.
- The **fusion of the *EWS1* gene to *FLI-1* gene → activation of C-myc promoter** → abnormal cell proliferation and survival.
- A less common translocation produces *EWS/ERG* fusion gene → produces a variant of EWS with a significantly worse prognosis.

MORPHOLOGY

Q.Write short note on morphology of Ewing sarcoma.

Location

Arise in the **diaphysis of the medullary cavity of long bones**.

Site

Ewing sarcoma and PNET involve the **long tubular bones in childhood**.

- **Long bones: Humerus, tibia** and **femur**.
- **Flat bones**: Pelvis

Gross

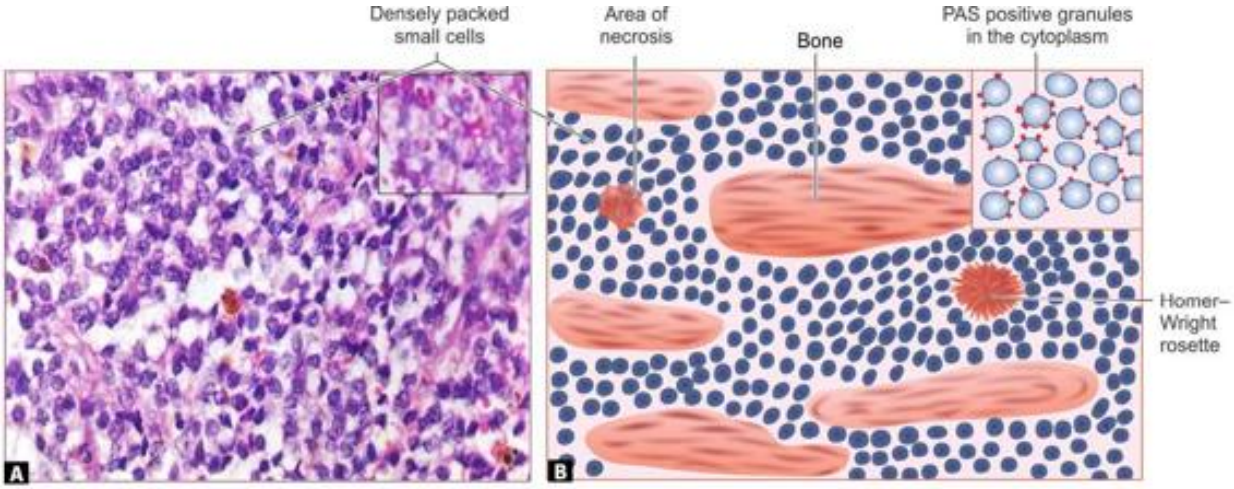
- Arise in the **diaphysis** and **may infiltrate the medullary spaces** without destroying the bony trabeculae.
- **Diffusely infiltrate the cortex** and the tumor **may also penetrate the periosteum** and spread into the soft tissues.
- **Soft, grayish white/tan-white**, and frequently show areas of **hemorrhage and necrosis.**

Microscopy (Figs. 25.10A and B)

- **Tumor cells**: They are **uniform, small and round.**
  - **Size:** Tumor cells are about **twice the size of a lymphocyte.**
  - **Cytoplasm: Scant or little** and **appears clear** because it is **rich in glycogen,** which is an important diagnostic feature. This **stains positive** with the **Periodic acid-Schiff (PAS) stain**.

Q. Write a short answer on Homer–Wright rosette.

- **Arrangement**: **Closely packed sheets** of cells.
- **Homer–Wright rosettes:** They may be seen. They consist of **tumor cells arranged in a circle about a central fibrillary space**. This is **indicative of neural differentiation**.
- **Mitoses are infrequent** even though the tumor is highly cellular.
- **Interstitial stroma: Little or absent** and the fibrous strands separate the sheets of tumor cells into irregular **nests or sheets**. **Necrosis** may be prominent.



Figs. 25.10A and B: (A) Photomicrograph; (B) Diagrammatic; Ewing sarcoma composed of sheets of closely packed uniform small round cells with scanty cytoplasm. The inset of B shows PAS positive granules in the cytoplasm.

Tumors with Homer–Wright rosette: Ewing sarcoma, neuroblastoma and medulloblastoma.

Immunohistochemistry

EWS cells express characteristic antigens, some of which are part of the translocation product (e.g., **FLI-1 and CD99**).

Radiographic Appearance

- It produces **destructive lytic lesion** in which the border between normal bone and the tumor is indistinct. The tumor may extend into the surrounding soft tissues.
- Characteristic circumferential, **discontinuous layers of periosteal new bone formation** produces **onion-skin pattern (Fig. 25.11)**.

Clinical Features

- **Painful enlarging masses** and the affected site is **tender, warm, and swollen**.
- **Systemic features**: It may develop in some and **mistaken for infection like osteomyelitis**.
  - Fever, weakness, and bone pain.
  - Hematological findings: Anemia, leukocytosis, and raised erythrocyte sedimentation rate.

Spread

- **Local spread:** It may spread from medullary cavity **into cortex, periosteum and surrounding soft tissue**.
- **Blood spread:** Lungs, brain and other bones (skull).

Treatment includes chemotherapy and surgical excision with or without irradiation.

Ewing sarcoma: Highly aggressive small round cell tumor affecting the diaphysis.

Location of genes:
- *EWS* on chromosome 22q12
- *FL1* gene on 11q24
- *ERG* gene on 21q22

Ewing sarcoma: Chromosomal abnormalities of EWS gene rearrangements
- Translocation of EWS gene
- Fusion gene *EWS/FLI-1*

Ewing sarcoma: Translocations of diagnostic importance
- t(11; 22) (q24;q12)
- t(21;22) (q22;q12)

Ewing sarcoma:
1. Uniform small round cells
2. PAS positive granules in the cytoplasm
3. Homer–Wright rosettes

Immunohistochemistry in EWS:
- FLI-1
- CD99

Ewing sarcoma: Onion-skin appearance on X-ray.

OSTEOARTHRITIS

Definition: Osteoarthritis (degenerative joint disease) is a **noninflammatory**, **slowly progressive joint disease**, mainly **involving the cartilage**.

- **Most common disease** characterized by the **progressive destruction of articular cartilage** of weight-bearing joints of **genetically susceptible older persons**.

- It leads to narrowing of joint, subchondral bone thickening, and finally **nonfunctioning, painful joint**.

Joints Affected

- **Weight-bearing joints** (knee, hips and cervical and lumbar segments of the spine).
- **Non-weight-bearing proximal and distal interphalangeal joints** of the fingers, first carpometacarpal joints, and first tarsometatarsal joints of the feet.

Types

- **Idiopathic or primary osteoarthritis**: It develops as aging process and may affect few (oligoarticular) or many joints.
- **Secondary osteoarthritis:** It appears in younger individuals with predisposing condition.
  - Previous injuries to a joint.
  - Congenital deformity of a joint(s).
  - Secondary to systemic disease (e.g., diabetes, marked obesity).

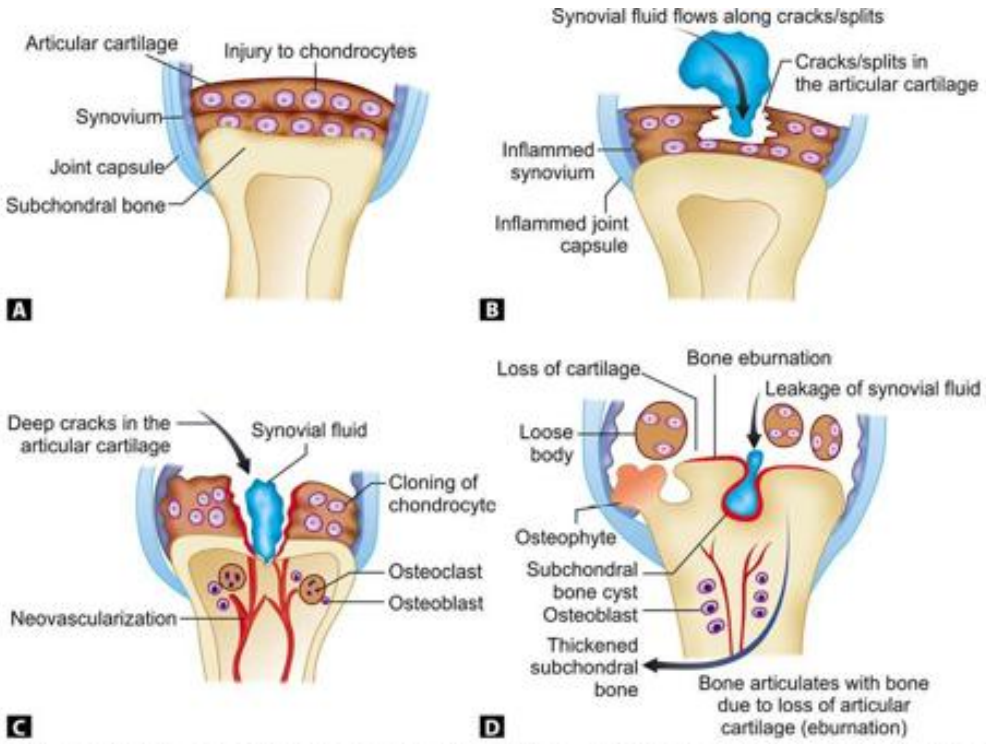
Pathogenesis and Morphology (Figs. 25.12A to D)

Osteoarthritis (OA) is a **multifactorial disease** having both genetic and environmental components.

- **Genetic factors**: Genes include those involved in prostaglandin metabolism and WNT signaling.



Fig. 25.11: X-ray of Ewing sarcoma involving the diaphysis of tibia.



Figs. 25.12A to D: Pathogenesis and morphological changes of osteoarthritis: (A) Aging together with genetic and biochemical factors initiate chondrocyte injury; (B) Death of chondrocytes leads to a crack in the articular cartilage and the synovial fluid flows into these cracks and leads to further loss and degeneration of cartilage; (C) Cartilage gradually worn away and new vessels grow (neovascularization) in from the epiphysis, and fibrocartilage is deposited; (D) The subchondral bone is exposed, which becomes thickened and eburnated. The broken pieces of cartilage form loose bodies (joint mice) in the synovial cavity. If there is a crack in this region, synovial fluid leaks into the marrow space and produces a subchondral bone cyst. Mushroom-shaped pearly-grayish bony spurs known as osteophytes develop at the periphery of the joint surface.

- **Environmental components:** The major factors are aging, biomechanical stress, obesity, muscle strength and joint stability.

Chondrocyte Injury

Aging together with genetic and biochemical factors initiate chondrocyte injury.

Changes in the Articular Cartilage

The injured chondrocytes proliferate and form clusters.

- **Cracks on superficial layers of the articular cartilage**: They develop following death of the injured chondrocytes (fibrillation).
- **Flow of synovial fluid along the cracks**: Synovial fluid penetrates deeper into the articular cartilage.
- **Inflammatory reaction**: This is initiated by inflammatory mediators secreted by injured chondrocyte. Repeated injury and chronic

inflammation lead to death of chondrocytes of the articular cartilage.

- **Grossly, the articular surface of involved bone appears soft and granular**.
- **Breaking off dead pieces of articular cartilage:** It produces inflammation and foreign-body giant cell reaction in the synovium. The process may lead to sloughing of full-thickness of the cartilage. The **broken pieces of cartilage form loose bodies** (**joint mice**) in the synovial cavity.

Changes in the Subchondral Bone

With sloughing of the full thickness of the articular cartilage; **the subchondral bone is exposed** and becomes the new articular surface.

- **Bone eburnation:** The friction of the opposing articular surface smoothens and burnishes the exposed subchondral bone. The subchondral bone appears thick, shiny, smooth giving it the appearance of polished ivory and known as **bone eburnation** (eburnated means ivory-like). New vessels growing from the epiphysis and fibrocartilage gets deposited.
- **Subchondral bone cyst:** The eburnated bone may crack in some areas (fracture gaps) and forces synovial fluid from the joint surface into the subchondral bone marrow regions but cannot exit. Because of this one-way ball valve-like mechanism, a **subchondral bone cyst** filled with synovial fluid is formed. The loculated fluid collection increases in size surrounded by reactive bone wall.
- **Development of osteophytes:** Mushroom-shaped pearly grayish bony outgrowths (spurs) known as **osteophytes** develop at the periphery of the joint surface. The synovium shows mild fibrosis and chronic inflammatory cells.

Clinical Features

- Osteoarthritis is an **insidious slowly progressive disease**, and causes long-term disability.
- Present with **deep, aching pain**, which worsens with joint movement and is relieved by rest.
- Involved **joints may be swollen, tender**, and may demonstrate crepitus.
- Osteophytes in spine can cause nerve root compression and neurologic deficits.

- **Prominent osteophytes** at the **distal interphalangeal joints** are known as **Heberden nodes**, and seen commonly in women.

Osteoarthritis: Most common degenerative joint disease mainly involving the cartilage.

RHEUMATOID ARTHRITIS

NC: Classify and describe the etiology, immunology, pathogenesis, manifestations, radiologic and laboratory features, diagnostic criteria and complications of rheumatoid arthritis.

Q. Write a short essay on rheumatoid arthritis.

Definition: Rheumatoid arthritis (RA) is a **systemic, chronic inflammatory disorder**, which mainly **affects the joints** but can affect many **other tissues and organs**.

Etiology

Q. Write short note on etiopathogenesis of rheumatoid arthritis.

Cause of rheumatoid arthritis remains **unknown**. Following three factors play an important role.

1. **Genetic factors:** Genetic susceptibility is a **major factor** in the pathogenesis of rheumatoid arthritis.
   - **HLA genes:** RA is linked to specific **HLA-DRB1 locus**.
   - **Non-HLA genes:** Polymorphism in *PTPN22* gene, which encodes a tyrosine phosphatase.
2. **Environmental arthritogen agents**: They are thought to **initiate the disease** process. **Smoking** and several **microbial agents** (e.g., virus, mycobacteria and *Mycoplasma)* have been suggested but not proved.
3. **Autoimmunity:** The initial inflammatory synovitis, an autoimmune reaction with T cells is responsible for the chronic destructive nature of rheumatoid arthritis.

Pathogenesis (Fig. 25.13)

- **Breakdown of self-tolerance**: Rheumatoid arthritis is initiated in a **genetically susceptible individual** by an **exposure to an unknown arthritogenic antigen** → which results in a breakdown of immunological self-tolerance.
- **Cytokine-mediated inflammation:** Loss of self-tolerance activates mainly **$CD4^+$ T cells** and initiates cytokine-mediated inflammation. The **principal source of cytokines is $CD4^+$ T cells** (T_H1 and T_H17). The **cytokines (e.g.,** interferon-γ TNF, IL-1, IL-8,IL-6 and IL-17) **recruit macrophages and other inflammatory cells**. The products of these inflammatory cells cause tissue injury as well as activation of resident synovial cells (synoviocytes).
- **Activation of B lymphocytes:** They mainly produce two types of autoantibodies.
  - **Anti-CCPs:** Many patients have **antibodies against cyclic citrullinated peptides (CCPs).**
    - CCPs include fibrinogen, type II collagen, α-enolase, and vientin.
    - CCPs are derived from proteins in which arginine residues are post-translationally converted to citrulline residues.
    - Antibodies against CCPs (anti-CCPs) form immune complexes and deposit in various tissues, main being the joints.
    - These antibodies are **useful for diagnosis** and may be involved in tissue injury.
  - **Rheumatoid factor:** About 80% of rheumatoid arthritis patients have autoantibodies called as **rheumatoid factor**.
    - **Rheumatoid factor is serum immunoglobulin M (IgM) autoantibody that binds to Fc portion of own/self-IgG.**
    - Rheumatoid factor may form immune complexes with self-IgG and can produce damage to joints and other tissues.
    - Rheumatoid factor is absent in some patients with rheumatoid arthritis (seronegative).
- **Production of proteolytic enzymes:** Inflammation causes tissue injury and activation of synoviocytes. Activated synoviocytes produce proteolytic enzymes, such as **collagenase**, **stromelysin, elastase**, etc., which **along with antigen–antibody complexes destroy the articular cartilage, ligaments and tendons of the joint**. TNF family of cytokines may cause increased activity of osteoclasts and produce destruction of bone.

- **Consequences**: The above actions bring out **edema, hyperplasia of synoviocytes** and **inflammatory infiltration in the synovium, which forms pannus**. The pannus adheres and grows over the articular surface causing **destruction of cartilage and erosion of adjacent subchondral bone**.

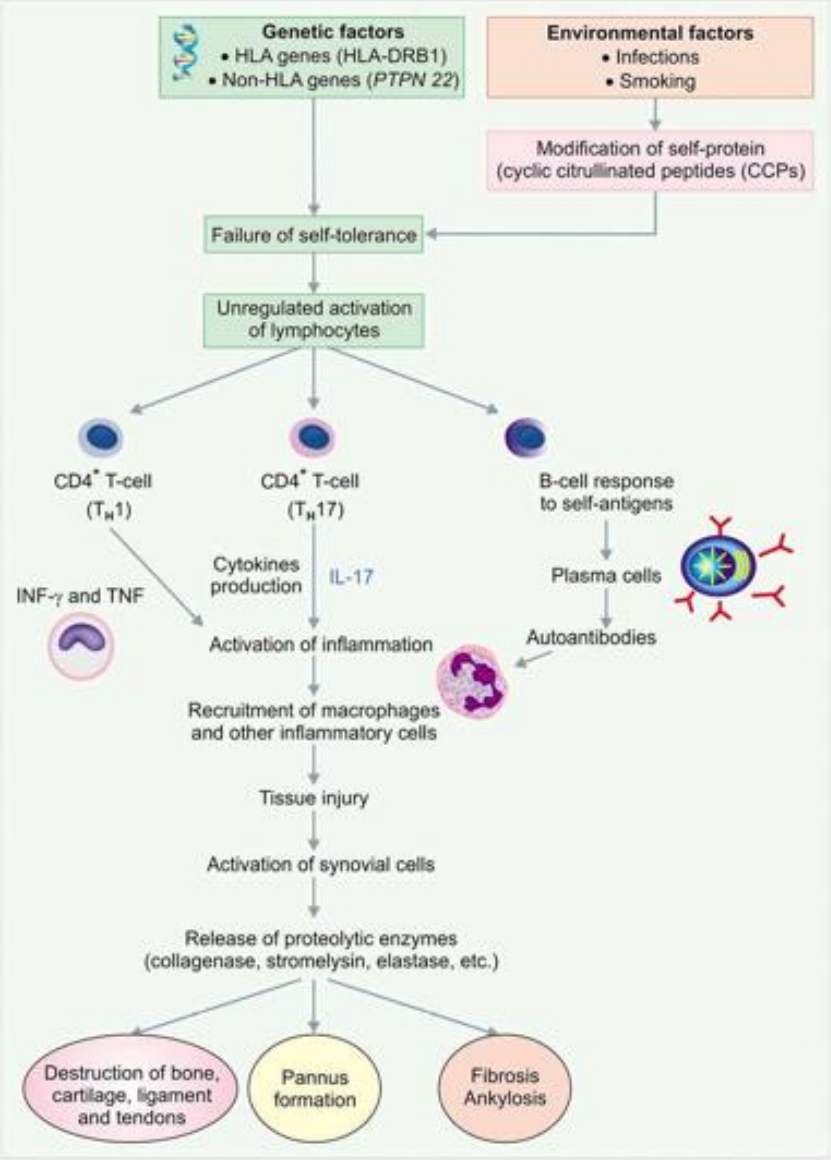


Fig. 25.13: Pathogenesis of rheumatoid arthritis.

Rheumatoid arthritis: Chronic autoimmune inflammatory disorder which mainly affects the joints.

RA: HLA-DRB1 and polymorphism in PTPN 22.

Rheumatoid arthritis: Loss of self-tolerance activates mainly CD4+ T cells and initiates cytokine-mediated inflammation.

Rheumatoid arthritis: Cytokine TNF plays main role.

Rheumatoid arthritis: B cells produce rheumatoid factor, which is an IgM antibody which has specificity against Fc portion of IgG.

Pannus is characteristic of: Rheumatoid arthritis.

Rheumatoid arthritis: Specific tests
- Rheumatoid factor
- Anti-CCP antibody

MORPHOLOGY

Joints (Figs. 25.14A to F)

Rheumatoid arthritis causes **most severe changes in the joints**.

- **Joints involved***:* **Symmetrical and bilateral**. Most commonly affected are **diarthrodial joints,** such as: **Proximal interphalangeal** and **metacarpophalangeal joints**, **elbows, knees, ankles**, and spine.
- **Joint lesions***:* It produces a **chronic nonsuppurative proliferative polyarthritis** and inflammatory **synovitis**. **As it progresses**, it **destroys the articular cartilage** and causes **ankylosis of the joints**.

Synovium (Fig. 25.14)

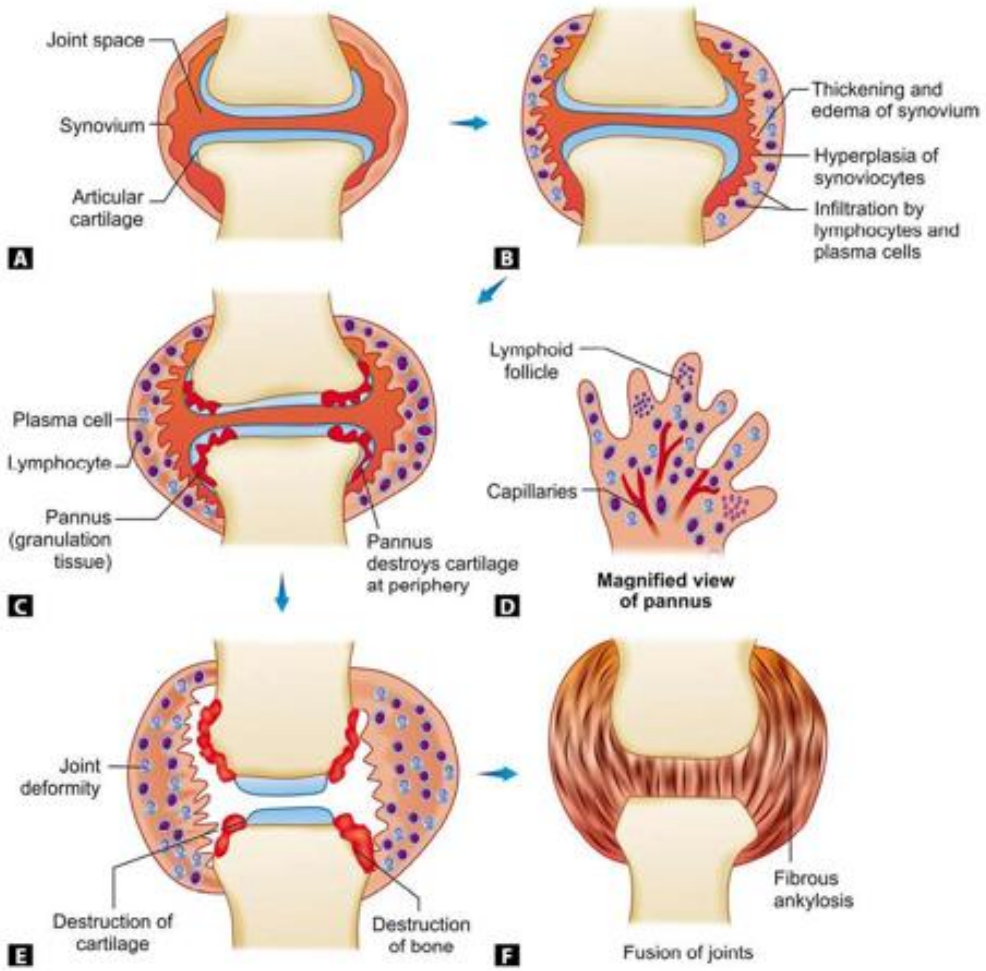
Gross

The involved synovium shows **edema, thickening, and hyperplasia of lining synoviocytes**. This results in conversion of smooth surfaced **synovium** into **delicate and bulbous papillary structures**.

Microscopy

Its characteristic histologic features include:

- **Synovial hyperplasia:** The lining synovial shows 6–10 layers of synoviocytes compared to normal 2–3 layers. These hyperplastic synoviocytes **may form villus or finger-like structures**.



Figs. 25.14A to F: Sequence of morphological changes in rheumatoid arthritis: (A) Normal joint; (B) Synovial cells proliferate and synovial tissue is infiltrated by lymphocytes and plasma cells; (C) The synovial hypertrophy and hyperplasia leads to formation of villi. This is accompanied by neovascularization with pannus formation (D), which covers the cartilage. The lymphoid cells form follicles; (E) Proliferating synovium extends into the joint space, destroys cartilage as well as the bone beneath the articular cartilage; (F) The joint is eventually destroyed and becomes fused, a condition termed ankylosis.

- **Dense inflammatory infiltrate:** Consisting of **lymphocytes** (mostly $CD4^+$ helper T cells), **B cells**, **plasma cells**, and **macrophages**. Lymphocytes may **aggregate to form lymphoid follicles.** Neutrophils in the synovial fluid and on the synovial surface.
- **Rice bodies: Fibrin** covering the synovium **may float in the joint space** as **rice bodies.**
- **Pannus formation**: Pannus is a **mass of synovium** and **synovial stroma** consisting of **inflammatory cells, granulation tissue**, and **synovial fibroblasts**. Pannus grows **over the articular cartilage and destroys it**.
- **Ankylosis**: After the destruction of cartilage, the **pannus bridges the opposing bones** to form a **fibrous ankylosis**, which may ossify resulting in **bony ankylosis**. **Destruction of joint followed by fusion is termed ankylosis**.
- **Osteoporosis:** Osteoclastic activity in underlying bone cause erosions and osteoporosis of the underlying bone.

Skin

Rheumatoid nodules are the **most common cutaneous lesion**.

- **Sites:** Develops **at sites to pressure**. For example, ulnar aspect of the forearm, elbows, occiput, and lumbosacral area.
- **Gross**: **Round to oval, firm, nontender**, nodules in the **subcutaneous tissue**.
- **Microscopy:** It consists of a **central zone of fibrinoid necrosis surrounded by epithelioid histiocytes (activated macrophages), lymphocytes and plasma cells**.

Blood Vessels

Rheumatoid **vasculitis** is a dangerous complication of rheumatoid arthritis, especially when it affects vital organs. It may also affect heart, lungs, and muscles.

Clinical Course

- **Age**: RA can occur at any age, but most common between **40 and 70 years**.
- **Sex:** Three to five times more often in **women than men** (3:1).
- **Slow and insidious in onset** and presents with malaise, fatigue, and generalized musculoskeletal pain.
- **Usually small joints are involved before larger ones** and are **symmetrical**. It affects hands (metacarpophalangeal and proximal interphalangeal joints) and feet, followed by the wrists, ankles, elbows, and knees. The affected joints are swollen, warm, painful, and stiff on arising or following inactivity.

Radiography

- Effusions into the joint.
- Narrowing of the joint space with loss of articular cartilage.
- Erosions and osteopenia of juxta-articular bone.

Deformities

- Destruction of tendons, ligaments, and joint capsules produces characteristic deformities. These consist of **radial deviation of the wrist**, **ulnar deviation of the fingers**, and flexion-hyperextension abnormalities of the fingers (**swan neck**).
- Deformed joints have no stability, lose their range of motion.

- Large synovial cysts, like the **Baker cyst** may develop on posterior knee, due to increased intra-articular pressure causing outside projection of the synovium.

Laboratory Findings

Rheumatoid factor and anti-CCP antibody are **specific for rheumatoid arthritis**.

GOUT AND GOUTY ARTHRITIS

Q. Write short note on gout/gouty tophi.

Definition: Gout is a **heterogeneous group of diseases** characterized by **hyperuricemia** (plasma urate level above6.8 mg/dL) and **urate crystal deposition in joints and kidneys**.

Classification (Box 25.1)

Depending on the etiology of the hyperuricemia.

- **Primary (idiopathic):** Hyperuricemia occurs without any other disease.
- **Secondary:** Hyperuricemia occurs in association with another illness.

Etiology

Q. Write a short note on causes/etiology of gout.

- Uric acid is the end product of purine metabolism, which is eliminated only in the urine. Humans do not have uricase, an enzyme which degrades uric acid. Usually, there is a balance between uric acid production and tissue deposition of urates.
- **Hyperuricemia** is necessary, but not sufficient for the development of gout. Several factors, which convert asymptomatic hyperuricemia into primary gout. These are as follows:
  - **Age:** Gout usually presents after 30 years of age.
  - **Duration of the hyperuricemia: Usually not<**20–30 years.
  - **Genetic predisposition:** Primary gout is of multifactorial inheritance and runs in families.
  - **Heavy alcohol intake**.
  - **Obesity**.
  - **Drugs** (e.g., thiazides) which reduce excretion of urate.

- **Lead toxicity**.

Pathogenesis

Q. Write short essay/note on pathogenesis of gouty arthritis.

Precipitation of monosodium urate (MSU) crystals into the joints.

- The solubility of urate in a joint is dependent on intra-articular concentration of urate and the temperature (less soluble in low temperature).
- Monosodium urate is less soluble in synovial fluid compared to plasma. So, they become easily supersaturated in the peripheral joints (ankles and toes), where temperatures are usually low.
- With hyperuricemia, precipitation of urate crystals develops in the synovium and in the joint cartilage.

Acute Arthritis

- Probably due to trauma, the precipitated crystals are released into the synovial fluid, which results in inflammatory response, which is characteristic of the acute attack.
- MSU crystals are phagocytosed by macrophages and release many cytokines (e.g., IL-1β,IL-18).

BOX 25.1: Classification of gout.

Primary gout (90%)

- Increased production of uric acid
  - Increased dietary intake of purine
  - Due to inborn errors of purine metabolism
- Decreased excretion of uric acid with normal production

Secondary gout (10%)

- Increased production of uric acid with increased urinary excretion
  - Increased catabolism of nucleic acid due to increased cell turnover (e.g., leukemias, cancers, and during tumor lysis induced by chemotherapy)
- Decreased excretion of uric acid with normal production
  - Chronic renal disease

- IL-1β stimulates the expression of adhesion molecules causing accumulation of neutrophils at the site of acute inflammation.

- The neutrophils release toxic-free radicals, leukotrienes (leukotriene B4), and lysosomal enzymes which aggravate inflammation and results in acute arthritis.
- Acute arthritis usually remits in days to weeks.

Chronic Arthritis

Repeated attacks of acute arthritis lead to chronic arthritis and tophi formation in the synovial membranes and periarticular tissue.

MORPHOLOGY (FIGS. 25.15A TO C)

Q. Write a short note on acute gout/gouty arthritis.

The morphological changes in gout are as follows:

1. Acute Arthritis

- **Acute inflammation**: It is characterized by edema, congestion and dense infiltration of synovium by neutrophils. Few lymphocytes, plasma cells, and macrophages may also be seen.
- **Monosodium urate (MSU) crystals:**
  - They are long, slender, and needle shaped.
  - They are found in the cytoplasm of the neutrophils and are arranged in small clusters in the synovium.
  - As the acute attack subsides, they may be resolubilized.

2. Chronic Tophaceous Arthritis

Q. Write a short note on gouty tophi.

- It follows the repeated acute attacks.
- The urates may heavily encrust the articular surfaces.
- The synovium shows fibrosis, thickening, and infiltration by inflammatory cells. It forms a pannus and destroys the cartilage, and causes juxta-articular bone erosions.

- When severe, fibrous or bony ankylosis may lead to loss of joint function.
- **Tophi (Figs. 25.15A to C):** Lesions consisting of large aggregates of urate crystals, surrounded by inflammatory reaction are known as tophi and are the pathognomonic of gout.

- **Sites:** Tophi are commonly found in the articular cartilage of joints and in the periarticular ligaments, tendons, and soft tissues, Achilles tendons, and earlobes.
- **Microscopy**: They consist of aggregations of urate crystals surrounded by macrophages, lymphocytes, and foreign- body giant cells.

Clinical Course

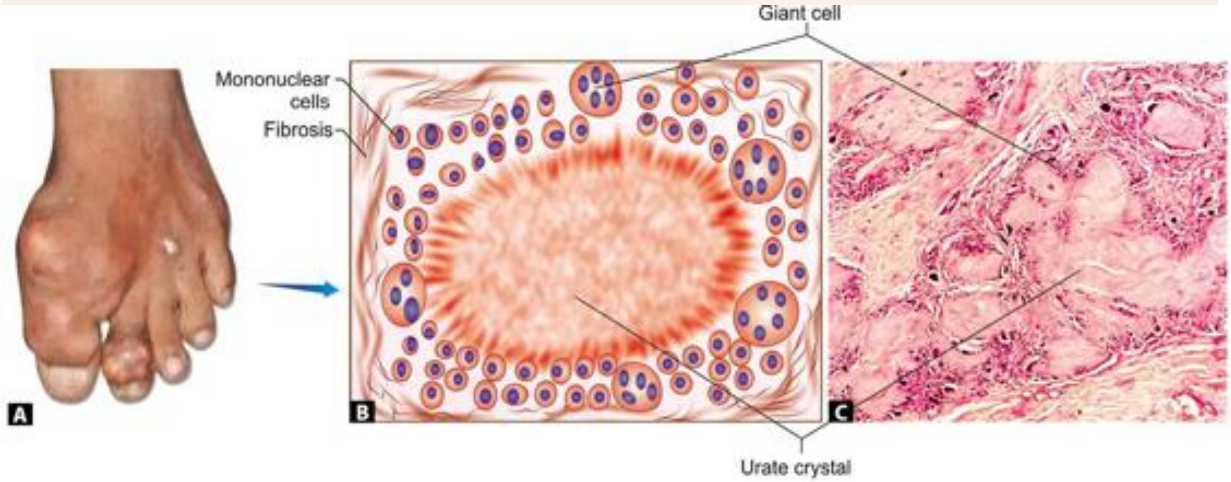
The gout has four stages:

1. **Asymptomatic hyperuricemia:** Appears at puberty in males and after menopause in females.
2. **Acute gouty arthritis:** It appears as sudden excruciating joint pain with tenderness. Even if not treated, acute attacks of gout are usually self-limited.
3. **Intercritical gout:** The intercritical period is the asymptomatic interval between the first acute attack and subsequent attacks.
4. **Chronic tophaceous gout:** After several years (about 12 years) of acute gouty arthritis, chronic tophaceous gout may develop, which may lead to severe crippling disease.

Prognosis: Gout does not reduce the life span, but may cause morbidity.

Tophi: Pathognomonic of gout.

Gout: Increased levels of uric acid (hyperuricemia) and urate crystal deposition in joints and kidneys.



Figs. 25.15A to C: (A) Usual site of gouty tophus; (B) Diagrammatic; (C) Photomicrograph; microscopy of a gouty tophus showing aggregates of dissolved urate crystals surrounded by fibroblasts, mononuclear inflammatory cells, and giant cells.

SOFT TISSUE TUMORS

CC: Classify and describe the etiology, pathogenesis, manifestations, radiologic and morphologic features and complications and metastases of soft tissue tumors.

Soft tissue is the term used for non-epithelial tissue excluding the skeleton, joints, central nervous system, hematopoietic and lymphoid tissues.

- Soft tissue tumors are mesenchymal neoplasms and **include tumors of skeletal muscle, fat, fibrous tissue peripheral nerve or blood vessels.** These neoplasms may arise anywhere in the body, but **most soft tissue occur in the extremities** especially the thigh.
- Soft tissue tumors may be **benign, locally aggressive or malignant**. **Benign soft tissue** tumors are **more** (100 times) **common** than malignant counterparts, except skeletal muscle neoplasms. **Malignant soft tissue tumors** are **termed as sarcomas**. Locally aggressive tumors (e.g., fibromatosis) invade the surrounding tissue and may recur locally. **Sarcomas** (malignant soft tissue tumors) **can metastasize to the lungs or bone** through blood vessels. Generally, **patients die of metastatic disease rather than local invasion** at the primary tumor site.
- About 15% develop in children and their incidence increases with age.

Etiology and Pathogenesis

Genetic disorders: Most sarcomas are sporadic without any predisposing cause. A small minority of soft tissue neoplasms are associated with genetic disorders. These include germline mutations in tumor suppressor genes (e.g., neurofibromatosis type1, Gardner syndrome, tuberous-sclerosis, Li–Fraumeni syndrome, Osler–Weber Rendu syndrome).

Environmental factors: Exposures to radiation (e.g., angiosarcoma, osteosarcoma or undifferentiated sarcoma), burns (fibroblastic tumors developing in burn scars), or toxins may be associated with few tumors. Trauma has no role in the development of soft tissue tumors, and it merely draws attention to a pre-existing tumor.

Origin: The origin of sarcomas is not known and does not usually arise from recognized precursor lesions. **Probably**, they **arise from pluripotent mesenchymal stem cells** that reside in soft tissues and bone marrow. These

stem cells acquire somatic "driver" mutations. Few generalizations can be made based on the genomic complexity of soft tissue tumors.

Simple karyotype (15–20% of sarcomas): Similar to many leukemias and lymphomas, sarcomas may be **euploid** (presence of chromosome number which is the multiple of the basic chromosome set) tumors with a single or limited number of chromosomal changes occurring during early tumorigenesis. They may be specific and useful as diagnostic markers. Such tumors usually arise in younger patients and microscopically have a monomorphic appearance. For examples, Ewing sarcoma and synovial sarcoma.

Complex karyotype (80%–85% of sarcomas): Usually, these tumors are **aneuploid or polyploid**. They show multiple chromosomal gains and losses, indicating genomic instability. For example, leiomyosarcoma and undifferentiated pleomorphic sarcoma. Such tumors sually arise in adults and microscopically have a pleomorphic appearance.

WHO classification of soft tissue tumors (Box 25.2).

BOX 25.2: WHO (2013) classification of tumors of soft tissue (abridged).

ADIPOCYTIC TUMORS

Benign

- Lipoma

Malignant

- **Liposarcoma: Well-differentiated, myxoid liposarcoma, pleomorphic liposarcoma**

FIBROBLASTIC/MYOFIBROBLASTIC TUMORS

Benign

- Nodular fasciitis

Intermediate (locally aggressive)

- Palmar/plantar fibromatosis
- Desmoid-type fibromatosis
- Abdominal (mesenteric) fibromatosis

Malignant

- Fibrosarcoma

SMOOTH MUSCLE TUMORS

Benign

- Deep leiomyoma

Malignant

- Leiomyosarcoma (excluding skin)

SKELETAL MUSCLE TUMORS

Benign

- Rhabdomyoma

Malignant

- Embryonal rhabdomyosarcoma (including botryoid, anaplastic)
- Alveolar rhabdomyosarcoma (including solid, anaplastic)
- Pleomorphic rhabdomyosarcoma

NERVE SHEATH TUMORS

Benign

- Schwannoma (including variants)
- Neurofibroma (including variants)

Malignant

- Malignant peripheral nerve sheath tumor

TUMORS OF UNCERTAIN DIFFERENTIATION

Benign

- Acral fibromyoma

Malignant

- Synovial sarcoma: Synovial sarcoma, spindle cell, synovial sarcoma, biphasic

UNDIFFERENTIATED/UNCLASSIFIED SARCOMAS

General principles of soft tissue tumors are presented in Box 25.3.

BOX 25.3: General principles of soft tissue tumors.

Location: Tumors that are superficial usually tend to be benign and deep lesions are often malignant.
Size: Large tumors are more likely malignant than small ones.
Growth: Rapidly growing tumors are more likely to be malignant than tumors that grow slowly.
Calcification: It may be seen in both benign and malignant tumors.
Vascularity: Benign tumors are relatively avascular, whereas most malignant tumors tend to be highly vascular.

TUMORS OF ADIPOSE TISSUE

Lipoma

- Lipoma is the **most common benign tumor of fat**.
- It is the **most common soft issue tumor of adulthood**.

Age: Occur most often during 4th to 5th (middle adulthood) decades of life.

Presentation: Lipomas are usually single, soft, mobile and painless (except angiolipoma). They are usually cured by simple excision.
Site: It can arise from any site in the body that contains adipose tissue. Usually arises in the subcutaneous tissues of the proximal extremities, neck, back, shoulder and trunk. Infrequently, lipomas are large and intramuscular.

MORPHOLOGY

There are several morphological variants such as conventional lipoma, fibrolipoma, angiolipoma, spindle cell lipoma and myelolipoma. The conventional lipoma is the most common subtype.

Gross (Fig. 25.16A)

It is usually a **well-encapsulated** (may be poorly circumscribed), small, round to oval mass. The cut surface is **soft, yellow, greasy and often lobulated**. They vary in size and may become very large. Infrequently, lipomas may be large, intramuscular, and poorly circumscribed.

Microscopy (Figs. 25.16B and C)

It shows **lobules of mature (**well-differentiated) **adipocytes** separated by delicate fibrous septa. The adipocytes **closely resemble normal fat,** A thin fibrous capsule is usually found surrounding the tumor

Liposarcoma

Liposarcoma is one of the most common soft tissue sarcomas (25% of malignant soft tissue tumor) of adulthood.
Age: It occurs mainly during 5th to 6th decades of life.
Sites: Deep soft tissues of the proximal extremities (deep thigh) and in the retroperitoneum.
Molecular pathogenesis:

- **Myxoid/round cell liposarcomas** have a translocation between chromosomes 12 and 16, [t (12;16) (q13; p11)]. It is characterized by fusion of *TLS/FUS* gene on chromosome 16 with the *DDIT3* gene on chromosome 12. The resultant fusion gene arrests adipose differentiation. The *TLS/FUS* gene product is similar to the EWS protein of Ewing sarcoma.

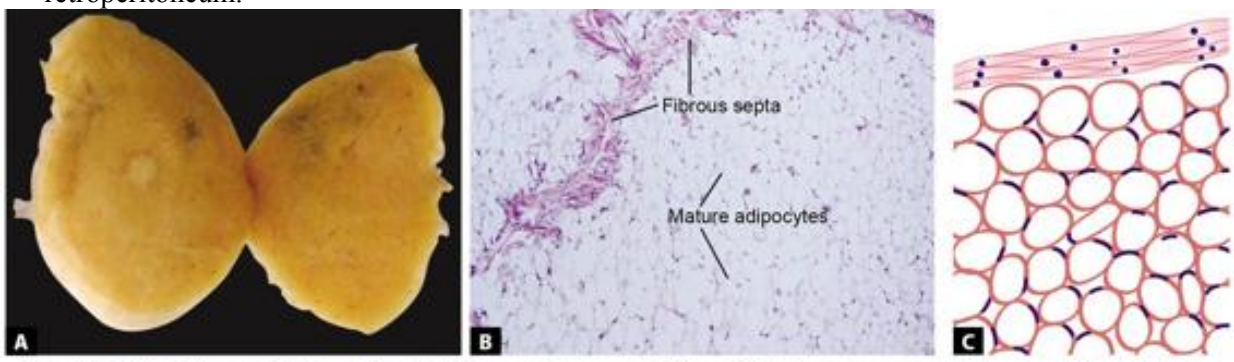
- **Well-differentiated liposarcomas** have amplification of the 12q13-q15 region, which includes the gene for MDM2 (encodes a potent inhibitor of p53).
- **Pleomorphic liposarcomas** have complex genomic rearrangements.

MORPHOLOGY

Gross

Gross appearances of liposarcoma depends on the subtypes. Usually, liposarcomas appear as nodular masses of 5 cm or more in diameter. Tumors usually appear circumscribed but infiltrate into surrounding tissue. Cut section show gray-white to yellow color with myxoid and gelatinous appearance. The gross appearance depends on the proportions of adipose, mucinous and fibrous tissue.

- Well-differentiated liposarcomas usually measure 5–10 cm in diameter. The retroperitoneal tumors may be large up to40 cm in diameter. They may resemble normal fat or may show fibrotic or gelatinous cut surfaces.
- Myxoid/round cell liposarcoma: They usually arise in the proximal extremities and are rare in the retroperitoneum.



Figs. 25.16A to C: Lipoma. (A) Gross; (B and C) Microscopically a well-differentiated tumor composed of lobules of fat cells that are identical in appearance to normal fat cells.

Microscopy

Lipoblasts may be seen microscopically in all types of liposarcoma. Lipoblasts are early adipocytes. They may be uninucleated or multinucleated having univacuolated or multivacuolated cytoplasmic fat vesicles indenting the nucleus. Lipoblasts are frequently seen in liposarcoma. However, **lipoblasts may be seen in reactive or regenerative conditions and are neither necessary nor sufficient for the diagnosis of liposarcoma**.

Liposarcomas are histologically divided into three morphologic subtypes:

1. **Well-differentiated liposarcoma:** Contains large amounts of mature adipocytes (fat cells) with scattered atypical spindle cells and lipoblasts. These may be confused with lipomas if inadequately sampled.
2. **Myxoid/round liposarcoma:** Most common histological subtype. It contains primitive ovoid to round cells at various stages of adipocyte differentiation reminiscent of fetal fat. Commonly show univacuolated "signet ring" lipoblasts. The tumor cells are embedded in

myxoid stroma with abundant basophilic extracellular matrix. The stroma also shows arborizing capillaries (**chicken-wire pattern**).

- **Round cell liposarcoma** represents a poorly differentiated form of myxoid liposarcoma. It shows high proportion of primitive uniform, round to oval cells with central hyperchromatic nuclei and a scant amount of myxoid stroma. The cytoplasm shows multiple vacuoles.

3. **Pleomorphic liposarcoma:** It is highly undifferentiated/anaplastic liposarcoma and consists of sheets of large, anaplastic cells, bizarre nuclei and variable amounts of immature adipocytes (lipoblasts).

Clinical course and behavior: All histological subtypes of liposarcoma recur locally and often repeatedly unless excised adequately excised. Well-differentiated liposarcoma is relatively indolent and almost never metastasizes. The pleomorphic variant usually is aggressive and frequently metastasizes with worst outcome. The myxoid/round cell liposarcoma is intermediate in its malignant behavior with risk of local recurrence and metastasis.

TUMORS AND TUMOR-LIKE CONDITIONSOF FIBROUS ORIGIN

Nodular Fasciitis

Nodular fasciitis is a **rapidly growing but self-limited fibroblastic and myofibroblastic** proliferation. It **typically affects superficial tissues of the upper extremities** (forearm, trunk and back) of **mostly young adults**. It is characterized by a **t (17;22) (p13; q13) translocation** producing a *MYH9-USP6* fusion gene. The fusion results in overexpression of USP6, an oncogenic protein. It presents as a rapidly growth tumor (a period of several weeks or months).

MORPHOLOGY

Gross

Nodular fasciitis may arise in the deep dermis, subcutis, fascia, or muscle. Grossly, it is < 5 cm, circumscribed, or slightly infiltrative.

Microscopy

It is highly cellular consisting of plump, immature appearing spindle-shaped fibroblasts and myofibroblasts. The stroma shows myxoid appearance. A gradient of maturation (zonation) from cellular, loose, and myxoid to organized and fibrous is characteristic of this tumor. The tumor cells vary in size and shape (spindle to stellate) and contain nuclei with conspicuous nucleoli. Numerous mitotic figures are seen. Lymphocytes and extravasated red blood cells are common.

Prognosis: Nodular fasciitis is self-limited lesion and can regress spontaneously. It is cured by surgical excision and it rarely recurs. Malignant transformation does not occur.

Fibromatoses

Fibromatosis (singular: fibromatosis; plural: fibromatoses) is a locally invasive, slowly growing fibroblastic proliferation that may occur anywhere in the body. They are divided into superficial and deep ("aggressive") fibromatoses depending on their location. Both superficial and deep fibromatoses are histologically similar, but there are genetic distinctions between them. Fibromatosis does not metastasize, but surgical resection of deep tumors may be followed by local recurrence.

Superficial Fibromatosis

Superficial fibromatosis is an infiltrative proliferation of fibroblasts. It **can cause local deformity but has a benign clinical course.** Males are more commonly affected than females. They consist of nodular or poorly defined broad fascicles of fibroblasts in long, sweeping fascicles, surrounded by abundant dense collagen. There are different forms or clinical subtypes of superficial fibromatosis depending on their characteristic locations:

- **Palmar (Dupuytren contracture) fibromatosis:** It is the most common form of fibromatosis. It may be unilateral or bilateral (50%). It consists of irregular or fibrous nodular thickening of the palmar fascia. Eventually its attachment to the overlying skin causes puckering and dimpling and leading to flexion contractures of the fingers, particularly the fourth and fifth fingers of the hand.
- **Plantar fibromatosis:** It is similar to palmar fibromatosis, except that it is less frequent, involves the plantar aponeurosis, common in young patients, unilateral and without contractures.
- **Penile fibromatosis (Peyronie disease):** It is the least common form of the localized fibromatoses. It is characterized by palpable induration or

mass on the dorsolateral aspect of the shaft of the penis. Over a span of years, it can cause abnormal curving of the shaft toward the affected side **(penile strabismus),** urethral obstruction and pain on erection.

Prognosis: In about one fourth of cases, the palmar and plantar fibromatoses stabilize and do not progress. In few cases it may spontaneously resolve. Some especially the plantar fibromatosis may recur after excision.

Deep Fibromatosis

Deep fibromatoses are also known as desmoid tumors that present as large and infiltrative masses. They frequently recur but do not metastasize.

Age and gender: They more common in the teenage years to 30s and more predominant in women (mainly abdominal fibromatosis).

Site: They frequently involve fascia and muscular aponeuroses of the extremities or anterior abdominal wall musculature. They may also arise in the limb girdles or mesentery.

Etiology: Deep fibromatoses may be associated with mutations in the *CTNNB1* (β-catenin) or *APC* genes, leading to increased Wnt signaling (*refer* **Fig. 18.44**). Most tumors are sporadic, but patients with familial adenomatous polyposis (Gardner syndrome) who have germline APC mutations are predisposed to deep fibromatosis (especially mesenteric fibromatosis).

Prognosis: They are highly infiltrative and difficult to resect completely. Hence, there is high rate of recurrence.

MORPHOLOGY

Gross

Fibromatoses vary in size from 1 to 15 cm in diameter. They are gray-white, rubbery, tough/firm and poorly demarcated masses with marked infiltration of surrounding muscle, nerve and fat. The cut surface shows whorled appearance.

Microscopy

It shows sheets and interdigitating fascicles of benign-appearing (bland) fibroblasts (spindle cells) with dense collagen. Mitotic activity is minimal to absent.

Prognosis: Since there will be extensive infiltration in deep fibromatosis, complete excision is often difficult. Recent efforts have concentrated on medical therapy and radiation as alternatives to surgery.

Fibrosarcoma

Fibrosarcomas are very rare m**alignant tumors of fibroblasts** because many of the tumors earlier diagnosed as fibrosarcomas presently belong to a group of tumors known as fibrous histiocytomas. Pure adult fibrosarcoma is a diagnosis of exclusion, Fibrosarcoma is a slow growing malignant tumor arising from fibrous tissue. It is often found between 4th to 7th decades of life.

Site: Most commonly they occur in the lower extremity (thigh and around knee), upper extremity, trunk, head and neck, and retroperitoneum.

MORPHOLOGY

Gross

They are circumscribed (sharply demarcated) gray-white, firm and lobulated tumors. Cut section has a **soft, fish flesh like-appearance**. Areas of hemorrhage and necrosis are usually seen.

Microscopy

The tumor consists of malignant-appearing spindle-shaped fibroblasts arranged in intersecting fascicles **(Fig. 25.17)**. Well-differentiated fibrosarcomas show characteristic **herring-bone pattern (herring = a type of sea-fish)**. Poorly-differentiated fibrosarcomas show pleomorphic fibroblasts with frequent mitotic figures.

SKELETAL MUSCLE TUMORS

Skeletal muscle neoplasms unlike other mesenchymal tumors, are almost all malignant. The benign tumor namely, rhabdomyoma occurs in individuals with tuberous sclerosis.

Rhabdomyosarcoma

It is a malignant mesenchymal tumor that shows **skeletal muscle differentiation**.

Subtypes: There are three main subtypes namely: embryonal (60%), alveolar (20%), and pleomorphic (20%).

Age: It is uncommon in mature adults. Alveolar and embryonal rhabdomyosarcoma are most **common soft tissue sarcoma of childhood and adolescence** (children between 3 and 12 years), usually presents before age 20. Pleomorphic rhabdomyosarcoma is seen mainly in adults.

Sites:

- **Embryonal rhabdomyosarcoma:** In children, embryonal rhabdomyosarcoma arises in the sinuses, head and neck and genitourinary tract. These locations do not normally contain much skeletal muscle. This points out that that sarcomas do not arise from mature, terminally differentiated mesenchymal cells.

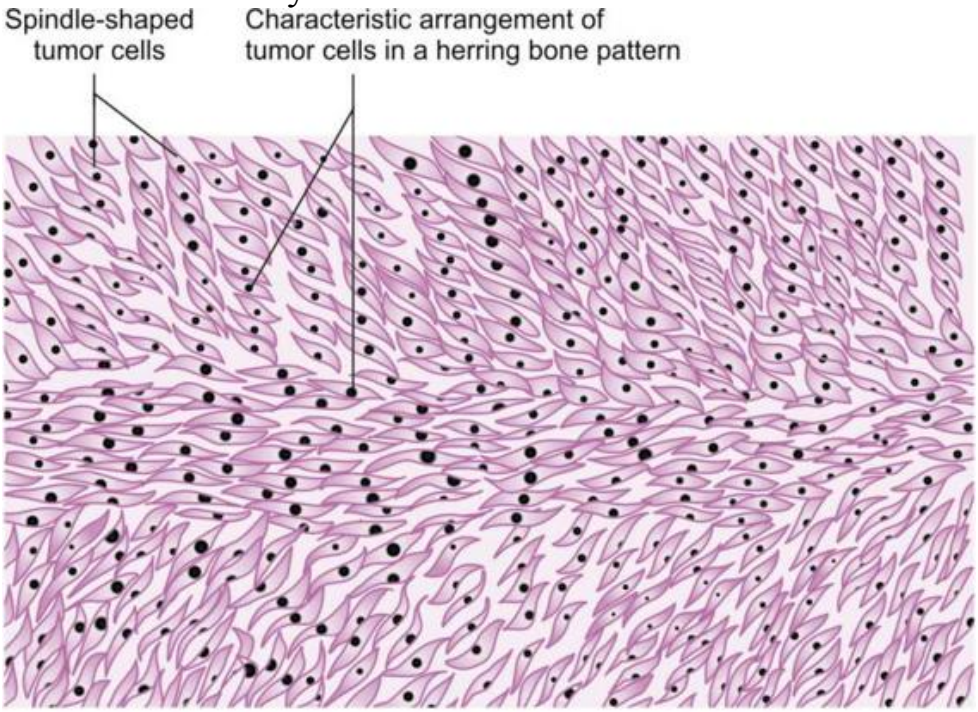


Fig. 25.17: Fibrosarcoma composed of spindle-shaped fibroblasts arranged in intersecting fascicles (characteristic herring-bone pattern).

- **Alveolar rhabdomyosarcoma:** It is less frequent than the embryonal type. It is mainly found in young individuals between 10 to 25 years of age and rarely in elderly patients. Most common site is upper and lower extremities.
- **Pleomorphic rhabdomyosarcoma:** It occurs in the skeletal muscles of older individuals (e.g., thigh).

MORPHOLOGY

1. **Embryonal (60%):**

   Gross: It presents as soft gray infiltrative mass.

   Microscopy: The tumor cells mimic skeletal muscle at various stages of embryogenesis. It consists of sheets of both primitive round and spindled cells in a myxoid stroma. Well-differentiated tumors may show **rhabdomyoblasts with strap like eosinophilic cytoplasm and cross-striations** (better seen with phosphotungstic acid [PTAH] stain). Poorly differentiated tumors show small cells.

   Sarcoma botryoides (botryoid embryonal rhabdomyosarcoma): It is a variant of embryonal rhabdomyosarcoma and is distinguished by the formation of **polypoid, grape-like tumor masses**. It occurs in the walls of hollow, mucosal-lined structures (e.g., nasopharynx, urinary bladder, common bile duct, vagina).

   Microscopy: The malignant cells are scattered in an abundant myxoid stroma. Where the tumors abut (touch) the mucosa of an organ, they form a submucosal zone of hypercellularity called the **cambium layer**.

2. **Alveolar (20%):** In these tumors a network of fibrous septae divide the tumor cells into clusters or aggregates producing an alveolar pattern (crudely resemble pulmonary alveoli). The tumor cells are uniform round, with scanty cytoplasm without cross striations. The tumors cells in the center of the aggregates are loose arranged (discohesive) producing an "alveolar" pattern whereas those at the periphery adhere to the septae.
3. **Pleomorphic (20%):** It is characterized by numerous, large, sometimes multinucleated, bizarre eosinophilic tumor cells. Microscopically, it can resemble other pleomorphic sarcomas.

Immunohistochemistry: All rhabdomyosarcoma shows immunohistochemical evidence of skeletal muscle (rhabdomyoblastic) differentiation. These include nonspecific myoid markers (e.g., actin and desmin) and skeletal muscle–specific markers (at least focal expression), such as the transcription factors myogenin and MyoD1.

Prognosis: Rhabdomyosarcomas are **aggressive malignant tumors.** They may be treated with surgery and chemotherapy, with or without radiation therapy. Factors that indicate worse prognosis include age older than 10, tumor size more than 5 cm, alveolar and pleomorphic histologic subtypes and advanced stage of disease. The botryoid rhabdomyosarcoma has the best prognosis, whereas the pleomorphic subtype is often fatal.

SMOOTH MUSCLE TUMORS

Smooth muscle cells are spindled cells with brightly eosinophilic cytoplasm and contain cylindrical nuclei. Immunohistochemically they **express smooth muscle actin, muscle-specific actin and desmin**.

Leiomyoma

Leiomyoma is a benign tumor of smooth muscle.

Site: It usually arises in sites associated with normal smooth muscle. It is most common in the uterus (most common neoplasm in females *refer* page 751) but can arise in any site having soft tissue (e.g., dermis, blood vessel walls in subcutaneous or deep somatic tissues and the muscular wall of the esophagus).

MORPHOLOGY

Gross

They appear as firm, gray-white, well-circumscribed nodules and usually measure 1–2 cm in diameter. Dermal or subcutaneous tumors may be painful.

Microscopy (*refer* Fig. 22.17)

They are composed of intersecting (intersect each other at right angles) fascicles of uniform densely eosinophilic spindled cells with cigar-shaped (blunt-ended, elongated) nuclei. Tumor cells show minimal atypia and few (very low) mitotic figures.

Treatment: Simple excision is curative.

Leiomyosarcoma

Leiomyosarcoma is a **malignant smooth muscle tumor that** accounts for about 10–20% of soft tissue sarcomas.

It occurs in adults and common in females than in males.

Site: Most common in the **deep soft tissues of the extremities and retroperitoneum**.

Leiomyosarcomas **present as painless firm masses**. Retroperitoneal tumors may be large and bulky and cause abdominal symptoms.

MORPHOLOGY

Gross

Leiomyosarcomas tend to be well circumscribed but are larger and softer than leiomyomas. Cut section may show areas of necrosis, hemorrhage and cystic degeneration.

Microscopy

They are composed of **eosinophilic spindle cells with blunt-ended, hyperchromatic nuclei.** Mitotic activity and necrosis are common. The tumor **cells are arranged in broad, interweaving fascicles**. Immunohistochemically, they are positive for smooth muscle actin, desmin and caldesmon.

They can spread to the lungs via hematogenous route. **Treatment:** It depends on tumor size, location, and grade.

PERIPHERAL NERVE SHEATH TUMORS

Common types of peripheral nerve sheath tumors are schwannoma, neurofibroma, and malignant peripheral nerve sheath tumor (MPNST).

Schwannoma (Neurilemmoma)

Schwannoma or neurilemmoma **is a benign encapsulated tumors of peripheral nerve sheath origin that shows Schwann cell differentiation**. Most schwannomas are sporadic. However, about 10% are associated with *familial neurofibromatosis type 2 (NF2)*.

Sites: It arise from the soft tissues, internal organs, or cranial or **spinal nerve roots**. Most common in the vestibular portion of the eighth cranial nerve (acoustic schwannoma).

MORPHOLOGY

Gross (Fig. 25.18A)

Most schwannomas are appear as a solid, well-circumscribed, encapsulated tumor abutting an adjacent nerve. They are firm and gray.

Microscopy (Fig. 25.18B)

Tumors usually show an admixture of dense and compact areas (called as Antoni A pattern) and loose acellular areas (called as Antoni B pattern). The tumor cells are neoplastic Schwann cells of peripheral nerve sheath.

- **Antoni A areas:** These dense areas contain spindle cells with buckled nuclei arranged in intersecting fascicles. The cells show palisading of nuclei and alternating bands of "nuclear-free zones" between the regions of nuclear palisading are termed **Verocay bodies.**

- **Antoni B areas:** These are loose, hypocellular areas in which the spindle cells are widely separated by a prominent myxoid extracellular matrix. Schwann cells are characterized by the presence of a spindled elongated nucleus having a wavy or buckled shape.

Blood vessels show thickening of wall and hyalinization. Hemorrhage or cystic changes may be sometimes seen.

Neurofibromas

Benign nerve sheath tumor: May be either sporadic or NF1-associated.

MORPHOLOGY

- **Localized cutaneous neurofibroma:**
  - Small, well-delineated but unencapsulated nodular lesions arise in the dermis **(Fig. 25.19)** and subcutaneous fat.
  - Relatively low cellular containing Schwann cells admixed with stromal cells.
  - Stroma contains loose collagen.
- **Diffuse neurofibroma:** Morphologically similar to those seen in localized cutaneous neurofibromas.

Plexiform neurofibroma: They grow within and expand nerve fascicles entrapping associated axons. The ropy thickening of multiple nerve fascicles produces a "bag of worms" appearance. Morphologically, it is similar to that of other neurofibromas.

Malignant Peripheral Nerve Sheath Tumors

Malignant peripheral nerve sheath tumor (MPNST) is a poorly differentiated, spindle cell sarcoma of peripheral nerve of uncertain histogenesis. It may arise de novo or from malignant transformation of a neurofibroma.

Age and site: MPNST are most common in adults. Commonly arises in larger nerves of the trunk or proximal limbs. They may arise from transformation of neurofibroma in patients with neurofibromatosis.

MORPHOLOGY

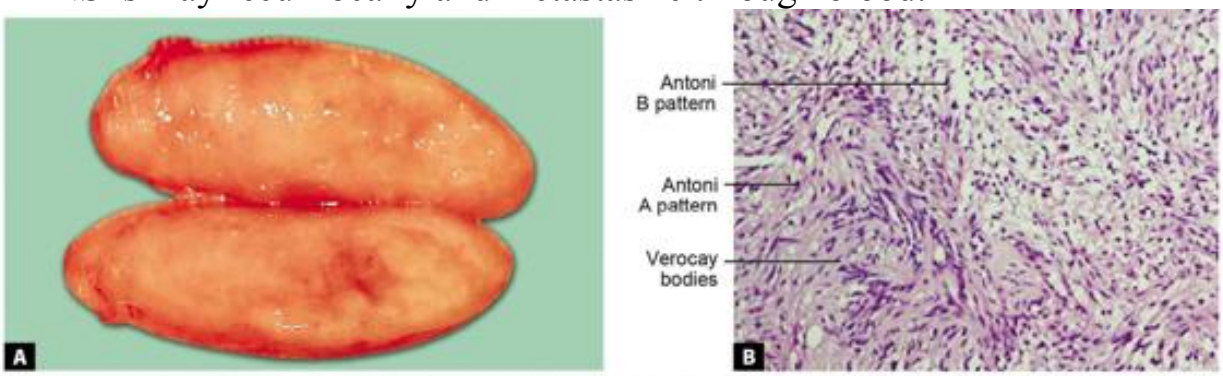
Gross

They appear as unencapsulated, fusiform enlargements. Sometimes a clear origin from a peripheral nerve of a nerve can be made.

Microscopy

They are highly cellular tumor composed of **spindle cells with buckled nuclei**. The tumor cells show cytological features of malignancy, including anaplasia (pleomorphism, nuclear atypia, mitotic figures), foci of necrosis, infiltrative growth pattern, pleomorphism, and high proliferative activity.

MPNSTs may recur locally and metastasize through blood.



Figs. 25.18A and B: (A) Cut section of Schwannoma; (B) Microscopic appearance of Schwannoma (neurilemmoma). It shows densely cellular (Antoni A) and loosely cellular (Antoni B) areas with characteristic nuclear palisading (Verocay bodies) in Antoni A area.

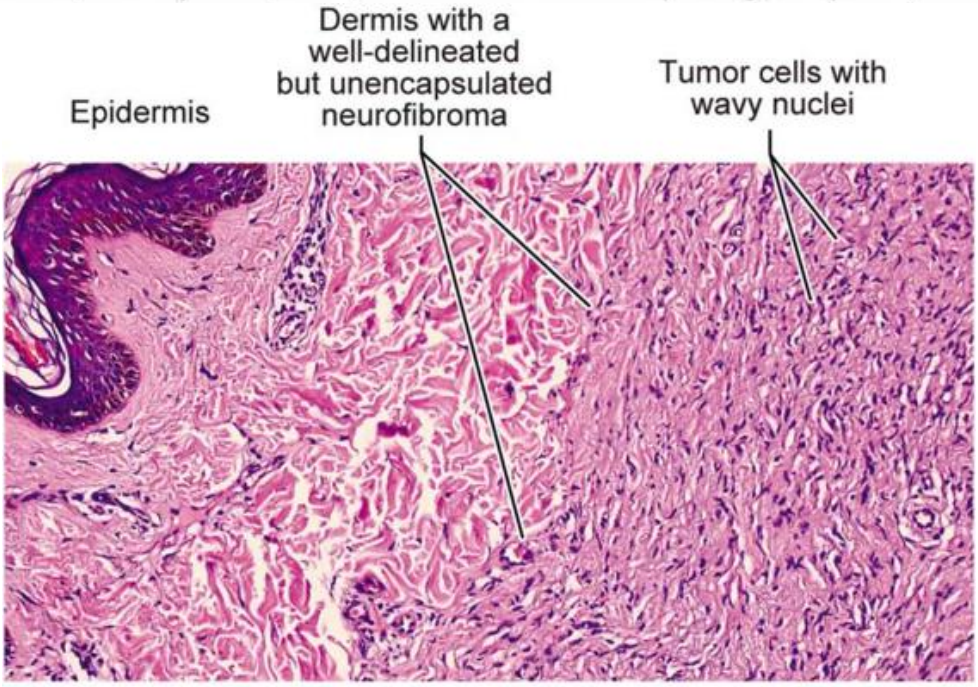


Fig 25.19: Neurofibroma composed of Schwann cells with wavy nuclei.

TUMORS OF UNCERTAIN ORIGIN

Many soft tissue tumors do not recapitulate any known mesenchymal lineage. One of the examples is synovial sarcoma.

Synovial Sarcoma

Synovial sarcoma was so-named because the first few cases described were observed in the soft tissues near the knee joint and was thought to arise from synovium. However, this term is a misnomer, because despite the name **these tumors does not arise from synovial tissues** and can occur in locations without synovium (e.g., chest wall, head and neck) and morphologically they do not show synoviocyte differentiation.

Synovial sarcoma is a highly malignant soft tissue tumor and account for about 10%of all soft tissue sarcomas.

Age: Most commonly it occurs in the age range of 20–40 years.

Site: They may arise anywhere in the body but are commonly **located in deep soft tissues near joints, tendon sheaths or joint capsules**.

Presentation: They usually present as a deep-seated painless mass of several years duration.

Molecular pathogenesis: Most synovial sarcomas show a characteristic balanced chromosomal translocation involving chromosomes X and 18 [t(x;18) (p11.2; q11.2)]. This translocation results in fusion of the *SS18/SYT* (synteny) gene on chromosome 18 to one of the three *SSX* gene (*SSX1*, *SSX2* or *SSX4)* on the X chromosome. This lead to production of a hybrid protein, SS18-SSX1, SS18-SSX2 or, rarely, SS18-SSX4. These fusion genes encode chimeric transcription factors that derail cell cycle.

MORPHOLOGY

Gross

They usually appear as circumscribed, round or multilobular masses attached to tendons, tendon sheaths or the exterior wall of the joint capsule. The tumors may be surrounded by a glistening pseudo capsule and may show cystic areas.

Areas of hemorrhage, necrosis and calcification may be seen. Their size ranges from small nodules to tumors measuring 15 cm or more in diameter (average 3–5 cm).

Microscopy

They may be monophasic or biphasic.

- **Monophasic synovial sarcoma:** It consists of uniform spindle cells with scant cytoplasm and dense chromatin. These tumor cells grow in short, tightly packed fascicles. Calcifications may be observed within the tumor.

- **Biphasic synovial sarcoma:** It contains gland like (glandular spaces) structures composed of cuboidal to columnar epithelioid cells (epithelium-like tumor cells) in addition to the spindle cell component (background) mentioned in monophasic type.

Immunohistochemistry: It helps in differentiating them from many other sarcomas. The tumor cells, especially in the biphasic type, are positive for epithelial antigens (e.g., keratins or epithelial membrane antigen). This supports the evidence of epithelial differentiation.

Treatment and prognosis: Synovial sarcomas are treated aggressively with limb sparing surgery and frequently chemotherapy. The recurrence rate is high, and metastases occur in more than 60% of cases. Common sites of metastases are the lung.

Undifferentiated Pleomorphic Sarcoma

Undifferentiated pleomorphic sarcoma (UPS) includes high-grade malignant mesenchymal tumors composed of pleomorphic cells. These cannot be classified into another category of mesenchymal tumors by morphology, immunophenotype, ultrastructure, and genetics.

Age: Commonly in middle aged or older adults.

Site: Most occur in the deep soft tissues of the extremity (e.g., thigh)

MORPHOLOGY

Gross

They usually appear as large, gray-white fleshy masses. The size varies and may be as large as 10–20 cm. Areas of necrosis and hemorrhage are common.

Microscopy

They are composed of sheets of large, anaplastic, spindled to polygonal cells with hyperchromatic irregular, sometimes bizarre nuclei. There will numerous mitotic figures (including atypical mitotic figures) and areas of necrosis. Tumor cells does not show differentiation along any of recognized lineages or characteristic genetic defects.

Behavior: These are aggressive malignant tumors that are treated with surgery and adjuvant chemotherapy and/or radiation. It has a poor prognosis, with metastases occurring in 30–50% of patients.

CHAPTER 26

Skin Disorders

MELANOCYTIC NEVUS (PIGMENTED NEVUS, MOLE)

Q. Write a short note on nevus.

- The term **mole or nevus** (plural nevi) is used for **any congenital skin lesion** (e.g., a birthmark).
- **Melanocytic nevus is a benign neoplasm (congenital/acquired) of melanocytes** and most nevi are acquired.

MORPHOLOGY (FIGS. 26.1A TO D)

Q. Write short note on junctional/compound/intradermal nevus.

Progressive Changes

Melanocytic nevi may progress through a series of morphologic changes ranging from junctional nevi to intradermal nevi.

Junctional Nevi (Fig. 26.2A)

It is the earliest lesions characterized by **aggregates or nests of round nevus cells along the dermoepidermal junction**. These nevus cells have round uniform nuclei with inconspicuous nucleoli, and may show little or no mitotic activity.

Compound Nevi

Most junctional nevi grow into the underlying dermis as nests or cords of cells and form **compound nevi**.

Intradermal Nevi (Figs. 26.2B and C)

As the compound nevi grow older, the **epidermal nests of nevus cells may be lost** and retain only the intradermal component to form pure **intradermal nevi**.

Clinically, compound and intradermal nevi appear as elevated lesions than junctional nevi.

Maturation

It is a process in which progressive growth of nevus cells occur from the dermoepidermal junction into the underlying dermis.

- **Superficial nevus cells** are larger, produce melanin, and grow in nests.
- **Deeper nevus cells** are smaller, produce little or no pigment, and appear as cords and single cells.

This sequence of maturation of nevus cells is of diagnostic use in differentiating benign nevi from melanomas. Melanoma usually does not show maturation.

- Melanocytic nevi are common and may be confused with melanoma.

Clinical Presentation

- **Acquired melanocytic nevi** are the **most common** skin lesions.
- **Tan to brown**, **uniformly pigmented**, and **small** (usually <6 mm across) lesions.
- **Solid** relatively **flat** (macules) **to elevated** skin (papules) lesions with **well-defined, rounded borders**.

Nevus cells: Modified melanocytes.

Nevus types: (1) Junctional; (2) Compound; (3) Intradermal

Maturation of nevus cells is used in differentiating benign nevi from melanomas.

MELANOMA

NC: Describe the distinguishing features between a nevus and melanoma. Describe the etiology, pathogenesis, risk factors morphology clinical features and metastases of melanoma.

Q. Write short note on malignant melanoma.

Q. Write a short note on sites of melanoma.

Melanoma is a **relatively common** neoplasm.

- **Site:**
  - **Skin**: It is the most common site and may develop in the trunk, leg, face, sole, palm and nail beds.
  - **Other sites**: Oral and anogenital mucosal surfaces, esophagus, leptomeninges, eye, and the substantia nigra.
- **Cell of origin**: Melanocytes.

Etiology and Pathogenesis

Q. Write a short essay on etiology and pathogenesis of malignant melanoma.

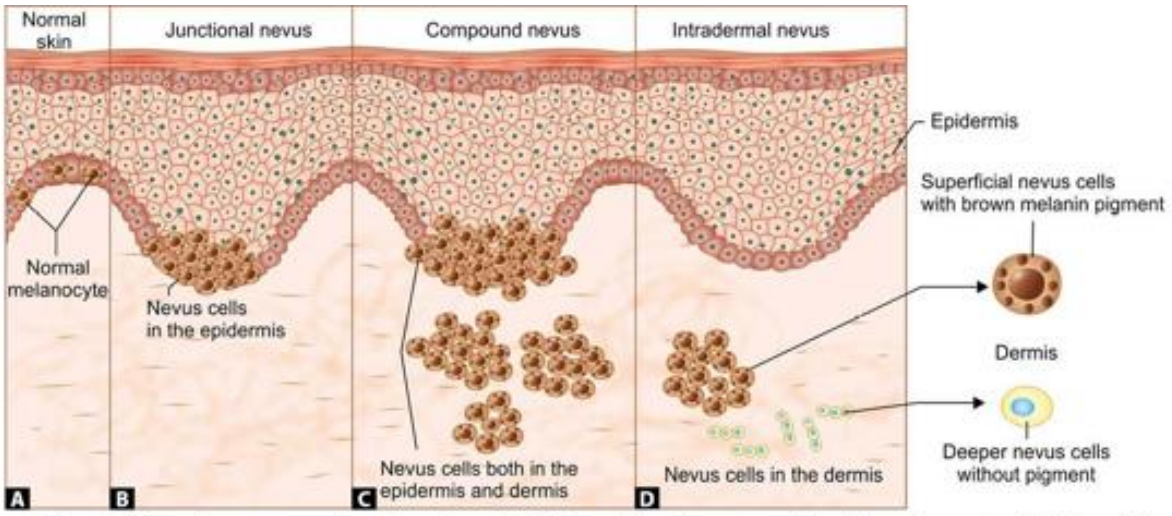
Predisposing factors include: (i) sun exposure and (ii) inherited genes.

Sun Exposure

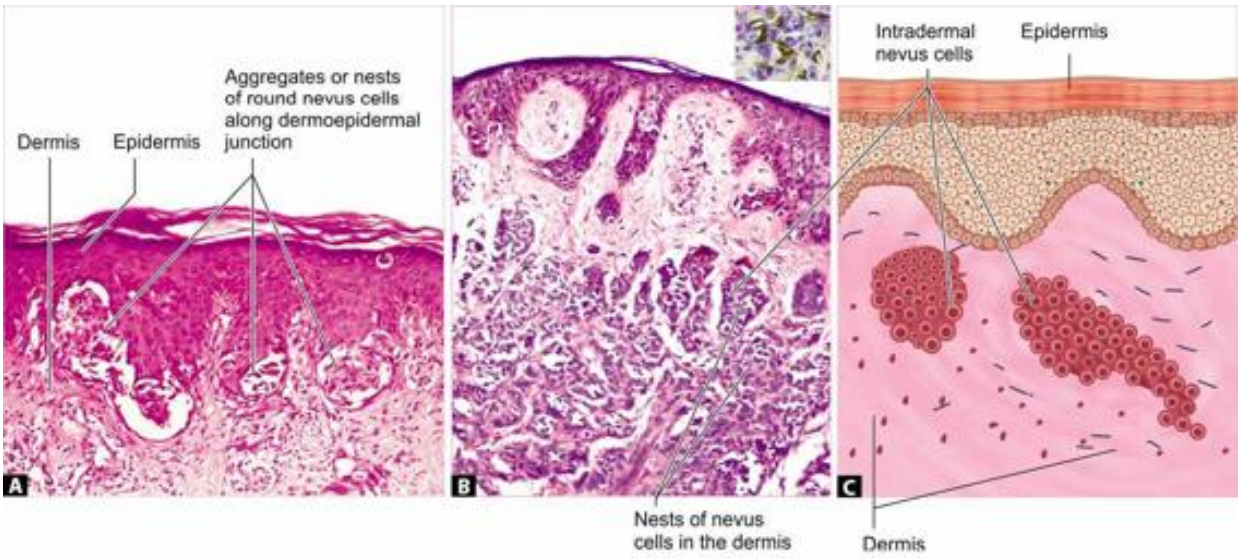
- **Melanomas most commonly develop on sun-exposed surfaces**, particularly the upper back in men and the back and legs in women.
- **Lightly pigmented individuals** are at **greater risk** than darkly pigmented individuals.
- Other environmental factors may also contribute to risk.

Inherited Genes

About 10–15% of melanomas are familial and the genetic abnormalities are as follow:



Figs. 26.1A to D: Maturation sequence of melanocytic nevi: (A) Normal skin with scattered dendritic melanocytes within the epidermal basal cell layer; (B) Junctional nevus; (C) Compound nevus; (D) Intradermal nevus.



Figs. 26.2A to C: Melanocytic nevus: (A) Junctional nevus shows aggregates or nests of round nevus cells along the dermoepidermal junction; (B) H & E; (C) Diagrammatic intradermal type showing nests of nevus cells in the dermis. Inset showing nevus cells with melanin pigment.

1. Mutations in Tumor Suppressor Gene

- **Mutations in *CDKN2A* gene:** It is observed in familial as well as in few sporadic melanomas.
  - *CDKN2A* is a complex locus, which encodes three different **tumor suppressors namely,** (1) *p15/INK4b*, (2) *p16/INK4a* and (3) *p14/ARF*.
  - C*DKN2A* gene is mutated in ~40% of familial melanoma and ~10% of sporadic melanomas. These mutations result in increased melanocytic proliferation and escape from oncogene-induced cellular senescence.
- **Mutations in *RB* gene**: These are common in both familial and sporadic melanomas.
- **Silencing of *PTEN* gene:** PTEN normally downregulate PI-3K/AKT signaling (*refer* **Fig. 24.10**). *PTEN* is **epigenetically silenced** in ~ 20% of melanomas, which leads to **activation of PI-3K/AKT signaling and proliferation of cells**.

2. Oncogene Activation

It may occur by **mutations in proteins involved in signal transduction**. *RAS* is a normal signal transduction protein, which stimulates downstream regulator RAS/RAF/MAP kinase cascade resulting in cell proliferation (*refer*

Figs. 7.16 and 24.10). Aberrant increase in RAS signaling may occur due to **mutations in** *RAS* or *BRAF*, which promote cell **growth and survival**.

- **Activating mutations in *NRAS*** (which is one type of RAS) leads to melanoma in 10–15% of tumors.
- **Activating mutations in *BRAF*** are seen in 60–70% of melanomas. *BRAF* (one of the member of RAF family) is an **intermediate signaling component in the MAP kinase pathway** (*refer* **Fig. 24.10**).

3. Mutations that Activate Telomerase (*refer* page 219)

MORPHOLOGY (FIGS. 26.3A TO C)

Q. Write short note on morphology of malignant melanoma. Mention its growth phases.

Growth Phases

1. Radial Growth Phase

During this phase, the **melanoma spread horizontally** within the **epidermis and superficial dermis**.

- It represents the **initial stage** where the tumor cells **lack the capacity to metastasize**.
- Tumors in radial growth phase **fall into different clinicopathologic classes**.
  - **Lentigo maligna:** It usually **presents as an indolent lesion** on the **face of older men**. The tumor may remain in the radial growth phase for **several decades**.
  - **Superficial spreading:** It is the **most common type** of melanoma, and **usually** involves the **skin exposed to sun**.
  - **Acral/mucosal lentiginous melanoma**: It is **not related to sun exposure** and usually seen in the **palm, sole or mucosa**.

2. Vertical Growth Phase

After a variable and unpredictable period, melanoma from the radial phase develops a **vertical growth phase.**

Characteristics: During this phase:

- Tumor cells **invade downward** into the deeper dermis.
- **Melanoma may appear as a nodule and develop clone of cells with metastatic potential**.
- **Maturation is absent from the deep invasive portion** of melanoma.

- **Risk of metastasis** correlates with the **depth of invasion**, which is the distance from the superficial epidermal granular cell layer to the deepest intradermal tumor cells. This measurement is known as the **Breslow thickness.**

Microscopy (Figs. 26.4A and B)

Q. Write a short note on microscopy of melanoma.

1. Tumor Cells

They have **similar appearance in both the radial and vertical phases** of growth.

- **Size**: Tumor cells are usually **larger than normal melanocytes** or nevus cells found in melanocytic nevi.

- **Nuclei:** They are **large with irregular contours**, **clumping of chromatin at the periphery** of the nuclear membrane, and **prominent red (eosinophilic) nucleoli**. **Mitotic figures** are often seen.

2. Pattern of Growth

Tumor cells are arranged in **solid masses, sheets, islands**, etc. Tumor invades upper epidermis as well as deeper dermis.

3. Melanin Pigment

It is **seen in melanoma.** The melanoma, **which does not show the pigment,** is known as **amelanotic melanoma**. Melanin is present **in the cytoplasm as uniform brown fine granules**.

4. Tumor Infiltrating Lymphocytes (TILs)

They may be seen in the **tissue surrounding the tumor**.

Clinical Features

- Melanoma of the skin is **usually asymptomatic**, but may present with itching or pain at the site of lesion.
- **Changes in pigmented lesions:** These are the **most important clinical signs** of melanoma and include:
  - **Color:** Unlike benign nevi, **melanomas show variations in color**, ranging from shades of black, brown, red, dark blue, and gray.
  - **Size:** Majority are **larger than 10 mm** in diameter at the time of diagnosis. But, if a pigmented lesion is greater than 6 mm diameter, any change in appearance, and new onset of itching or pain should raise the suspicion of malignancy.

- **Border:** The borders of melanomas are **irregular and often notched**, whereas they are smooth, round, and uniform in melanocytic nevi.

Prognostic Factors (Table 26.1)

Sentinel Lymph Node Biopsy

- Most melanomas metastasize to regional lymph nodes.
- As in breast cancer, sentinel lymph node biopsy may provide additional prognostic information. **Presence of even small number of melanoma cells** (micrometastases) indicates a **worse prognosis**.
- The degree and number of lymph nodes involved correlate well with overall survival.

Distinguishing features between a nevus and melanoma are presented in **Table 26.2**.

Melanoma: Excessive exposure to sunlight at early age is the most important risk factor.

Malignant melanoma: Mutations in

- *CDKN2A*
- *RB*
- *NRAS*
- *BRAF*

Melanoma: Two growth phases.

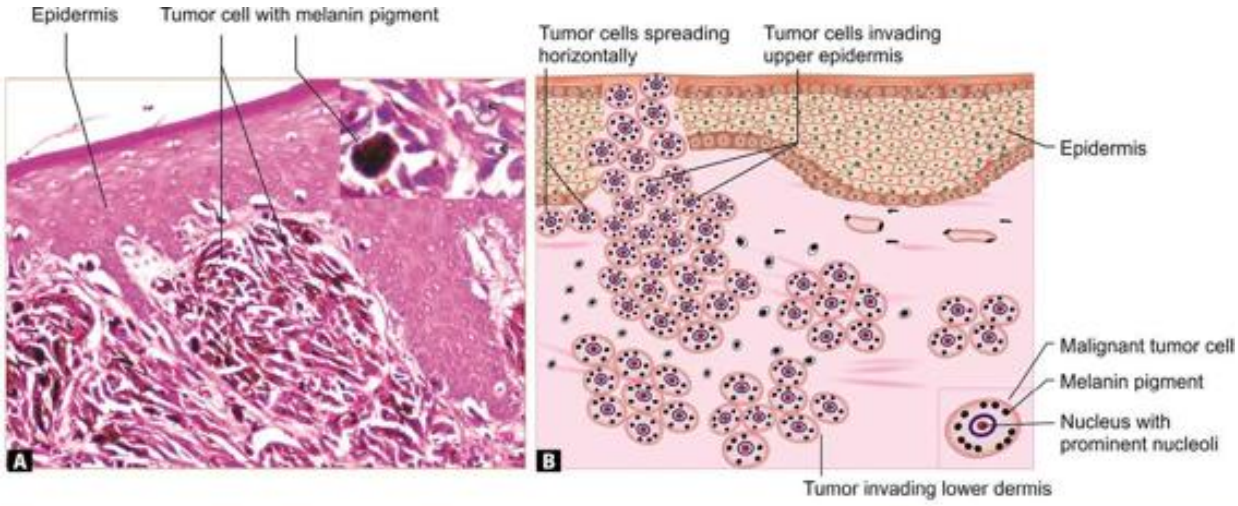
- Initial radial growth/phase: Spreads laterally in the papillary dermis with no metastatic potential
- Later vertical growth phase: Penetrates reticular dermis with metastatic potential



Breslow thickness:

- Determines the risk of metastasis
- Measurement for depth of invasion
- Distance from the superficial epidermal granular cell layer to the deepest intradermal tumor cells

Figs. 26.3A to C: Gross features of malignant melanoma (arrows): (A) Nodular growth projecting from the skin; (B) Cut section of the same shows blackish pigmentation in the tumor; (C) Lymph node with extensive metastasis from malignant melanoma (black color).



Malignant melanoma: (1) Highly aggressive; (2) Small tumors can produce deadly metastases; (3) Can metastasize any site.

Figs. 26.4A and B: (A) Photomicrograph of malignant melanoma showing nests of tumor cells in the upper dermis containing melanin pigment. Inset shows tumor cell with melanin pigment; (B) Diagrammatic microscopic appearance of malignant melanoma showing nests of tumor cells infiltrating epidermis (radial growth phase) and dermis (vertical growth phase).

TABLE 26.1: Prognostic factors in malignant melanoma.

Prognostic factors	***Favorable prognosis***
• Tumor depth (the Breslow thickness)	• Tumor depth of less than 1.7 mm
• Number of mitoses	• No or very few mitoses
• Evidence of tumor regression (may be due to the host immune response)	• Absence of regression
• Presence and number of tumor infiltrating lymphocytes (TILs)	• Brisk TIL response
• Gender	• Female gender
• Location (central body or extremity)	• Location on an extremity

TABLE 26.2: Distinguishing features of nevus and melanoma.

Feature	***Nevus***	***Melanoma***
Clinical features		
Border	Smooth, round, and uniform	Irregular and often notched border
Color	Uniformly pigmented	Variation in color ranging from shades of black, brown, red, dark blue, and gray
Size	Usually small, less than 6 mm	Diameter more than 6 mm
Solar damage	None	Solar elastosis

Symmetry	Symmetrical	Asymmetry
Common locations	Skin of face, mucosa	Skin; mucosa of nose, bowel, anal region
Microscopy		
Maturation	Superficial cells are larger and contain pigment and deeper cells are smaller and produce little or no pigment	No maturation
Pattern of growth	Nests or aggregates of nevus cells	Various patterns: solid masses, sheets, islands, etc.
Cell morphology	Uniform nevus cells	Cells are larger than nevus cells. Nuclei are large irregular with clumping of chromatin at the periphery of the nuclear membrane. Prominent eosinophilic nucleoli and mitotic figures often seen.
Melanin pigment	Irregular, coarse clumps	Fine granules, uniformly distributed
Edge of lesion	Similar	Dissimilar
Tumor infiltrating lymphocytes (TILs)	Not usual	May be seen
Spread	No spread and may be cosmetic problem only	Early hematogenous and/or lymphatic spread

Special stain for melanin: **Masson–Fontana or DOPA-oxidase reaction**.

HMB 45 is a tumor marker for: Malignant melanoma.

Q. What is ABCD of melanoma?

ABCD warning signs of melanoma:

- **A**symmetry
- Irregular **b**orders
- Variegated **c**olor
- Increased **d**iameter

Prognosis of melanoma: Depth of invasion is most important factor.

Sentinel lymph node: It is the initial site of drainage of intratumoral lymphatic vessels.

PREMALIGNANT EPIDERMAL TUMORS

Q. Write a short note on precancerous lesions of skin.

Actinic Keratosis

- Malignant epidermal tumors may be preceded by precursor lesions, similar to squamous cell carcinoma of the uterine cervix.
- In the skin, these **precursor lesions** are known as **actinic keratoses**; which usually develop in sun-damaged skin.
- Occur more frequently in **light-pigmented individuals**.
- Exposure to **ionizing radiation**, **industrial hydrocarbons** and **arsenicals** may produce similar lesions.

MORPHOLOGY

Gross

- **Size:** It usually **less than 1 cm** in diameter.
- **Color: Tan-brown, red, or skin-colored**.
- **Consistency:** Rough, **sandpaper-like consistency**.
- **Sites: Sun-exposed sites,** such as face, arms, dorsum of hands.

Microscopy

- **Cytologic atypia** is seen in the **lowermost basal cells of the epidermis** and these atypical cells have pink or reddish cytoplasm.
- **Superficial dermis** show **thickened, blue-gray elastic fibers** (elastosis).

\

Actinic keratosis:
- Develop on sun-exposed skin
- Cytological atypia in lowermost epidermis
- Infrequently progress to carcinoma in situ

NC: (Show How): Identify, distinguish and describe common tumors of the skin.
(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

SQUAMOUS CELL CARCINOMA

CC: Describe the risk factors pathogenesis, pathology and natural history of squamous cell carcinoma of the skin.

Q. Write short note on squamous cell carcinoma of skin.

- **Second most common tumor** arising **on sun-exposed sites**.
- **Sex:** More **common in men** than in women.
- **Clinical presentation:** Appear as sharply defined, red, scaling plaques.

Etiology and Pathogenesis

Q. Write a short note on etiology of squamous cell carcinoma of skin.

Risk Factors

- **Exposure to UV light:** It **may produce DNA damage** and extremely high mutational loads. It is the **most important cause** of squamous cell carcinoma of skin. The risk is proportional to the degree of lifetime sun exposure.
- **Chronic immunosuppression:** It may be due to **chemotherapy or organ transplantation** and may contribute to carcinogenesis. The immunosuppression reduces host surveillance and increases the susceptibility of keratinocytes to infection and transformation by oncogenic viruses. These oncogenic viruses include human papilloma virus (HPV) subtypes 5 and 8. Apart from damaging DNA, sunlight can cause a defect in cutaneous immunity by reducing the immune surveillance function of epidermal Langerhans cells.
- **Other risk factors:**
  - **Industrial carcinogens**: **Tars and oils**.
  - **Chronic non-healing ulcers:** Chronic osteomyelitis.
  - **Old burn scars (e.g., Marjolin's ulcers)**.
  - **Ingestion of arsenicals**.
  - **Ionizing radiation**.
  - Tobacco and betel nut chewing in the oral cavity.

Genetics of Squamous Cell Carcinoma

- **Mutations in tumor suppressor *TP53* gene**:

- **Ultraviolet (UV) light** present in the sunlight damages DNA. Squamous cell carcinoma induced by sunlight, ***TP53* mutation** occurs as an **early event**. **Cells with DNA damage are arrested in the G_1** phase of the cell cycle by normal *TP53* → DNA may be either repaired by DNA repair genes or cells undergo apoptosis if the damage is beyond repair.
- Mutated ***TP53*** results in **loss of** these **protective functions** and DNA mutations induced by UV light are passed down to daughter cells.

- **Mutations in DNA repair genes: Xeroderma pigmentosum** is a disorder characterized by **inherited mutations in DNA repair genes**, and these **patients are susceptible to squamous cell carcinoma**.
- **Other genes**: These include activating mutations in *RAS* and loss of function mutations in Notch receptors. Normally, these transmit signals that regulate orderly differentiation of normal squamous epithelia.

MORPHOLOGY

Squamous Cell Carcinoma In Situ

- Characterized by **cells with atypical** (enlarged and hyperchromatic) **nuclei involving all levels** of **epidermis**.
- **No invasion** through the basement membrane of the dermoepidermal junction.

Q. Write a short note on morphology of squamous cell carcinoma.

Invasive Squamous Cell Carcinoma

Gross (Fig. 26.5)

Invasive lesions are more advanced lesions, which appear as **nodular growth and may ulcerate**. The ulcer is **surrounded by a wide, elevated, indurated border**.

Microscopy

Tumor consists of **irregular masses of epidermal cells** that **proliferate downward into the dermis**. They show variable degrees of differentiation, ranging from well to poorly differentiated.

Well-differentiated squamous cell carcinoma(Figs. 26.6A and B)

They are composed of **polygonal squamous tumor cells arranged in orderly lobules** and **produce large amounts of keratin**. Some of this keratin form **epithelial or squamous pearls** and are characteristically seen in well-differentiated tumors.

Moderately differentiated squamous cell carcinoma

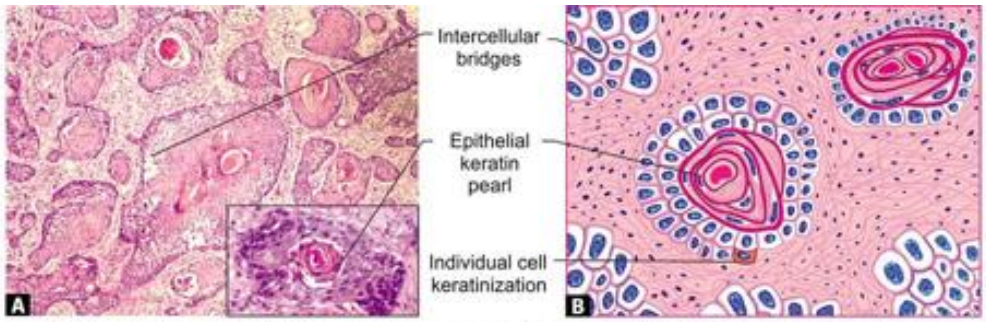
They consist of **anaplastic squamous cells, which show single-cell keratinization** (dyskeratosis). It may be associated with areas of geographic necrosis.

Poorly differentiated squamous cell carcinoma

They consist of **highly anaplastic cells.** Poorly differentiated tumor **may require immunohistochemical stains for keratins to confirm the diagnosis**.



Fig. 26.5: Squamous cell carcinoma (arrow) of foot (gross).



Figs. 26.6A and B: (A) Photomicrograph; (B) Diagrammatic representation: Well-differentiated squamous cell carcinoma composed of polygonal squamous tumor cells arranged in orderly lobules and produce large amounts of keratin. Some of this keratin form epithelial or squamous pearls (inset of A).

Well-differentiated squamous cell carcinoma: Epithelial or squamous pearls.

Poorly differentiated tumor: May require immunohistochemical stains for keratins to confirm the diagnosis.

Squamous cell carcinoma of skin—etiological factors:
- Exposure to UV light
- Chronic immunosuppression
- Chronic non-healing ulcers and scars

Xeroderma pigmentosum:
- Inherited mutations in DNA repair genes
- Susceptible to:
  - Squamous cell carcinoma
  - Basal cell carcinoma

BASAL CELL CARCINOMA

CC: Describe the risk factors pathogenesis, pathology and natural history of basal cell carcinoma of the skin.

Q. Write short note on basal cell carcinoma (rodent ulcer) of skin.

- Basal cell carcinoma is the most common, **slow-growing invasive cancer** that **rarely metastasizes**.
- **Sites:** Occur at **sun-exposed sites** and in fair-skinned people. Usual site is **above a line drawn from angle of mouth to the pinna of the ear**.
- **Origin:** Basal cell carcinoma derives its name from the cytological similarity of the tumor cells to the normal basal cells of the epidermis. They are believed to be **primitive 'adnexal' carcinomas probably of indubitable hair follicle derivation**.

Etiology and Pathogenesis

Q. Write a short note on etiology of basal cell carcinoma of skin.

The incidence of basal cell carcinoma is also **more in patients with immunosuppression** and **inherited defects in DNA repair,** such as

xeroderma pigmentosum.

Genetics of Basal Cell Carcinoma

Majority of basal cell carcinomas have mutations which causes unbridled hedgehog signaling.

1. **Mutation in tumor suppressor gene:**
   - **Mutation in *PTCH*: *PTCH* acts as tumor suppressor gene that negatively regulates Hedgehog signaling. Loss of function mutations in PTCH leads to constitutive activation of Hedgehog pathway.** This in turn activates many downstream genes involved in cell proliferation and survival. Mutations of genes belonging to the *PTCH* signaling pathway are important for the development of both common **sporadic and inherited** form of basal cell carcinoma.
     - **Sporadic form:** *PTCH* mutations are found in ~30% of sporadic basal cell carcinomas, and may be due to UV damage.
     - **Germline mutation: Nevoid basal cell carcinoma syndrome** (NBCCS; also known as **basal cell nevus or Gorlin syndrome**) is an autosomal dominant disorder characterized by multiple basal cell carcinomas. Patients with NBCCS are born with a germline mutation in one of the *PTCH* alleles; the second normal allele is inactivated in tumors by exposure to mutagens (such as UV light).
   - ***TP53* mutations:** It is caused by UV light and is common in both familial and sporadic basal cell carcinoma.
2. **Defect in DNA repair genes: Xeroderma pigmentosum** is a disorder of DNA repair, associated with increased incidence of basal cell carcinoma.

Clinical Presentation

- Appear as **pearly papules** often containing prominent, dilated subepidermal blood vessels.
- Some tumors may contain melanin and may resemble melanocytic nevi or melanomas.
- Advanced tumors **may ulcerate, and locally invade and erode the underlying bone or facial sinuses like a rodent** and are known as **rodent ulcers**.

MORPHOLOGY

Q. Write a short note on morphology of basal cell carcinoma.

Gross

- **Appearance varies** and may be **nodular, ulcerative (Fig. 26.7), superficial or erythematous**.
- **Nodulo-ulcerative** basal cell carcinoma is the **most common type** and present as a nodule that increases slowly in size and undergoes central ulceration. A **typical lesion** consists of a **slowly enlarging ulcer surrounded by a pearly, rolled border**. This represents the so-called rodent ulcer (**erodes the underlying structures similar to a rodent**).

Microscopy (Figs. 26.8A and B)

Tumor cells: They **resemble the normal basal cell layer** of the epidermis and referred to by some as basaloma (germinative) cells. The tumor cells are **deeply basophilic epithelial cells** and have a **large, oval, or elongated nucleus with narrow rim of cytoplasm**.

- **Arrangement**: Tumors cells are arranged in **nests** and are attached to the epidermis and protrude into the subjacent papillary dermis.
- **Peripheral palisading**: At the periphery of each nest, the **columnar cells are arranged radially with their long axes in parallel alignment** known as peripheral **palisading**.
- **Clefting artifact between tumor islands and adjacent stroma**: It may **sometimes help distinguish BCC from other adnexal neoplasms displaying basaloid cell proliferation**.
- **Two patterns of growth:**
  - **Multifocal growths**: These tumors originate from the epidermis and extend over several square centimeters or more of skin surface (multifocal superficial type).
  - **Nodular lesions**: These tumors grow deeply downward into the dermis as cords and islands of variably basophilic cells with hyperchromatic nuclei.

Basal cell carcinoma: Mutations in
- *PTCH*
- *TP53*
- *DNA repair genes*

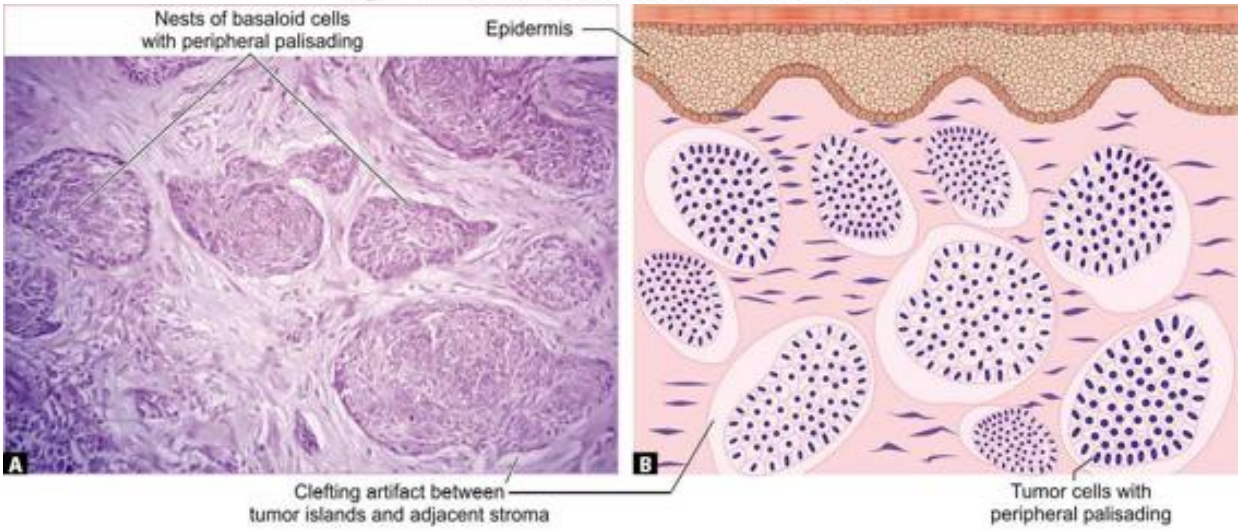
Basal cell carcinoma:
- Erodes the underlying tissue like a rodent
- Locally invasive
- Metastasis very rare

Basal cell carcinoma: Usual site is above a line drawn from angle of mouth to the pinna of the ear.

Basal cell carcinoma: Resemble the normal basal cell layer of the epidermis and show peripheral palisading.



Fig. 26.7: Basal cell carcinoma of skin.



Figs. 26.8A and B: Basal cell carcinoma. Composed of nests of uniformly atypical basaloid cells within the dermis, that are often separated from the adjacent stroma by thin clefts: (A) Photomicrograph; (B) Diagrammatic representation.

CHAPTER 27

Disorders of Central Nervous System and Eye

CEREBROVASCULAR DISEASES

Definition: Cerebrovascular disease is the term for **group of disorders of the brain** caused by **pathological process involving the blood vessels of the brain**.

Pathogenic Mechanism

- **Thrombosis and embolism** → occlusion of blood vessel → infarction or ischemia injury.
- **Rupture of blood vessel** → hemorrhage → direct damage to parenchyma.

Stroke is characterized by sudden onset and cause neurologic damage due to focal ischemia or hemorrhage.

Effects of Cerebrovascular Diseases

(1) Hypoxia, ischemia, and infarction or (2) hemorrhage (*refer* intracranial hemorrhage, pages 871-2).

1. **Global cerebral ischemia** (diffuse ischemic/hypoxic encephalopathy)
   - Ischemic damage occurs in the entire brain.
   - **Causes:** Generalized reduction of oxygenated blood to the brain as in shock (hemorrhagic shock), severe systemic hypotension (e.g., cardiac arrhythmia, myocardial infarction or cardiac arrest).
   - **Clinical outcome:**
     - **Mild:** Transient ischemia → confusion state followed by complete recovery.
     - **Severe**: Ischemia → results in death of neurons and the patient (if survives) may remain in a vegetative state.

2. **Focal cerebral ischemia: It may cause infarction.** Majority are caused by thrombosis and embolism.
   - **Thrombosis: Atherosclerosis** → predisposes to thrombus formation → occlude the vascular lumen.
   - **Embolic arterial occlusion:** More common. The origin of emboli may be:
     - **Heart:** Predisposing factors are myocardial infarct, valvular disease, and atrial fibrillation.
     - **Arteries:** Atheromatous plaques within the carotid arteries.
     - **Other sources of emboli:** Paradoxical emboli (e.g., in children with cardiac anomalies), emboli following cardiac surgery, and other emboli like tumor, fat, or air.
   - **Generalized arterial disease: Infectious vasculitis** (syphilis and tuberculosis), polyarteritis nodosa and other noninfections vasculitides.

Classification of Infarcts

1. **Hemorrhagic (red) infarction**: It is characterized by multiple, petechial hemorrhages and is seen with **embolic events**.
2. **Nonhemorrhagic (pale, bland, anemic) infarcts**: It is usually associated with **thrombosis**.

Microscopy: It shows liquefactive necrosis.

Clinical Features

- Defects associated with infarction depend on the region of brain involved rather than the cause.
- Neurologic symptoms referable to the area of injury usually develop rapidly, over minutes, and may continue to evolve over hours.

INTRACRANIAL HEMORRHAGE

Hemorrhages can occur at any site within the CNS. Depending on the anatomic location they are subdivided into:

1. **Epidural hemorrhage**] Usually associated with trauma
2. **Subdural hemorrhage**
3. **Subarachnoid hemorrhage:** It is usually due to ruptured aneurysm, vascular malformations.
4. **Intraparenchymal hemorrhage:** It is usually spontaneous (nontraumatic) due to rupture of intraparenchymal vessel (e.g., hypertension and cerebral amyloid angiopathy).

Epidural Hemorrhage

Definition: Accumulation of **blood between skull bone and the dura mater**.

Causes: (1) Road accidents, (2) fracture of skull bones, and (3) fall.

Subdural Hemorrhage

Definition: Subdural hematoma is defined as **accumulation of blood in the subdural space**, i.e., between the inner surface of the dura mater and the outer arachnoid layer of the leptomeninges.

Causes: It is usually due to tear in the veins at the point where they penetrate the dura mater. The causes may be: (1) fall, (2) road traffic accidents, (3) assaults, and (4) sport accidents.

Subarachnoid Hemorrhage and Ruptured Saccular Aneurysms

Q. Write a short note on subarachnoid hemorrhage.

Definition: Accumulation of blood in the subarachnoid space, i.e., between the inner surface of the arachnoid and pia mater.

Causes

- Rupture of a saccular (berry) aneurysm
- Trauma
- Rupture of a hypertensive intracerebral hemorrhage into the ventricular system
- Hematologic disorders

- Vascular malformation
- Tumors.

Saccular Aneurysms

Q. Write short note on berry aneurysm.

It is one of the cause of subarachnoid hemorrhage.

Pathogenesis

It is found in **association with polycystic kidney disease**. There is **congenital defect in the smooth muscle of the tunica media** at the site of arterial bifurcation, where local hemodynamic factors act to produce a slowly enlarging saccular aneurysm. Common sites of berry aneurysm are shown in **Figure 27.1**.

MORPHOLOGY

Gross

- **Size:** Few **millimeters to 2 or 3 cm** in diameter.
- **Appearance: Bright red, shiny surface** and **thin translucent wall**.
- **Location:** At an **arterial branch point along the circle of Willis**.

Microscopy

Absence of internal elastic lamina in the neck of the aneurysm.

Clinical Features

- Most frequent in the **fifth decade** and is slightly **more frequent in females**.
- Presents with sudden, severe headache followed by loss of consciousness.

Intracerebral (Intraparenchymal) Hemorrhage (Fig. 27.2)

Definition: Hemorrhages within the brain parenchyma. Usually spontaneous and occur in middle to late adult life (peak during 60 years of age).

Etiology: Most are caused by **rupture of a small intraparenchymal vessel**. The two major causes are **hypertension and cerebral amyloid angiopathy** (CAA).

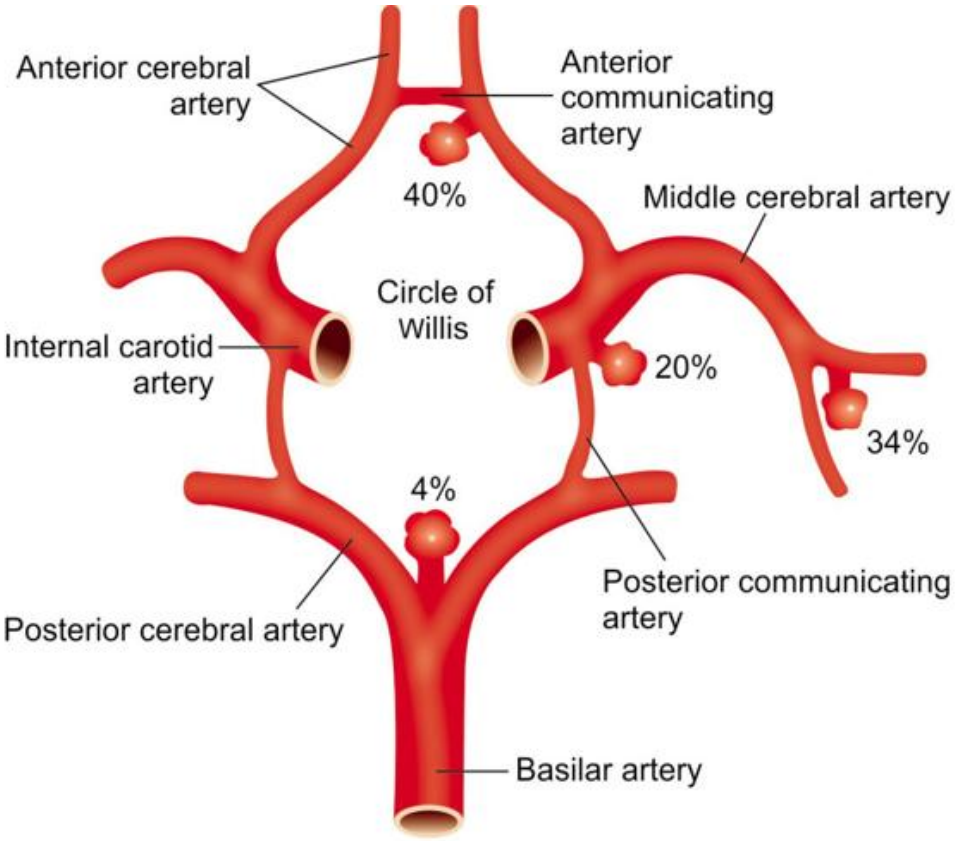


Fig. 27.1: Blood supply to the brain and circle of Willis with common sites of saccular (berry) aneurysms.

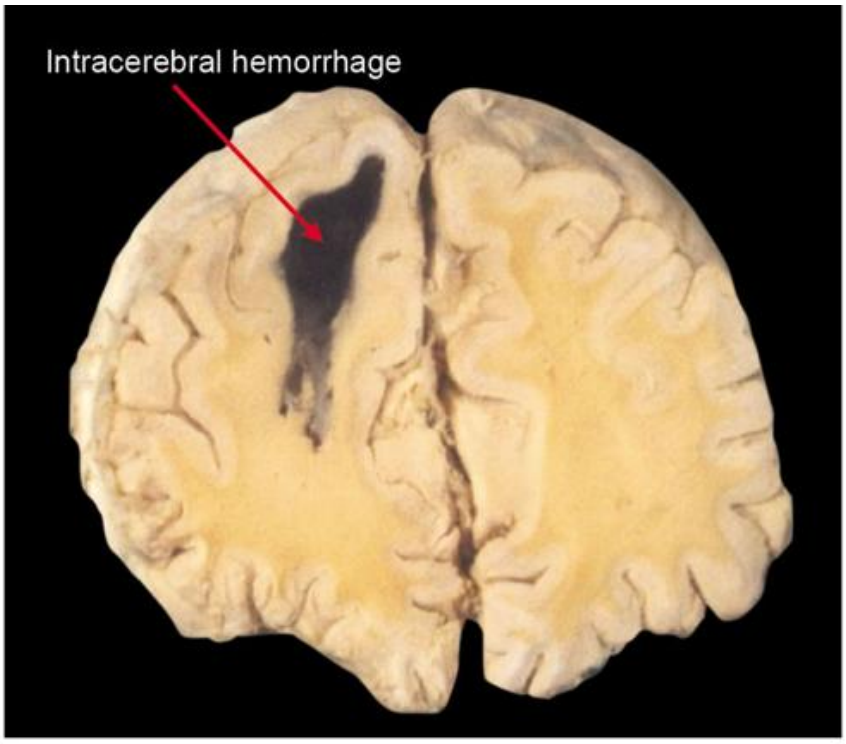


Fig. 27.2: Intraparenchymal hemorrhage on the left cerebral hemisphere appears as dark-colored area.

Blood vessel changes in hypertension:

- Atherosclerosis in larger arteries.
- Hyaline arteriolosclerosis in smaller vessels → weaken the vessels → more vulnerable to rupture.
- Malignant hypertension → proliferative changes and frank fibrinoid necrosis of arterioles.

Epidural hemorrhage: Fracture of skull and tear in the middle meningeal artery.

Subdural hemorrhage: Venous bleeding between dura and arachnoid mater. Most commonly occurs due to rupture of bridging veins.

Subarachnoid hemorrhage: Rupture of congenital berry aneurysm. Berry aneurysm is associated with adult polycystic kidney (ADPKD).

Intracerebral hemorrhage: Complication of hypertension.

MENINGITIS

CC: Describe the etiology, types and pathogenesis, differentiating factors, CSF findings in

meningitis.

Q. Write short note on meningitis.

Meninges have three layers and from inside out are pia, arachnoid and dura mater.

Leptomeningitis: Meningitis is the term usually used for inflammation **within subarachnoid space between pia and arachnoid mater** and known as leptomeningitis.

Meningitis is a **dangerous infection** caused by a variety of microorganisms and CSF acts an excellent culture medium for most of these causative organisms.

Causes

- **Infectious meningitis**:
  - **Acute: (**1) Pyogenic (usually bacterial), (2) aseptic (usually viral).
  - **Chronic:** (1) Tuberculous, (2) syphilitic and (3) cryptococcal.
- **Chemical meningitis:** Due to a nonbacterial irritant introduced into the subarachnoid space.

CSF in acute pyogenic meningitis:
- Cloudy
- Proteins increased
- Sugar decreased
- Numerous polymorphonuclear leukocytes

Waterhouse-Friderichsen syndrome:
- Meningitis
- Septicemia
- Hemorrhagic infarction of the adrenal glands
- Cutaneous petechiae

Bacterial meningitis: Majority of organisms originate in nasophayrnx.

Acute Pyogenic (Bacterial/Purulent) Meningitis

Q. Write short note on acute pyogenic meningitis.

Etiology

- **Neonates**: ***Escherichia coli*** and the **group B streptococci**
- **Infants and children:** *Haemophilus influenzae*
- **Adolescents and in young adults**: *Meningococcus* (***Neisseria meningitides)*** is the most common pathogen.
- **Extremes of life:** *Streptococcus pneumoniae* and *Listeria monocytogenes*.

Routes of Infection

Infectious microbes may enter the meninges by following routes.

- **Hematogenous route**
- From an **adjacent focus of infection**
- **Iatrogenic**: Through lumbar puncture.

MORPHOLOGY

Gross

Thick creamy exudate is seen within the leptomeninges **over the surface of the brain** (cerebral hemispheres). The **meninges** appear **opaque**.

Location of the Exudate

- *H. influenzae* meningitis—usually basal.
- Pneumococcal meningitis—over the cerebral convexities near the sagittal sinus.

Microscopy

- Exudates in the subarachnoid space: Consists of dense collections of neutrophils and fibrin
- **Gram stain** may reveal the causative organism.

Cerebrospinal Fluid (CSF) Changes

CC: Identify the etiology of meningitis based on given cerebrospinal fluid (CSF) parameters.

Q. Write short note on CSF changes in pyogenic meningitis.

Presence of polymorphonuclear leukocytes in the CSF is the most definitive feature of meningitis.

- **Appearance:** Normally CSF is clear. In acute meningitis, it becomes **cloudy or frankly purulent**.
- **Pressure: Increased** (>180 mm of water).
- **Cells: Increased neutrophils** (may be as many as 90,000 per cubic millimeter).
- **Protein concentration**: **Increased** (>50 mg/dL).
- **Glucose**: **Markedly reduced** (<40 mg/dL).
- **Smear: Bacteria** may be seen on a smear (Gram stain) or can be cultured.

Complications of Bacterial Meningitis

- **Obstructive hydrocephalus**
- **Thrombophlebitis of leptomeningeal veins** may lead to

TABLE 27.1: Cerebrospinal fluid findings in meningitis.

	Normal	*Acute pyogenic*	*Acute viral*	*Tuberculous*
Physical examination	Clear and colorless	Turbid and forms coagulum	Clear	Clear and colorless, forms cobweb on standing due to coagulation of fibrinogen
CSF pressure	60–150 mm of H_2O	Raised above 180 mm of H_2O	Raised above 250 mm of H_2O	Raised above 300 mm of H_2O
Total protein	20–40 mg/100 mL	50–200 mg/100 mL	>40 mg/100 mL	50–150 mg/100 mL
Glucose	45–80 mg/100 mL	0–20 mg/l00 mL	Normal	20–50 mg/100 mL
Chlorides	720–750 mg/100 mL	600–700 mg/100 mL	Normal	450–600 mg/100 mL
Cells				
Polymorphs	Usually absent	150–2000/µL	Absent	0–5 cells/µL
Lymphocytes	0–5 cells/µL	5–50 cells/µL	10–100 cells/µL	500–700 cells/µL
Gram stain/ZN stain	-	Bacteria +	–	AFB +

- **Venous thrombosis**
- **Cerebral infarction**
- Focal infection of the underlying brain parenchyma.

- **Chronic adhesive arachnoiditis**
- **Cerebral abscess**
- **Subdural empyema**
- **Epilepsy**
- **Waterhouse–Friderichsen syndrome:** It results from **meningitis-associated septicemia** with **hemorrhagic infarction of the adrenal glands** and **cutaneous petechiae**. It occurs most often with meningococcal and pneumococcal meningitis.

Clinical Features

- Symptoms: **Headache, vomiting, fever and convulsions** (especially in children).
- Classic signs of meningitis
  - **Cervical rigidity/neck stiffness**
  - **Kernig sign: Knee pain with hip flexion**
  - **Brudzinski sign: Knee/hip flexion when the neck is flexed**.

Untreated pyogenic meningitis can be fatal.

Meningitis: Inflammation of pia and arachnoid.

Meningitis: Different pathogens can reach through different routes.

Acute Aseptic (Viral) Meningitis

Symptoms of acute meningitis with **no identifiable organisms**.

Etiology: Enterovirus, HIV, mumps virus, EB virus and herpes simplex virus.

Age: Common in children and young adults.

CSF Findings

- Appearance: Clear.
- Protein: Moderately increased (>40 mg/100 mL).
- Glucose: Always normal.
- Microscopy: 10–100 lymphocytes/mL.

Clinical features: Fever and headache.

Prognosis: Usually self-limiting.

CSF findings in various types of meningitis are summarized in **Table 27.1.**

Viral meningitis: Most often transmitted by fecal-oral route.

Chronic Meningitis

Types

- Bacterial: (1) Tuberculous caused by *M. tuberculosis* and (2) syphilitic caused by *T. pallidum*.
- Fungal: Cryptococcal.

Tuberculous Meningitis

Q. Write short note on tuberculous meningitis.

Infection of the meninges by tubercle bacilli.

Etiology: *Mycobacterium tuberculosis* **human type is the most common cause**.

Mode of Infection

- **Hematogenous route** from other site (most commonly from lung)
- **Miliary spread**
- **Direct spread** from adjacent site such as vertebral body.

MORPHOLOGY

Gross

- **Subarachnoid space** contains a **greenish, gelatinous or fibrinous exudate**, most **prominent at the base of the brain surrounding cranial nerves.**
- Leptomeninges may show discrete, white granules of tubercle.

Microscopy

- **Granuloma** consisting of epithelioid cells, Langhans giant cells surrounded by lymphocytes. It may show central area of caseous necrosis
- AFB stain may show acid-fast bacilli.

Clinical Features

Includes headache, malaise, mental confusion, and vomiting. On examination, there will be neck rigidity.

Cerebrospinal Fluid Findings

Q. Write short note on CSF changes in tuberculous meningitis.

- **Physical examination**: **Clear and colorless, forms cobweb on standing** due to coagulation of fibrinogen.
- **CSF pressure: Raised above 300 mm of H_2O**
- **Protein: Raised ranges from 50–150 mg/100 mL**
- **Glucose: Moderately reduced or normal (20–50 mg/100 mL)**
- **Chloride: Decreased (450–600 mg/100 mL)**
- **Cells: Moderate CSF pleocytosis** (500–700 cells/µL), **mainly lymphocytes**.

Complications: (1) Hydrocephalus, (2) nerve root damage, and (3) tuberculous encephalitis.

Tuberculoma

It is another manifestation of tuberculosis of CNS.

MORPHOLOGY

Gross

- **Number: Single or multiple** well circumscribed intraparenchymal mass (tuberculoma).
- **Size:** Varies and may be as large as several centimeters in diameter, and may present as intracranial space-occupying lesion.

Microscopy

Shows **epithelioid granuloma** consisting of central core of caseous necrosis surrounded by epithelioid cells, Langhans giant cell and lymphocytes.

Tuberculous meningitis:
- Greenish, gelatinous or fibrinous exudate
- Most prominent at the base of the brain surrounding cranial nerves.

CSF in tuberculous meningitis:

- Forms cobweb on standing
- Proteins increased
- Sugar decreased
- Chlorides decreased
- Lymphocytes

Meningitis: Neck rigidity.

Neurosyphilis

Develops in the tertiary stage of syphilis and occurs in about 10% of untreated patients.

Major Patterns of CNS Involvement

- **Meningovascular neurosyphilis:** Chronic meningitis.
  - Involves the base of the brain and may also involve the cerebral convexities and the spinal leptomeninges.
  - Obliterative endarteritis (Heubner arteritis) characterized by a distinctive perivascular inflammatory reaction rich in plasma cells and lymphocytes.
  - Cerebral gumma is mass rich in plasma cell seen in parenchyma and may also be seen in the meninges.
- **General paresis of the insane:** Due to invasion of the brain by *Treponema pallidum*

  Insidious in onset but progressive mental deficits with mood alterations that terminate in severe dementia **(general paresis of the insane)**.

Microscopy

(1) Loss of neurons, (2) proliferations of microglia (rod cells); and (3) gliosis. Iron deposits mainly in the perivascular region and in the neurophil, which stain positive with the Prussian blue stain.

- **Tabes dorsalis**: Due to damage to the sensory nerves in the dorsal roots.

  Clinical features:
  - Impaired joint position sense and resultant ataxia (locomotor ataxia).
  - Loss of pain sensation, leading to skin and joint damage (Charcot joints).

- Other sensory disturbances: Lightning pains and absence of deep tendon reflexes.

Microscopy

Loss of both axons and myelin in the dorsal roots resulting in atrophy of dorsal columns of the spinal cord.

INTERPRETATION CEREBROSPINAL FLUID FINDINGS

CC: S – Skill, P – Perform independently: Identify the etiology of meningitis based on given CSF parameters. Skill Assessment: Procedure require certification.

(DOAP: **D**emonstrate (by Student), **O**bserve, **A**ssist, **P**erform)

Case 1

History: A 24-year-old male presented to an emergency department for the complaint of fever and severe headache of 1-day duration. The headache has rapidly become more intense. He had vomited twice, appears drowsy and confused. He finds bright lights uncomfortable. There was no significant previous medical history.

Examination: His temperature is 39.2°C. He has stiffness on passive flexion of his neck. His consciousness level is decreased, but he is rousable to command; there are no focal neurological signs.

Investigations

Hematological tests: WBC count of 16, 200/µL (normal 4,000–11,000/µL) and platelet count of 310, 000/µL (150,000–450,000/µL).

Cerebrospinal fluid (CSF) findings

Physical appearance	Turbid and formed coagulum (clear and colorless)
Pressure	210 mm of H_2O (60–150 mm of H_2O)
Protein	150 mg/100 mL (20–40 mg/100 mL)
Glucose	8 mg/100 mL (45–80 mg/100 mL)
Chlorides	645 mg/100 mL (720–750 mg/100 mL)
Microscopy	
• Leukocytes	1500 cells/µL (Usually absent)
• Lymphocytes	8 cells/µL (0–5 cells/µL)

• Gram stain	Demonstrated intracellular diplococci

Note: Reference values in brackets.

Questions

1. What is the probable diagnosis?
2. What is/are the cause/s of this condition?
3. What are the complications?

Answers

1. Probable diagnosis is acute bacterial meningitis. The clinical features which favors the diagnosis are sudden onset of severe headache, vomiting, confusion, photophobia and neck stiffness. The presence of leukocytosis suggests acute bacterial infection rather than viral meningitis. Normally CSF is clear. In acute meningitis, it becomes **cloudy or frankly purulent**. The combination of 1,500 neutrophils/µL CSF (most definitive feature of pyogenic meningitis), decreased CSF glucose (8 mg/100 mL) and a raised CSF protein of 150 mg/100 mL is strongly suggestive of bacterial meningitis. Glucose is reduced because the organisms use glucose for their multiplication. The gram stain and culture will give the definitive diagnosis. In this case, the Gram stain demonstrated Gram-negative cocci consistent with *Neisseria meningitidis* infection.
2. The most likely causative bacteria are *Neisseria meningitidis* (meningococcal meningitis), *Haemophilus influenzae* and *Streptococcus pneumoniae*. Meningitis may be caused by virus, fungi and *Mycobacterium tuberculosis* apart from bacteria.
3. Complications (*refer* pages 873-4).

Case 2

History: A 10-year-old male child presented to an emergency department for the complaint of fever and severe headache of 1-day duration. He had vomited twice, appears drowsy and confused. He finds bright lights uncomfortable. There was no significant previous medical history.

Examination: His temperature is 38.8°C. He has stiffness on passive flexion of his neck. His consciousness level is decreased, but he is rousable to command; there are no focal neurological signs.

Investigations

Cerebrospinal fluid (CSF) findings

Physical examination	Clear
CSF pressure	270 mm of H_2O (60–150 mm of H_2O)
Total protein	80 mg/100 mL (20–40 mg/100 mL)
Glucose	50 mg/mL (45–80 mg/100 mL)
Chlorides	730 mg/mL (720–750 mg/100 mL)
Cells	
Polymorphs	Absent (usually absent)
Lymphocytes	70 cells/μL (0–5 cells/μL)
Gram stain/ZN stain	No organism detected

Note: Reference values in brackets.

Questions

1. What is the probable diagnosis?
2. What are the features that helps in diagnosis?
3. What is/are the cause/s of this condition?

Answers

1. Probable diagnosis is acute aseptic (viral) meningitis. It is common in children and young adults.
2. Feature that favors the diagnosis: The clinical features which favors the diagnosis of meningitis are sudden onset of severe headache, vomiting, confusion, photophobia and neck stiffness. The presence of lymphocytes in CSF suggests acute viral infection rather than bacterial meningitis. The combination of 70 lymphocytes/μL in CSF, normal CSF glucose (50 mg/100 mL) and a moderate increase of CSF protein of 80 mg/100 mL are strongly suggestive of acute viral meningitis. Symptoms of acute meningitis with **no identifiable organisms** on Gram stain/ZN stain will give the definitive diagnosis. Culture will be negative for bacteria.

3. The e**tiological agents include** enterovirus, HIV, mumps virus, EB virus and herpes simplex virus.

Case 3

History: A 10-year-old boy was brought to an emergency department for the complaint of fever and severe headache of 2 days duration. The headache has rapidly become more intense. He had vomited twice, appears drowsy and confused. He finds bright lights uncomfortable. His father is having chronic cough, evening rise of temperature and taking treatment for the past 3 months for a chronic disease of the lung.
Examination: His temperature is 38.0°C. He has stiffness on passive flexion of his neck (on neck rigidity). His consciousness level is decreased, but he is rousable to command; there are no focal neurological signs.

Investigations

Cerebrospinal fluid (CSF) findings

Physical examination	Clear and colorless, forms cobweb on standing due to coagulation of fibrinogen
CSF pressure	320 mm of H_2O (60–150 mm of H_2O)
Total protein	130 mg/100 mL (20–40 mg/100 mL)
Glucose	40 mg/100 mL (45–80 mg/100 mL)
Chlorides	550 mg/100 mL (720–750 mg/100 mL)
Cells	
Polymorphs	2 cells/μL (usually absent)
Lymphocytes	600 cells/μL (0–5 cells/μL)
ZN stain	AFB +

Note: Reference values in brackets.

Questions

1. What is the probable diagnosis?
2. What are the features that helps in diagnosis?
3. What are the complications?

Answers

1. Probable diagnosis is tuberculous meningitis. It is caused by *Mycobacterium tuberculosis* **human type**.
2. Feature that favors the diagnosis: The clinical features which favors the diagnosis of meningitis is sudden onset of severe headache, vomiting, confusion, photophobia neck stiffness and raised **CSF pressure. Physical examination of clear and colorless CSF which forms cobweb on standing** due to coagulation of fibrinogen and the presence of lymphocytes (600 cells/μL) in CSF (which is more than seen in acute viral infection) suggests tuberculous meningitis. The combination of 600 lymphocytes/μL CSF, mildly reduced CSF glucose (40 mg/100 mL) an increase of CSF protein of 130 mg/100 mL and **decreased chloride** is strongly suggestive of tuberculous meningitis. ZN stain for AFB will give the definitive diagnosis.
   Mode of infection: (i) **Hematogenous route** from other site (most commonly from lung); (ii) **Miliary spread or (iii) Direct spread** from adjacent site such as vertebral body.
3. **Complications:** (1) Hydrocephalus, (2) nerve root damage, and (3) tuberculous encephalitis.

Brain Abscesses

Q. Write a short note on brain abscess.

Brain abscesses are caused mainly by bacterial infections.

Mode of Infection

- Direct implantation of organisms
- Local extension from adjacent foci (mastoiditis, paranasal sinusitis)
- **Blood spread:** Primary site may be in the heart, lungs, or distal bones, or after tooth extraction. Predisposing conditions include: acute bacterial endocarditis, cyanotic congenital heart disease, chronic pulmonary infections (e.g., bronchiectasis).

Microscopy

Abscesses appear as discrete lesions with central area of liquefactive necrosis (*refer* **Fig. 2.15**). These are surrounded by granulation tissue composed of capillaries and fibrous tissue. This in turn surrounded by a zone of reactive gliosis.

Clinical Features

It presents with progressive focal deficits and signs due to increased intracranial pressure. CSF is not useful for the diagnosis. If abscess produces a significant mass effect lumbar puncture can result in downward herniation of cerebral contents and neurologic deterioration. Culture of drained abscess may be useful.

Complications

If it increases the intracranial pressure, it may cause fatal brain herniation. Rupture of abscess can lead to ventriculitis, meningitis, and venous sinus thrombosis.

TUMORS OF CENTRAL NERVOUS SYSTEM (CNS)

CC: Classify and describe the etiology, genetics, pathogenesis, pathology, presentation, sequelae and complications of CNS tumors.

Classification of important CNS tumors is presented in **Box 27.1**.

BOX 27.1: Classification (abridged) of important CNS tumors.

Diffuse astrocytic and oligodendroglial tumors

- Diffuse astrocytoma, IDH-mutant
- Diffuse astrocytoma, IDH-wildtype
- Diffuse astrocytoma, NOS
- Anaplastic astrocytoma, IDH-mutant
- Anaplastic astrocytoma, IDH-wildtype
- Anaplastic astrocytoma, NOS
- Glioblastoma, IDH-wildtype
- Glioblastoma, IDH-mutant
- Glioblastoma, NOS
- Diffuse midline glioma, H3 K27M-mutant
- Oligodendroglioma, IDH-mutant 1p/19q-codeleted
- Oligodendroglioma, NOS
- Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted
- Anaplastic oligodendroglioma, NOS

Other astrocytic tumors

- Pilocytic astrocytoma

- Ependymoma

Neuronal tumors

- Central neurocytomas
- Ganglioglioma

Embryonal tumors

- Medulloblastomas

Meningiomas

- Meningioma

Metastatic tumors

(IDH: isocitrate dehydrogenase; NOS: not otherwise specified)

DIFFUSE ASTROCYTIC AND OLIGODENDROGLIAL TUMORS

Gliomas are the **most common group of primary brain tumors**. The tumors are classified histologically on the resemblance of cells to glial cells. **Major tumors includes:** (1) **astrocytomas,** (2) **oligodendrogliomas and** (3) **ependymomas**.

Astrocytoma

Q. Write short note on astrocytoma.

Astrocytoma is a **glioma derived from astrocytes**.

Diffuse Astrocytomas

- Form—**80% of primary brain tumors in adults.**
- **Site:** Usually in the **cerebral hemispheres.** Other sites include: cerebellum, brainstem, and spinal cord.
- **Age**: Usually **fourth to sixth decades.**

Definition: *A diffuese astrocytoma is characterized by a mutation in either the IDH1 or IDH2 gene.* IDH-mutant diffuse astrocytoma is usually consists of moderately pleomorphic cells, a high degree of cellular differentiation and slow growth.
The diagnosis is supported by the presence of *ATRX* and *TP53* mutation.

Classification

Diffuse astrocytomas can be further categorized into:

- Diffuse astrocytoma, IDH-mutant

- Diffuse astrocytoma, IDH-wildtype
- Diffuse astrocytoma, NOS

There are **no WHO grade I** infiltrating astrocytomas.

Anaplastic Astrocytoma

This includes: (i) Anaplastic astrocytoma IDH-mutant,(ii) Anaplastic astrocytoma, IDH-wildtype and (iii) Anaplastic astrocytoma, NOS.

Glioblastoma

Classification of glioblastoma: Before modern advances in genetic analyses, glioblastoma were divided into:

1. **Primary glioblastoma:** It **arises de novo** as new onset disease, without any pre-existing low-grade astrocytoma and occurs at older age.
2. **Secondary glioblastoma:** It arises **in patient who had lower-grade astrocytoma earlier** and occur in younger patients.

Presently it is classified as (i) Glioblastoma, IDH-wildtype; (ii) Glioblastoma, IDH-mutant and (iii) Glioblastoma, NOS.

Molecular Genetics

Mutations in *isocitrate dehydrogenase (IDH)* genes are the most common genetic alterations in grade II astrocytoma and oligodendroglioma. Mutations can occur in *IDH1* or *IDH2* genes. These mutations lead to increased production of 2-hydroxyglutarate, which in turn interferes with the activity of several enzymes that regulate gene expression. Glioblastomas can be divided into *IDH*-wildtype (without IDH mutations) or IDH mutant. IDH-wildtype glioblastomas is the most common (90%) and most malignant astrocytic glioma.

Mutations in the promoter for *telomerase*: This causes immortalization of tumor cells and can be seen in glioblastomas and other astrocytic tumors. In tumors with IDH mutations, telomerase mutations are uncommon; instead, they have loss of function mutations in *ATRX*, which normally suppresses recombination events that can preserve telomere length. This mechanism is termed as alternative lengthening of telomeres.

Other genetic alterations: These are common in tumors outside the CNS and may be observed in CNS tumors. These include mutations that lead to overexpression of the EGF receptor and other receptor tyrosine kinases or disable *p53* or RB.

MORPHOLOGY

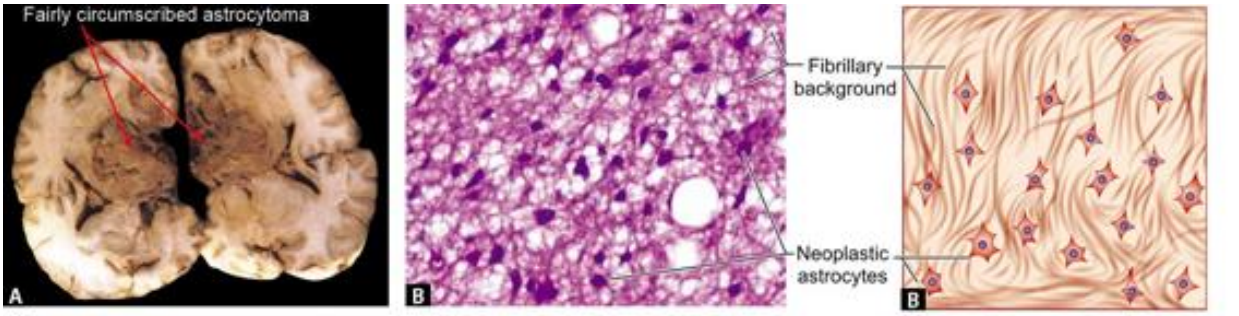
Diffuse Astrocytoma

Gross (Fig. 27.3A)

- **Poorly demarcated, infiltrative tumor**
- **Size:** Range from a few centimeters to large lesions.
- Cut surface: **Gray, firm or soft and gelatinous**. May show cystic degeneration.

Microscopy (Figs. 27.3B and C)

- **Mild to moderate cellularity** due to increase in the astrocytic glial tumor cells.
- Variable degree of nuclear pleomorphism.
- **Fibrillary background**: Between the nuclei of tumor cells, extensive network of fine, GFAP-positive astrocytic processes



Figs. 27.3A to C: Astrocytoma involving cerebrum. Microscopic appearance of diffuse astrocytoma: (B) Photomicrograph; (C) Diagrammatic.

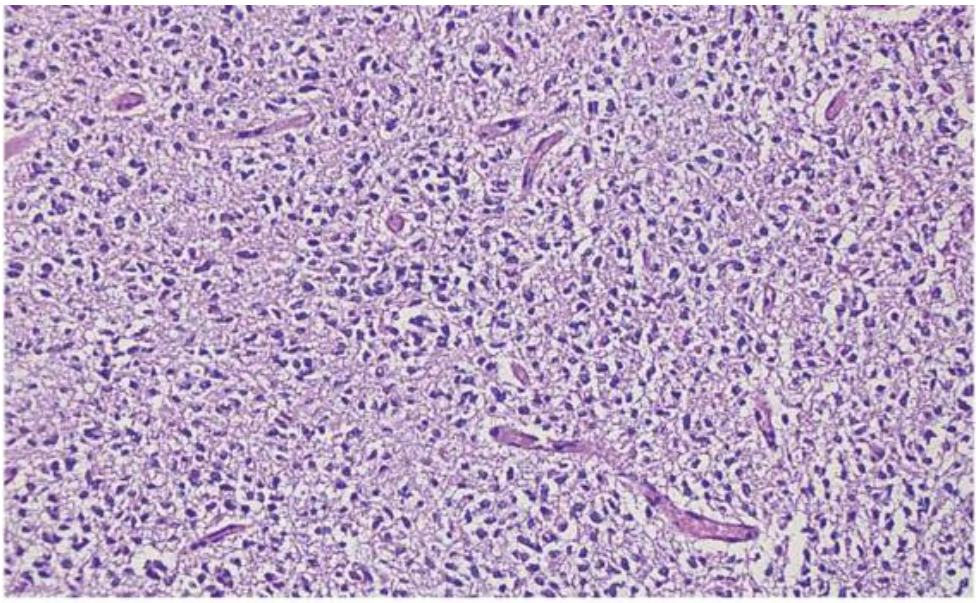


Fig. 27.4: Anaplastic astrocytoma showing increased cellularity, cellular and nuclear pleomorphism and anaplasia.

produces a fibrillary background appearance. The demarcation between neoplastic and normal tissue is indistinct, and tumor cells infiltrate surrounding normal tissue, some distance away from the main tumor.

- **Immunohistochemistry:** These glial neoplasms show **immunopositivity for GFAP (glial fibrillary acid protein)**.

Anaplastic Astrocytomas (Grade III) (Fig. 27.4)

- Increased cellularity
- **Cellular and nuclear pleomorphism**
- **Anaplasia**
- **Presence of mitotic figures**
- **Rapid growth of the tumor**.

Glioblastoma

Q. Write short note on glioblastoma.

Gross

- **Consistency and color varies.**
- **Variegated appearance:** Some areas are firm and white, others are soft and yellow due to necrosis.
- Colors represent multiple areas of **recent (red) and old (yellow) hemorrhage.**
- Show cystic degeneration.

Microscopy (Figs. 27.5A and B)

Similar to anaplastic astrocytoma with necrosis or vascular/endothelial cell proliferation.

- Increased cellularity
- Marked cellular and nuclear pleomorphism
- Anaplasia
- Frequent mitoses
- **Necrosis: (1) Serpentine (snake like) pattern of necrosis** and (2) **edges of the necrotic area** is surrounded by the palisading tumor cells, which is known as **pseudopalisading (garlanding)**.
- **Vascular or endothelial cell proliferation:** It is characterized by **tufts of piled-up endothelial cells** (at least two layers), which **protrude into the lumen**. Marked endothelial cell proliferation may form structure similar to glomerulus and known as glomeruloid body.

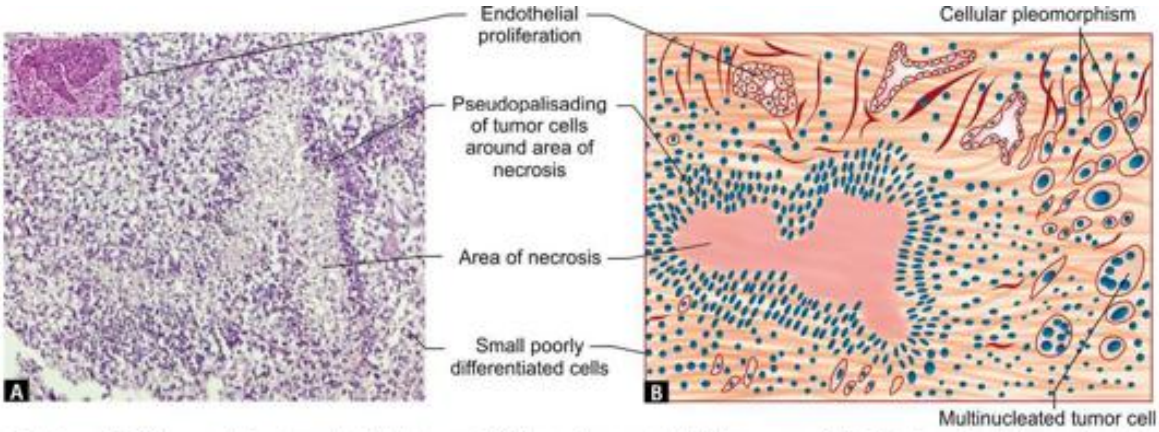
Gliomatosis cerebri: It is a diffuse glioma showing extensive infiltration of multiple regions of the brain (or the entire brain). It is aggressive and a grade III/IV lesion.

Clinical features: Seizures and headaches. Symptoms depend on the location and growth rate of the tumor. Well-differentiated diffuse astrocytomas may remain stable or progress slowly. Clinical deterioration invariably occurs due to the more rapid growth and higher histological grade.

Radiologic Studies

Show mass effect and **edema of brain adjacent to the tumor**. **High-grade astrocytomas** have abnormal vessels that are "leaky" and demonstrate **contrast enhancement** on imaging studies.

Prognosis: Diffuse astrocytomas may remain static or progress only slowly over a number of years. **Glioblastoma** has a **very poor prognosis**.



Figs. 27.5A and B: Microscopic features of glioblastoma: (A) Photomicrograph; (B) Diagrammatic. Foci of necrosis with pseudopalisading of tumor nuclei and endothelial cell proliferation are seen. Inset in A shows vascular endothelial proliferation. The microscopic features of glioblastoma are: Increased cellularity, pleomorphism, serpentine necrosis, pseudopalisading and endothelial cell proliferation.

Oligodendroglioma (WHO Grade II/IV)

Q. Write short note on oligodendroglioma.

- Constitute 5–15% of gliomas. These are infiltrating gliomas composed of tumor cells that resemble oligodendrocytes.
- **Age**: Most common during **fourth and fifth decades**.

Molecular Genetics

Most common genetic alterations are mutations of the isocitrate dehydrogenase a gene (*IDH1* and *IDH2*) observed in about 90% of cases and has a better prognosis. Loss of 9p, loss of 10q, and mutations in *CDKN2A* occur with progression to anaplastic oligodendroglioma. Tumors with co-deletion of 1p/19q (in ~ 80% of cases) (IDH-mutant and 1p/19q-codeleted) respond well to chemotherapy and radiation, and those without loss of 1p or 19q appear to be resistant to chemotherapy regimens.

MORPHOLOGY

Gross

- **Site:** Common in the **white matter of the cerebral hemispheres**
- **Well-circumscribed, gelatinous**, and gray
- May show **cysts, areas of hemorrhage**, and **calcification**.

Microscopy (Figs. 27.6A and B)

- Sheets of **small, round, and regular cells**.

- **Nuclei: Spherical nuclei with fine granular chromatin.**
- **Cytoplasm:** Nuclei are surrounded by a **clear halo of cytoplasm** giving rise to **fried egg appearance** to the cell.
- **Stroma:** It consists of **delicate network of anastomosing capillaries** (**chicken-wire appearance**).
- **Calcification** (calcospherites) is common.
- Mitotic figure is usually not seen.

Prognosis: Better than astrocytomas.

OTHER ASTROCYTIC TUMORS

Pilocytic Astrocytoma (Grade I/IV)

Pilocytic astrocytomas are **relatively benign (grade I/IV)**, grow very slowly and have an **excellent prognosis**. They are **distinguished from the other types of astrocytoma by their gross and microscopic appearance**. Unlike other astrocytomas, *TP53* mutations are rare.

Age: Occur in **children and young adults**.

Site: Usually located in the **cerebellum, floor and walls of the third ventricle** and optic nerves.

MORPHOLOGY

Gross

Usually cystic or solid and **well circumscribed**.

Microscopy

Usually shows **biphasic pattern**.

- **Fibrillary areas:** They are composed of **bipolar astrocytes with abundant, long, thin hair-like glial processes**, which form dense fibrillary meshworks. These **processes are GFAP-positive**.
- **Loose microcystic areas**
- Cells typically contain **Rosenthal fibers** and eosinophilic granular bodies.
- **Do not show infiltration** into the surrounding brain.

EPENDYMAL TUMORS

Ependymoma

Tumor **arising from ependymal cells**, which normally line ventricular system or central canal of the spinal cord. Hence, they are usually found next to the ependyma-lined ventricular system, including the oft-obliterated central canal of the spinal cord.

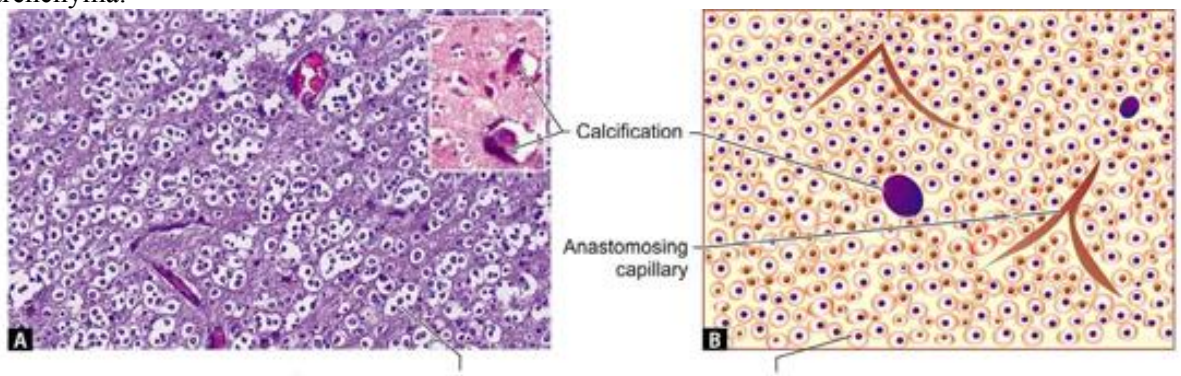
Sites

- First 2 decades of life: **Fourth ventricle**.
- **Adults**: **Spinal cord**—frequently associated with neurofibromatosis type 2 (NF2).

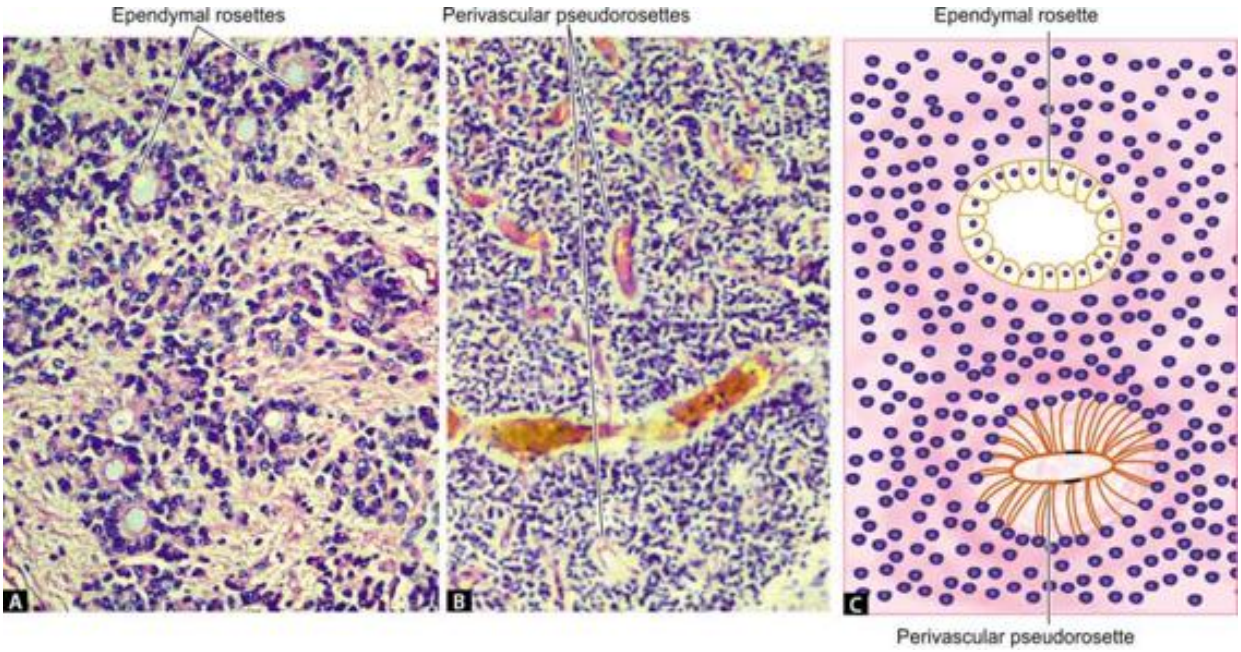
MORPHOLOGY

Gross

Usually **solid and homogeneous** or papillary. **Moderately well demarcated** from adjacent brain parenchyma.



Figs. 27.6A and B: (A) (Photomicrograph) and (B) (Diagrammatic), microscopy of oligodendroglioma. Tumor cells are small, round, regular, having clear cytoplasm forming "halos" around nuclei. The stroma shows thin-walled capillaries and foci of calcification (Inset in A).



Figs. 27.7A to C: Microscopic appearance of ependymoma: (A and B) Hematoxylin and eosin (H & E); and (C) diagrammatic, showing ependymal rosettes and perivascular pseudorosettes.

Microscopy (Figs. 27.7A to C)

Tumor Cells

- **Resemble normal ependymal cells**
- Cells are regular having well-defined cell membranes.
- Nuclei are round to oval having abundant granular chromatin.
- Variable amount of dense fibrillary background.

Rosettes (Figs. 27.7A to C)

- **Ependymal rosettes:** Tumor **cells may form gland like round or elongated structures, which resemble the embryologic ependymal canal** → known as ependymal rosettes or canals, and show long, delicate processes extending into a lumen.
- **Perivascular pseudorosettes:** Tumor **cells are arranged around vessels** to form perivascular pseudorosettes. More frequent than true ependymal rosettes.

Special Stain

PTAH may reveal **PTAH-positive blepharoplasts**, which represent basal bodies of cilia of ependymal cells.

Immunohistochemistry

GFAP is positive in most ependymomas.

Grade: Most are **well differentiated** and behave as WHO grade II/IV lesions.

Clinical features: Posterior fossa ependymomas usually manifest with hydrocephalus secondary to obstruction.

Spread: Through CSF is common and **is associated with a poor prognosis**.

EMBRYONAL TUMORS

Embryonal tumors are of **neuroectodermal origin**, which **consists of primitive, undifferentiated cells**. The most common is the medulloblastoma.

Medulloblastoma

Q. Write short note on medulloblastoma.

- **Highly malignant undifferentiated or embryonal tumor**. Neuronal and glial markers may be expressed.
- **Age:** Occurs **predominantly in children** (majority at the end of the first decade). Constitutes about 20% of the brain tumors in children.

Molecular Genetics

Depending on the molecular alterations, medulloblastoma can be divided into four groups:

1. **WNT type:** It is characterized by mutations in the WNT signaling pathway. It develops in older children and shows classic histological features of medulloblastoma. It shows monosomy of chromosome 6 and nuclear expression of β-catenin. It has best prognosis.
2. **SHH type:** It is characterized by mutations involving the sonic hedgehog signaling pathway. It is seen in infants or young adults and histologically shows nodular desmoplastic appearance. It may show amplification of MYCN. Prognosis is intermediate between the WNT subtype and groups 3 and 4.

3. **Group 3 medulloblastoma:** It usually shows MYC amplification and isochromosome 17 (i17q). It usually found in infants and children. Microscopically, it may show a classic or large cell histology and has worst prognosis.
4. **Group 4:** It is characterized by an i17q cytogenetic alteration, classic or large cell histology and without MYC amplification. Prognosis is intermediate.

MORPHOLOGY

Site

Exclusively occurs in the **cerebellum**.

- **Children:** In the midline of the cerebellum
- **Adults:** Lateral locations in the cerebellar hemispheres.

Gross

- Well circumscribed
- Gray and friable.

Microscopy (Figs. 27.8A and B)

- **Highly cellular**, composed of **sheets of anaplastic (small blue) cells.**
- **Tumor cells: Small, with little cytoplasm** and **hyperchromatic nuclei** (elongated or crescent shaped).
- **Numerous mitotic figures**
- **Homer–Wright (neuroblastic) rosette:** It is characterized by **central neurophil** (delicate pink material formed by neuronal processes) **surrounded by primitive tumor cells** may be seen.

Immunohistochemistry: GFAP+

Nodular/desmoplastic variant: Characterized by nodular, reticulin-free zones (pale islands) surrounded by densely packed highly proliferative tumor cells. These tumor cells have hyperchromatic and moderately pleomorphic nuclei and they produce a collagen and dense intercellular reticulin fiber network.

Large cell variant: It is characterized by monomorphic cells with large, round, vesicular nuclei, prominent nucleoli, and frequent mitoses and apoptotic cells. These cells show variable amount of eosinophilic cytoplasm.

Spread

- **Through the CSF is common**, may present as nodular masses anywhere in the CNS.
- **Metastases to the cauda equina** are sometimes termed **drop metastases**.

Clinical Features

- Cerebellar dysfunction.
- **Hydrocephalus** due to occlusion of CSF flow caused by rapid growth of tumor.

Prognosis

- **Poor** for untreated patients.
- ***MYC* amplification** is associated with **poor prognosis**.

Gliomas: Most common primary brain tumors includes:
- Astrocytomas
- Oligodendrogliomas
- Ependymomas

Astrocytoma: Most common primary brain tumors in adults.

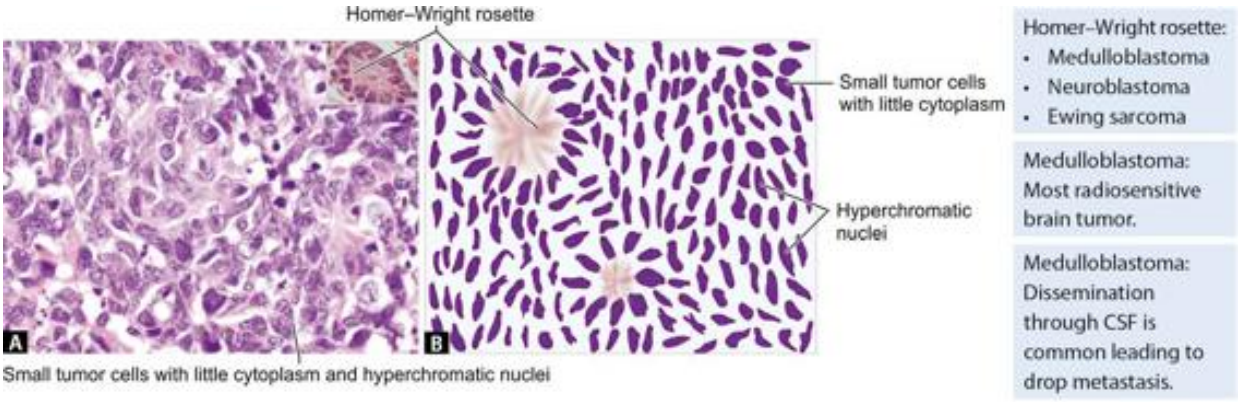
Glioblastoma multiforme: High-grade astrocytoma with worst prognosis.

Oligodendroglioma 3 Cs:
1. Clear halo of cytoplasm (fried egg appearance) in the tumor cells
2. Calcification
3. Chicken-wire appearance of anastomosing capillaries

Oligodendroglioma: Deletion of chromosome 1p and 19q.

Most common site of ependymoma:
- In children: Typically near fourth ventricle
- In adults: Spinal cord

Ependymoma:
- Ependymal rosettes
- Perivascular pseudorosettes



Figs. 27.8A and B: Microscopic: (A) Photomicrograph; (B) Diagrammatic appearance of medulloblastoma. Inset shows Homer–Wright rosette.

Medulloblastoma: Highly malignant undifferentiated/embryonal tumor predominant in children. Occurs exclusively in cerebellum.
Most common site vermis of cerebellum (70%). Most common site in adults is lateral cerebellar hemisphere.

Tumors which histologically appear similar to medulloblastoma in sites other than CNS are known as primitive neuroectodermal tumor (PNET).

Medulloblastoma:
- Highly cellular
- Small cells with little cytoplasm
- Homer–Wright rosettes

Glioblastoma: Previously known as glioblastoma multiforme (GBM), because of variation in the gross appearance of the tumor from region to region.

MENINGIOMAS

Q. Write short note on meningioma.

Meningiomas are **benign intracranial tumors** that **arise from** the **meningothelial cell** of the arachnoid mater.

Incidence: 20% of all primary intracranial neoplasms.

Age: Peak during **fourth to fifth decades**.

Sex: **Female predominance** (female-to-male ratio is 3:2).

Molecular Genetics

Most consistent cytogenetic abnormality is deletion/loss of chromosome 22 (especially the long arm-22q). Deletions of the region of 22q12 which contains the *NF2* gene (encodes the protein merlin) are a common finding in meningioma developing in mutation of *NF2* gene. In sporadic meningiomas 50–60% has mutations in the *NF2* gene. Higher grade meningiomas are more often associated with *NF2* mutations, loss of chromosome 22, and chromosomal instability.

MORPHOLOGY

Q. Write short note on morphology of meningioma.

Site

Anywhere in intracranial site both on external surfaces of the brain as well as within the ventricular system. **Most common sites** include **parasagittal regions of the cerebral hemispheres**, dura over the **lateral convexity, olfactory groove**, etc.

Gross

- **External surface: Well-circumscribed** (usually encapsulated), **smooth, rounded, bosselated** or polypoid masses **(Fig. 27.9)**
- **Size: Variable**
- **Consistency:** Range from **firm** and fibrous to finely gritty (in the presence of psammoma bodies).
- **Cut surface: Gray without necrosis or hemorrhage.**
- **Base: Usually attached to the dura**. They compress the underlying brain but do not infiltrate.

Microscopy (Figs. 27.10A and B)

The **characteristic features** of meningiomas are a **whorled pattern** of arrangement of meningothelial cells and the presence of **psammoma bodies** (laminated, spherical calcospherites).

Types

Q. Write short note on histological subtypes of meningioma.

Various histological types do not differ in the biological behavior and has no prognostic significance.

- **Syncytial (meningothelial):** It shows **whorled clusters of polygonal** cells **without visible cell membranes** (syncytial). The tumor cells have **centrally placed oval nuclei**.
- **Fibroblastic:** It consists of **spindle-shaped elongated cells**, which are **arranged in the interlacing or parallel bundles** with abundant collagen deposition in between the cells.
- **Transitional/mixed:** It shows **features of the syncytial and fibroblastic types**. The tumor cells show whorled pattern often around a central capillary-sized blood vessel. The center of some of the whorls **may show psammoma bodies**.
- **Psammomatous:** It shows **numerous psammoma bodies**.
- **Secretory:** It consists of **gland-like PAS-positive**, **eosinophilic secretions** (pseudopsammoma bodies).
- **Microcystic:** It is composed of **microcystic spaces** with a loose, spongy appearance.
- **Angiomatous:** It shows numerous blood vessels.

Immunohistochemistry: Negative for GFAP and keratins, but **positive for epithelial membrane antigen.**

Grade: Most meningiomas are considered as WHO grade I/IV.

Atypical Meningioma (WHO Grade II/IV)

It is **locally aggressive tumor** with a **higher rate of recurrence** than meningioma.

They show either four or more mitoses/10 high power fields or at least 3 atypical features (increased cellularity, small cells with a high nuclear-to-cytoplasmic ratio, prominent nucleoli, or necrosis).

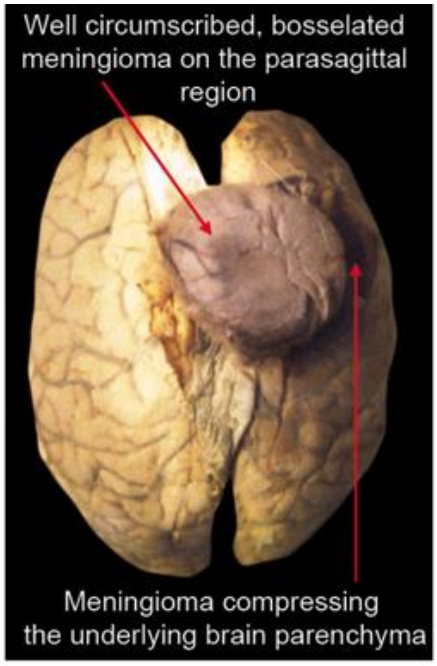
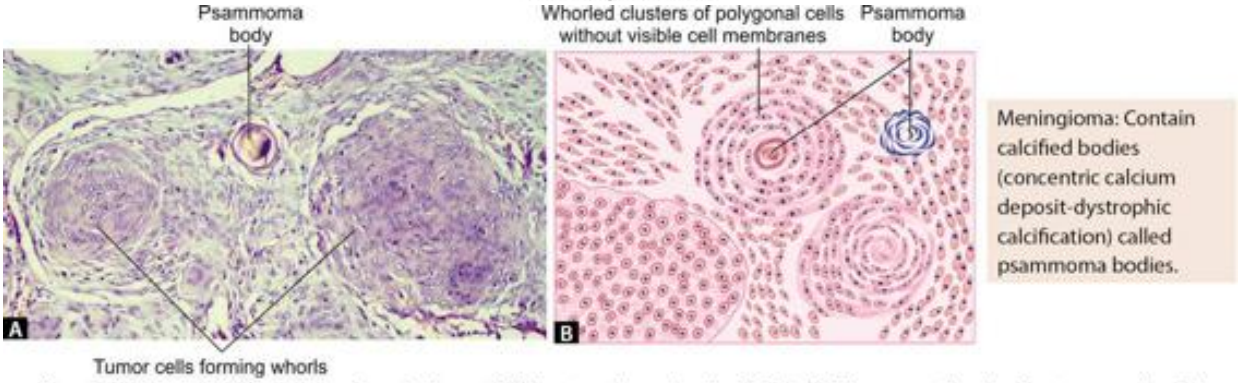


Fig. 27.9: Meningioma on the parasagittal region of the cerebral hemispheres.



Figs. 27.10A and B: Microscopy of meningioma. (A) Hematoxylin and eosin (H & E); (B) Diagrammatic, showing tumor cells with indistinct cytoplasm arranged in whorls.

Anaplastic (Malignant) Meningioma (WHO Grade III/IV)

It is a highly aggressive tumor and histologically appear like high-grade sarcoma.

Clinical Features

Meningiomas are **usually slow-growing tumors**. They produce **symptoms by compressing underlying brain** tissue and depend on the site.

Meningioma:
- Benign tumor of meningothelial cell
- Female predominance

Meningioma: Most common intracranial, extra-axial dural-based neoplasms.

Meningioma:
- Whorled pattern of arrangement of tumor cells
- May show psammoma bodies

METASTATIC TUMORS

Metastatic tumors are the **most common intracranial neoplasms**.

Primary site: Mostly carcinomas and the **five most common primary sites are: (1) lung, (2) breast, (3) skin (melanoma), (4) kidney, and (5) gastrointestinal tract**.

Route of spread: **Through the bloodstream**, generally in patients with advanced cancer.

MORPHOLOGY

Gross

- Intraparenchymal metastases in contrast with a primary glioma, form **multiple, sharply demarcated masses (Fig. 27.11)**, usually surrounded by a prominent zone of edema.
- The **boundary between metastatic tumor and surrounding brain parenchyma is sharp** and well defined both grossly and microscopically.
- They are **usually seen at the junction of gray matter and white matter**.

Microscopy

It is similar to that of primary tumor.

Choriocarcinoma has high likelihood of metastasizing to brain whereas carcinoma prostate almost never grow in the brain.

Metastatic tumors: Most common primary sites are:
- Lung
- Breast
- Skin (melanoma)

- Kidney
- Gastrointestinal tract

EYE

Retinoblastoma

NC: Describe the etiology, genetics, pathogenesis, pathology, presentation, sequelae and complications of retinoblastoma.

Q. Write short note on retinoblastoma.

Retinoblastoma is the **most common primary intraocular malignant tumor occurring in children.**

Cell of origin of retinoblastoma is a **neuronal progenitor.**

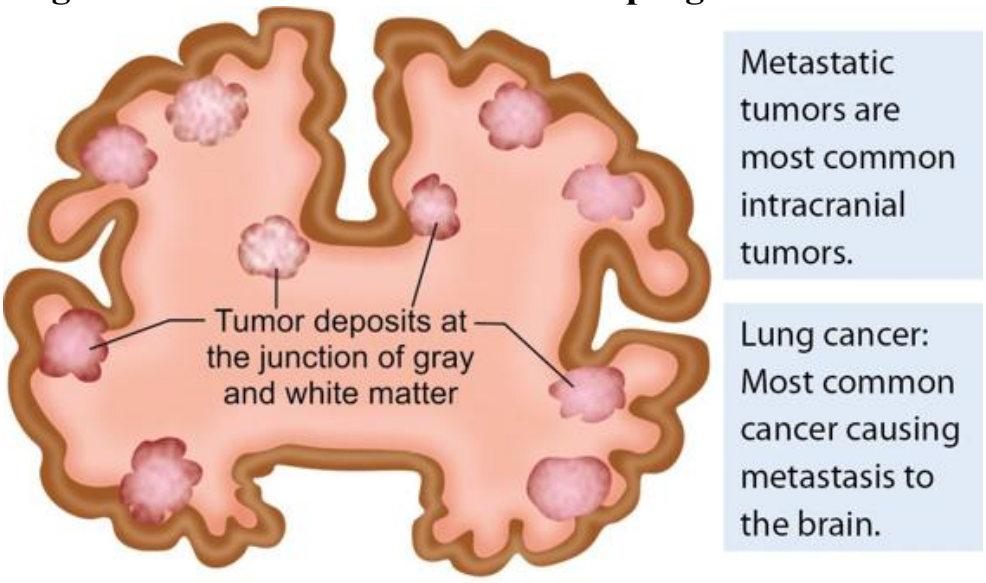


Fig. 27.11: Sites of deposit of metastatic tumors in brain.

Etiology and Pathogenesis

- **Familial cases**: Features of familial retinoblastoma are:
  - About **40% of cases** occurs as familial/hereditary cases in individuals who **inherit a germline mutation of one *RB* allele**.
  - **Usually are associated with** development of **multiple tumors (multifocal)** that are **bilateral**, but may be unifocal and unilateral.
  - Have an **increased risk for** the development of second tumors, such as **osteosarcoma** and other soft tissue tumors.

- **Sporadic cases**: The remaining **60%** of the tumors develop sporadically. In these cases, **both RB alleles are lost by somatic mutations.** All of the sporadic, nonheritable tumors are **unilateral and unifocal**.

MORPHOLOGY

Morphological features are same in both hereditary and sporadic retinoblastoma.

Gross

Retinoblastomas appear as nodular masses, usually in the posterior retina, often with satellite seedings.

Microscopy

Tumors **may contain both undifferentiated and differentiated elements (Fig. 27.12)**.

- **Undifferentiated elements:** It consists of collections of **small, round cells with large hyperchromatic nuclei and scant cytoplasm**. They resemble undifferentiated retinoblasts.
- **Well-differentiated tumors:** Differentiated structures are found within many retinoblastomas. However, the degree of tumor differentiation does not have any prognostic value. The most characteristic is **Flexner–Wintersteiner rosettes** and fleurettes that reflects photoreceptor differentiation**.**
  - **Flexner–Wintersteiner rosettes:** These structures **consist of clusters of cuboidal or short columnar cells arranged around a central lumen.** This is in contrast with the pseudorosettes of neuroblastoma which does not have a central lumen. The nuclei are displaced away from the lumen, and microscopically appears to have a limiting membrane resembling the external limiting membrane of the retina.

The molecular genetics of retinoblastoma is discussed on pages 212-4.

Spread

Retinoblastoma spread through the optic nerve or subarachnoid space. The most common sites of distant metastases are CNS (brain), skull, distal bones, bone marrow, and lymph nodes.

Clinical Features

- **Clinical presentation**: It presents with poor vision, strabismus, pain and tenderness in the affected eye. On examination it shows a whitish hue to the pupil (“cat’s eye reflex”).

- **Age**: Median age of presentation is 2 years, though the tumor may be present at birth.
- **Prognosis**: Some tumors spontaneously regress. If not treated, they are usually fatal. Survival is the rule, when treated with chemotherapy, radiotherapy, and (in locally advanced tumors) enucleation.

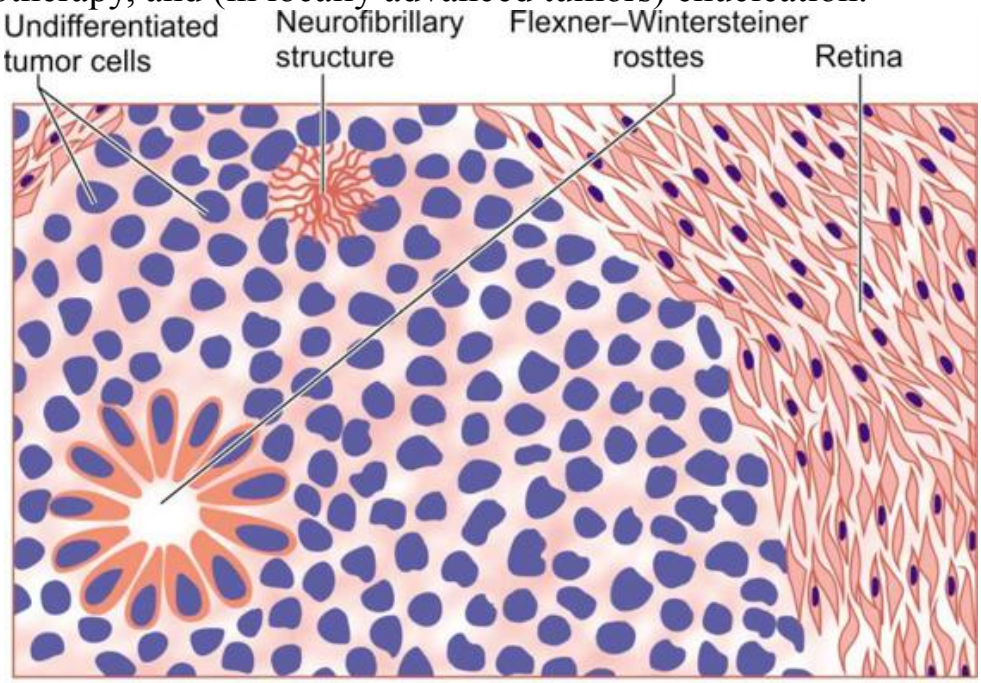


Fig. 27.12: Retinoblastoma. The tumor shows undifferentiated elements cells and the typical Flexner–Wintersteiner rosette.

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APPENDIX 1: IMPORTANT BODIES AND ITS ASSOCIATED CONDITIONS

Various Important Bodies and its Associated Conditions (Table A-1.1)

TABLE A-1.1: . Various important bodies and its associated conditions.

Name of the body	*Associated condition*
Aschoff body	Rheumatic fever
Asbestos body	Asbestosis
Ferruginous body	
Asteroid body	Sarcoidosis and sporotrichosis
Call-Exner body	Granulosa cell tumor
Civatte (colloid) body	Lichen planus
Councilman body	Acute hepatitis
Creola body	Asthma
Donovan body	Granuloma inguinale
Gamna Gandy body	Congestive splenomegaly
Halberstaedter–Prowazek's (HP) body	Trachoma
Heinz body	G6PD deficiency
Hirano body	Alzheimer disease
Howell–Jolly body	After splenectomy, asplenia, megaloblastic anemia, severe hemolytic anemia

TABLE A-1.1: . Various important bodies and its associated conditions.	
Name of the body	***Associated condition***
Leishman–Donovani (LD) body	Kala-azar
Lewy body	Parkinsonism
Mammillary body (Rokitansky tubercle)	Benign cystic teratoma of ovary
Michaelis–Gutmann body	Malakoplakia
Mallory bodies	Alcoholic hepatitis, Wilson disease, hepatocellular carcinoma, primary biliary cirrhosis, etc.
Negri body	Rabies (intracytoplasmic)
Pick body	Pick disease
Psammoma body (calcospherites)	Papillary carcinoma of thyroid, serous papillary cystadenoma and papillary carcinoma of ovary, meningioma, papillary renal cell carcinoma (RCC)
Russell body (cytoplasmic) and Dutcher body (nuclear)	Multiple myeloma
Schaumann body	Sarcoidosis
Schiller–Duval body	Yolk sac (endodermal sinus) tumor
Verocay body (“Antoni A”)	Neurilemmoma (Schwannoma)
Weibel–Palade body	Endothelial cells
Zebra body	Metachromatic leukodystrophy

APPENDIX 2: IMPORTANT CELLS AND PATHOGNOMONIC STRUCTURES

Important Cells in Various Lesions (Table A-2.1)

TABLE A-2.1: Important cells in various lesions.	
Name of the cell	***Associated conditions***
Anitschkow cell (plump activated macrophage)	Rheumatic heart disease

TABLE A-2.1: Important cells in various lesions.	
Name of the cell	***Associated conditions***
Burr cell/helmet cell/triangle cell	Uremia, hemolytic uremic syndrome (HUS), microangiopathic hemolytic anemia (MAHA)
Bite cell	G6PD deficiency
Foam cell (lipid–filled macrophage)	Atheromatous plaque, storage disorders, xanthoma
Flame/mott cell (plasma cells with glycoprotein globules)	Multiple myeloma
Glitter cell (leukocyte with visible movement of cytoplasmic process)	Pyelonephritis
Reed–Sternberg cell, Hodgkin cell, lacunar cell, mummified cell	Hodgkin lymphoma
Hürthle cells	Hashimoto thyroiditis, Hürthle cell adenoma of thyroid
Ito cell (space of Disse in liver) stores vitamin A	Secretes collagen in cirrhosis
LE cell (neutrophil with phagocytosed nuclear chromatin)	Systemic lupus erythematosus (SLE)
Tart cell	
Langerhans cell	Antigen presenting cell in the epidermis
Merkel cell (present in lower layer of epidermis)	Merkel cell carcinoma
Target cell	Thalassemia, HbS, HbC, liver disease

Pathognomonic Structures in Diseases (Table A-2.2)

TABLE A-2.2: Pathognomonic structures in diseases.	
Pathognomonic characteristics	***Diseases***
Aschoff body and Anitschkow cell	Rheumatic fever
Auer rod in myeloblast	Acute myeloid leukemia
Cytoplasmic Birbeck granules on ultrastructural examination	Langerhans cell histiocytosis
Mosaic pattern of lamellar bone	Paget disease of bone
Negri body	Rabies

TABLE A-2.2: Pathognomonic structures in diseases.	
Pathognomonic characteristics	***Diseases***
Owl's eye appearance of intranuclear inclusion body	Cytomegalovirus
Proliferation center	Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL)
Reed–Sternberg (RS) cell	Hodgkin lymphoma
Spongiform transformation of the cerebral cortex	Creutzfeldt–Jakob disease
Tophi	Gout
Warthin–Finkeldey cell	Measles
White blood cell (WBC) cast	Pyelonephritis

APPENDIX 3: PERIPHERAL BLOOD SMEAR

PREPARATION (FIG. A-3.1)

- A drop of blood is placed on a clean grease free glass slide 1 cm away from one end.
- Take a spreader (either another slide or a narrower piece of slide with smooth edge) and place its smooth edge over the drop of blood so as to spread the blood along its edge.
- Place the slide at about 30–40° angle and make the smear with a smooth (not jerky) forward movement. The resulting ideal smear should be about 2.5–3.5 cm in length.
- Allow it to dry at room temperature and label.

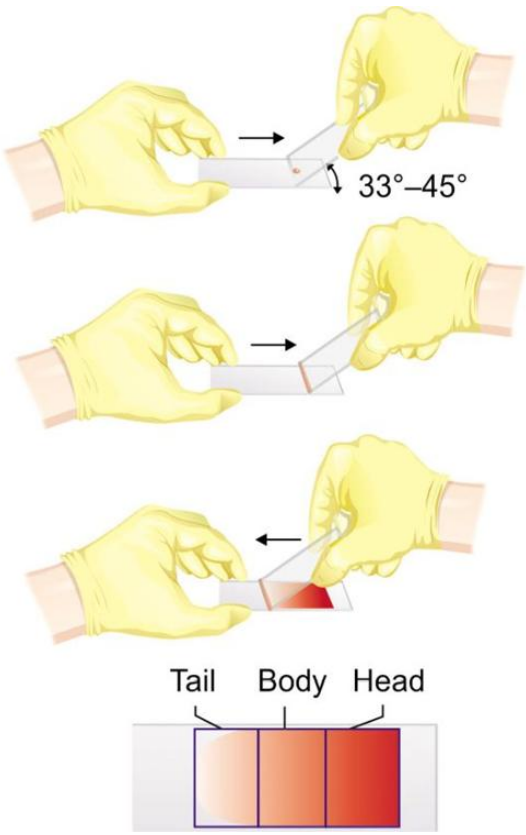


Fig. A-3.1: Different steps in the preparation of peripheral blood smear and appearance of a well-made smear.

Features of a Well-made Peripheral Smear

- **Length**: Smear should be 2/3 to 3/4 of the length of the slide.
- **Shape**: The smear should be either tongue or finger-shaped (smooth without any irregularities or holes).
- **Well-made smear has three parts** namely, head, body and tail.

Sources of Error

- Presence of grease on the slide causes blank oval areas in the smear.
- If spreader edge is not smooth, the tail of the smear shows ragged edges and most of the neutrophils accumulate in the tail end. The rest of the smear shows paucity of neutrophils, a process termed as tailing of the smear. This results in erroneous differential counts.

FIXATION OF THE SMEAR

- The blood smears should be fixed within 4 hours for good staining.
- Blood films are 'fixed' by **acetone-free methyl alcohol** which is present in most of the commonly used stains, namely Leishman or Wright or Giemsa stain.
- It can also be fixed by dipping the slide 8–10 times in a coplin jar with methyl alcohol.

STAINING OF THE SMEAR

Staining of the smears is carried out using *Romanowsky stains (refer* page 384) which stain red cells, white cells, platelets and parasites.

Procedure of Staining with Leishman Stain

- Keep the peripheral smear on the staining rack.
- **Pour the undiluted stain** on the slide with a dropper so that stain covers the entire upper surface of slide.
- Wait for **2 minutes** for the methyl alcohol to **fix the smear**. Then pour double the amount of buffer water on the slide. Gently mix using a dropper.
- Wait for another **8–10 minutes** (time depends on the batch of stain) for **staining**. Look for greenish metallic scum to appear.
- Displace the stain by pouring running water from one side of the slide so that the stain scum is drained off the slide and no more stain is left on the slide.
  - **Precaution:** Do not discard the stain from slide by just tilting it, since it will result in stain precipitates over the smear.
- Remove the slide from staining rack and keep it in slanting position to dry it up.
- Examine the slide under microscope.

Sources of Error

- Buffered pH of 6.8 is important for good quality of staining.
  - Acidic pH red cells appear pink and WBCs takes up very light stain.
  - Basic pH red cells appear blue and WBCs appear bluish-black.
- Staining errors:

- If smear is overstained (due to more alkaline pH/too long period of staining), wash the smears with undiluted Leishman stain or methanol for few seconds.
- If smear is under stained (due to more acidic pH/too short period of staining), restain by adding both Leishman stain and buffer for required period depending on the extent of under staining.

Procedure of Staining with Giemsa Stain

- Peripheral smear prepared is fixed by dipping 8–10 times in a coplin jar containing methanol.
- Allow the slide to dry.
- Pour the diluted stain (1 in 10) and wait for 20–30 minutes.
  Note: The amount of dilution and time for staining varies with each batch of stain.
- Wash the slide (as in staining by Leishman) and dry it.

EXAMINATION OF A PERIPHERAL BLOOD SMEAR

A well-prepared peripheral smear usually has three parts namely, head, body and tail. The best area of the smear to study the morphology of cells is the body, where the cells are well separated out and touch one another without overlapping. Apply a thin layer of oil over the smear which makes the appearance of cells very clear.

First look under low power, followed by high power and oil immersion. Observe the following:

- The **quality of the film** and uniformity of staining.
- **Red blood cells:** Select an area (best between tail and body of the smear) suitable for examination of red cells in the thin area of the smear where the red cells are evenly placed and just touch each other. Look for number, distribution and staining of the red cells, degree of rouleaux formation if present.
- **White blood cells:** Number and distribution of white cells.
- **Platelets:** Number and distribution.
- **Hemoparasites** (such as malaria, microfilaria).

- **Any abnormal cells**.

Red Blood Cells

Red cells are examined for: (1) size, (2) shape, (3) color, (4) inclusions and (5) other abnormalities.

1. **Size:** In the peripheral smear, size of the red blood cells is compared with that of a small lymphocyte which has size of about 7–8 μm. The red cells are called microcytic if smaller than small lymphocyte and macrocytic if larger than 9 μm. Appearance of size variation (anisocytosis) and conditions responsible for these variations are shown in **Figure 14.1**.
2. **Shape:** Variation in red cell shape (poikilocytosis) and conditions responsible for these variations are shown in **Figure 14.2.**
3. **Color:** Variations in red cell color are depicted in **Figure 14.3.**
4. Sometimes red cells appear to be hyperchromic without a central area of pallor. This is not actually due to an increase in the concentration of hemoglobin in the cell, but due to change in cell shape and increase in the thickness of the cell membrane in macrocytes.
5. **Inclusions in the red cells:** Look for any inclusions in red blood cells. Inclusions in red blood cells and associated conditions are presented in **Figure A-3.2**.
6. **Other abnormalities:** Example, rouleaux formation.

White Blood Cells

White cells (leukocytes) are examined for the following features:

- **Number:** Note whether the number is decreased, normal or increased. Assessment of approximate total white cell count: Observe at least 10 high power fields in the thick portion of the smear where red blood cells overlap. Estimate the approximate number of WBCs/high power filed. Each WBC/high power field is approximately equivalent to 2,000 WBC/cu mm.
- **Abnormal or immature forms**.
- **Predominant cell type:** This is determined by performing the differential white cell count. At least 100 white cells should be counted. For normal range of differential leukocyte count, *refer* **Table 12.1**.

Platelets

A single platelet under oil immersion is equivalent to 15,000–20,000 platelets/cu mm. They are examined for the following features under oil immersion.

- **Number:** Normal/increased/decreased.
- **Form:** Note the size and shape.
- **Aggregation:** Smears made from finger-prick show aggregation of platelets or platelet clumps.

Hemoparasites (refer page 385)

These include: (1) malarial parasites, (2) microfilaria, (3) trypanosomes, (4) *Leishmania donovani* and (5) babesiosis.

Any Abnormal Cells

For example, myeloblast, lymphoblast.

Reporting of peripheral smear: Format for peripheral smear reporting is shown in **Table A-3.1**.

Term	Description	Appearance	Condition
Basophilic stippling (punctate basophilia)	• Red cells contain small, blue black inclusions and represent precipitated ribosomal RNA		• Lead poisoning • Megaloblastic anemias • Hemolytic anemias like thalassemias • Sideroblastic anemias • Alcoholism
Howell–Jolly bodies	• Small, round densely staining dark blue particles and represent nuclear remnants		• Megaloblastic anemias • Acute hemolytic anemias • Post-splenectomy state
Cabot rings	• Rings or 'figure of eight' shaped and are probably remnants of the nuclear membrane		• Hemolytic anemia • Megaloblastic anemia • Leukemia • Rarely after splenectomy
Pappenheimer bodies/siderotic granules (RBCs with Pappenheimer bodies are called siderocytes)	• Aggregates of ferritin and appear pale blue, but are easily demonstrable with Perl's stain (Prussian blue reaction)		• Sideroblastic anemias • Megaloblastic anemias • Hemolytic anemias • Post-splenectomy
Hemoglobin H inclusions	• Free β chains in red cells. Small, regular, multiple and diffusely distributed (golf ball like appearance). Stained by supravital stains		• α-thalassemias
Heinz bodies	• Refractive, single or multiple rounded inclusions of denatured globin detected by supravital stains		• Intake/exposure to oxidizing drugs or chemicals • G6PD deficiency • Unstable hemoglobin disease
Hemoglobin C crystals	• Crystallization of hemoglobin C		• Splenectomy in homozygous hemoglobin C

CC: Perform and show how to prepare a peripheral blood smear and identify hemolytic anemia from it. Skill assessment: Procedure require certification.

Fig. A-3.2: Inclusions in red blood cells and associated conditions.
(RBCs: red blood cells; RNA: ribonucleic acid; G6PD: glucose-6-phosphate dehydrogenase)

TABLE A-3.1: Format for reporting peripheral blood smear.

1. RBCs: Comment on the following features	
– Size	Normocytic (normal), microcytic or macrocytic (predominant feature to be mentioned)
– Chromasia	Normochromic (normal), hypochromic, absence of central pallor
– Anisocytosis	Present (slight/marked) or absent
– Poikilocytosis	Present (slight/marked) or absent
– Other abnormalities	Spherocytosis, sickle cells, target cells, etc.
– Polychromatophilia	Present or absent
– Nucleated red cells	Normoblasts—per 100 leukocytes
2. WBCs: Comment on total WBC count as normal/increased/decreased	

– Differential count (on 100 cells)	
– Type of WBC (percentage)	Neutrophils, eosinophils, basophils, lymphocytes, monocytes, immature leukocytes (specify different cells)
3. Platelets	Approximate judgment on adequate/increased/reduced
4. Hemoparasites	

(RBCs: red blood cells; WBCs: white blood cells)

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