LECTURE NOTES

For Health Science Students

General Pathology

Ethiopia Public Health
Training Initiative

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LETTER TO THE STUDENT

Dear Student,

Welcome to the exciting & fascinating world of pathology! Up to now in your studies, you have been learning the normal features of human beings (i.e. anatomy, physiology, etc…). Now it is time to introduce you to the abnormalities that can occur in humans – i.e. to diseases. Pathology is a scientific study of diseases. In this book, you will learn the basic mechanisms of diseases. Pathology is divided into general & systemic pathology for pedagogical reasons. General pathology covers the basic mechanisms of diseases whereas systemic pathology covers diseases as they occur in each organ system. This book covers only general pathology. And it is divided into ten chapters on - Introduction, Cell injury, Inflammation, Healing, Hemodynamic disorders, Genetic diseases, Immunopathology, Neoplasia, Metabolic diseases, & Selected infectious diseases. Most of these topics represent the major categories of diseases that can occur in different organ systems. For example, acute inflammation can occur in different organs but wherever it occurs its mechanism is the same. That is, an acute inflammation in the skin has the same mechanisms & features as an acute inflammation of the meninges. The same principle applies to the other topics covered in general pathology. Therefore, if one knows general pathology well, one can apply this knowledge to diseases in the various organ systems. Hence, your general pathology knowledge will facilitate your understanding of systemic diseases (Systemic Pathology). Therefore, the whole purpose of general pathology is to help you understand systemic diseases – i.e. Systemic Pathology. So, even though, you will understand the basic mechanisms of diseases common to various types of illnesses, it doesn't mean that this book has covers all of pathology in as much as it didn't cover systemic pathology. Therefore, after reading this book, you are encouraged to read books on systemic pathology. The reason for not including systemic pathology in this book was because the book conceived when the previous curriculum was being implemented. At this juncture, we would like to call up on all professional colleagues to include systemic pathology in the pathology lecture for Health Officer students since this is very basic for understanding clinical medicine. We would also like to mention that the new curriculum for Health Officer students includes systemic pathology. We also call up on all those concerned to write a book on systemic pathology for health science students. General pathology is necessary but not sufficient for understanding clinical medicine.

The main reason for writing this book is the absence of standardized uniformity in the pathology courses given to health science students. Health science students* here means health officer, pharmacy, dentistry, midwifery, anesthesiology, nursing (B.SC.), & physiotherapy students. There was no uniformity in what was taught to these students in the various institutions in Ethiopia. We hope this book will alleviate this problem regarding general pathology.

In writing this book, we have tried to make it as clear & as brief as possible. Since too much brevity may compromise understanding, we have been a bit “liberal” in some areas in including some details which are necessary for the student’s understanding. This is done to encourage understanding rather than memorization.

This book is intended to be a textbook of general pathology for health science students. However, medical students & even those beyond can use this book for review.

Abiye Tesfaye, MD, Editor

ACKNOWLEDGEMENTS

Proper training of health care workers is the starting point for improving the health status of a nation. Having good standardized textbooks contributes a lot to the proper training of health care workers. The Carter Center in Addis Ababa initiated the idea of writing standardized textbooks for health science students in Ethiopia to tackle the current critical lack of such books. It funded everything from the inception of this book up to its completion. In addition to initiating the idea of writing the book, The Carter Center paid allowances to the authors, arranged appropriate & conducive environment for the writing & reviewing process, & covered all the publishing cost. By doing so, we think, The Carter Center has contributed a lot to the improvement of the health science education & thereby to the betterment of the public health status in Ethiopia. For all of these reasons, our gratitude to the Carter Center in Addis Ababa is immense & deep! We immeasurably thank Ato Aklilu… (of The Carter Center in Addis Ababa) for his immense understanding, fatherly guidance, encouragement, & patience. Without his frequent stirring, this work would not have come into fruition.

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At the end, even though we tried our best to be as accurate as possible, we bear all the responsibilities for any inadvertent mistakes this book may have.

Your comments regarding this book are very much welcome. And you can send them to the following address:

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The preparation of this book went through many stages. First, each author wrote his chapter. Then all of the authors met in the offices of The Carter Center in Addis Ababa to collectively revise & comment on each other's writings. Then all the chapters were given to Dr. Abiye Tesfaye for compilation & overall edition.

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CHAPTER ONE
INTRODUCTION TO PATHOLOGY

I. Learning Objectives

Upon completing this chapter students should be able to:

1. Define pathology
2. Discuss the core aspects of disease in pathology
3. Know the diagnostic techniques used in pathology
4. Know the various categories of the causes of diseases
5. Know the course, outcome, consequences of diseases

II. The core aspects of diseases in pathology

Pathology is the study of disease by scientific methods. The word pathology came from the Latin words “patho” & “logy”. ‘Patho’ means disease and ‘logy’ means study, therefore pathology is a scientific study of disease. Diseases may, in turn, be defined as an abnormal variation in structure or function of any part of the body. Pathology gives explanations of a disease by studying the following four aspects of the disease.

1. Etiology,
2. Pathogenesis,
3. Morphologic changes and
4. Functional derangements and clinical significance.

1. Etiology

Etiology of a disease means the cause of the disease. If the cause of a disease is known it is called primary etiology. If the cause of the disease is unknown it is called idiopathic. Knowledge or discovery of the primary cause remains the backbone on which a diagnosis can be made, a disease understood, & a treatment developed. There are two major classes of etiologic factors: genetic and acquired (infectious, nutritional, chemical, physical, etc). Detailed discussion will be given in subsequent topics. The etiology is followed by pathogenesis.

2. Pathogenesis

Pathogenesis means the mechanism through which the cause operates to produce the pathological and clinical manifestations. The pathogenetic mechanisms could take place in the latent or incubation period. Pathogenesis leads to morphologic changes.

3. Morphologic changes

The morphologic changes refer to the structural alterations in cells or tissues that occur following the pathogenetic mechanisms. The structural changes in the organ can be seen with the naked eye or they may only be seen under the microscope. Those changes that can be seen with the naked eye are called gross morphologic changes & those that are seen under the microscope are called microscopic changes. Both the gross & the microscopic morphologic changes may only be seen in that disease, i.e. they may be specific to that disease. Therefore, such morphologic changes can be used by the pathologist to identify (i.e. to diagnose) the disease. In addition, the morphologic changes will lead to functional alteration & to the clinical signs & symptoms of the disease.

4. Functional derangements and clinical significance

The morphologic changes in the organ influence the normal function of the organ. By doing so, they determine the clinical features (symptoms and signs), course, and prognosis of the disease.

In summary, pathology studies:-

Etiology ➔ Pathogenesis ➔ Morphologic changes ➔ Clinical features & Prognosis of all diseases.

Understanding of the above core aspects of disease (i.e. understanding pathology) will help one to understand how the clinical features of different diseases occur & how their treatments work. This understanding will, in turn, enable health care workers to handle & help their patients in a better & scientific way. It is for these reasons that the health science student should study pathology. In addition, the pathologist can use the morphologic changes seen in diseases to diagnose different diseases. There are different diagnostic modalities used in pathology. Most of these diagnostic techniques are based on morphologic changes.

III. Diagnostic techniques used in pathology

The pathologist uses the following techniques to the diagnose diseases:

a. Histopathology
b. Cytopathology
c. Hematopathology
d. Immunohistochemistry
e. Microbiological examination
f. Biochemical examination
g. Cytogenetics
h. Molecular techniques
i. Autopsy

A. **Histopathological techniques**

Histopathological examination studies *tissues* under the microscope. During this study, the pathologist looks for abnormal structures in the tissue.

Tissues for histopathological examination are obtained by biopsy. Biopsy is a tissue sample from a living person to identify the disease. Biopsy can be either incisional or excisional.

Once the tissue is removed from the patient, it has to be immediately fixed by putting it into adequate amount of 10% Formaldehyde (10% formalin) before sending it to the pathologist. The purpose of fixation is:

1. to prevent autolysis and bacterial decomposition and putrefaction
2. to coagulate the tissue to prevent loss of easily diffusible substances
3. to fortify the tissue against the deleterious effects of the various stages in the preparation of sections and tissue processing.
4. to leave the tissues in a condition which facilitates differential staining with dyes and other reagents.

Once the tissue arrives at the pathology department, the pathologist will exam it macroscopically (i.e. naked-eye examination of tissues).

Then the tissue is processed to make it ready for microscopic examination. The whole purpose of the tissue processing is to prepare a very thin tissue (i.e. five to seven μm or one cell thick tissue) which can be clearly seen under the microscope. The tissue is processed by putting it into different chemicals. It is then impregnated (embedded) in paraffin, sectioned (cut) into thin slices, & is finally stained. The stains can be Hematoxylin/Eosin stain or special stains such as PAS, Immunohistochemistry, etc... The Hematoxylin/Eosin stain is usually abbreviated as H&E stain. The H&E stain is routinely used. It gives the nucleus a blue color & the cytoplasm & the extracellular matrix a pinkish color. Then the pathologist will look for abnormal structures in the tissue. And based on this abnormal morphology he/she will make the diagnosis. Histopathology is usually the gold standard for pathologic diagnosis.

B. Cytopathologic techniques

Cytopathology is the study of cells from various body sites to determine the cause or nature of disease.

Applications of cytopathology:

The main applications of cytology include the following:

1. Screening for the early detection of asymptomatic cancer

For example, the examination of scrapings from cervix for early detection and prevention of cervical cancer.

2. Diagnosis of symptomatic cancer

Cytopathology may be used alone or in conjunction with other modalities to diagnose tumors revealed by physical or radiological examinations.

It can be used in the diagnosis of cysts, inflammatory conditions and infections of various organs.

3. Surveillance of patients treated for cancer

For some types of cancers, cytology is the most feasible method of surveillance to detect recurrence. The best example is periodic urine cytology to monitor the recurrence of cancer of the urinary tract.

Advantages of cytologic examination

Compared to histopathologic technique it is cheap, takes less time and needs no anesthesia to take specimens. Therefore, it is appropriate for developing countries with limited resources like Ethiopia. In addition, it is complementary to histopathological examination.

Cytopathologic methods

There are different cytopathologic methods including:

1. Fine-needle aspiration cytology (FNAC)

In FNAC, cells are obtained by aspirating the diseased organ using a very thin needle under negative pressure. Virtually any organ or tissue can be sampled by fine-needle aspiration. The aspirated cells are then stained & are studied under the microscope. Superficial organs (e.g. thyroid, breast, lymph nodes, skin and soft tissues) can be easily aspirated. Deep organs, such as the lung, mediastinum, liver, pancreas, kidney, adrenal gland, and retroperitoneum are aspirated with guidance by fluoroscopy, ultrasound or CT scan. FNAC is cheap, fast, & accurate in diagnosing many diseases.

2. Exfoliative cytology

Refers to the examination of cells that are shed spontaneously into body fluids or secretions. Examples include sputum, cerebrospinal fluid, urine, effusions in body cavities (pleura, pericardium, peritoneum), nipple discharge and vaginal discharge.

3. Abrasive cytology

Refers to methods by which cells are dislodged by various tools from body surfaces (skin, mucous membranes, and serous membranes). E.g. preparation of cervical smears with a spatula or a small brush to detect cancer of the uterine cervix at early stages. Such cervical smears, also called Pap smears, can significantly reduce the mortality from cervical cancer. Cervical cancer is the most common cancer in Ethiopian women.

C. Hematological examination

This is a method by which abnormalities of the cells of the blood and their precursors in the bone marrow are investigated to diagnose the different kinds of anemia & leukemia.

D. Immunohistochemistry

This is a method is used to detect a specific antigen in the tissue in order to identify the type of disease.

E. Microbiological examination

This is a method by which body fluids, excised tissue, etc. are examined by microscopical, cultural and serological techniques to identify micro-organisms responsible for many diseases.

F. Biochemical examination

This is a method by which the metabolic disturbances of disease are investigated by assay of various normal and abnormal compounds in the blood, urine, etc.

G. Clinical genetics (cytogenetics),

This is a method in which inherited chromosomal abnormalities in the germ cells or acquired chromosomal abnormalities in somatic cells are investigated using the techniques of molecular biology.

H. Molecular techniques

Different molecular techniques such as fluorescent in situ hybridization, Southern blot, etc... can be used to detect genetic diseases.

I. Autopsy

Autopsy is examination of the dead body to identify the cause of death. This can be for forensic or clinical purposes.

The relative importance of each of the above disciplines to our understanding of disease varies for different types of diseases. For example, in diabetes mellitus, biochemical investigation provides the best means of diagnosis and is of greatest value in the control of the disease. Whereas in the diagnosis of tumors, FNAC & histopathology contribute much. However, for most diseases, diagnosis is based on a combination of pathological investigations.

IV. The causes of disease

Diseases can be caused by either environmental factors, genetic factors or a combination of the two.

A. Environmental factors

Environmental causes of disease are many and are classified into:

1. Physical agents
2. Chemicals
3. Nutritional deficiencies & excesses
4. Infections & infestations
5. Immunological factors
6. Psychogenic factors

1. Physical agents

These include trauma, radiation, extremes of temperature, and electric power. These agents apply excess physical energy, in any form, to the body.

2. Chemicals

With the use of an ever-increasing number of chemical agents such as drugs, in industrial processes, and at home, chemically induced injury has become very common. Their effects vary:

- Some act in a general manner, for example cyanide is toxic to all cells.
- Others act locally at the site of application, for example strong acids and caustics.

- Another group exhibit a predilection for certain organs, for example – the effect of paracetamol and alcohol on liver. Many toxic chemicals are metabolized in liver and excreted in kidney, as a result, these organs are susceptible to chemical injury.

3. Nutritional deficiencies and excesses

Nutritional deficiencies may arise as a result of poor supply, interference with absorption, inefficient transport within the body, or defective utilization. It may take the form of deficiency either of major classes of food, usually protein and energy, or vitamins or elements essential for specific metabolic processes, e.g. iron for haemoglobin production. Often, the deficiencies are multiple and complex.

On the other hand, dietary excess plays an important role in diseases in Western countries. Obesity has become increasingly common, with its attendant dangers of type 2 diabetes, high blood pressure and heart disease.

4. Infections and infestations

Viruses, bacteria, fungi, protozoa, and metazoa all cause diseases. They may do so by causing cell destruction directly as in virus infections (for example poliomyelitis) or protozoal infections (for example malaria). However, in others the damage is done by toxins elaborated by the infecting agent as in diphtheria and tetanus. Like chemicals, they may have a general effect or they may show a predilection for certain tissues.

5. Immunological factors

The immune process is essential for protection against micro-organisms and parasites. However, the immune system can be abnormal which can lead to diseases. The abnormalities of the immune system include:

A. Hypersensitivity reaction

   This is exaggerated immune response to an antigen. For example, bronchial asthma can occur due to exaggerated immune response to the harmless pollen.

B. Immunodeficiency

   This is due to deficiency of a component of the immune system which leads to increased susceptibility to different diseases. An example is AIDS.

C. Autoimmunity

This is an abnormal (exaggerated) immune reaction against the self antigens of the host. Therefore, autoimmunity is a hypersensitivity reaction against the self antigens. For example, type 1 diabetes mellitus is caused by autoimmune destruction of the beta cells of the islets of Langerhans of the pancreas.

6. Psychogenic factors

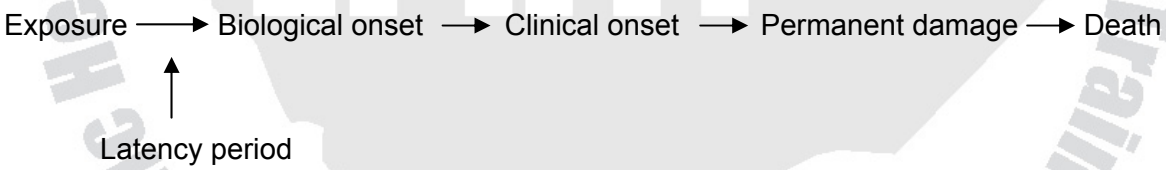
The mental stresses imposed by conditions of life, particularly in technologically advanced communities, are probably contributory factors in some groups of diseases.

B. Genetic Factors

These are hereditary factors that are inherited genetically from parents. Detailed discussion will be done on this topic in a subsequent chapter.

V. Course of disease

The course of disease is shown with a simplified diagram as follows.



The course of a disease in the absence of any intervention is called the natural history of the disease. The different stages in the natural history of disease include:

a) Exposure to various risk factors (causative agents)
b) Latency, period between exposure and biological onset of disease
c) Biological onset of disease; this marks the initiation of the disease process, however, without any sign or symptom. Following biological onset of disease, it may remain asymptomatic or subclinical (i.e. without any clinical manifestations), or may lead to overt clinical disease.
d) Incubation (induction) period refers to variable period of time without any obvious signs or symptoms from the time of exposure.

e) The clinical onset of the disease, when the signs and symptoms of the disease become apparent. The expression of the disease may be variable in severity or in terms of range of manifestations.
f) The onset of permanent damage, and
g) Death

Natural recovery, i.e. recovery without any intervention, can occur at any stage in the progression of the disease.

VI. Outcome and consequences of disease

Following clinical onset, disease may follow any of the following trends:

a) Resolution can occur leaving no sequelae,
b) The disease can settle down, but sequelae are left, or
c) It may result in death.

VII. Clinical & biologic death

Clinical death

Clinical death is the reversible transmission between life and biologic death. Clinical death is defined as the period of respiratory, circulatory and brain arrest during which initiation of resuscitation can lead to recovery.

Clinical death begins with either the last agonal inhalation or the last cardiac contraction.
Signs indicating clinical death are

- The patient is without pulse or blood pressure and is completely unresponsive to the most painful stimulus.
- The pupils are widely dilated
- Some reflex reactions to external stimulation are preserved. For example, during intubations, respiration may be restored in response to stimulation of the receptors of the superior laryngeal nerve, the nucleus of which is located in the medulla oblongata near the respiratory center.
- Recovery can occur with resuscitation.

Biological Death

Biological death (sure sign of death), which sets in after clinical death, is an irreversible state of cellular destruction. It manifests with irreversible cessation of circulatory and respiratory functions, or irreversible cessation of all functions of the entire brain, including brain stem.

However, one should notice that there are internationally accepted criteria to diagnose biological death.

VIII. Exercises

1. What is pathology? What core aspects of disease does it study?
2. Why should you study pathology?
3. What is pathogenesis?
4. What are the diagnostic modalities used by the pathologist?
5. What are the purposes of tissue fixation?
6. Describe the course of disease.

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CHAPTER TWO
CELLULAR REACTIONS TO INJURY

I. Learning objectives

At the end of this chapter, the student should be able to:

1. Define hyperplasia, hypertrophy, atrophy, & Metaplasia & list some of their causes.
2. Know the differences between reversible & irreversible forms of cell injury.
3. Describe the mechanisms of necrosis.
4. Describe the various types of necrosis & know some of their causes.

II. Introduction

Cell injury underlies all diseases. So to understand diseases one, has to start by knowing what cell injury is. When a cell is exposed to an injurious agent (i.e. the causes of diseases discussed in chapter one), the possible outcomes are:

1. The cell may adapt to the situation or
2. They cell may acquire a reversible injury or
3. The cell may obtain an irreversible injury & may die. The cell may die via one of two ways: either by necrosis or by apoptosis.

Which of these outcomes occur depends on both the injurious agent & on cellular factors. In other words, the result depends on the type, severity, & duration of the injury & on the type of the cell.

This chapter covers the types of cellular adaptation, reversible cell injury, & cell death in that order.

III. Types of cellular adaptation

The types of cellular adaptation include hypertrophy, atrophy, hyperplasia, & metaplasia.

A. Hypertrophy

Hypertrophy is increase in the size of cells. Increased workload leads to increased protein synthesis & increased size & number of intracellular organelles which, in turn, leads to increased cell size. The increased cell size leads to increased size of the organ.

Examples: the enlargement of the left ventricle in hypertensive heart disease & the increase in skeletal muscle during sternous exercise.

B. Hyperplasia

Hyperplasia is an increase in the number of cells. It can lead to an increase in the size of the organ. It is usually caused by hormonal stimulation. It can be physiological as in enlargement of the breast during pregnancy or it can pathological as in endometrial hyperplasia.

C. Atrophy

Atrophy is a decrease in the size of a cell. This can lead to decreased size of the organ. The atrophic cell shows autophagic vacuoles which contain cellular debris from degraded organelles.

Atrophy can be caused by:

1. Disuse
2. Undernutrition
3. Decreased endocrine stimulation
4. Denervation
5. Old age

D. Metaplasia

Metaplasia is the replacement of one differentiated tissue by another differentiated tissue. There are different types of metaplasia. Examples include:

1. Squamous metaplasia

This is replacement of another type of epithelium by squamous epithelium. For example, the columnar epithelium of the bronchus can be replaced by squamous epithelium in cigarette smokers

2. Osseous metaplasia

This replacement of a connective tissue by bone, for example at sites of injury.

IV. Reversible cellular changes & accumulations

Even though there are many different kinds of reversible cellular changes & accumulations, here we will only mention fatty change & accumulation of pigments.

1. Fatty change

This is accumulation of triglycerides inside parenchymal cells. It is caused by an imbalance between the uptake, utilization, & secretion of fat. Fatty change is usually seen in the liver, heart, or kidney. Fatty liver may be caused by alcohol, diabetes mellitus, malnutrition, obesity, & poisonings. These etiologies cause accumulation of fat in the hepatocytes by the following mechanisms:

a. Increased uptake of triglycerides into the parenchymal cells.
b. Decreased use of fat by cells.
c. Overproduction of fat in cells.
d. Decreased secretion of fat from the cells.

2. The accumulations of pigments

Pigments can be exogenous or endogenous. Endogenous pigments include melanin, bilirubin, hemosiderin, & lipofuscin. Exogenous pigments include carbon. These pigments can accumulate inside cells in different situations.

a. Melanin

Melanin is a brownish-black pigment produced by the melanocytes found in the skin. Increased melanin pigmentation is caused by suntanning & certain diseases e.g. nevus, or malignant melanoma. Decreased melanin pigmentation is seen in albinism & vitiligo.

b. Bilirubin

Bilirubin is a yellowish pigment, mainly produced during the degradation of hemoglobin. Excess accumulation of bilirubin causes yellowish discoloration of the sclerae, mucosae, & internal organs. Such a yellowish discoloration is called jaundice.

Jaundice is most often caused by

1. Hemolytic anemia

   Hemolytic anemia is characterized by increased destruction of red blood cells.

2. Biliary obstruction

   This is obstruction of intrahepatic or extrahepatic bile ducts. It can be caused by gallstones.

3. Hepatocellular disease

   This is associated with failure of conjugation of bilirubin.

C. Hemosiderin

Hemosiderin is an iron-containing pigment derived from ferritin. It appears in tissues as golden brown amorphous aggregates & is identified by its staining reaction (blue

color) with the Prussian blue dye. Hemosiderin exists normally in small amounts within tissue macrophages of the bone marrow, liver, & spleen as physiologic iron stores. It accumulates in tissues in excess amounts in certain diseases.This excess accumulation is divided into 2 types:

1. Hemosiderosis

   When accumulation of hemosiderin is primarily within tissue macrophages & is not associated with tissue damage, it is called hemosiderosis.

2. Hemochromatosis

   When there is more extensive accumulation of hemosiderin, often within parenchymal cells, which leads to tissue damage, scarring & organ dysfunction, it is called hemochromatosis.

V. Cell death

Cells can die via one of the following two ways:

1. Necrosis
2. Apoptosis

1. Necrosis

In necrosis, excess fluid enters the cell, swells it, & ruptures its membrane which kills it. After the cell has died, intracellular degradative reactions occur within a living organism. Necrosis does not occur in dead organisms. In dead organisms, autolysis & heterolysis take place.

Necrosis occurs by the following mechanisms:

A. Hypoxia
B. Free radical-induced cell injury
C. Cell membrane damage
D. Increased intracellular calcium level

A. Hypoxia

Hypoxia is decreased oxygen supply to tissues. It can be caused by:

1. Ischemia

   Ischemia is decreased blood flow to or from an organ. Ischemia can be caused by obstruction of arterial blood flow – the most common cause, or by decreased perfusion of tissues by oxygen-carrying blood as occurs in cardiac failure, hypotension, & shock.

2. Anemia

   Anemia is a reduction in the number of oxygen-carrying red blood cells.

3. Carbon monoxide poisoning

   CO decreases the oxygen-capacity of red blood cells by chemical alteration of hemoglobin.

4. Poor oxygenation of blood due to pulmonary disease.

   The cell injury that results following hypoxia can be divided into early & late stages:

   1. Early (reversible) stages of hypoxic cell injury

      At this stage, hypoxia results in decreased oxidative phosphorylation & ATP synthesis. Decreased ATP leads to:

      a. Failure of the cell membrane Na – K pump, which leads to increased intracellular Na & water, which cause cellular & organelle swelling. Cellular swelling (hydropic change) is characterized by the presence of large vacuoles in the cytoplasm. The endoplasmic reticulum also swells. The mitochondria show a low amplitude swelling. All of the above changes are reversible if the hypoxia is corrected.

      b. Disaggregation of ribosomes & failure of protein synthesis.

   2. Late (irreversible) stages of hypoxic cell injury.

      This is caused by severe or prolonged injury. It is caused by massive calcium influx & very low pH, which lead to activation of enzymes, which damage the cell membrane& organelle membranes. Irreversible damage to the mitochondria, cell membranes, & the nucleus mark the point of no return for the cell, that is after this stage, the cell is destined to die.

Release of aspartate aminotransferase (AST), creatine phosphokinase(CPK), & lactate dehydrogenase (LDH) into the blood is an important indicator of irreversible injury to heart muscle following myocardial infarction.

B. Free radical-induced injury

Free radical is any molecule with a single unpaired electron in the outer orbital. Examples include superoxide & the hydroxyl radicals. Free radicals are formed by normal metabolism, oxygen toxicity, ionizing radiation, & drugs & chemicals, & reperfusion injury. They are degraded by spontaneous decay, intracellular enzymes such as glutathione peroxidase, catalase, or superoxide dismutase, & endogenous substances such as ceruloplasmin or transferrin. When the production of free radicals exceeds their degradation, the excess free radicals cause membrane pump damage, ATP depletion, & DNA damage. These can cause cell injury & cell death.

a. Cell membrane damage

Direct cell membrane damage as in extremes of temprature, toxins, or viruses, or indirect cell membrane damage as in the case of hypoxia can lead to cell death by disrupting the homeostasis of the cell.

b. Increased intracellular calcium level

Increased intracellular calcium level is a common pathway via which different causes of cell injury operate. For example, the cell membrane damage leads to increased intracellular calcium level. The increased cytosolic calcium, in turn, activates enzymes in the presence of low pH. The activated enzymes will degrade the cellular organelles.

Types of necrosis

The types of necrosis include:

1. Coagulative necrosis
2. Liquefactive necrosis
3. Fat necrosis
4. Caseous necrosis
5. Gangrenous necrosis

1. Coagulative necrosis

Cogulative necrosis most often results from sudden interruption of blood supply to an organ, especially to the heart. It is, in early stages, characterized by general preservation of tissue architecture. It is marked by the following nuclear changes: Pyknosis (which is chromatin clumping & shrinking with increased basophilia), karyorrhexis (fragmentation of chromatin), & karyolysis (fading of the chromatin material).

2. Liquefactive necrosis

Liquefactive necrosis is characterized by digestion of tissue. It shows softening & liquefaction of tissue. It characteristically results from ischemic injury to the CNS. It also occurs in suppurative infections characterized by formation of pus.

3. Fat necrosis

Fat necrosis can be caused by trauma to tissue with high fat content, such as the breast or it can also be caused by acute hemorrhagic pancreatitis in which pancreatic enzymes diffuse into the inflamed pancreatic tissue & digest it. The fatty acids released from the digestion form calcium salts (soap formation or dystrophic calcification). In addition, the elastase enzyme digests the blood vessels & cause the hemorrhage inside the pancreas, hence the name hemorrhagic pancreatitis.

4. Caseous necrosis

Caseous necrosis has a cheese-like (caseous, white) appearance to the naked eye. And it appears as an amorphous eosinophilic material on microscopic examination. Caseous necrosis is typical of tuberculosis.

5. Gangrenous necrosis

This is due to vascular occlusion & most often affects the lower extremities & the bowel. It is called wet gangrene if it is complicated by bacterial infection which leads to superimposed liquefactive necrosis. Whereas it is called dry gangrene if there is only coagulative necrosis without liquefactive necrosis.

Necrosis can be followed by release of intracellular enzymes into the blood, inflammation or dystrophic calcification. Inflammation will be discussed in the next chapter.

2. Apoptosis

Apoptosis is the death of single cells within clusters of other cells. (Note that necrosis causes the death of clusters of cells.) In apoptosis, the cell shows shrinkage & increased acidophilic staining of the cell. This is followed by fragmentation of the cells. These fragments are called apoptotic bodies. Apoptosis usually occurs as a physiologic process for removal of cells during embryogenesis, menstruation, etc… It can also be seen in pathological conditions caused by mild injurious agents.

Apoptosis is not followed by inflammation or calcification. The above mentioned features distinguish apoptosis from necrosis.

VI. Pathologic calcification

Pathologic calcification is divided into 2 types:

1. Metastatic calcification

This is caused by hypercalcemia, resulting from hyperparathyroidism, milk-alkali syndrome, sarcoidosis etc…

2. Dystrophic calcification

This occurs in previously damaged tissue, such as areas of old trauma, tuberculous lesions, scarred heart valves, & atherosclerotic lesions.

Unlike metastatic calcification, it is not caused by hypercalcemia. Typically, the serum calcium level is normal.

VII. Exercises

1. Why should you study cell injury?
2. Compare & contrast the various types of cellular adaptation.
3. Describe the various mechanisms of necrosis.
4. Mention some of the causes of each of the various types of necrosis.
5. Compare & contrast necrosis & apoptosis.
6. Contrat metastatic & dystrophic calcification.

CHAPTER THREE
INFLAMMATION

I. LEARNING OBJECTIVES:

At the end of the chapter the student is expected to

1. Know the causes of inflammation
2. Understand the process of inflammations
3. Comprehend the etiopathogeneses of granulomatous inflammations
4. Contrast the differences between acute and chronic inflammations

II. INTRODUCTION

Definition: Inflammation is a local response (reaction) of living vasculaized tissues to endogenous and exogenous stimuli. The term is derived from the Latin "inflammare" meaning to burn. Inflammation is fundamentally destined to localize and eliminate the causative agent and to limit tissue injury.

Thus, inflammation is a physiologic (protective) response to injury, an observation made by Sir John Hunter in 1794 concluded: “inflammation is itself not to be considered as a disease but as a salutary operation consequent either to some violence or to some diseases”.

Causes:

Causes of inflammation are apparently causes of diseases such as

- **physical agents** - mechanical injuries, alteration in temperatures and pressure, radiation injuries.
- **chemical agents**- including the ever increasing lists of drugs and toxins.
- **biologic agents (infectious)**- bacteria,viruses,fungi, parasites
- **immunologic disorders**- hypersensitivity reactions, autoimmunity, immunodeficiency states etc
- **genetic/metabolic disorders**- examples gout, diabetes mellitus etc…

Nomenclature:

- The nomenclatures of inflammatory lesion are usually indicated by the suffix 'itis'. Thus, inflammation of the appendix is called appendicitis and that of meninges as meningitis, etc.… However, like any rule, it has its own exceptions examples pneumonia, typhoid fever, etc….

Classification:

Inflammation is classified crudely based on duration of the lesion and histologic appearances into acute and chronic inflammation.

III. ACUTE INFLAMMATION

- Acute inflammation is an immediate and early response to an injurious agent and it is relatively of short duration, lasting for minutes, several hours or few days.
- It is characterized by exudation of fluids and plasma proteins and the emigration of predominantly neutrophilic leucocytes to the site of injury.

The five cardinal signs of acute inflammation are

- **Redness** (rubor) which is due to dilation of small blood vessels within damaged tissue as it occurs in cellulitis.
- **Heat** (calor) which results from increased blood flow (hyperemia) due to regional vascular dilation
- **Swelling** (tumor) which is due to accumulation of fluid in the extravascular space which, in turn, is due to increased vascular permeability.
- **Pain** (dolor), which partly results from the stretching & destruction of tissues due to inflammatory edema and in part from pus under pressure in as abscess cavity. Some chemicals of acute inflammation, including bradykinins, prostaglandins and serotonin are also known to induce pain.
- **Loss of function**: The inflammed area is inhibited by pain while severe swelling may also physically immobilize the tissue.

Events of acute inflammation:

Acute inflammation is categorized into an early vascular and a late cellular responses.

1) The **Vascular response** has the following steps:

a) Immediate (momentary) vasoconstriction in seconds due to neurogenic or chemical stimuli.

b) Vasodilatation of arterioles and venules resulting in increased blood flow.

c) After the phase of increased blood flow there is a slowing of blood flow & stasis due to increased vascular permeability that is most remarkably seen in the post-capillary venules. The increased vascular permeability oozes protein-rich fluid into extra-vascular tissues. Due to this, the already dilated blood vessels are now packed with red blood cells resulting in stasis. The protein-rich fluid which is now found in the extravascular space is called exudate. The presence of the exudates clinically appears as swelling. Chemical mediators mediate the vascular events of acute inflammation.

2) **Cellular response**

The cellular response has the following stages:

A. Migration, rolling, pavementing, & adhesion of leukocytes
B. Transmigration of leukocytes
C. Chemotaxis
D. Phagocytosis

- Normally blood cells particularly erythrocytes in venules are confined to the central (axial) zone and plasma assumes the peripheral zone. As a result of increased vascular permeability (See vascular events above), more and more neutrophils accumulate along the endothelial surfaces (peripheral zone).

A) **Migration, rolling, pavementing, and adhesion of leukocytes**

- Margination is a peripheral positioning of white cells along the endothelial cells.
- Subsequently, rows of leukocytes tumble slowly along the endothelium in a process known as rolling
- In time, the endothelium can be virtually lined by white cells. This appearance is called pavementing
- Thereafter, the binding of leukocytes with endothelial cells is facilitated by cell adhesion molecules such as selectins, immunoglobulins, integrins, etc which result in adhesion of leukocytes with the endothelium.

B). **Transmigration of leukocytes**

- Leukocytes escape from venules and small veins but only occasionally from capillaries. The movement of leukocytes by extending pseudopodia through the vascular wall occurs by a process called diapedesis.
- The most important mechanism of leukocyte emigration is via widening of inter-endothelial junctions after endothelial cells contractions. The basement membrane is disrupted and resealed thereafter immediately.

C). **Chemotaxis:**

- A unidirectional attraction of leukocytes from vascular channels towards the site of inflammation within the tissue space guided by chemical gradients (including bacteria and cellular debris) is called chemotaxis.
- The most important chemotactic factors for neutrophils are components of the complement system (C5a), bacterial and mitochondrial products of arachidonic acid metabolism such as leukotriene B4 and cytokines (IL-8). All granulocytes, monocytes and to lesser extent lymphocytes respond to chemotactic stimuli.
- How do leukocytes "see" or "smell" the chemotactic agent? This is because receptors on cell membrane of the leukocytes react with the chemoattractants resulting in the activation of phospholipase C that ultimately leads to release of cytocolic calcium ions and these ions trigger cell movement towards the stimulus.

D) **Phagocytosis**

- Phagocytosis is the process of engulfment and internalization by specialized cells of particulate material, which includes invading microorganisms, damaged cells, and tissue debris.
- These phagocytic cells include polymorphonuclear leukocytes (particularly neutrophiles), monocytes and tissue macrophages.

Phagocytosis involves three distinct but interrelated steps.

1). **Recognition and attachment** of the particle to be ingested by the leukocytes: Phagocytosis is enhanced if the material to be phagocytosed is coated with certain plasma proteins called **opsonins**. These opsonins promote the adhesion between the particulate material and the phagocyte's cell membrane. The three major opsonins are: the Fc fragment of the immunoglobulin, components of the complement system C3b and C3bi, and the carbohydrate-binding proteins – lectins.

Thus, IgG binds to receptors for the Fc piece of the immunoglobulin (FcR) whereas 3cb and 3bi are ligands for complement receptors CR1 and CR2 respectively.

2). **Engulfment:** During engulfment, extension of the cytoplasm (pseudopods) flow around the object to be engulfed, eventually resulting in complete enclosure of the particle within the phagosome created by the cytoplasmic membrane of the phagocytic cell.

As a result of fusion between the phagosome and lysosome, a phagolysosome is formed and the engulfed particle is exposed to the degradative lysosomal enzymes.

3) **Killing or degradation**

The ultimate step in phagocytosis of bacteria is killing and degradation. There are two forms of bacterial killing

a). **Oxygen-independent mechanism:**

- This is mediate by some of the constituents of the primary and secondary granules of polymorphonuclear leukocytes. These include:

Bactericidal permeability increasing protein (BPI)
Lysozymes
Lactoferrin
Major basic protein
Defenses

- It is probable that bacterial killing by lysosomal enzymes is inefficient and relatively unimportant compared with the oxygen dependent mechanisms. The lysosomal enzymes are, however, essential for the degradation of dead organisms within phagosomes.

b) **Oxygen-dependent mechanism**:

There are two types of oxygen- dependent killing mechanisms

i) **Non-myeloperoxidase dependent**

- The oxygen - dependent killing of microorganisms is due to formation of reactive oxygen species such as hydrogen peroxide (H2O2), super oxide (**O2)** and hydroxyl ion (HO-) and possibly single oxygen (1O2). These species have single unpaired electrons in their outer orbits that react with molecules in cell membrane or nucleus to cause damages. The destructive effects of **H2O2** in the body are gauged by the action of the glutathione peroxidase and catalase.

ii) **Myloperoxidase–dependent**

- The bactericidal activity of **H2O2** involves the lysosomal enzyme myeloperoxidase, which in the presence of halide ions converts H2O2 to hypochlorous acid (HOCI). This **H2O2** – halide - myecloperoxidease system is the most efficient bactericidal system in neutrophils. A similar mechanism is also effective against fungi, viruses, protozoa and helminths.

Like the vascular events, the cellular events (i.e. the adhesion, the transmigration, the chemotaxis, & the phagocytosis) are initiated or activated by chemical mediators. Next, we will focus on the sources of these mediators.

IV. Chemical mediators of inflammation

Chemical mediators account for the events of inflammation. Inflammation has the following sequence:

Cell injury → Chemical mediators → Acute inflammation (i.e. the vascular & cellular events).

Sources of mediators:

The chemical meditors of inflammation can be derived from plasma or cells.

a) Plasma-derived mediators:

i) Complement activation

- increases vascular permeability (C3a,C5a)
- activates chemotaxis (C5a)
- opsoninization (C3b,C3bi)

ii) Factor XII (Hegman factor) activation

Its activation results in recruitment of four systems: the kinin, the clotting, the fibrinolytic and the compliment systems.

b) Cell-derived chemical mediatos:

Cell-derived chemical mediators include:

Cellular mediators	Cells of origin	Functions
Histamine	Mast cells, basophiles,	Vascular leakage & platelets
Serotonine	Platelets	Vascular leakage
Lysosomal enzymes	Neutrophiles, macrophages	Bacterial & tissue destruction
Prostaglandines	All leukocytes	Vasodilatation, pain, fever
Leukotriens	All leukocytes	LB4
	Chemoattractant LC4, LCD4, & LE4	Broncho and vasoconstriction
Platlete activating factor	All leukocytes	Bronchoconstriction and WBC priming
Activated oxygen species	All leukocytes	Endothelial and tissue damage
Nitric oxide	Macrophages	Leukocyte activation
Cytokines	Lymphocytes, macrophages	Leukocyte activation

- Most mediators perform their biologic activities by initially binding to specific receptors on target cells. Once activated and released from the cells, most of these mediators are short lived. Most mediators have the potential to cause harmful effects.

V. Morphology of acute inflammation

- Characteristically, the acute inflammatory response involves production of exudates. An exudate is an edema fluid with high protein concentration, which frequently contains inflammatory cells.
- A transudate is simply a non-inflammatory edema caused by cardiac, renal, undernutritional, & other disorders.

The differences between an exudate and a transudate are

	EXUDATE	**TRANSUDATE**
Cause:	Acute inflammation	Non-inflammatory disorders
Appearance	Colored, turbid, hemorrhagic	Clear, translucent or pale yellow
Specific gravity:	Greater than or equal to 1.020	Much less
Spontaneous coagulability:	Yes	No
Protein content:	>3gm %	
Cells:	Abundant WBC, RBC, & Cell debris usually present	Only few mesothelial cells
Bacteria:	Present	Absent.

There are different morphologic types of acute inflammation:

1) Serous inflammation

- This is characterized by an outpouring of a thin fluid that is derived from either the blood serum or secretion of mesothelial cells lining the peritoneal, pleural, and pericardial cavities.
- It resolves without reactions

2) Fibrinous inflammation

- More severe injuries result in greater vascular permeability that ultimately leads to exudation of larger molecules such as fibrinogens through the vascular barrier.
- Fibrinous exudate is characteristic of inflammation in serous body cavities such as the pericardium (butter and bread appearance) and pleura.

Course of fibrinous inflammation include:

- Resolution by fibrinolysis
- Scar formation between perietal and visceral surfaces i.e. the exudates get organized
- Fibrous strand formation that bridges the pericardial space.

3) Suppurative (Purulent) inflammation

This type of inflammation is characterized by the production of a large amount of pus. Pus is a thick creamy liquid, yellowish or blood stained in colour and composed of

- A large number of living or dead leukocytes (pus cells)
- Necrotic tissue debris
- Living and dead bacteria
- Edema fluid

There are two types of suppurative inflammation:

A) **Abscess formation**:

- An abscess is a circumscribed accumulation of pus in a living tissue. It is encapsulated by a so-called pyogenic membrane, which consists of layers of fibrin, inflammatory cells and granulation tissue.

B) **Acute diffuse (phlegmonous) inflammation**

- This is characterized by diffuse spread of the exudate through tissue spaces. It is caused by virulent bacteria (eg. streptococci) without either localization or marked pus formation. Example: Cellulitis (in palmar spaces).

4) Catarrhal inflammation

- This is a mild and superficial inflammation of the mucous membrane. It is commonly seen in the upper respiratory tract following viral infections where mucous secreting glands are present in large numbers, eg. Rhinitis.

5) Pseudomembranous inflammation

- The basic elements of pseudomembranous inflammation are extensive confluent necrosis of the surface epithelium of an inflamed mucosa and severe acute inflammation of the underlying tissues. The fibrinogens in the inflamed tissue coagulate within the necrotic epithelium. And the fibrinogen, the necrotic epithelium, the neutrophilic polymorphs, red blood cells, bacteria and tissue debris form a false (pseudo) membrane which forms a white or colored layer over the surface of inflamed mucosa.
- Pseudomembranous inflammation is exemplified by Dipthetric infection of the pharynx or larynx and Clostridium difficille infection in the large bowel following certain antibiotic use.

VI. Effects of acute inflammation:

A. Beneficial effects

- **Dilution of toxins**: The concentration of chemical and bacterial toxins at the site of inflammation is reduced by dilution in the exudate and its removal from the site by the flow of exudates from the venules through the tissue to the lymphatics.
- **Protective antibodies**: Exudation results in the presence of plasma proteins including antibodies at the site of inflammation. Thus, antibodies directed against the causative organisms will react and promote microbial destruction by phagocytosis or complement-mediated cell lysis.
- **Fibrin formation**: This prevents bacterial spread and enhances phagocytosis by leukocytes.
- **Plasma mediator systems provisions**: The complement, coagulation, fibrinolytic, & kinin systems are provided to the area of injury by the process of inflammation.

- **Cell nutrition**: The flow of inflammatory exudates brings with it glucose, oxygen and other nutrients to meet the metabolic requirements of the greatly increased number of cells. It also removes their solute waste products via lymphatic channels.
- **Promotion of immunity**: Micro-organisms and their toxins are carried by the exudates, either free or in phagocytes, along the lymphaics to local lymph nodes where they stimulate an immune response with the generation of antibodies and cellular immune mechanisms of defence.

B. Harmful effects

- **Tissue destruction** Inflammation may result in tissue necrosis and the tissue necrosis may, in turn, incite inflammation.
- **Swelling:** The swelling caused by inflammation may have serious mechanical effects at certain locations. Examples include acute epiglottitis with interference in breathing; Acute meningitis and encephalitis with effects of increased intracranial pressure.
- **Inappropriate response:** The inflammatory seen in hypersensitivity reactions is inappropriate (i.e. exaggerated).

VII. Course of acute inflammation

Acute inflammation may end up in:

- Resolution: i.e. complete restitution of normal structure and function of the tissue, eg. lobar pneumonia.
- Healing by fibrosis (scar formation).
- Abscess formation {Surgical law states -Thou shallt (you shold) drain all abscesses.} However, if it is left untouched, it may result in

  - **Sinus formation** - when an abscess cavity makes contact with only one epithelial lining.
  - **Fistula formation**: when an abscess tract connects two epithelial surface.

  Or very rarely to septicemia or

  Pyemia with subsequent metastatic abscess in heart, kidney, brain etc.

VIII.CHRONIC INFLAMMATION

Definition: Chronic inflammation can be defined as a prolonged inflammatory process (weeks or months) where an active inflammation, tissue destruction and attempts at repair are proceeding simultaneously.

Causes of chronic inflammation:

1. **Persistent infections**
   - Certain microorganisms associated with intracellular infection such as tuberculosis, leprosy, certain fungi etc characteristically cause chronic inflammation.
   - These organisms are of low toxicity and evoke delayed hypersensitivity reactions.

2. **Prolonged exposure to nondegradable but partially toxic substances** either endogenous lipid components which result in atherosclerosis or exogenous substances such as silica, asbestos.

3. **Progression from acute inflammation:** Acute inflammation almost always progresses to chronic inflammation following:

   a. Persistent suppuration as a result of uncollapsed abscess cavities, foreign body materials (dirt, cloth, wool, etc), sequesterum in osteomylitis, or a sinus/fistula from chronic abscesses.

4. **Autoimmuniy.** Autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosis are chronic inflammations from the outset.

Morphology:

Cells of chronic inflammation:

- **Monocytes and Macrophages** are the prima Dona (primary cells) in chronic inflammation. Macrophages arise from the common precursor cells in the bone marrow, which give rise to blood monocytes. These cells are then diffusely scattered in various parts of the body, in the liver (Kupffer cells), spleen, lymph nodes (sinus histiocytes), lungs (alviolar macrophages), bone marrow, brain (microglia), skin

(Langerhan's cells), etc.…. These cells constitute the mononuclear- phagocytic system.

- Macrophages are scavenger cells of the body.

Other cells in chronic inflammation:

1. **T-Lymphocytes** are primarily involved in cellular immunity with lymphokine production, and they are the key regulator and effector cells of the immune system.
2. **B-lymphocytes and Plasma cells** produce antibody directed either against persistent antigen in the inflammatory site or against altered tissue components.
3. Mast cells and eosinophils appear predominantly in response to parasitic infestations & allergic reactions.

Though neutrophils are hallmarks of acute inflammatory reactions, large numbers of neutrophils may be seen in some forms of chronic inflammation, notably chronic osteomylitis, actinomycosis, & choric lung diseases induced by smoking and other stimuli.

Thus, the overall differentiation points between acute and chronic inflammations include:

Characteristics	Acute inflammation	Chronic inflammation
Duration	Short	Relatively long
Pattern	Stereotyped	Varied
Predominant cell	Neutrophils	Macrophages,
	Lymphocytes	plasma cells
Tissue destruction	Mild to moderate	Marked
Fibrosis	Absent	Present
Inflammatory reaction	Exudative	Productive

Classification of chronic inflammation:

Chronic inflammation can be classified into the following two types based on histologic features:

1) **Nonspecific chronic inflammation:** This involves a diffuse accumulation of macrophages and lymphocytes at site of injury that is usually productive with new fibrous tissue formations. E.g. Chronic cholecystitis.

2) Specific inflammation (granulomatous inflammation):

Definition: Granulomatous inflammation is characterized by the presence of granuloma. A granuloma is a microscopic aggregate of epithelioid cells. Epithelioid cell is an activated macrophage, with a modified epithelial cell-like appearance (hence the name epithelioid). The epitheloid cells can fuse with each other & form multinucleated giant cells. So, even though, a granuloma is basically a collection of epithelioid cells, it also usually contains multinucleated giant cell & is usually surrounded by a cuff of lymphocytes and occasional plasma cells. There are two types of giant cells:

a. **Foreign body-type giant cells** which have irregularly scattered nuclei in presence of indigestible materials.
b. **Langhans giant cells** in which the nuclei are arranged peripherally in a horse -shoe pattern which is seen typically in tuberculosis, sarcoidosis etc…

Giant cells are formed by fusion of macrophages perhaps by a concerted attempt of two or more cells to engulf a single particle.

Pathogenesis:

There are two types of granulomas, which differ in their pathogenesis.

A. **Foreign body granuloma**

These granulomas are initiated by inert foreign bodies such as talc, sutures (non-absorbable), fibers, etc… that are large enough to preclude phagocytosis by a single macrophage and do not incite an immune response.

B. **Immune granulomas**

Antigen presenting cells (macrophages) engulf a poorly soluble inciting agent. Then, the macrophage processes and presents part of the antigen (in association with MHC type2 molecules) to CD4+T helper 1 cells which become activated. The activated CD4+ T-cells produce cytokines (IL-2 and interferon gamma).The IL-2 activates other CD4+T helper cells and perpetuates the response while IFN-**γ** is important in transforming macrophages into epitheloid cells and multinucleated giant cells. The cytokines have been implicated not only in the formation but also in the maintenance of granuloma.

Macrophage inhibitory factor helps to localize activated macrophages and epitheloid cells.

Diagram

Causes:

Major causes of granulomatious inflammation include:

a) **Bacterial:** Tuberculosis, Leprosy, Syphilis, Cat scratch disease, Yersiniosis
b) **Fungal**: Histoplasmosis, Cryptococcosis, Coccidioidomycosis, Blastomycosis
c) **Helminthic**: Schistosomiasis
d) **Protozoal:** Leishmaniasis, Toxoplasmosis
e) **Chlamydia:** Lymphogranuloma venerum
f) **Inorganic material**: Berrylliosis
g) **Idiopathic:** Acidosis, Cohn's disease, Primary biliary cirrhosis

I. SYSTEMIC EFFECTS OF INFLAMMATIONS

The systemic effects of inflammation include:

a. Fever
b. Endocrine & metabolic responses
c. Autonomic responses
d. Behavioral responses
e. Leukocytosis
f. Leukopenia
g. Weight loss

a. **Fever**

Fever is the most important systemic manifestation of inflammation. It is coordinated by the hypothalamus & by cytokines (IL -1, IL-6, TNF-α) rekeased from macrophages and other cells.

b. **Endocrine and metabolic responses** include:

- The liver secrets acute phase proteins such as:
  C-reactive proteins
  Serum Amyloid A
  Complement and coagulation proteins
- Glucocorticoids (increased)
- Vasopressin (decreased)

c. **Autonomic** responses include:

- Redirection of blood flow from the cutaneous to the deep vascular bed.
- Pulse rate and blood pressure (increased)
- Sweating (decreased)

d. **Behavioral** responses include:

- Rigor, chills, anoroxia, somnolence, and malaise.

e. **Leucocytosis is** also a common feature of inflammation, especially in bacterial infections. Its usual count is 15,000 to 20,000 cells/mm3. Most bacterial infections induce neutrophilia. Some viral infections such as infectious mononucleosis, & mumps cause **lymphocytosis.** Parasitic infestations & allergic reactions such as bronchial ashma & hay fever induce **eosinophilia**.

f. **Leukopenia** is also a feature of typhoid fever and some parasitic infections.

g. **Weight loss** is thought to be due to the action of IL-1 and TNF-α which increase catabolism in skeletal muscle, adipose tissue and the liver with resultant negative nitrogen balance.

IX. Exercises

1. Discuss the pathopysiology of the cardinal sign of acute inflammation.
2. Enumerate the sequential steps in both the vascular and cellular responses of acute inflammation.
3. Why is inflammation a largely protective response?
4. List the morphologic changes in acute inflammation.
5. Discuss the squelae of suppurative inflammation.
6. Enumerate the causes of chronic inflammation.
7. Define gramulomatous inflammation
8. Discuss the pathogenesis and morphologic changes of granulomatous inflammation
9. Compare & contrast acute and chronic inflammations.
10. Enumerate the systemic effects of inflammations.

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CHAPTER FOUR

HEALING

I. Learning objectives

Upon completing the chapter, students should be able to:

1. Describe the processes of healing.
2. Specify the patterns of wound healing.
3. List the factors that influence wound healing.
4. Discuss the complications of wound healing.
5. Understand fracture healing.

II. Definition of healing

The word *healing*, used in a pathological context, refers to the body's replacement of destroyed tissue by living tissue.

III. Processes of healing

The healing process involves two distinct processes:

- Regeneration, the replacement of lost tissue by tissues similar in type and

- Repair (healing by scaring), the replacement of lost tissue by granulation tissue which matures to form scar tissue. Healing by fibrosis is inevitable when the surrounding specialized cells do not possess the capacity to proliferate.

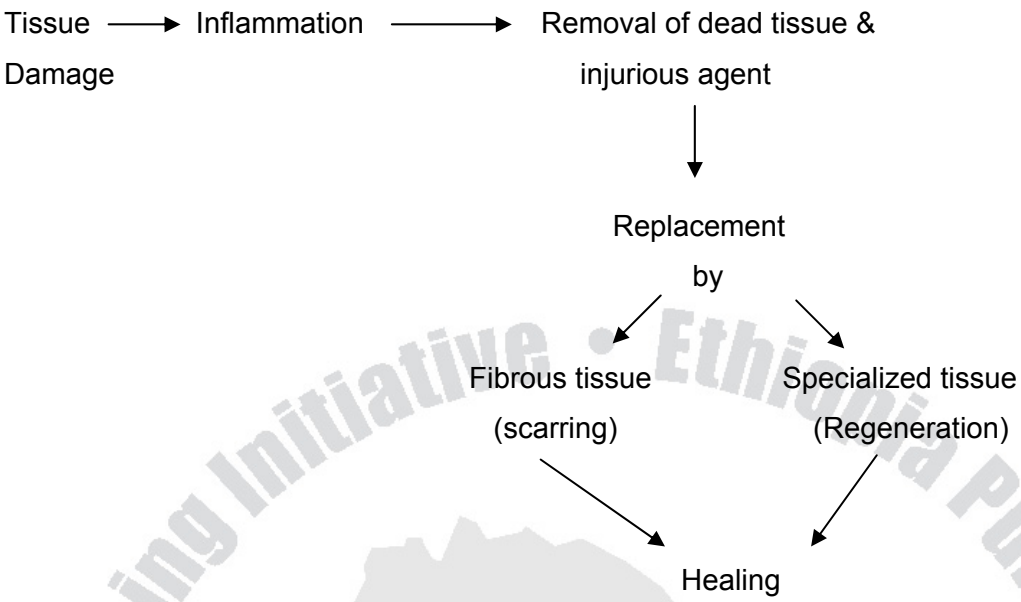

Figure 4.1 Processes of healing: Removal of dead tissue & injurious agent and replacement occur simultaneously.

Whether healing takes place by regeneration or by repair (scarring) is determined partly by the type of cells in the damaged organ & partly by the destruction or the intactness of the stromal frame work of the organ. Hence, it is important to know the types of cells in the body.

Types of cells

Based on their proliferative capacity there are three types of cells.

1. Labile cells

These are cells which have a continuous turn over by programmed division of stem cells. They are found in the surface epithelium of the gastrointestinal treat, urinary tract or the skin. The cells of lymphoid and haemopoietic systems are further examples of labile cells. The chances of regeneration are excellent.

2. Stable cells

Tissues which have such type of cells have normally a much lower level of replication and there are few stem cells. However, the cells of such tissues can undergo rapid division in response to injury. For example, mesenchymal cells such as smooth muscle cells, fibroblasts, osteoblasts and endothelial cells are stable cells which can proliferate. Liver,

endocrine glands and renal tubular epithelium has also such type of cells which can regenerate. Their chances of regeneration are good.

3. Permanent cells

These are non-dividing cells. If lost, permanent cells cannot be replaced, because they don not have the capacity to proliferate. For example: adult neurons, striated muscle cells, and cells of the lens.

Having been introduced to the types of cells, we can go back to the two types of healing processes & elaborate them.

a. Healing by regeneration

Definition: Regeneration (*generare=bring to life*) is the renewal of a lost tissue in which the lost cells are replaced by identical ones.

Regeneration involves two processes

1. Proliferation of surviving cells to replace lost tissue
2. Migration of surviving cells into the vacant space.

The capacity of a tissue for regeneration depends on its

1) proliferative ability,
2) degree of damage to stromal framework and
3) on the type and severity of the damage.

Tissues formed of labile and stable cells can regenerate provided that stromal framework are intact.

b. Repair (Healing by connective tissue)

Definition:- Repair is the orderly process by which lost tissue is eventually replaced by a scar.

A wound in which only the lining epithelium is affected heals exclusively by regeneration. In contrast, wounds that extend through the basement membrane to the connective tissue, for example, the dermis in the skin or the sub-mucosa in the gastrointestinal tract, lead to the

formation of granulation tissue and eventual scarring. Tissues containing terminally differentiated (permanent) cells such as neurons and skeletal muscle cells can not heal by regeneration. Rather the lost permanent cells are replaced by formation of granulation tissue.

In granulation-tissue formation, three phases may be observed.

1. Phase of inflammation

At this phase, inflammatory exudate containing polymorphs is seen in the area of tissue injury. In addition, there is platelet aggregation and fibrin deposition.

2. Phase of demolition

The dead cells liberate their autolytic enzymes, and other enzymes (proteolytic) come from disintegrating polymorphs. There is an associated macrophage infiltration. These cells ingest particulate matter, either digesting or removing it.

3. Ingrowth of granulation tissue

This is characterized by proliferation of fibroblasts and an ingrowth of new blood vessels into the area of injuty, with a variable number of inflammatory cells.

Fibroblasts actively synthesize and secrete extra-cellular matrix components, including fibronectin, proteoglycans, and collagen types I and III. The fibronectin and proteoglycans form the ‘scaffolding’ for rebuilding of the matrix. Fibronectin binds to fibrin and acts as a chemotactic factor for the recruitment of more fibroblasts and macrophages. The synthesis of collagen by fibroblasts begins within 24 hours of the injury although its deposition in the tissue is not apparent until 4 days. By day 5, collagen type III is the predominant matrix protein being produced; but by day 7 to 8, type I is prominent, and it eventually becomes the major collagen of mature scar tissue. This type I collagen is responsible for providing the tensile strength of the matrix in a scar.

Coincident with fibroblast proliferation there is angiogenesis (neovascularization), a proliferation and formation of new small blood vessels. Vascular proliferation starts 48 to 72 hours after injury and lasts for several days.

With further healing, there is an increase in extracellular constituents, mostly collagen, with a decrease in the number of active fibroblasts and new vessels. Despite an increased collagenase activity in the wound (responsible for removal of built collagen), collagen accumulates at a steady rate, usually reaching a maximum 2 to 3 months after the injury. **The tensile strength of the wound continues to increase many months after the collagen content has reached a maximum.** As the collagen content of the wound increases, many of the newly formed vessels disappear. This vascular involution which takes place in a few weeks, dramatically transforms a richly vascularized tissue in to a pale, avascular scar tissue.

Wound contraction

Wound contraction is a mechanical reduction in the size of the defect. The wound is reduced approximately by 70-80% of its original size. Contraction results in much faster healing, since only one-quarter to one-third of the amount of destroyed tissue has to be replaced. If contraction is prevented, healing is slow and a large ugly scar is formed.

Causes of contraction

It is said to be due to contraction by myofibroblasts. Myofibroblasts have the features intermediate between those of fibroblasts and smooth muscle cells. Two to three days after the injury they migrate into the wound and their active contraction decrease the size of the defect.

Summary

Following tissue injury, whether healing occurs by regeneration or scarring is determined by the degree of tissue destruction, the capacity of the parenchymal cells to proliferate, and the degree of destructon of stromal framework as illustrated in the diagram below (See Fig. 4.2). In the above discussion, regeneration, repair, and contraction have been dealt with separately. Yet they are not mutually exclusive processes. On the contrary, the three processes almost invariably participate together in wound healing.

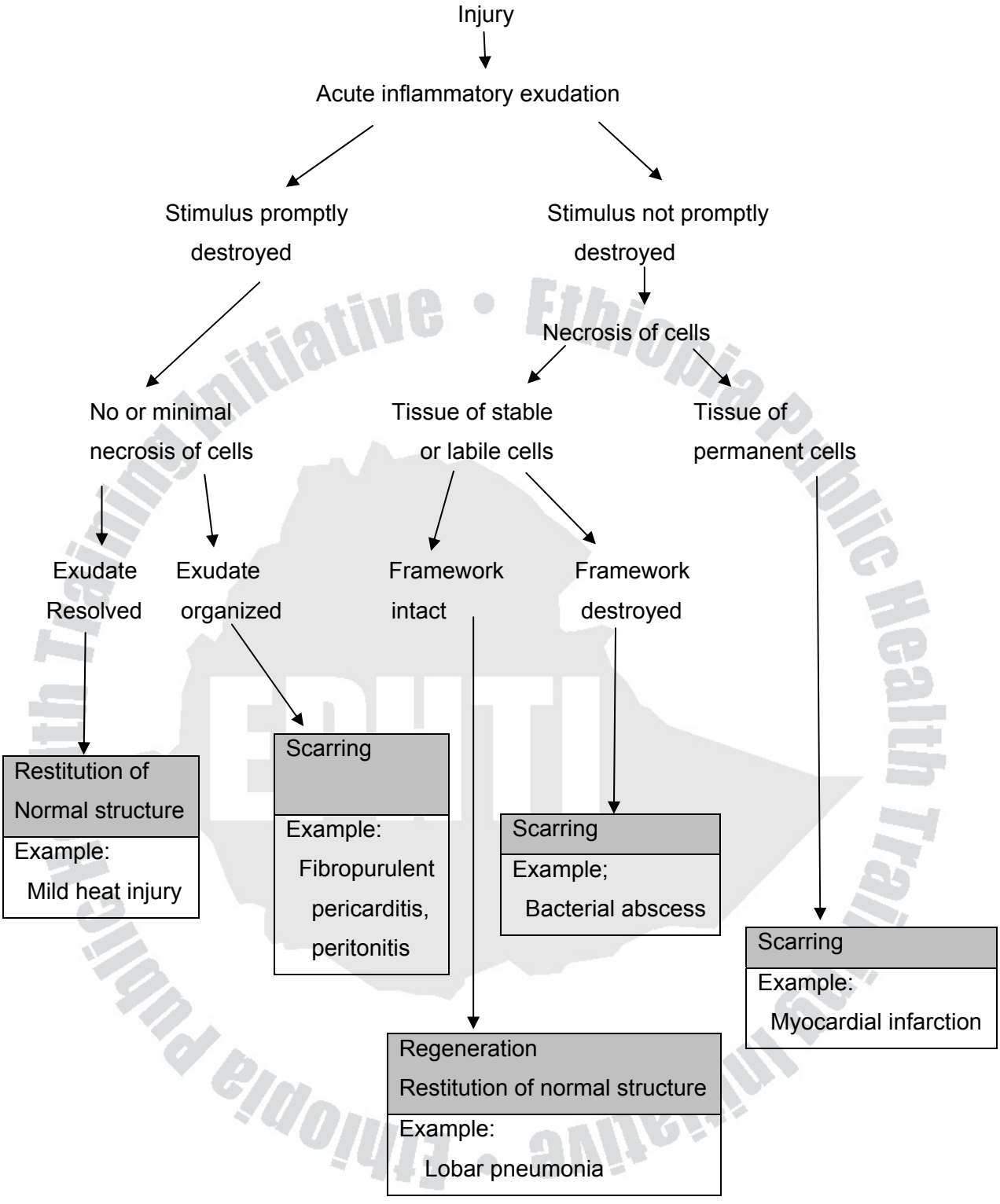

Figure 4.2 Diagram showing healing process following acute inflammatory injury.

IV. Molecular control of healing process

As seen above, healing involves an orderly sequence of events which includes regeneration and migration of specialized cells, angiogenesis, proliferation of fibroblasts and related cells, matrix protein synthesis and finally cessation of these processes.

These processes, at least in part, are mediated by a series of low molecular weight polypeptides referred to as **growth factors**.

These growth factors have the capacity to stimulate cell division and proliferation. Some of the factors, known to play a role in the healing process, are briefly discussed below.

Sources of Growth Factors:

Following injury, growth factors may be derived from a number of sources such as:

1. Platelets, activated after endothelial damage,
2. Damaged epithelial cells,
3. Circulating serum growth factors,
4. Macrophages, or
5. Lymphocytes recruited to the area of injury

The healing process ceases when lost tissue has been replaced. The mechanisms regulating this process are not fully understood. TGF-β acts as a growth inhibitor for both epithelial and endothelial cells and regulates their regeneration.

The summary of molecular control of the healing process is illustrated with the diagram shown below.

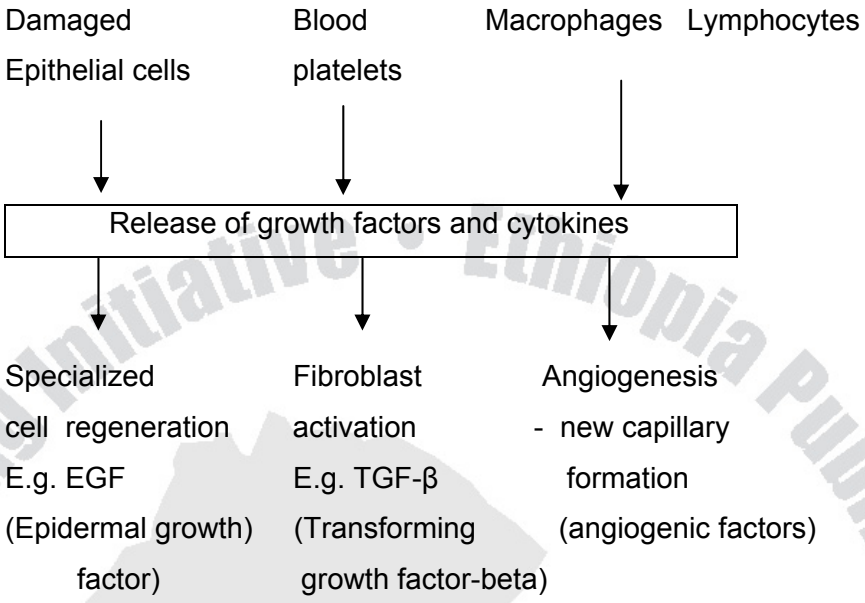

Fig. 4-3 Diagram showing sources of growth factors and their effect.

VI. Wound Healing

The two processes of healing, described above, can occur during healing of a diseased organ or during healing of a wound. A wound can be accidental or surgical. Now, we will discuss skin wound healing to demonstrate the two basic processes of healing mentioned above.

Healing of a wound demonstrates both epithelial regeneration (healing of the epidermis) and repair by scarring (healing of the dermis).

There are two patterns of wound healing depending on the amount of tissue damage:

1. Healing by first intention (Primary union)
2. Healing by second intention

These two patterns are essentially the same process varying only in amount.

1. Healing by first intention (primary union)

The least complicated example of wound healing is the healing of a clean surgical incision (Fig. 4-4, left). The wound edges are approximated by surgical sutures, and healing occurs with a minimal loss of tissue. Such healing is referred to, surgically, as “primary union” or “healing by first intention”. The incision causes the death of a limited number of epithelial cells as well as of dermal adnexa and connective tissue cells; the incisional space is narrow and immediately fills with clotted blood, containing fibrin and blood cells; dehydration of the surface clot forms the well-known scab that covers the wound and seals it from the environment almost at once.

Within 24 hours, neutrophils appear at the margins of the incision, moving toward the fibrin clot. The epidermis at its cut edges thickens as a result of mitotic activity of basal cells and, within 24 to 48 hours, spurs of epithelial cells from the edges both migrate and grow along the cut margins of the dermis and beneath the surface scab to fuse in the midline, thus producing a continuous but thin epithelial layer.

By day 3, the neutrophils have been largely replaced by macrophages. Granulation tissue progressively invades the incisional space. Collagen fibers are now present in the margins of the incision, but at first these are vertically oriented and do not bridge the incision. Epithelial cell proliferation continues, thickening the epidermal covering layer.

By day 5, the incisional space is filled with granulation tissue. Neovascularization is maximal. Collagen fibrils become more abundant and begin to bridge the incision. The epidermis recovers its normal thickness and differentiation of surface cells yields a mature epidermal architecture with surface keratinization.

During the second week, there is continued accumulation of collagen and proliferation of fibroblasts. Leukocytic infiltrate, edema, and increased vascularity have largely disappeared. At this time, the long process of blanching begins, accomplished by the increased accumulation of collagen within the incisional scar, accompanied by regression of vascular channels.

By the end of the first month, the scar comprises a cellular connective tissue devoid of inflammatory infiltrate, covered now by an intact epidermis. The dermal appendages that have been destroyed in the line of the incision are permanently lost. Tensile strength of the wound increases thereafter, but it may take months for the wounded area to obtain its maximal strength.

2. Healing by second intention (secondary union)

When there is more extensive loss of cells and tissue, such as occurs in infarction, inflammatory ulceration, abscess formation, and surface wounds that create large defects, the reparative process is more complicated. The common denominator in all these situations is a large tissue defect that must be filled. Regeneration of parenchymal cells cannot completely reconstitute the original architecture. Abundant granulation tissue grows in from the margin to complete the repair. This form of healing is referred to as “secondary union” or “healing by second intention.”

Secondary healing differs from primary healing in several respects:

1. Inevitably, large tissue defects initially have more fibrin and more necrotic debris and exudate that must be removed. Consequently, the inflammatory reaction is more intense.
2. Much larger amounts of granulation tissue are formed. When a large defect occurs in deeper tissues, such as in a viscus, granulation tissue bears the full responsibility for its closure, because drainage to the surface cannot occur.
3. Perhaps the feature that most clearly differentiates primary from secondary healing is the phenomenon of wound contraction, which occurs in large surface wounds.
4. Healing by second intention takes much longer than when it occurs by first intention.

VI. Factors that influence wound healing

A number of factors can alter the rate and efficiency of healing. These can be classified in to those which act locally, and those which have systemic effects. Most of these factors have been established in studies of skin wound healing but many are likely to be of relevance to healing at other sites.

Local Factors

- **Type, size, and location of the wound**

A clean, aseptic wound produced by the surgeon’s scalpel heals faster than a wound produced by blunt trauma, which exhibits aboundant necrosis and irregular edges.Small

blunt wounds heal faster than larger ones.Injuries in richly vascularized areas (e.g., the face) heal faster than those in poorly vascularized ones (e.g., the foot).

In areas where the skin adheres to bony surfaces, as in injuries over the tibia, wound contraction and adequate apposition of the edges are difficult. Hence, such wounds heal slowly.

- **Vascular supply**

Wounds with impaired blood supply heal slowly. For example, the healing of leg wounds in patients with varicose veins is prolonged. Ischemia due to pressure produces bed sores and then prevents their healing. Ischemia due to arterial obstruction, often in the lower extremities of diabetics, also prevents healing.

- **Infection**

Wounds provide a portal of entry for microorganisms. Infection delays or prevents healing, promotes the formation of excessive granulation tissue (proud flesh), and may result in large, deforming scars.

- **Movement**

Early motion, particularly before tensile strength has been established, subjects a wound to persistent trauma, thus preventing or retarding healing.

- **Ionizing radiation**

Prior irradiation leaves vascular lesions that interfere with blood supply and result in slow wound healing. Acutely, irradiation of a wound blocks cell proliferation, inhibits contraction, and retards the formation of granulation tissue.

Systemic Factors

- **Circulatory status**

Cardiovascular status, by determining the blood supply to the injured area, is important for wound healing. Poor healing attributed to old age is often due, largely, to impaired circulation.

- **Infection**

Systemic infections delay wound healing.

- **Metabolic status**

Poorly controlled diabetes mellitus is associated with delayed wound healing.The risk of infection in clean wound approaches five fold the risk in non- diabetics.

In diabetic patients, there can be impaired circulation secondary to arteriosclerosis and impaired sensation due to diabetic neuropathy. The impaired sensation renders the lower extremity blind to every day hazards. Hence, in diabetic patients, wounds heal the very slowly.

- **Nutritional deficiencies**

Protein deficiency

In protein depletion there is an impairment of granulation tissue and collagen formation, resulting in a great delay in wound healing.

Vitamine deficiency

Vitamin C is required for collagen synthesis and secretion. It is required in hydroxylation of proline and lysine in the process of collagen synthesis. Vitamin C deficiency (scurvy) results in grossly deficient wound healing, with a lack of vascular proliferation and collagen deposition.

Trace element deficiency

Zinc (a co-factor of several enzymes) deficiency will retard healing by preventing cell proliferation. Zinc is necessary in several DNA and RNA polymerases and transferases; hence, a deficiency state will inhibit mitosis. Proliferation of fibroblasts (fibroplasia) is, therefore, retarded.

- **Hormones**

Corticosteroids impair wound healing, an effect attributed to an inhibition of ollagen synthesis. However, these hormones have many other effects, including anti-inflammatory actions and a general depression of protein synthesis. It also inhibits fibroplasia and neovascularization. Both epithelialization and contraction are impaired. It is, therefore, difficult to attribute their inhibition of wound healing to any one specific mechanism.

Thyroid hormones, androgens, estrogens and growth hormone also influence wound healing. This effect, however, may be more due to their regulation of general metabolic status rather than to a specific modification of the healing process.

- **Anti-inflammatory drugs**

Anti-inflammatory medications do not interfere with wound healing when administered at the usual daily dosages. Asprin and indomethalin both inhibit prostaglandin synthesis and thus delay healing.

VII. Complications of Wound Healing

Abnormalities in any of the three basic healing processes – contraction, repair, and regeneration – result in the complications of wound healing.

1. Infection

A wound may provide the portal of entry for many organisms. Infectrion may delay healing, and if severe stop it completely.

2. Deficient Scar Formation

Inadequate formation of granulation tissue or an inability to form a suitable extracellular matrix leads to deficient scar formation and its complications. The complications of deficient scar formation are:

a. Wound dehiscence & incitional hernias
b. Ulceration

a. Wound Dehiscence and Incisional Hernias:

Dehiscence (bursting of a wound) is of most concern after abdominal surgery. If insufficient extracellular matrix is deposited or there is inadequate cross-linking of the matrix, weak scars result. Dehiscence occurs in 0.5% to 5% of abdominal operations.Inappropriate suture material and poor surgical techiniques are important factors. Wound infection, increased mechanical stress on the wound from vomiting, coughing, or ileus is a factor in most cases of abdominal dehiscence. Systemic factors that predispose to dehiscence include poor metabolic status, such as vitamin C deficiency, hypoproteinemia, and the general inanition

that often accompanies metastatic cancer. Dehiscence of an abdominal wound can be a life-threatening complication, in some studies carrying a mortality as high as 30%.

An incisional hernia, usually of the abdominal wall, refers to a defect caused by poor wound healing following surgery into which the intestines protrude.

b. Ulceration:

Wounds ulcerate because of an inadequate intrinsic blood supply or insufficient vascularization during healing. For example, leg wounds in persons with varicose veins or severe atherosclerosis typically ulcerate. Nonhealing wounds also develop in areas devoid of sensation because of persistent trauma. Such **trophic or neuropathic** ulcers are occasionally seen in patients with leprosy, diabetic peripheral neuropathy and in tertiary syphilis from spinal involvement (in tabes dorsalis).

3. Excessive Scar Formation

An excessive deposition of extracellular matrix at the wound site results in a hypertrophic scar or a keloid (See Figure 4-5 and 4-6). The rate of collagen synthesis, the ratio of type III to type I collagen, and the number of reducible cross-links remain high, a situation that indicates a “maturation arrest”, or block, in the healing process.

Keloid Formation

An excessive formation of collagenous tissue results in the appearance of a raised area of scar tissue called keloid. It is an exuberant scar that tends to progress and recur after excision. The cause of this is unknown. Genetic predisposition, repeated trauma, and irritation caused by foreign body, hair, keratin, etc., may play a part. It is especially frequent after burns. It is common in areas of the neck & in the ear lobes.

Hypertrophic Scar

Hypertrophic scar is structurally similar to keloid. However, hypertrophic scar never gets worse after 6 months unlike keloid, which gets worse even after a year and some may even progress for 5 to 10 years. Following excision keloid recurres, whereas a hypertrophic scar does not.

4. Excessive contraction

A decrease in the size of a wound depends on the presence of myofibroblasts, development of cell-cell contacts and sustained cell contraction. An exaggeration of these processes is termed **contracture (cicatrisation)** and results in severe deformity of the wound and surrounding tissues. Contracture (cicatrisation) is also said to arise as a result of late reduction in the size of the wound. Interestingly, the regions that normally show minimal wound contraction (such as the palms, the soles, and the anterior aspect of the thorax) are the ones prone to contractures. Contractures are particularly conspicuous in the healing of serious burns. Contractures of the skin and underlying connective tissue can be severe enough to compromise the movement of joints. Cicatrisation is also important in hollow viscera such as urethra, esophagus, and intestine. It leads to progressive stenosis with stricture formation. In the alimentary tract, a contracture (stricture) can result in an obstruction to the passage of food in the esophagus or a block in the flow of intestinal contents.

Several diseases are characterized by contracture and irreversible fibrosis of the superficial fascia, including Dupuytren disease (palmar contracture), plantar contracture (Lederhosen disease), and Peyronie disease (contracture of the cavernous tissues of the penis). In these diseases, there is no known precipitating injury, even though the basic process is similar to the contracture in wound healing.

5. Miscellaneous

Implantation (or epidermoid cyst: Epithelial cells which flow into the healing wound may later sometimes persist, and proliferate to form an epidermoid cyst.

VIII. Fracture Healing

The basic processes involved in the healing of bone fractures bear many resemblances to those seen in skin wound healing. Unlike healing of a skin wound, however, the defect caused by a fracture is repaired not by a fibrous “scar” tissue, but by specialized bone-forming tissue so that, under favorable circumstances, the bone is restored nearly to normal.

Structure of bone

Bone is composed of calcified osteoid tissue, which consists of collagen fibers embedded in a mucoprotein matrix (osteomucin). Depending on the arrangement of the collagen fibers, there are two histological types of bone:

1. Woven, immature or non-lamellar bone

   This shows irregularity in the arrangement of the collagen bundles and in the distribution of the osteocytes. The osseomucin is less abundant and it also contains less calcium.

2. Lamellar or adult bone

   In this type of bone, the collagen bundles are arranged in parallel sheets.

Stages in Fracture Healing (Bone Regeneration)

Stage 1: Haematoma formation. Immediately following the injury, there is a variable amount of bleeding from torn vessels; if the periosteum is torn, this blood may extend into the surrounding muscles. If it is subsequently organized and ossified, myositis ossificans results.

Stage 2: Inflammation. The tissue damage excites an inflammatory response, the exudate adding more fibrin to the clot already present. The inflammatory changes differ in no way from those seen in other inflamed tissues. There is an increased blood flow and a polymorphonuclear leucocytic infiltration. The haematoma attains a fusiform shape.

Stage 3: Demolition. Macrophages invade the clot and remove the fibrin, red cells, the inflammatory exudate, and debris. Any fragments of bone, which have become detached from their blood supply, undergo necrosis, and are attacked by macrophages and osteoclasts.

Stage 4: Formation of granulation tissue. Following this phase of demolition, there is an ingrowth of capillary loops and mesenchymal cells derived from the periosteum and the endosteum of the cancellous bone. These cells have osteogenic potential and together with the newly formed blood vessels contribute to the granulation – tissue formation.

Stage 5: Woven bone and cartilage formation. The mesenchymal “osteoblasts” next differentiate to form either woven bone or cartilage. The term “callus”, derived from the Latin and meaning hard, is often used to describe the material uniting the fracture ends regardless of its consistency. When this is granulation tissue, the “callus” is soft, but as bone or cartilage formation occurs, it becomes hard.

Stage 6: Formation of lamellar bone. The dead calcified cartilage or woven bone is next invaded by capillaries headed by osteoclasts. As the initial scaffolding (“provisional callus”) is removed, osteoblasts lay down osteoid, which calcifies to form bone. Its collagen bundles are now arranged in orderly lamellar fashion, for the most part concentrically around the blood vessels, and in this way the Haversian systems are formed. Adjacent to the periosteum and endosteum the lamellae are parallel to the surface as in the normal bone. This phase of formation of definitive lamellar bone merges with the last stage.

Stage 7: Remodelling. The final remodeling process involving the continued osteoclastic removal and osteoblastic laying down of bone results in the formation of a bone, which differs remarkably little from the original tissue. The external callus is slowly removed, the intermediate callus becomes converted into compact bone containing Haversian systems, while the internal callus is hollowed out into a marrow cavity in which only a few spicules of cancellous bone remain.

IX. Exercises

1. Compare & contrast wound healing by primary & secondary union
2. State the factors that influence fracture healing.
3. Discuss the complications of wound healing.
4. What are the two basic processes of healing? What factors determine which of these occurs?

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CHAPTER FIVE
HEMODYNAMIC DISORDERS

I. Learing objectives

Upon completion of this chapter, students should be able to:

1. Explain how fluid balance is maintained across the arteriolar & venular end of the vasculature by Starling forces
2. Know the pathologic conditions occurring when the balance between the above forces is disrupted across the vascular wall under different conditions, i.e. edema.
3. Understand and explain the cause and pathogenesis of clinical conditions like myocardial infarction, deep venous thrombosis, pulumonary thromboembolism, etc….
4. Know the pathogenesis of edema of congestive heart failure, nephrotic syndrome, cirrosis, and other clinical conditions
5. Have the basic knowledge about various types of shock, their pathogenesis, manifestations, and complications.

II. Introduction

The health and well-being of cells & tissues depend not only on an intact circulation to deliver nutrients but also on normal fluid hemostasis. This chapter reviews the major disturbances involving the hemodynamic system.

III. Edema

Definition: Edema is increased fluid in the interstitial tissue spaces or it is a fluid accumulation in the body cavities in excessive amount.

Depending on the site, fluid accumulation in body cavities can be variously designated as:

a) Hydrothorax – fluid accumulation in pleural cavity in a pathologic amount.
b) Hydropericardium – pathologic amount of fluid accumulated in the pericardial cavity.
c) Hydroperitoncum (ascites) – fluid accumulation in peritoneal cavity.

d) Ancsarca – is a severe & generalized edema of the body with profound subcutaneous swelling.

Mechanism of edema formation:

Approximately 60% of the lean body weight is water, two-thirds of which is intracellular with the remainder in the extracellular compartment.

The capillary endothelium acts as a semipermeable membrane and highly permeable to water & to almost all solutes in plasma with an exception of **proteins**. Proteins in plasma and interstial fluid are especially important in controlling plasma & interstitial fluid volume.

Normally, any outflow of fluid into the interstitium from the arteriolar end of the microcirculation is nearly balanced by inflow at the venular end. Therefore, normally, there is very little fluid in the interstitium.

Edema formation is determined by the following factors:

1) Hydrostatic pressure
2) Oncotic pressure
3) Vascular permeability
4) Lymphatic channels
5) Sodium and water retention

We will discuss each of the above sequentially.

1) Hydrostatic and oncotic pressures:

The passage of fluid across the wall of small blood vessels is determined by the balance between **hydrostatic** & **oncotic** pressures.

There are four primary forces that determine fluid movement across the capillary membrane. Each of them can be listed under the above two basic categories, the hydrostatic pressure & the oncotic pressure. These four primary forces are known as **Starling forces** & they are:

a. The capillary hydrostatic pressure (Pc)

This pressure tends to force fluid outward from the intravascular space through the capillary membrane to the interstitium.

b. The interstial fluid hydrostatic pressure (Pif)

This pressure tends to force fluid from the interstitial space to the intravascular space.

c. The plasma colloid osmotic (oncotic) pressure (Πp)

This pressure tends to cause osmosis of fluid inward through the capillary membrane from the interstitium. The plasma oncotic pressure is caused by the presence of plasma proteins.

d. The interstial fluid colloid osmotic (oncotic) pressure (Πif)

This pressure tends to cause osmosis of fluid outward through the capillary membrane to the interstitium.

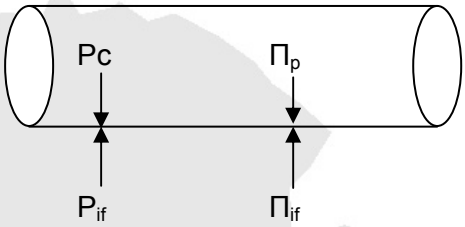


Fig. 5.1 The forces that determine the movement of fluid across the capillary wall.

The net filtration pressure can be calculated as

$$F_{Net} = K [Pc + \Pi_{if}] - [\Pi_p + Pif]$$

In addition, some fluid is normally derained by the lymphatic channels.Usually, excess fluid will accumulate in the interstitium (i.e. edema is formed) when the capillary hydrostatic pressure is increased or when the plama oncotic pressure is decreased or when the lymphatic drainage is blocked.

Hence, basically, one can divide pathologic edema into **two broad categories:**

A. Edema due to **decreased plasma oncotic pressure.** The plasma oncotic pressure is decreased when the plasma proteins are decreased in various diseases such as**:**

1. Protein loosing glomerulopathies like nephroticsyndrome with leaky glomerulus.
2. Liver cirrhosis which leads to decreased protein synthesis by the damaged liver.
3. Malnutrition
4. Protein loosing enteropathy.

B. Edema resulting from **increased capillary hydrostatic pressure** as in the following diseases:

1. Deep venous thrombosis resulting in impaired venous return.
2. Pulmonary oedema
3. Cerebral oedema
4. Congestive heart failure

Clinical classification of edema:

One can also clinically classify edema into localized & generalized types.

A) Localized	**B) Generalized**
1) Deep venous thrombosis	1) Nephrotic syndrome
2) Pulmonary edema	2) Liver cirrhosis
3) Brain edema	3) Malnutrition
4) Lymphatic edema	4) Heart failure
	5) Renal failure

Next, we will elaborate on some of the above examples.

1. Localized edema

a. Edema of the brain:

- May be localized at the site of lesion e.g neoplasm, trauma.
- May be generalized in encephalitis, hypertensive crisis, & trauma
- Narrowed sulci & distended gyri.
- ↑ Edema → compression of medulla towards formen magnum → compression of vital centers lead to →- Hernation of the brain

↓

Patient dies

b. Pulmonary edema

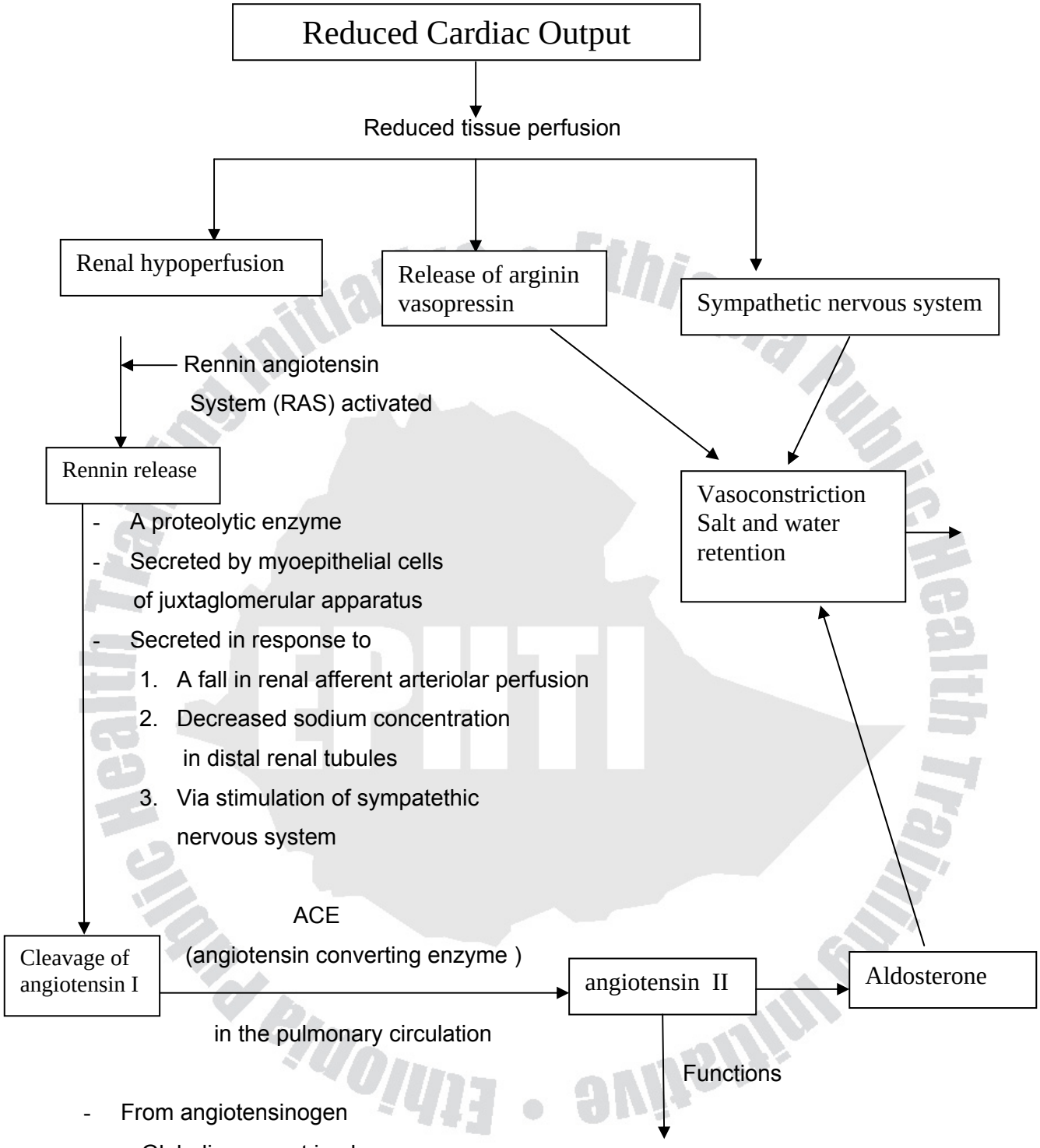
- Usually occurs in left ventricular failure.
- May occur in adult respiratory distress syndrome (ARDS).
- lung ↑ 2.3x its weight.

2. Generalized edema (anasarca) occurs due to

a. Reduction of albumin due to excessive loss or reduced synthesis as is caused by:
   1) Protein loosing glomerulopathies like nephrotic syndrome
   2) Liver cirrhosis
   3) Malnutrition
   4) Protein-losing enteropathy

b. Increased volume of blood secondary to sodium retention caused by congestive heart failure:

Fig. 5.2 **Mechanism of edema formation in congestive heart failure:**



NB: If perfusion fails to improve, this cycle will operate continuously further exacerbating the edema resulting in **anasarca.**

This ends our discussion of the first two factors (listed near the beginning of this chapter) which determine edema formation. We will now go on to discuss the other factors.

2) Vascular permeability:

Increased vascular permeability usually occurs due to acute inflammation. In inflammation, chemical mediators are produced. Some of these mediators (See the chapter on inflammation) cause increased vascular permeability which leads to loss of fluid & high molecular weight albumin and globulin into the interstitium. Such edema (i.e. that caused by increased vascular permeability) is called inflammatory edema. Inflammatory edema differs from non-inflammatory edema by the following features

a) Inflammatory edema (exudate)

⇒ Due to inflammation-induced increased permeability and leakage of plasma proteins.
⇒ Forms an exudate [protein rich]
⇒ Specific gravity > 1.012

b) Non-inflammatory oedema (transudate)

⇒ A type of edema occurring in hemodynamic derangement (i.e. increased plasma hydrostatic pressure & decreased plasma oncotic pressure. See above)
⇒ Formed transudate [protein poor]
⇒ Specific gravity < 1.012

3) Lymphatic channels:

Also important is the lymphatic system which returns to the circulation the small amount of proteinaceous fluid that does leak from the blood into the interstial spaces. Therefore, obstruction of lymphatic channels due to various causes leads to the accumulation of the proteinaceous fluid normally drained by the lymphatic channels. Such kind of edema is called lymphatic edema.

Lymphatic edema occurs in the following conditions:

1) Parasitic infection. E.g filariasis which causes massive lymphatic and inguinal fibrosis
2) Lymphatic obstruction secondary to neoplastic infiltration. E.g. breast cancer
3) post surgical or post irradiation, i.e surgical resection of lymphatic channels or scarring after irradiation

4) Sodium and water retention:

Sodium & subsequently water retention occurs in various clinical conditions such as congetive heart failure (See Fig.5.2, above) & renal failure. In these conditions, the retained sodium & water result in increased capillary hydrostatic pressure which leads to the edema seen in these diseases.

Morphology of edema

Microscopy

- Manifests only as subtle cell swelling. Clearing & separation of extracellular matrix.

IV. Hypermia and Congestion

Definition: Both of them can be defined as a local increase in volume of blood in a particular tissue.

Hypermia

- is an active process resulting from an increased inflow of blood into a tissue because of arteriolar vasodilation.
- commonly occurs in exercising skeletal muscle or acute inflammation.
- Affected tissue becomes red as there is engorgement with oxygenated blood.

Congestion

- is a passive process resulting from impaired outflow of blood from a tissue.
- occurs systemically as in cardiac failure or locally as in isolated venous obstruction.
- Affected tissue appears blue-red due to accumulation of deoxygenated blood.

- In long-standing congestion (also called chronic passive congestion states), poorly oxygenated blood causes hypoxia → results in parenchyma cell degeneration or cell death.

a) Pulmonary congestion

Cut surface: hemorrhagic & wet.

1. Acute pulmonary congestion:
   - Alveolar capillaries engorged with blood
   - Septal edema

2. Chronic pulmonary congestion:
   - Thickened & fibrotic septa
   - Alveolar spaces contain hemosiderin-laden macrophages resulting in an appearance termed brown indurations.
   - Can result in pulmonary hypertension.

b) Hepatic congestion

1) Acute hepatic congestion:
- Central vein & sinusoids are distended
- There may be even central hepatocyte degeneration.
- Peripheral hepatocytes better oxygenated & develop only fatty changes.

2) Chronic passive congestion of liver:
   - Central lobules grossly depressed because of loss of cells & appear red brown (**nutmeg liver**).
   - Hemosiderin laden macrophages
   - In longstanding hepatic congestion, commonly associated with cardiac failure, there is a grossly evident hepatic fibrosis called **cardiac cirrhosis**

V. Haemorrhage

Definition:

Hemorrhage is extravasation of blood outside the blood vessel.

Causes:

- Physical trauma – Stabbing
  - Stick injury
  - Gunshot
  - Motor vehicle accident

- Inadequacies in blood clotting which can be due to:

  A. Too few or poorly functioning platelets (i.e. qualitative & quantitative defect of platelets)
  B. Missing or low amount of clotting factors
     E.g. Low levels of prothrombin, fibrinogen & other precursors.
     Inadequate vitamin K leads to clotting factor deficiency because this vitamin is important in the synthesis of the clotting factors by the liver.

Terminology:

1) Haemorrhage enclosed within a tissue or a cavity is knownas **hematoma.**
2) Minute 1-2 mm hemorrhages occurring in the skin, mucosal membrane, or serosal surface are called **petechiae.**
3) Slightly > 3mm hemorrhage occurring in the skin is referred to as **purpura.**
4) Larger than 1-2cm subcutaneous hematoma is called **eccymosis** (bruises). It is typical after trauma.

Effects of haemorrhage: depend on the rate and amount of blood loss:

- If > 20% the total blood volume is rapidly lost from the body, it may lead to hypovolumic shock & death.
- Chronic loss of blood leads to anaemia.

VI. Hemostasis and Blood Coagulation

Hemostasis

Definition: Hemostasis is the maintainence of the clot-free state of blood & the prevention of blood loss via the formation of hemostatic plug.

Hemostasis depends on three general components:

a) Vascular wall
b) Platelets
c) Coagulation pathways

Whenever a vessel is ruptured or severed, hemostasis is achieved by several mechanisms:

A. Vascular spasm
B. Formation of platelet plug
C. Formation blood clot as a result of blood coagulation
D. Eventual growth of fibrous tissue in to the blood clot to close the hole in the vessel permanently.

Remark: The student is advised to revise his physiology lecture note on the above topics.

VII. Thrombosis

Under this topic, we will discuss the **definition, pathogenesis, morphology, fates, & clinical significance of thrombi, in this order**.

Definition: Thrombosis is defined as the formation of a solid or semisolid mass from the constituents of the blood within the vascular system during life.

Pathogenesis:

- There are three factors that predispose to thrombus formation. These factors are called Virchow's triad:
  A: Endothelial injury
  B: Stasis or turbulence of blood flow
  C: Blood hypercoagulability

A: Endothelial injury

- It is the most important factor in thrombus formation and by itself can lead to thrombosis.
- Endothelial injury is particularly important in thrombus formation in the heart & arterial circulation.
- Some Examples:
  - Endocardial injury during myocardial infarction & eosinophilic endocarditis in which eosiophils release from their granules crystals called Charcot – Leyden damaging the endocardial endothelium.
  - Injury over ulcerated plaque in severely atherosclerotic arteries.
  - In hemodynamic stress like severe hypertension & turbulence of flow over scarred valves directly damaging the endothelium.
  - Bacterial endtoxin & hyperchloestrolemia, radiation & cigarette smoking may be sources of endothelial injury.
- Irrespective of endothelial damage, the final event is exposure of the highly thrombogenic subendothelial extracellular matrix, mainly collagen & tissue factors up on which platelets undergo adherence & contact activation.

B: Turbulence or Stasis (Alterations in normal blood flow)

Under physiologic conditions normal blood flow is laminar, that is, the cellular elements flow centrally in the vessel lumen separated from endothelium by slowing moving clear zone of plasma. Stasis & turbulence therefore:

a. Disrupt the laminar flow and bring platelets in to contact with the endothelium
b. Prevent dilution of activated clotting factors by freshly flowing blood
c. Retard or make a time lag in the inflow of clotting factor inhibitors and permit the build up of thrombi.
d. Turbulence causes reduction in endothelial PGI_2 and tissue-type plasminogen activator (t-PA) which has fibrinolytic activity causing endothelial cell activation. ???

- Stasis is a major factor in the development of venous thrombi while turbulence contributes to arterial & cardiac thrombosis by causing direct endothelial injury or by forming countercurrents & local pockets of stasis.

- Examples:
    a) Ulcerated atherosclerotic plaque, which forms a sort of irregularity on endothelial surface, not only exposes subendothelial extracellular matrix but are also sources of local turbulence.
    b) Aneurysms are favoured sites of stasis
    c) Myocardial infarction not only has endothelial injury but also has a region of noncontractile myocardium, creating an area of stasis resulting in mural thrombus formation.
    d) Mitral valve stenosis after chronic rheumatic fever may result in left atrial dilation, usually associated with arterial fibrillation. A dilated left atrium is a site of stasis & a prime location of thrombus development.
    e) Hypervisicosity syndrome, i.e an increase in hematocrit in excessive amount due to various reasons such as polycythemia causes stasis in small vessels.

C: Hypercoagulablity

Definition: Hypercoagulability is any alteration of the coagulation pathway that predisposes to thrombosis. Hypercoagulability is a less common cause of thrombosis & & it can be divided into:

1. Primary (Genetic)

- Mutations in factor V[Lieden factor]
- Anti thrombin III deficiency
- Protein C or S deficiency

2. Secondary (Acquired) which, in turn, can be categorized into:

A: **High-risk for hypercoagulablity**

- prolonged bed rest or immobilization
- Myocardial infarction
- Tissue damage (surgery, fracture, burns)
- ancers (Cancers release procoagulant tissue products to cause thrombosis)
- Prosthetic cardiac valves
- Disseminated intra vascular coagulation

B: **Low risk factor for hypercoagulablity**

- A trial fibrillation
- Cardiomyopathy
- Nephrotic syndrome
- Smoking
- Oral contraceptives
- Hyperestrogenic state eg. Pregnancy.

Morphology of Thrombi

- Thrombi may develop any where in the cardiovascular system.
- According to their location, thrombi can be divided into venous & arterial thrombi. (Cardiac thrombi can be considered as arterial thrombi because of certain similarities between the two). The differences between arterial & venous thrombi are:

Arterial thrombi	**Venous thrombi**
a) Arise at the site of endothelial injury	a) Arise at area of stasis
b) Grow in a retrograde fasion, against site of attachment.flow towards the heart	b) Grow in the direction of blood flow from its
c) Has firm attachment	c) Has loose attachment, hence, propagating tail may undergo fragmentation.
d) They usually occlude the blood flow	d) Almost invariably occlusive

- The most common site of arterial thrombi in descending order are:
  - Coronary arteries
  - Cerebral arteries
  - Temporal arteries

- Damaged valves can be infected by bacteria or fungi (infective endocarditis) which leads to the development of small infected thrombi on the valves. These small infected thrombi (vegetations) can further damage the valve.

Fates of a thrombus

A thrombus can have one of the following fates:

A: Propagation:

The thrombus may accumulate more platelets and fibrin & propagate to cause vessel obstruction.

B: Embolization:

The thrombus may dislodge and travel to other sites in the vasculature. Such a traveling thrombus is called an embolus. An embolus may obstruct a vessel. The obstruction leads to the death of the tissue supplied by the blood vessel. Death of a tissue due to a decreased blood supply or drainage is called infarction. Therefore, an embolus can eventually lead to an infarction of an organ. E.g cerebral infarction can be caused by a thromboembolus.
We will discuss embolism & infarction shortly (See p.).

C: Dissolution:

The thrombus may be removed by fibrinolytic activity.

D: Organization and recanalization

Organization refers to the ingrowth of endothelial cells, smooth muscle cells, and fibroblasts into the fibrin-rich thrombus. Organization is accompanied by the formation of capillary channels across the thrombus, re-establishing lumen continuity to some extent. This is known as **recanalization.** The recanalization eventually converts the thrombus into a vasscularized mass of tissue which is later on incorporated as a subendothelial swelling of the vessel wall.

Clinical significance of thrombi

- Thrombi are significant clinically because:
  - They cause obstruction of arteries and veins &
  - They are possible source of emboli.

We will discuss the clinical effects of venous & arterial thrombi separately.

A. Venous Thrombosis (Phlebothrombosis)

Venous thrombosis affects veins of the lower extremity in 90% of cases. It can be divided into superficial & deep vein thrombosis:

1. Superficial venous thrombosis

- Usually occurs in saphenous venous system, particularly when there are varicosities.
- Rarely embolizes
- Causes local edema, pain, and tenderness (i.e. it is symptomatic)
- Local edema due to impaired venous drainage predisposes the involved overlying skin to infection after slight trauma leading to a condition known as **varicose ulcer.**

2. Deep venous thrombosis (DVT)

- May embolize, hence, is more serious.
- Usually starts in deep veins within the calf muscles.
- Although they may cause local pain & edema, unlike superficial veinous thrombosis, they are entirely asymptomatic in approximately 50% of patients. This is because deep venous obstruction is rapidly offset or releaved by collateral bypass channels.
- Has higher incidence in middle aged & elderly people due to increased platelet aggregation & reduced PGI_2 production by the endothelium.
- Has the following **predisposing factors:**

1. Trauma, surgery, burns which usually result in:-

   a:Reduced physical activity leading to stasis
   b:Injury to vessels
   c:Release of procagulant substance from the tissue
   d:Reduced t-PA activity (fibrinolysis)

2. Pregnancy & puerperal states increase coagulation factors & reduce the synthesis of antithrombotic substances. Myocardial infarction & heart failure cause venous stasis to the left side.

3. Malnutrition, debilitating conditions and wasting diseases such as cancer. DVT due to these conditions is known as **marantic thrombosis.**

4. Inflammation of veins (thrombophlebitis) also predisposes to thrombosis.

5. Migratory thrombophlebitis is a condition that affects various veins throughout the body & is usually of obscure aetiology, but sometimes it is associated with cancer, particularly pancreatic cancer. Migratory thrombophlebitis is also known as **Trosseau syndrome.**

B. Arterial Thrombosis

- The rapid flow of arterial blood prevents the occurrence of thrombosis unless the vessel wall is abnormal.
- In western society atheroma is by far the commonest predisposing lesion for arterial thrombosis. Atheromatous plaques produce turbulence and may ulcerate & cause endothelial injury, both of which can lead to thrombosis. These thrombi may narrow or occlude the lumen of arteries such as the coronary and cerebral arteries. Occlusion of these arteries will lead to myocardial infarction (MI) & cerebral infarction respectively.
- Cardiac thrombi can be caused by infective endocarditis, atrial fibrillation,& myocardial infarcion.
- Cardiac thrombosis is common on the heart valves & in the auricular appendages (especially, of the right atrium). A thrombus develops in the atrium in patients with atrial fibrillation & dilatation superimposed on mitral stenosis.
- Myocardial infarction causes dyskinetic myocardial contraction & damage to the endocardium, which usually result in mural thrombi in the ventricles.
- Apart from obstructive features, arterial thrombi (especially, cardiac mural thromi) may embolize to any tissue, but, particularly, commonly to the brain, kidney, & spleen because of large volume of blood flow to these organs.

VIII. Embolism

Definition:-

An embolus is a detached intravascular solid, liquid or gaseous mass that is carried by blood to sites distant from its point of origin. After traveling via the blood, the embolus can obstruct a vessel.

Causes of embolism:

An embolus can arise from:

- Thrombus (99% of emboli arise from a thrombus. Such an embolus is called thromboembolus)
- Platelets aggregates
- Fragment of material from ulcerating atheromatous plaque
- Fragment of a tumour
- Fat globules
- Bubbles of air
- Amniotic fluid
- Infected foreign material
- Bits of bone marrow
- Others.

Unless otherwise specified, the term embolism should be considered to mean thromboembolism. This is because thromboembolism is the commonest form of embolism. Next, we will discuss it in more detail.

Thromboembolism

Based on its sites of origin & impaction, thromboembolism can be divided into:

a) Pulmonary thromboembolism (PTE)

- PTE is refers to the impaction of an embolus in the pulumonary arteries & their branches. Such an embolus is derived from a thrombus in the systemic veins or the right side of the heart.

b) Systemic thromboembolism

- Systemic emboli arise from the left side of the heart or from thrombi & atheromatous debris in large arteries. And they impact in the systemic arteries.

c) Crossed embolism (Paradoxical embolism)

- This occurs in the presence of patent foremen ovale when an embolus is transferred from the right to the left side of the heart, then into the systemic circulation.

Now, we will elaborate the first two.

a) Pulmonary thrombeomblism (PTE)

95% of PTE arise from thromi in the deep leg veins. The thromboembolus will travel long with the venous return & reach the right side of the heart. From there, it will go into the pulmonary trunk & pulmonary arteries. Depending on the size of the embolus and on the state of pulumonary circulation, the pulmonary embolism can have the following effects:

1. If the thrombus is large, it may block the outflow tract of the right ventricle or the bifurcation of the main pulumonary trunk (**saddle embolus**) or both of its branches, causing sudden death by circulatory arrest. Sudden death, right side heart failure (cor pulmonale), or cardiovascular collapse occurs when 60% or more of the pulumonary circulation is obstructed with emboli.
2. If the embolus is very small (as in 60-80% of the cases), the pulmonary emboli will be clinically silent. Embolic obstruction of medium sized arteries manifests as pulmonary haemorrhage but usually does not cause infarction because of dual blood inflow to the area from the bronchial circulation.
3. If the cardiorespiratory condition of the patient is poor (i.e., if the patient previously had cardiac or pulmonary disease), then obstruction of a medium sized pulmonary artery by a medium-sized embolus can lead to pulmonary infarction.
4. Recurrent thromboembolism can lead to pulmonary hypertension in the long run.

A patient who has had one pulmonary embolus is at high risk of having more.

b) Systemic thromboembolism

- Systemic thromboembolism refers to emboli travelling within arterial circulation & impacting in the systemic arteries.

- Most systemic emboli (80%) arise from intracardiac mural thrombi. In turn, two thirds of intracardiac mural thrombi are associated with left ventricular wall infarcts and another quarter with dilated left atria secondary to rheumatic valvular heart disease.
- The remaining (20%) of systemic emboli arise from aortic aneurysm, thrombi on ulcerated athrosclerotic plaques, or fragmentation of valvular vegetation.
- Unlike venous emboli, which tend to lodge primarily in one vascular bed (the lung), arterial emboli can travel to a wide variety of sites. The major sites for arteriolar embolization are the lower extremities (75%) & the brain (10%), with the rest lodging in the intestines, kidney, & spleen. The emboli may obstruct the arterial blood flow to the tissue distal to the site of the obstruction. This obstruction may lead to infarction. The infarctions, in turn, will lead to different clinical features which vary according to the organ involved.

Next, we will briefly touch upon some rare forms of embolism.

Fat Embolism

Fat embolism usually follows fracture of bones and other type of tissue injury. After the injury, globules of fat frequently enter the circulation. Although traumatic fat embolisms occur usually it is as symptomatic in most cases and fat is removed. But in some severe injuries the fat emboli may cause occlusion of pulmonary or cerebral microvasculature and fat embolism syndrome may result. Fat embolism syndrome typically begins 1 to 3 days after injury during which the raised tissue pressure caused by swelling of damaged tissue forces fat into marrow sinsosoid & veins. The features of this syndrome are a sudden onset of dyspnea, blood stained sputum, taccycardia, mental confusion with neurologic symptoms including irritability & restlessness, sometimes progress to delirium & coma.

5. Air embolism

Gas bubbles within the circulation can obstruct vascular flow and cause distal ischemic injury almost as readily as thrombotic masses. Air may enter the circulation during:

- Obstetric procedures
- Chest wall injury
- In deep see divers & under water construction workers.
- In individuals in unpressurized aircraft

- Neck wounds penetrating the large veins
- Cardio thoracic surgery.
- Arterial catheterisation& intravenous infusion.
- Etc.

Generally, in excesses of 100cc is required to have a clinical effect and 300cc or more may be fatal. The bubbles act like physical obstructions and may coalesce to form a frothy mass sufficiently large to occlude major vessels.

Amniotic fluid embolism

It is a grave but un common, unpredictable complication of labour which may complicate vaginal delivery, caesarean delivery and abortions. It had mortality rate over 80%. The amniotic fluid containing fetal material enters via the placental bed & the ruptured uterine veins. The onset is characterized by sudden severe dyspnea, cyanosis, hypotensive shock followed by seizure & coma of the labouring mother. If the patient survives the initial crisis, pulmonary oedema typically develops & 50% of the cases will develop DIC due to activation of the coagulation cascade by fetal material.

As discussed in this & the previous subtopics, the potential consequence of thromboembolic events is ischemic necrosis of distal tissue, known as **infarction.** Therefore, it is appropriate to discusss it next.

IX. Infarction

Definition: An infract is an area of ischemic necrosis caused by occlusion of either the arterial supply or venous drainage in a particular tissue.

Nearly 99% of all infarcts result from thrombotic or embolic events. Other mechanisms include [almost all of them are arterial in origin]:

- Local vasospasm
- Expansion of atheroma due to hemorrage in to athermotous plaque.
- External compression of the vessels. e.g trauma
- Entrapment of vessels at hernial sacks etc.

The development & the size of an infarct are determined by the following factors:

A. The nature of the vascular supply
B. The rate of development of occlusion
C. Suceptibility of the tissue for hypoxia
D. Oxygen content of the blood
E. The severity & duration of ischemia

A. **The nature of vascular supply**

The following organs have a dual blood supply.

- Lung → pulumonary artery
  → Bronchial artery
- Liver → hepatic artery
  → Portal vein
- Hand & forearm
  → Radial arteries
  → Ulnar arteries.

The effect of such a dual blood supply is that if there is obsrtuction of one of the arterial supplies, the other one may offset the rapid occurrence of infarction in these organs unlike the renal & splenic circulations which have end arterial supply.

Infarction caused by venous thrombosis is more likely to occur in organs with single venous outflow channels, such as testis &ovary.

B: Rate of development occlusion

Slowly developing occlusions are less likely to cause infraction since they provide time for the development of collaterals.

C: Tissue suceptibility to hypoxia:

The susceptibility of a tissue to hypoxia influences the likelihood of infarction. Neurons undergo irreversible damage when deprived of their blood supply for only 3 to 4 minutes. Myocardial cells die after 20-30 minutes of ischemia. Fibroblasts are more resistant, especially those in the myocardium.

D: Oxygen content of blood

Partial obstruction of the flow of blood in an anaemic or cyanotic patient may lead to tissue infarction.

E: The severity & duration of ischemia.

Types of infarcts

Infarcts are classified depening on:

A) the basis of their colour (reflecting the amount of haemorrhage) into:

1. Hemorrhagic (Red) infarcts
2. Anemic (White) infarcts

B) the presence or absence of microbial infection into:

1. Septic infarcts
2. Bland infarcts

1. Red infarcts occur in:

a) Venous occlusions as in ovarian torsion
b) Loose tissues such as the lung which allow blood to collect in infarct zone.
c) Tissues with dual circulations (eg. the lung), permitting flow of blood from unobstructed vessel in to necrotic zone.
d) In tissues that were previously congested because of sluggish outflow of blood.
e) When blood flow is reestablished to a site of previous arterial occlusion & necrosis.

2. **White infarcts** occur in:

a) Arterial occlusion in organs with a single arterial blood supply.
b) Solid organs such as the heart, spleen, & kidney, where the solidity of the tissue limits the amount of hemorrage that can percolate or seep in to the area of ischemic necrosis from the nearby capillaries.

Morphology of infarcts

Gross: All infarcts are wedge-shaped with the occluded vessel at the apex and the periphery of the organ forming the base of the wedge. THe infarction will induce inflammation in the tissue surrounding the area of infarction. Following inflammation, some of the infarcts may show recovery, however, most are ultimately replaced with scars except in the brain.

Microscopy:

The dominant histologic feature of infarction is ischemic coagulative necrosis. The brain is an exception to this generalization, where liquifactive necrosis is common.

Clinical examples of infarction:

A. Myocardial infarction

- Usually results from occlusive thrombosis supervening on ulcerating atheroma of a major coronary artery.
- Is a white infarct.
- Can cause sudden death, cardiac failure, etc...

B. Cerebral infarcts

- May appear as pale or hemorrhagic
- A fatal increase in intracranial pressure may occur due to swelling of large cerebral infarction, as recent infarcts are raised above the surface since hypoxic cells lack the ability to maintain ionic gradients & they absorb water & swell.
- Is one type of cerebrovascular accidents (CVA) or stroke which has various clinical manifestations.

C. Lung infarcts

- Are typically dark red & conical (wedge-shaped).
- Can cause chest pain, hemoptysis, etc…

D. Splenic infarcts

- Conical & sub capsular
- Initially dark red later turned to be pale.

X. Disseminated Intravascular Coagulation (DIC)

Definition: -DIC is an acute, or chronic thrombohemorrhagic disorder occurring as a result of progressive activation of coagulation pathway beyond physiologic set point secondary to a variety of diseases resulting in failure of all components of hemostasis. Hence the other term for DIC is **consumption coagoulopathy.**

Etiology andPathogensis

At the outset, it must be emphasize that DIC is not a primary disease. It is a coagulopathy that occurs in the course of variety of clinical conditions. DIC follows massive or prolonged release of soluble tissue factors & /or endothelial-derived thromboplastin into the circulation with generalized (pathologic) activation of coagulation system.

Therefore, DIC results from pathologic activation of the extrinsic &/or intrinsic pathways of coagulation or impairment of clot inhibiting influences by different causes. Two major mechanisms activating the coagulation pathway to cause DIC are: (1) release of tissue factor or thromboplastic substance into the circulation (2) widespread injury to the endothelial cells.

1. Tissue thromboplastin substance may be derived from a variety of sources such as:

   A: Massive trauma, severe burns & extensive surgery. The major mechanism of DIC is believed to be autoinfusion of thromboplastin from the tissues.

   B: Obstetric conditions in which thromboplastin derived from the placenta, dead retained fetus, or amniotic fluid may enter the circulation. .

C: Cancers such as acute promyelocytic leukaemia, adenocarcinoma of the lung in which a variety of thromboplastin substances like mucus are released which directly activate factor X, VII, & proteolytic enzymes.

D: Gram negative sepsis (an important cause of DIC) in which bacterial endoxins cause increased synthesis, membrane exposure, & release of tissue factor from monocytes. Furthermore, activated monocysts release intereukin-1 (IL-I), TNF-α, both of which:

- Increase expression of tissue factor in endothelial membrane.
- Decrease expression of thrombmodulin which is a potent activator of protein C- an anti coagulant
- TNF-α, an extremely important mediator of septic shock, in addition to the above, up regulates the expression of adhesion molecules on endothelial cells and favours adhesion of leukocytes, with subsequent damage of endothelial cells by free radicals & preformed proteases.

2. Endothelial injury: Widespread endothelial injury may result from:
   - Deposition of antigen-antibody complexes as it occurs in systemic lupus erythematosus
   - Extreme temperature eg. Heat stroke, burns
   - Hypoxia, acidosis, shock

Clinical Course

The consequences of DIC are two fold. First, there is a widespread deposition of fibrin within the microcirculation. This may lead to ischemia of the more severely affected or more vulnerable organs and hemolytic anemia resulting from fragmentation of led cells as they squeeze through the narrowed microvasculature (Microangiopathic haemolytic anaemia). Second, a hemorrhagic diathesis may dominate the clinical picture because of consumption of the coagulation factors and increased fibrinolysis.

The onset may be fulminant when caused by endotoxic shock or amniotic fluid embolism or it may be chronic in the case of carcinomatosis or retention of dead fetus. The clinical presentation varies with stage & severity of the syndrome. Overall 50% of patients with DIC are obstetric patients & about 33% of patients have carcinomatosis.

Clinically, patients with DIC may present with extensive skin & mucus membrane bleeding and haemorrhage from multiple sites, usually from surgical incision, vein punctures, or

catheter sites. Respiratory symptoms such as dyspnea, cyanosis may occur. They may present with convulsion & coma in the case of CNS bleeding or with acute renal failure with oliguria. Less often, they may present with acrocyanosis, pre-gangrenous changes in the digits, genitalia, & nose areas where blood flow may be markedly decreased. Circulatory failure may appear suddenly & may be progressing. The presentations of acute DIC, as it occurs in case of trauma or obstetric conditions, is dominated by bleeding diathesis.

Laboratory manifestations include thrombocytopenia secondary to platelets aggregation in the thrombus, schistocytes or fragmented RBCs, prolonged PT, PTT, thrombin time & reduced fibrinogen from depleted coagulation proteins. There is also increased fibrin degradation product (FDP) from intense fibrinolysis. The cardinal manifestation of DIC, which correlates most closely with bleeding is plasma fibrinogen level, i.e. low fibrinogen means increased tendency of bleeding.

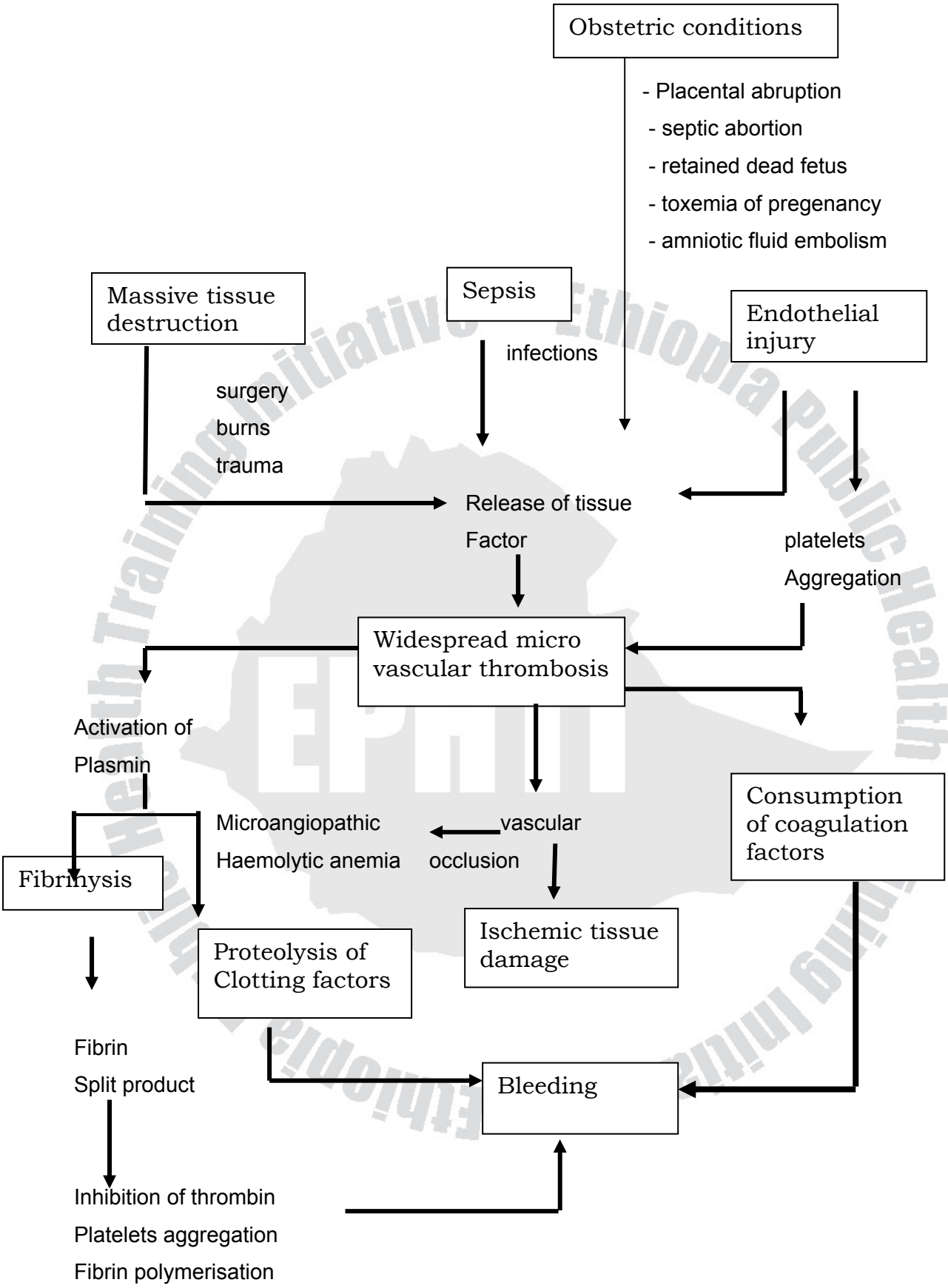


Fig. 5.3 Patholphysiology of DIC

XI. Shock

Definition: Shock is a state in which there is failure of the circulatory system to maintain adequate cellular perfusion resulting in widespread reduction in delivery of oxygen & other nutrients to tissues. In shock, the mean arterial pressure is less than 60 mmHg or the systolic blood pressure is less than 90 mmHg.

- Regardless of the underlying pathology, shock constitutes systemic hypoperfusion due to reduction either in cardiac out put or in the effective circulating blood volume. The end results are hypotension followed by impaired tissue perfusion and cellular hypoxia.
- Adequate organ perfusion depends on arterial blood pressure (BP) which, in turn, depends on:
  1. Cardiac output (CO)
  2. Peripheral vascular resistance (PVR)

- CO = stroke volume X heart rate
  In turn, stroke volume depends on:
  a) Preload i.e. blood volume,
  b) Afterload i.e. arterial resistance, &
  c) Myocardial contractility.

- Therefore, shock (i.e. widespread decreased perfusion of tissues) occurs when the preload (i.e. the blood volume) is decreased, or when the afterload (the peripheral vascular resistance) is decreased, or when the myocardium fails to contract. These basic mechanisms of shock are used to classify it. Next, we will look at the classification of shock.

Classification of shock

Shock can be divided into:

A. Hypovolemic shock
B. Cardiogenic shock
C. Distributive shock

A. Hypovolemic shock

Definition: This is shock caused by reduced blood volume. Reduction in circulating blood volume results in the reduction of the preload which leads to inadequate left ventricular filling, reflected as decreased left & right ventricular end diastolic volume and pressure. The reduced preload culminates in decreased cardiac out put which leads to widespread tissue perfusion (shock).

Causes of hypovolumic shock include:

a) Haemorrhage
b) Diarrhoea & vomiting
c) Burns
d) Trauma
e) etc

The effect of haemorrhage depends on the rate and amount of blood loss. Hypovolumic shock is the most common type of shock in clinical medicine .A normal healthy adult can lose 550ml (10%of blood volume) without significant symptoms.

But loss of 25% or more of the blood volume (N=1250ml) results in significant hypovolemia.

B. Cardiogenic shock

Definition: This is shock that results from severe depression of cardiac performance. It primarily results from pump failure [myocardial failure].

- Cardiogenic shock is hemodynamically defined as:

  - DBP<60mm Hg
  - Left ventricle filling pressure > 18mm Hg
  - Cardiac index< 1.8 l/min/m^2
  - Usually pulmonary oedema coexists.

Causes of cardiogenic shock can be divided into:

A. Myopathic
B. Mechanical

A) Myopathic causes of cardiogenic shock include:

1. Acute myocardial infraction. Usually shock occurs in this conditioin if ≥ 40% of the left ventricular mass & more on the right ventricle is involved by infarction.
2. Mycocarditis
3. Dilated cardiomyopathy/hypertrophic cardiomyopathy
4. Myocardial depression in septic shock
5. Etc….

B) Mechanical

i) Intracardiac

a) Left ventricle outflow obstruction E.g.Aortic stenosis, hypertrophic cardiomyopathy

b) Reduction in forward cardiac output E.g. Aortic or mitral regurgitation

c) Arrhythmia

ii) Extracardiac

This can be called <u>obstructive shock.</u> The extracardiac causes of cardiogenic shock can be caused by:

a) Pericardial tamponade (gross fluid accumulation in the pericardial space) results in a decreased ventricular diastolic filling → ↓CO
b) Tension pneumothorax (gas accumulation in pleural space)
   This decreases the venous return by creating a positive pressure.
c) Acute massive pulumonary embolism occupying 50-60% of pulumonary vascular bed.
d) Severe pulumonary hypertension (1^0 pulmonary hypertension).

C. Distributive shock

Definition: Distributive shock refers to a group of shock subtypes caused by profound peripheral vasodilatation despite normal or high cardiac output.

Causes of distributive shock

1) Septic shock – the commonest among the group & clinically very important.

2) Neurogenic shock

- Usually occurs in the setting of anaesthetic procedure [cephalo-caudal migration of anaesthetic agent] or spinal cord injury owing to loss of vascular tone & peripheral pooling of blood.

3) Anaphylactic shock

- Initiated by generalized IgE – mediated hypersensitivity response, associated with systemic vasodilatation & increased vascular permeability.

4) Endocrine shock

- This is a type of shock that typically occurs in adrenal insufficiency.

Next, we will discuss septic shock in some detail. But before discussing septic shock in detail it would be useful to know some aspects of sepsis briefly. **Bactermia** is the presence of viable bacteria in the blood as evidenced by blood culture. **Septicemia** is systemic infection due the presence of microbes and their toxin the blood. **Sepsis** is a systemic response to severe infection mediated via macrophage-derived cytokines that target end organ receptors in response to infection. It is also called SIRS.

Septic shock

Definition: This is a kind of shock caused by systemic microbial infection, most commonly by gram – negative infection (endotoxic shock) but can also occur with gram – positive or fungal infections.

or

It can be defined as sepsis with

1. Hypotention, arterial blood pressure less than 90mmHg or 40mmHg less than the patient's normal blood pressure,
2. Organ dysfunction, &
3. Unresponsiveness to fluid administration.

Pathogenesis of septic shock:

Septic shock has a mortality rate of over 50% ranking the first among the causes of death in intensive care units. It results from the spread & expansion of an initially localized infection like pneumonia into the blood stream.

Most causes of septic shock (~70%) are caused by endotoxin-producing gram-negative bacilli, hence the term endotoxic shock. **Endotoxins** are bacterial wall lipopolyschardes (LPS) released when cell walls are degraded. Analogues molecules in the walls of gram-positive bacteria & fungi can also elicit septic shock. LPS bind with CD14 molecule on leucocytes, especially monocytes & macrophages, endothelial cells & others. Depending on the dosage of LPS – protein complex, initiation of a cascade of cytokine-mediated events take place.

The mononuclear phagocytes respond to LPS by producing TNF which, in turn, induces IL – 1 synthesis. TNF & IL-1 both act on endothelial cells to produce further cytokines like IL-6, IL-8, & secondary effectors like NO & PAF (platelet aggregating factor).

- **High levels of the above molecules or mediators (TNF-α, IL-1, etc…)** cause septic shock by acting on:

→ The heart – causing decreased myocardial contractility which results in low cardiac output,

→ Blood vessel – causing systemic vasodilation which decreases the peripheral arteries. The mediators also cause widespread endothelial injury & activation of the coagulation system resulting in DIC, &

→ Lung – causing alveolar capillary damage resulting in adult respiratory distress syndrome (ARDS).

Stages of shock

Uncorrected shock passes through 3 important stages:

1) An initial nonprogressive phase

- It is also called a period of early compensatory period, during which compensatory mechanisms are activated & perfusion of vital organs maintained.

Mechanisms

- A variety of neurohumoral mechanisms operate:
  i) A decrease in cardiac output will stimulate peripheral & central baro receptors with subsequent intense sympatho-adrenal stimulation. This sometimes leads to up to 200 fold increase in plasma catecholamine level. The net effect is → Tachycardia, ↑ HR → ↑ CO → Peripheral vasoconstriction → ↑ BP. This is a major autocompensatory response.
  ii) The fall in renal perfusion stimulates the renin – aldosterone secretion mechanism → renal conservation of fluid.

2. Progressive stage (Established shock)

- This is characterized by tissue hypoperfusion with onset of worsening circulatory & metabolic imbalances including acidosis.
- There is a widespread tissue hypoxia.
- Anaerobic gycolysis results in excessive lactic acid production. The lactic acid reduces tissue PH & blunts vasomotor response. The hypoxic cells leak glucose leading to insulin-resistant hyperglycaemia and increased glycogenolysis. Impaired carbohydrate metabolism causes a fall in production of ATP, failure in function of Na^+ - K^+ ATPase, result in Na & water enterance into the cell, causing cellular swelling also called **sick cell syndrome**. Anoxic injury to endothelial cells results in DIC.

3. An irreversible stage

- A sage at which, even if hemodynamic disorders are corrected survival is not possible.
- Transition to irreversible damage is mediated via various mechanisms.

Morphology of septic shock:

- All organs are affected in severe shock. In shock, there is widespread tissue hypoperfusion involving various organs such as the heart, brain, & kidney. This leads to widespread hypoxic tissue necrosis. The widespread tissue necrosis manifests as multiple organ dysfunction [MODS]. Various organs may fail to perform their normal functions. And lungs may show ARDS or Shock lung.

Clinical course of shock

- Patient with shock may manifest as having a weak and rapid pulse, tachypenia, & cool, clammy, cyanotic skin. In septic shock, the skin will initially be warm & flushed because of peripheral vasodilation. The patient may present with confusion, restlessnes, decreased urine output, coma, and death.

XII. Excercises

1. a) What is edema?
   b) Enumerate four clinical conditions that cause generalized body oedema. Discuss their pathogeneses.
   c) Enumerate the causes of oncotic and nononcotic oedema.

2. Case study I: A 40 year old patient got a car accident and he was found to have femoral shaft fracture & then he suddenly developed dyspnea, cyanosis, and shock and passed away immediately after surgery. There was no massive blood loss at the time of trauma or during surgery. The probable cause of death is:
   a) Shock
   b) Arterial emboli
   c) Fat embolism
   d) Stress
   e) None

3. a) Define infarction.
   b) Briefly discuss the difference between venous & arterial thromboses
   c) Enumerate the difference between red & white infracts & the organs in which they commonly occur.

4. Case study II: A 28 year old female patient presented with fever, chills, decreased urine output, offensive vaginal discharge, and abdominal pain. One week earlier she had an abortion attended by a non-medical personnel with metallic materials. The doctor found out that her blood pressure was 60/20 mmHg, that she has altered consciousness, & a temperature of 38.9^0c.
   a) What is the primary problem?
   b) What is the complication of the primary problem? Discuss the pathogenesis of this complicatioin.
   c) What organisms are the most likely causes of the disease?
   d) What are the morphologic changes & their complications that will be seen in different organs in this patient?

5. a) Describe the coagulation system & its normal purpose (function).
   b) Enumerate vitamin K dependent coagulating factors.
   c) Enumerate the cause of DIC

6. Give a brief explanation for the following questions.

   a) Why is venous embolus is almost always occlusive compared to arterial emboli?
   b) What is the pathogenesis shared by both myocardial infarction and ulcerated atherosclerotic plaque in causing thrombosis?
   C] Why is superficial phlebothrombosis more symptomatic than deep vein thrombosis?

7. Discuss the causes of hypovolemic & cardiogenic shock.

CHAPTER SIX
GENETIC DISEASES

I. Learning objectives:

At the end of this chapter, the student should be able to:-

1. Know the basis of genetic diseases.
2. Know the 4 major categories of genetic diseases.
3. Know the categories of mendelian disorders based on their pattern of inheritance & give some examples of each category.
4. Know the categories of mendelian disorders based on the type of protein involved (i.e. the biochemical mechanism) & give some examples of each category.
5. Know the different types of chromosomal disorders & give examples for each type.
6. Know multifactorial disorders.

II. Introduction

A knowledge of the normal human genetics will facilitate the understanding of genetic diseases. Hence, the student is advised to revise the normal human genetics before reading this chapter. Here, only brief highlights of the normal are given. Genetic diseases are often said to be difficult tostudents. We have tried to dispell this wrong notion & to make genetic as clear as possible at the cost of brevity. we did in order to facilitate the student's understanding.

Genetic information is stored in DNA. The typical normal human cell contains 46 chromosomes {i.e. 23 pairs of chromosomes: 22 homologous pairs of autosomes & one pair of sex chromosomes (XX or XY)}. Members of a pair (described as homologous chromosomes or homologs] carry matching genetic information. I.e. they have the same gene loci in the same sequence, though at any specific locus they may have either identical or slightly different forms, which are called alleles. One member of each pair of chromosomes is inherited from the father, the other from the mother.Each chromosome is in turn composed of a very long unbranched molecule of DNA bound to histones & other proteins. This interaction between the long DNA molecule & the histones decreases the space occupied by the long DNA. I.e. this interaction packages the long DNA into the shorter chromosomes.

Each chromosome contains a single continuous DNA molecule. DNA is composed of two very long complementary chains of deoxynucleotides. The 2 chains (strands) of DNA wind around each other i.e. twist about each other forming a double helix – "the twisted ladder model". Each deoxynucleotide, in turn, is composed of a nitrogenous base {i.e. adenine (A), or guanine (G), or cytosine (C), or thymine (T)} bound to deoxyribose & phosphate.

DNA has two basic functions:

1. It codes for the proteins which are important for the metabolic & structural functions of the cell. I.e. it provides the genetic information for protein synthesis.
2. It transmits the genetic information to the daughter cells & to the offsprings of the individual.

Hence, the central dogma of molecular biology is:-

Figer 6-1 showing the central dogma of molecular biology

- DNA →→ transcription →→ RNA→→ translation →→ PROTEIN.
  ↓
  ↓ replication
  DNA

DNA stores genetic information. This is done by the sequence of the nucleotides in the DNA. The portion of DNA that is required for the production of a protein is called a gene. A gene has exons (coding sequences) & introns (intervening sequences). The transcription of a gene is regulated by a promoter region, enhancer region, etc…. The sequence of nucleotides in a gene determines the sequence of amino acids in a specific protein. Three consecutive nucleotides form a code word or codon. Each codon signifies a single amino acid. Since the number of condons (64) outnumbers the number of amino acids (20), most amino acids are specified by more than 1 condon, each of which is completely specific.

To translate its genetic information into a protein, a segment of DNA (i.e. a gene) is first transcribed into mRNA. The mRNA contains a sequence of nucleotides that is complementary to the nucleotides of the DNA. Each DNA triplet codon is converted into a corresponding RNA triplet codon. Then each mRNA codon codes for a specific amino acid. Hence, the sequences of the RNA codons is translated into a sequence of amino acids (i.e. protein). Therefore, the sequence of the amino acids in the protein is determined by the sequence of the codons in the mRNA which in turn is determined by the sequence of nucleotides in the DNA.

In summary, the primary sequence of bases in the coding regions of DNA determines the sequence of amino acids in the protein. Hence, any alteration in the sequence of bases in the normal gene causes an alteration of the protein at a specific point in its sequence. Such alteration is called mutation & is the basis of genetic diseases.

Genetic information is transmitted to the daughter cells under two circumstances:

1. Somatic cells divide by mitosis, allowing the diploid (2n) genome to replicate itself completely in conjunction with cell division.

2. Germ cells (sperm & ova) undergo meiosis – a process that enables the reduction of the diploid (2n) set of chromosomes to the haploid state (1n).When the egg is fertilized by the sperm, the 2 haploid sets are combined, thereby restoring the diploid state in the zygote.

Terminology:-

- Hereditary (familial) disorders are disorders derived from one's parents.
- Congenital means "born with." It may be genetic, for example Down's syndrome. Or it may not be genetic, for example congenital syphilis. Not all genetic diseases are congenital, for example patients with Huntington's disease begin to manifest their disease in the 3rd or 4th decades.
- Genotype means the genetic constitution (genome).
- Phenotype means the observed biochemical, physiological, & morphological characteristics of an individual as determined by his/her genotype & the environment in which it is expressed.
- Allele means one of the alternative versions of a gene that may occupy a given locus.
- Gene, as already stated, is the portion of DNA that codes for a protein.

III. Mutations

- are the bases of genetic diseases.
- are defined as permanent changes in the primary nucleotide sequence of DNA regardless of its functional significance.
- occur spontaneously during cell division or are caused by mutagens such as radiation, viruses, & chemicals.
- can occur in germ line cells (sperm or oocytes) or in somatic cells or during embryogenesis. Germline mutations can be passed from one generation to the next & thus cause inherited disease. Somatic mutations do not cause hereditary disease but

they may cause cancer (because they confer a growth advantage to cells) & some congenital malformations. Mutations that occur during development (embryogenesis) lead to mosaicism. Mosaicism is a situation in which tissues are composed of cells with different genetic constitutions. If the germ line is mosaic, a mutation can be transmitted to some progeny but not others. This can sometimes lead to confusion in assessing the patterns of inheritance.

- affect the various levels of protein synthesis.
- can be classified into the following three categories based on the extent of the genetic damage:

2. Genome mutations

- are due to chromosome missegregation.
- are gain or loss of one or more whole chromosomes.
- are exemplified by aneuploidy & polyploidy.
- are often incompatible with survival.

3. Chromosome mutations

- are due to rearrangement of genetic material in a chromosome which results in structural changes in the chromosome.
- can be seen by the microscope.
- are exemplified by translocations.
- are infrequently transmitted because most are incompatible with survival (like genome mutations).

4. Gene mutations

- cause most of the hereditary diseases.
- are submicroscopic (i.e. cannot be seen by the microscope).
- may affect a single base (more common) or they may affect a larger portion of a gene.

- have the following types:

A. Single base pair change (Point Mutation)

B. Deletions & Insertions

C. Expansions of repeat sequences

Each of these types are discussed below.

A. Point mutation (Single base pair change)

- is the substitution of one base for another.
- includes the following types:-
    1. Silent mutations
    2. Missense mutations
    3. Nonsense mutations

1. Silent (Synonymous) mutation.

The genetic code is redundant (i.e. there is more than one codon for most amino acids) & therefore a change in one base may result in no change in the amino acid sequence of the protein. The base replacement does not lead to a change in the amino acid but only to the substitution of a different codon for the same amino acid. For example, the change of the codon UUU which codes for phenylalanine to UUC (i.e. the replacement of U by C) is a silent mutation because the new codon (UUC) also codes the same amino acid (phenylalanine).

2. Missense mutations

- changes the codon for one amino acid to the codon for another amino acid.
- is exemplified by the mutation which causes sickle cell anemia.

Hemoglobin is composed of a heme, two α-globin polypeptide chains, & two β-globin polypeptide chains. In normal individuals, the codon GAG codes for glutamic acid in the 6^{th} position of the β-globin polypeptide chain. But in sickle cell anemia this codon is changed to GUG which codes valine. Hence, as a result of this single base substitution, valine substitutes glutamic acid in the β-globin chain. This amino acid substitution alters the physicochemical properties of hemoglobin, which is now called Hemoglobin S. This leads to sickle cell anemia.

3. Nonsense mutation

- changes the codon for an amino acid to a stop codon, leading to termination of translation of the mRNA transcript & a truncated protein.
- is exemplified by the mutation which causes β^o – thalassemia. In this, a substitution of U for C in the codon 39 of the β globin chain of hemoglobin (i.e. the change of CAG to UAG) converts the codon for glutamine to a stop codon. This results in premature termination of the β globin gene translation. I.e. protein synthesis stops at the 38^{th} amino

acid. This results in short peptide which is rapidly degraded leading to the absence of β-globin chains. This leads to β^o – thalassemia.

B. Deletions & insertions

- can occur within coding sequences or within noncoding sequences.

i. Deletions & insertions of one or two bases within coding sequences lead to frameshift mutations because they alter the reading frame of the triplet genetic code in the mRNA so that every codon distal to the mutation in the same gene is read in the wrong frame. This leads to altered amino acid sequence & usually premature termination of the peptide chain because of the occurrence of a termination codon in the altered reading frame.

ii. Deletion or insertion of three or a multiple of three base pairs within coding sequences does not cause frameshift mutation, instead it results in abnormal protein missing one or more amino acid.

iii. Deletions affecting the promoter/enhancer sequences (i.e. in the noncoding regions) leads to promoter / enhancer mutations.

C. Expansion of repeat sequences (trinucleotide repeat mutations)

- show expansion of a sequence of 3 nucleotides. Normally, 3 nucleotides are repeated 20-30 times. Trinucleotide repeat mutation is when there is expansion of these normally repeated sequences to more than 100 repeats.
- The mechanism leading to an increase in the number of repeats is not clear. It is also not clear how the increase leads to disease.
- cause myotonic dystrophy, Huntington's disease, fragile X syndrome, etc…are not stable (i.e. they are dynamic) (i.e. the degree of amplification increases during gametogenesis). This leads to the phenomenon of anticipation (i.e. the disease worsens during the subsequent generations).

Summary:-

- Mutations can interfere with normal protein synthesis at various levels:-

1. Promoter/enhancer mutations → No transcription/ increased transcription → No protein/increased protein.
2. Missense mutation → Abnormal protein with a different amino acid → A protein altered with function or loss of function
3. Nonsense mutation → Affects translation → Truncated protein → Rapidly degraded protein → Absence of the protein.

Many different proteins are synthesized in each cell of the body. These proteins include enzymes & structural components responsible for all the developmental & metabolic processes of an organism. Mutation can result in abnormality in any of these protiens. Mutation → Abnormal protein/No protein/ Increased protein → Abnormal metabolic processes → Tissue injury →Genetic diseases.

IV. Categories of genetic diseases

Genetic diseases generally fall into one of the following 4 categories:

a. Mendelian disorders
b. Chromosomal disorders
c. Multifactorial disorders
d. Single gene diseases with nonclassic patterns of inheritance.

Each of these categories is discussed below.

V. Mendelian disorders

- Each medelian disorder is caused by a single mutant gene.
  → affects transcription, mRNA processing, or translation
  → abnormal protein or decreased protein
  → may affect any type of protein →Disease.
- show the classic mendelian patterns of inheritance.
- are also called monogenic mendelian disorders.
- are uncommon.
- can be classified into the following based on their patterns of inheritance:
  1. **Autosomal dominant inheritance**
  2. **Autosomal recessive inheritance**
  3. **X-linked recessive inheritance**

The mode of inheritance for a given phenotypic trait/disease is determined by pedigree analysis in which all affected & unaffected individuals in the family are recorded in a pedigree using standard symbols & indicating the sex, the generation, & biologic relationship among the family members. In all mendelian disorders, the distribution of the parental alleles to their offspring depends on the combination of the alleles present in the parents.

1. Autosomal dominant disorders

- will be discussed under the following 4 headings:-
  a. The criteria for autosomal inheritance
  b. Additional features of autosomal dominant disorders
  c. Pathogenesis
  d. Clinical examples

- Dominant implies that the disease allele needs to be present only in a single copy (as in the heterozygote) to result in the phenotype.

a. The criteria of autosomal inheritance include:

i. The transmission of the trait is from generation to generation without skipping. In a typical dominant pedigree, there can be many affected family members in each generation.

ii. Except for new mutation, every affected child will have an affected parent Some patients do not have affected parents because the disease in such cases is due to new mutations in the sperm/ovum from which the patients were derived. New germ line mutations occur more frequently in fathers of advanced age.

Iii. In the mating of an affected heterozygote to a normal homozygote (the usual situation), each child has a 50% chance to inherit the abnormal allele & be affected & a 50 % chance inherit the normal allele. See Fig.1 below.

iv. The 2 sexes are affected in equal numbers (because the defective gene resides on one of the 22 autosomes (i.e. nonsex chromosomes). The exceptions to this rule are the sex-limited disorders such as breast & ovarian cancers in females & familial male precocious puberty in boys.

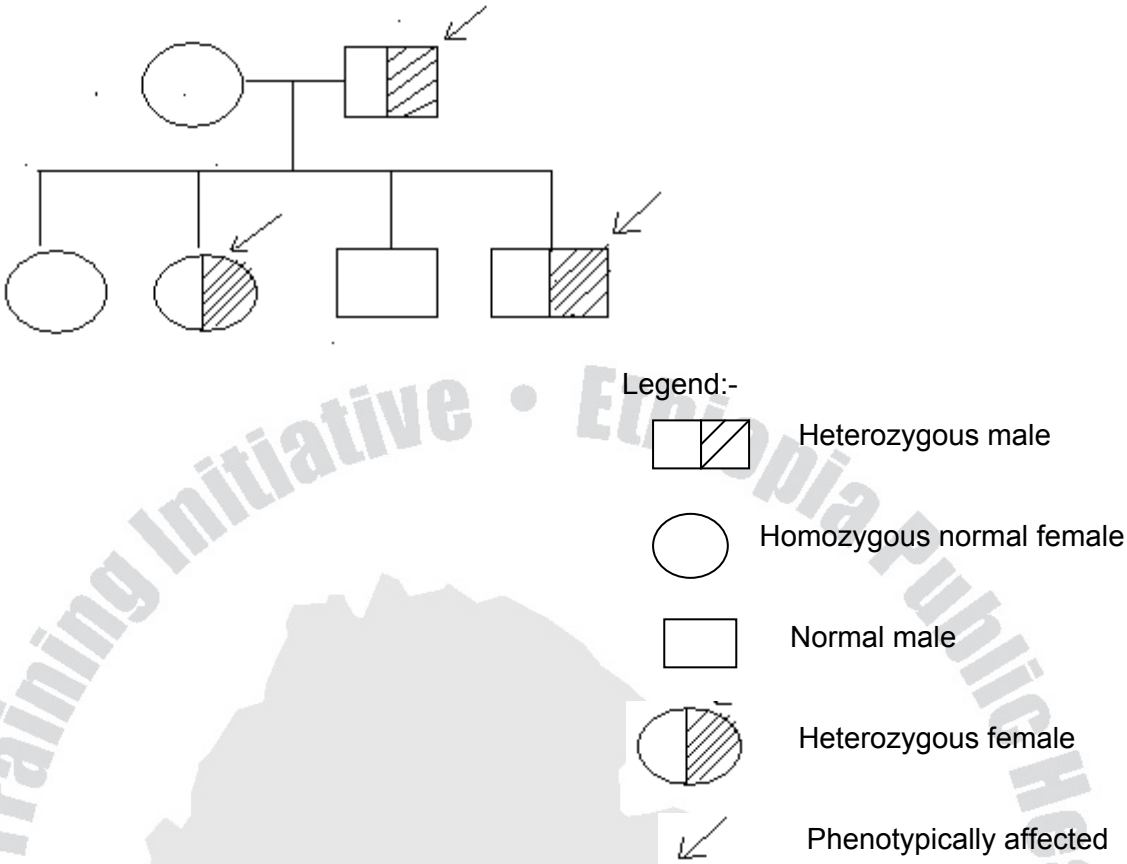


Fig 6.2. The pedigree for autosomal dominant pattern of inheritance. This figure shows the pedigree for a normal female parent & an affected male parent & their four children. Vertical distribution of the condition through successive generations occurs when the trait does not impair reproductive capacity.

b. Additional features of autosomal dominant disorders

Each of the following may alter the idealized dominant pedigree (& they should be considered to provide the most accurate counselling):-

i. Autosomal dominant disorders can sometimes be caused by new mutations. New mutations may give rise to an isolated case of a dominant disorder. New mutations are more often seen with diseases that are so severe that people who are affected by them are less likely to reproduce than normal. For example, the majority of cases of achondroplasia are the results of new mutations.

ii. Autosomal dominant disorders can show reduced penetrance (i.e. some individuals inherit the mutant gene but are phenotypically normal). Penetrance is the probability of expressing the phenotype given a defined genotype. Penetrance is expressed as the percentage of individuals who have the mutant allele & are actually phenotypically affected. For example, 25% penetrance indicates that 25% of those who have the gene

express the trait. Penetrance can be complete or incomplete. Reduced (incomplete) penetrance is when the frequency of expression of a genotype is < 100%. Nonpenetrance is the situation in which the mutant allele is inherited but not expressed.

iii. Autosomal dominant disorders commonly show variable expressivity.Variable expressivity is the ability of the same genetic mutation to cause a phenotypic spectrum. It is when the trait is seen in all individuals carrying the mutant gene but is expressed differently among individuals. For example, some patients with neurofibromatosis type 1 (which is an autosomal dominant disorder) have only brownish spots (café au lait spots) on their skin whereas other patients with the same disease have multiple skin tumors & skeletal deformities. Therefore, neurofibromatosis is said to show variable expressivity. Variable expressivity most likely results from the effects of other genes or environmental factors that modify the phenotypic expression of the mutant allele. For example, individuals with familial hypercholesterolemia who take cholesterol-rich diet have a higher risk of manifesting with atherosclerosis than those individuals with hypercholesterolemia & who take low cholesterol diet. Hence, the variable expressivity in this case is brought about by the influence of an environmental factor (i.e. the diet).In general, variable expressivity & reduced penetrance can modify the clinical picture of autosomal dominant disorders.

c. Pathogenesis of autosomal dominant disorders

Autosomal dominant disorders are caused by 2 types of mutations:

1. **Loss of function mutations**
2. **Gain of function mutations**

1. **Loss of function mutations** cause autosomal dominant disorders when they result in inactive or decreased amount of **regulatory proteins** (e.g. cell membrane receptors such as LDL receptor), or **structural proteins** (e.g. collagen, fibrillin, spectrin, dystrophin).

A 50% reduction in the levels of such **nonenzyme proteins** results in an abnormal phenotype (i.e. the heterozygote, who produces this much amount, will manifest the disorder). This can sometimes be explained by the dominant negative effect of the mutant allele (i.e. product of the mutant allele impairs the function of the product of the normal allele).

2. **Gain of function** mutations are much less common than loss of function mutations. In such cases, the mutant gene produces a **toxic protein** (i.e. the protein will have a new toxic function). This is exemplified by Huntington disease. Gain of function mutations almost always have autosomal dominant pattern.

d. Clinical examples of autosomal dominant disorders:

- Marfan syndrome*
- Some variants of Ehlers – Danlos syndrome
- Osteogenesis imperfecta
- Achondroplasia
- Huntington disease
- Neurofibromatosis*
- Tuberous sclerosis
- Myotonic dystrophy
- Familial hypercholesterolemia*
- Hereditary spherocytosis
- Familial polyposis coli
- Polycystic kidney disease

* Only these are briefly described here.

Marfan syndrome

- is a defect of connective tissue characterized by faulty scaffolding.
- is caused by mutations of FBN1 gene → Abnormal fibrillin (which is a structural protein) No normal microfibrils in the extracellular matrix→ No scaffolding on which tropoelastin is deposited to form elastic fibers → Marfan's syndrome.Microfibrils are normally abundant in the aorta, ligaments, & ciliary zonules of the lens where they support the lens. Hence, Marfan syndrome (in which there is deficiency of normal fibrillin & microfibrils) mainly involves these tissues.
- is characterized by defects in skeletal, visual, & cardiovascular structures:-
    i. Patients are tall & thin with abnormally long legs & arms, spider like fingers (arachnodactyly), hyperextensible joints.
    ii. Dislocation of the ocular lens (Ectopia lentis) is frequent.

iii. Cardiovascular changes include:

a. Mitral valve prolapse due to loss of connective tissue support in the mitral valve leaflets.
b. Dilatation of the ascending aorta due to cystic medionecrosis (→lack of medial support).Dilatation of the aortic valve ring & the root of the aorta → Aortic regurgitation.
c. Dissecting aneurysm of the aorta due to medial necrosis & intimal tear.

Familial hypercholesterolemia

- is possibly the most frequent mendelian disorder
- is caused by mutation of the gene for LDL (low density lipoprotein) receptor → Decreased functional LDL receptor → Increased plasma cholesterol → Premature atherosclerosis → Increased risk of myocardial infarction & other complications of atherosclerosis/ The occurrence of xanthomas (which are raised yellow lesions filled with lipid-laden macrophages in the skin & tendons).
- is best understood by knowing the normal process of cholesterol metabolism & transport which is briefly described below.7% of the body's cholesterol circulates in the plasma, predominantly in the form of LDL. The level of plasma cholesterol is influenced by its synthesis & catabolism. The liver plays an important role in both these processes. The following flow chart illustrates the normal cholesterol metabolism. Abbreviations used in this flow chart
- TG = Triglyceride VLDL = Very Low Density Lipoprotein = has a lot of triglyceride (TG), very little cholesterol, & 3 apoproteins. IDL = Intermediate Density Lipoprotein= has less TG & more cholesterol than VLDL & also has 2 apoproteins.LDL = Low Density Lipoprotein = has much more cholesterol than IDL.

Liver cell Secrets ↓ VLDL ↓

VLDL is transported to the capillaries of adipose tissue or muscle which contain lipoprotein lipase. The lipoprotein lipase degrades the VLDL into TG & IDL.

a. The TG is stored in fat cells or is used for energy in skeletal muscle.
b. The IDL follows 2 pathways:-

i. 50% of plasma IDL is cleared by the liver. **The liver uses *LDL receptors* to remove plasma IDL.**
ii. The rest of IDL is converted to plasma LDL (which is cholesterol-rich).↓Plasma LDL is removed by the following 2 pathways:-

1. The scavenger receptor pathway:- In which oxidized LDL or acetylated LDL is removed by a scavenger receptor on the cells of the mononuclear phagocyte system, &
2. Hepatic clearance:- **70% of plasma LDL is removed by the liver (because LDL binds with LDL receptors** which are concentrated in certain regions (called the coated pits) of the cell membrane of the hepatocyte). Then, the coated vesicles containing the bound LDL fuse with the lysosomes.↓ in the lysosomes, LDL is degraded into free cholesterol which enters the cytoplasm.

There, **cholesterol** does the following things:-

i.It is used for the synthesis of cell membrane & bile acids.

ii. It stimulates storage of excess cholesterol

iii. **It inhibits the synthesis of LDLreceptors** thus protects the cell from excessive accumulation of cholesterol.

Familial hypercholesterolemia

- is caused by different types of mutations in the gene for LDL receptor → No functional LDL receptor → Leads to:-
  i. Impaired plasma LDL clearance. This leads to the accumulation of LDL in plasma (i.e. hypercholesterolemia).
  ii. Impaired IDL uptake by the liver (because IDL uses hepatic LDL receptors for this uptake).→ Diversion of a greater proportion of plasma IDL into the precursor pool for plasma LDL. → Hypercholesterolemia.
  iii. Increased scavenger receptor – mediated clearance of LDL into the cells of the mononuclear phagocyte system & possibly the vascular walls. This leads to xanthomas & contributes to premature atherosclerosis.

The hypercholesterolemia & the accumulation of LDL inside macrophages produced by the above mechanisms lead to premature atherosclerosis & xanthomas.

This knowledge of the pathogenesis of familial hypercholesterolemia has led to a logical discovery of its treatment. We have said that the basic problem in this disease is absence of LDL receptors. Hence, the logical treatment is to increase the number of LDL receptors. (i.e. to remove the basic problem). This can be done by:-

1. Statins
   - are drugs which inhibit hepatic HMG CoA reductase→ Inhibits intracellular cholesterol synthesis→ leads to greater synthesis of LDL receptors (See the normal cholesterol metabolism above)
2. Gene therapy (under investigation)
   - by giving normal LDL receptor genes via a viral vector.

This illustrates that knowing the pathogenesis of diseases greatly helps not only in understanding their morphologic & clinical features but also in the logical discovery of their treatment.

Neurofibromatosis:-

- is a familial neoplasm. Familial neoplasms have neoplasm-causing mutations ransmitted through the germ line. Familial neoplasms account for about 5% of all cancers & they are mendelian disorders. They are often inherited in autosomal dominant pattern with few exceptions. They are caused by mutations that affect proteins which regulate cell growth. And they are exemplified by neurofibromatosis types 1 & 2. It should be noted that most cancers are not familial & these non-familial cancers are caused by mutations of tumor-suppressor genes, proto-oncogenes, & apoptosis- regulating genes in somatic cells. Hence, these mutations are not passed in the germ line. Therefore, most cancers are not mendelian disorders i.e. they are sporadic or nonfamilial disorders. Here, neurofibromatosis which is a mendelian neoplasm is discussed.

1. Neurofibromatosis type 1

- was previously called von Recklinghausen disease.
- has autosomal dominant transmission in 50% of cases. (The rest 50% are due to new mutations.
- has extremely variable expressivity but the penetrance is 100%.
- is due to a mutation in the NF1 gene (which is a tumor-suppressor gene).
- mainly shows neurofibromas in the skin & other locations, café au lait spots (i.e. light brown skin pigmentations), & pigmented iris hamartomas (Lisch nodules). The benign neurofibromas can sometimes become malignant.
- may also show skeletal disorders such as scoliosis & bone cysts & increased incidence of other tumors especially pheochromocytoma & malignancies such as Wilm's tumor, rhabdomyosarcoma, & leukaemia.

2. Neurofibromatosis type 2

- was in the past called acoustic neurofibromatosis.
- is much less common than neurofibromatosis type 1.
- is an autosomal dominant disorder.
- is due to a mutation of the NF-2 gene (which is a tumor suppressor gene)
- shows bilateral acoustic schwannomas, multiple meningiomas, & gliomas (typically ependymomas of the spinal cord).

Autosomal recessive disorders

- will be discussed under the following headings:-

a. Criteria

b. Additional features

c. Pathogenesis

d. Clinical examples

In autosomal recessive disorders, the phenotype is usually observed only in the homozygote. The typical pedigree shows affected male & female siblings with normal parents & offspring. Recessive inheritance is suspected when parents are consanguineous; it is considered proven when the corresponding enzyme levels are low or absent in affected individuals & are at half normal values in both parents.

a. Criteria

i. If the trait is rare, parents & relatives other than siblings are usually normal

ii. In the mating of 2 phenotypically normal heterozygotes, the segregation frequency with each pregnancy is 25% homozygous normal, 50% heterozygous normal, & 25% homozygous affected. See Fig. 2 below.

iii. All children of two affected parents are affected.

iv. Both sexes are affected in equal numbers

v. If the trait is rare in the population, the probability of parenta consanguinity is increased.

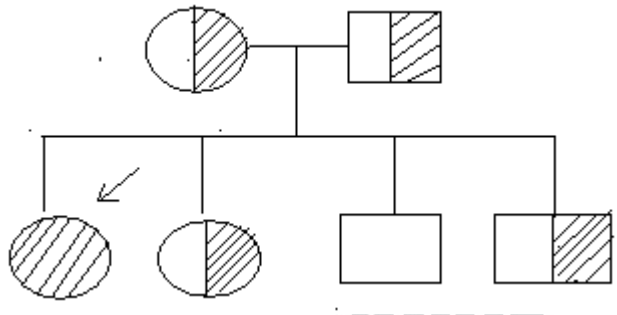


Fig 6.3. Autosomal recessive pattern. This diagram shows the pedigree for 2 heterozygous parents & their 4 children. Only siblings are affected. Vertical distribution of affected individuals is not usually seen. Horizontal distribution & consanguinity are seen in a multiplex pedigree. (Note: This is not a multiplex pedigree). See the legend of Fig. 1.

b. Additional features

i. Autosomal recessive disorders show more uniform expression of the trait than autosomal dominant disorders. (i.e. they don't show variable expressivity).

ii. They commonly show complete penetrance.

iii. They frequently show signs & symptoms early in life, whereas many autosomal dominant disorders have delayed onset e.g. Huntington disease clinically manifests for the first time during adulthood.

c. Pathogenesis

Many autosomal recessive disorders are caused by **loss of function mutations** which result in **decreased enzyme proteins**.

Homozygotes → No normal enzyme → Disease.

Heterozygotes →Equal amounts of normal & defective enzymes→Cells with half the normal amount of the enzyme function normally →No disease.

d. Clinical examples include:-

- Sickle cell anemia
- Thalassemias
- Congenital adrenal hyperplasia
- Cystic fibrosis Wilson disease
- HemochromatosisMendelian disorders associated with enzyme defects:*
    - o Phenylketonuria
    - o Galactosemia
    - o Homocystinuria
    - o Lysosomal storage diseases
    - o Alpha 1 antitrypsin deficiency
    - o Glycogen storage disease

    * These will be discussed further.

- Mendelian disorders associated with enzyme defects.

- have mutations → decreased amount of a normal enzyme or abnormal enzyme with decreased activity→ Metabolic block → Consequences → Disease.
- show autosomal recessive pattern of inheritance because half the normal amount of enzyme is enough for normal function. Hence, heterozygotes (who produce this amount) do not manifest the disease. I.e. the inheritance is autosomal recessive.
- is illustrated by the following model of a metabolic pathway:- Substrate ↓

Enzyme 1↓ Intermediate 1↓ Enzyme 2↓Intermediate 2 →→ M1 →→ M2 ↓ (Minor pathway products) Enzyme 3 ↓ Final product If an enzyme of the above pathway is defective, then the consequences may be:

1. Accumulation of the substrate, &/or one or both of the intermediates, & the products of the minor pathway depending on the level of the block. These substances may be toxic in high concentrations & result in tissue damage. This mechanism occurs in the following diseases:
    - Lysosomal storage diseases
    - Galactosemia
    - Phenylketonuria

2. Decreased amount of the final end product.
    - This is exemplified by albinism.

3. Failure to inactivate a toxic substrate. E.g. Hereditary alpha -1 antitrypsin deficiency.

Mendelian disorders associated with enzyme defects include most inborn errors of metabolism such as:

- Lysosomal storage diseases (E.g. Gaucher disease)
- Phenylketonuria
- Severe combined immunodeficiency disease
- Alpha 1 antitrypsin deficiency
- Albinism
- Lesch – Nyhan syndrome

In order to illustrate the basic principles of this category, only the first two disorders from the above list are discussed below in moderate depth.

1. Lysosomal storage diseases

- result from lack of any protein essential for the normal function of lysosomes. Lysosomes are intracellular organelles used for degrading a variety of complex substrates. They do so by means of a variety of enzymes. The following figure compares the normal lysosomal degradation pathway with that of lysosomal storage disease.Complex substrate

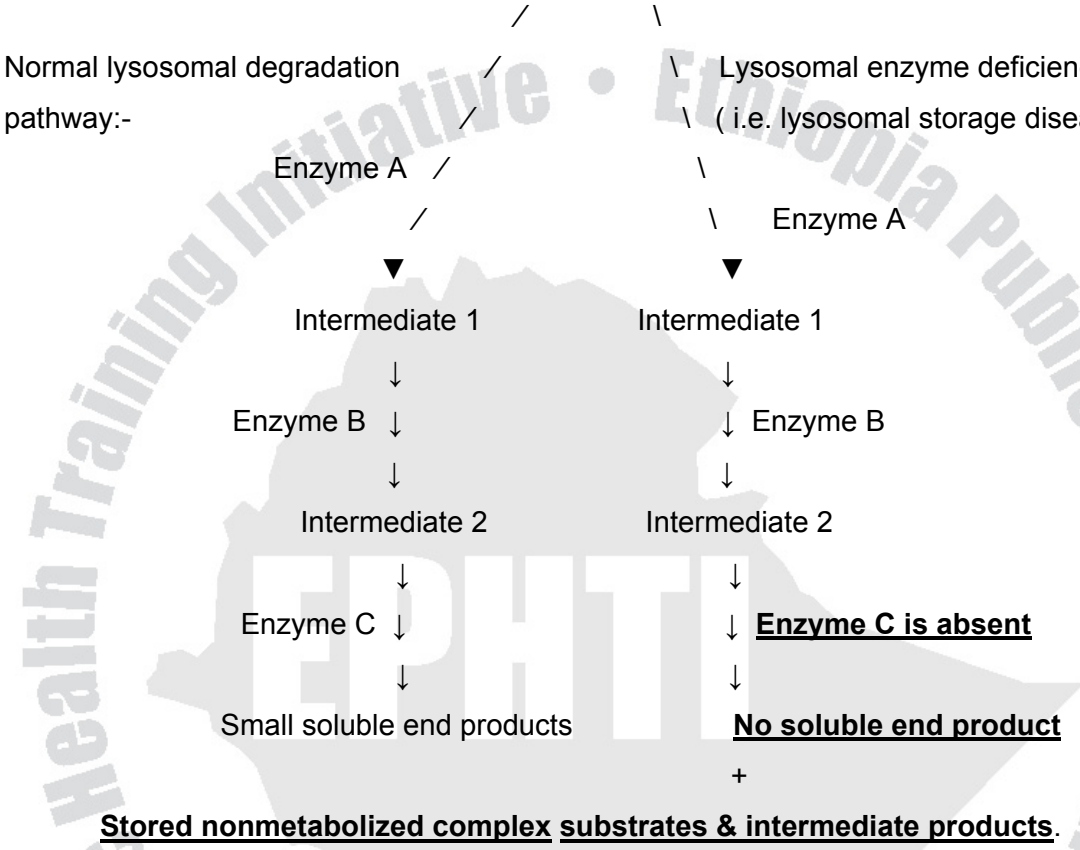


Fig. 6. 4. Normal lysosomal degradation Vs lysosomal storage diseases.

Lysosomal storage diseases can be divided into the following subgroups based on the nature of the accumulated substance:

a. Sphingolipidoses
   e.g. Tay-Sachs disease in which there is deficiency of the alpha subunit of the enzyme – hexosaminidase leading to the accumulation of GM2 gangliosides.
b. Sulfatidoses e.g. 1. Gaucher disease e.g. 2. Niemann-Pick disease types A & B (have deficiency of sphingomyelinase resulting in the accumulation of sphingomyelin).
c. Muopolysacharidoses (MPS)
d. Mucolipidoses (ML)
e. Type 2 glycogenosis (Pompe disease)
f. etc…

The organs affected in lysosomal storage diseases (i.e. the distribution of the stored material) are determined by the following 2 factors:

i. The site where most of the material to be degraded is found.

E.g.1. Brain is rich in gangliosides, hence defective degradation of gangliosides as in Tay-Sachs disease results in the storage of gangliosides within neurons leading to neurologic symptoms. E.g.2. Mucopolysaccharides are widely distributed in the body. Hence, mucopolysacharidoses (i.e. defects in the degradation of polysaccharides) affect virtually any organ.

ii. The location where most of the degradation normally occurs.

Organs rich in phagocytic cells such as the spleen & liver are frequently enlarged in several forms of lysosomal storage diseases. This is because cells of the mononuclear phagocytic system are rich in lysosomes & are involved in the degradation of a variety of substrates.

From among the various types of lysosomal storage diseases listed above, only Gaucher disease is discussed here to illustrate the basic principles of lysosomal storage diseases.

Gaucher disease

- is the most common lysosomal storage disorder.
- is a disorder of lipid metabolism caused by mutations in the gene encoding glucocerebrosidase.→ Deficiency of glucocerebrosidase→ Accumulation of glucocerebroside mainly in the cells of the mononuclear phagocyte system & sometimes in the central nervous system. Glucocerebrosides are continually formed from the catabolism of glycolipids derived mainly from the cell membranes of old red blood cells & white blood cells.
- morphologically shows Gaucher cells (distended phagocytic cells with a distinctive wrinkled tissue paper cytoplasmic appearance).
- - has 3 clinical subtypes : Type I (chronic nonneuronopathic), Type II (acute neuronopathic) , & Type III (Juvenile).

Type I (Chronic non-neuronopathic form) (Adult Gaucher disease):-

- accounts for 99% of the cases.
- is found mainly in European Jews.
- does not involve the brain.
- shows accumulation of gluccocerebrosides only in the cells of the mononuclear phagocytic system throughout the body.

- Hence, it shows Gaucher cells in the spleen, liver, lymph nodes, & bone marrow.
- clinically manifests by
    - Splenomegaly→Hypersplenism→Pancytopenia.
    - Hepatomegaly
    - Generalized lymphadenopathy
    - Pathologic fractures & bone pain due to erosion of the bone.
    - First appearance of sings & symptoms in adult life.
    - Progressive disease which is compatible with long life.

Phenylketonuria (PKU)

- is caused by mutation of the phenylalanine hydroxylase gene → Phenylalanine hydroxylase deficiency → Failure of conversion of phenylalanine to tyrosine in the liver →High serum concentration of phenylalanine which is neurotoxic →Progressive cerebralmyelination

In addition the minor pathways of phenylalanine metabolism produce phenyl pyruvic acid ("phenylketone") & phenyl acetic acid which are excreted via the urine.

- is clinically characterized by:
    - Progressive mental deterioration usually pronounced by age 1.
    - Seizures.
    - Hyperactivity & other neurologic abnormalities.
    - Decreased pigmentation of hair, eyes, & skin. (Children are characteristically blond & blue-eyed).
    - Mousy body odour from phenylacetic acid in urine & sweat.
- can be successfully treated by a phenylalanine–free diet.
- Screening tests for serum phenylalanine or urinary catabolites are ordinarily performed on the 3rd or 4th day of life . But this is not currently done in Ethiopia.

3. X-linked recessive inheritance

All sex-linked disorders are X-linked. There is no Y-liked inheritance because Y-linked mutations result in infertility. X-linked disorders can be either recessive (almost all) or dominant (rare).

X-linked recessive inheritance:-

- is suspected when several male relatives in the female line of the family are affected.

a. Criteria:-

i. In the mating of a heterozygous carrier female parent & a normal male parent (the most frequent setting), the sons are hemizygous affected 50% of the time (i.e. the sons have 50% chance of being affected) & the daughters are normal heterozygous carriers 50% of the time & normal homozygotes 50% of the time. See Fig.3 below.

ii. Affected daughters are produced by matings of heterozygous females with affected males.

iii. No male-to-male (i.e. father-to-son) transmission of the trait (in all sex-linked inheritance). This is because a male contributes his Y chromosome to his son & does not contribute an X-chromosome to his son. On the other hand, since a male contributes his sole X-chromosome to each daughter, all daughters of a male with an X-linked disorder will inherit the mutant allele. All female offspring of affected males are carriers if the mother is normal.

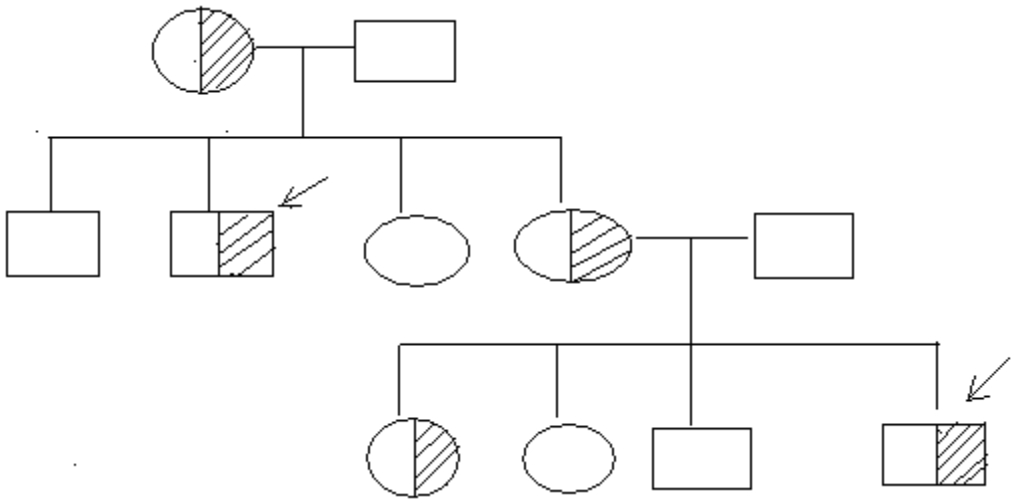


Fig.6.5. X-linked recessive pattern.

This figure shows an extended pedigree of an X-linked recessive disorder in which the male parents (in both generations) are normal & the female parents carriers. In contrast to the vertical distribution in dominant traits (parents & children affected) & the horizontal distribution in autosomal recessive traits (sibs affected), the pedigree pattern in X –linked recessive traits tends to be oblique, i.e. the trait manifests in the maternal uncles of affected males & in male cousins who are descended from the mother's sisters who are carriers.

b. Pathogenesis of X-linked recessive disorders

The genes responsible for X-linked disorders are located on the X-chromosome, & the clinical risks are different for the 2 sexes.

Since a female has 2 X chromosomes, she may be either homozygous or heterozygous for a mutant gene, & the mutant allele may demonstrate either dominant or recessive expression.

The homozygous female (i.e. having the mutation in both the X chromosomes) will express the full phenotypic change of the disease

Clinical expression of X-linked recessive disorders in heterozygous females is often variable & is influenced by the normal random X-chromosome inactivation (i.e. lyonization) (See below). Normally, one of the two X-chromosomes in females is randomly inactivated. Therefore, in heterozygous females carrying X-linked recessive mutations, some cells have one active normal X chromosome & other cells have an active abnormal X chromosome containing the mutant allele. I.e. such females have a variable proportion of cells in which the mutant X-chromosome is active. Often the mutant allele is activated in only some of the cells. Therefore, the heterozygous female expresses the disorder partially & with less severity than hemizygous men. I.e. she usually does not express the full phenotypic change. E.g. G6PD deficiency. Very rarely, the mutant allele may be activated in most cells & this results in full expression of a heterozygous X-linked recessive condition in the female.

In males, the Y chromosome is not homologous to the X-chromosome. So mutant genes on the X are not paired with alleles on the Y. The male is, therefore, said to be hemizygous (& not heterozygous) for the X-linked mutant genes. **Males have only oner X-chromosome, so they will clinically show the full phenotype of X-linked recessive diseases, regardless of whether the mutation produces a recessive or dominant allele in the female.** Thus, the terms X-linked dominant or X-linked recessive refer only to the expression of the mutations in women.

c. Clinical examples of X-linked recessive disorders include:

- Hemophilia A & B
- Chronic granulomatous disease of childhood
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency
- Agammaglobulinemia
- Wiskott -Aldrich syndrome

- Diabetes insipidus
- Lesch-Nyhan syndrome
- Fragile X syndrome
- Duchenne muscular dystrophy

X-linked dominant inheritance

- is a rare variant of X-linked inheritance.
- is when heterozygous females & hemizygous males phenotypically manifest the disorder.
- is caused by dominant disease alleles on the X-chromosome.
- is transmitted by an affected heterozygous female to half her sons & half her daughters.
- is transmitted by an affected male parent to all his daughters but none of his sons, if the female parent is unaffected.
- is exemplified by vitamin D-resistant rickets.

Mitochondrial inheritance

- is mediated by maternally transmitted mitochondrial genes, which are inherited exclusively by maternal transmission.
- is a rare form of inheritance mentioned here just for the sake of completeness.

VII. Chromosomal disorders (Cytogenetic disorders)

- are caused by chromosome & genome mutations (i.e. abnormal structure & number of chromosomes respectively).
- are not uncommon. They are found in 50% of early spontaneous abortuses, in 5% of stillbirths, & in 0.5 -1% of live born infants.
- may, therefore, be suspected in the following clinical situations:
    - Spontaneous abortion
    - Stillbirth
    - Abnormal live births
    - Infertile couple

The following subtopics will be discussed below:

A. Normal karyotype

- Chromosome identification & nomenclature

B. Types of chromosomal abnormalities

C. Abnormalities of autosomal chromosomes

E.g. Down syndrome

D. Abnormalities of sex chromosomes

E.g. 1. Klinefelter syndrome

E.g. 2. Turner syndrome

A. The normal karyotype

Chromosome classification & nomenclature:

Karyotype is the chromosome constitution of an individual. The term is also used for a photomicrograph of the chromosomes of an individual arranged in the standard classification (i.e. metaphase chromosomes arranged in order of decreasing length).

Karyotyping means the process of preparing such a photomicrograph. I.e. it is the study of chromosomes. Karyotyping uses many types of techniques of which G-banding is the most common procedure.

G-banding has the following steps:-

- Arrest dividing cells in metaphase by using colchicine.
- Stain the metaphase chromosomes using Giemsa stain, hence thename G-banding.→The metaphase chromosomes will show alternating dark staining & light-staining bands. The dark bands are by convention called G bands, & the light bands are R bands. About 400 -800 dark & light bands can be seen in a haploid set of chromosomes using G banding.
- Each chromosome can be identified based on its banding pattern & length. The chromosome pairs are arranged in decreasing order of their length. And the first chromosome in such an arrangement is called chromosome 1, the 2nd chromosome is called chromosome 2, etc…. up to chromosome 23. The banding can also identify breakpoints & other chromosomal alterations.

Metaphase chromosomes are divided longitudinally into 2 sister chromatids held together at the centromere, which delineates the chromosome into a short arm (p) & a long arm (q).

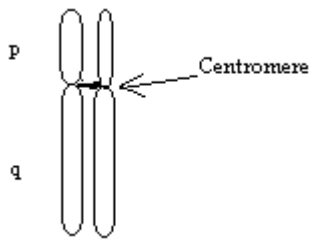


Fig. 6.6. A pair of chromosomes.

The position of the centromere is used in the morphologic description of a chromosome:

Metacentric chromosomes have more central centromeres.

Acrocentric chromosomes have a centromere that is very close to one end. E.g. chromosomes 13 & 21.

In a banded karyotype, each arm of the chromosome is divided into 2 or more regions. The regions are numbered e.g. 1, 2, and 3 from the centromere progressing to the telomere. Each region is further subdivided into bands & sub bands which are also similarly numbered. Thus, Xq22.1 refers to a segment located on the long arm of the X chromosome in region 2 band 2, sub band 1. The following figure illustrates this.

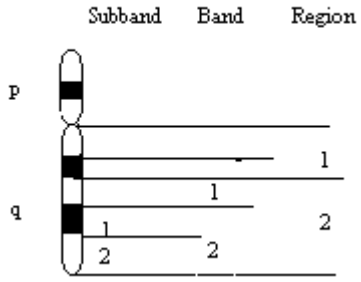


Fig. 6.7. Nomenclature of a chromosome showing the division of the long arm (q) of the chromosome into regions 1 & 2. Region 2 is further subdivided into bands 1 & 2. Band 2 is further subdivided into sub bands 1 & 2. Even though not shown in this figure, the other bands of this q arm & the p arm are similarly divided & numbered.

Karyotypes are usually described using a shorthand system of notations. The following order is used to describe karyotypes:

- First the total number of chromosomes is given.
- Second the sex chromosome constitution is given.
- Finally any abnormality is described.

  E.g. 1. A normal female karyotype is 46,XX. E.g. 2. A female with trisomy 21 is described as 47,XX,+21.

B. Types of chromosomal anomalies

- Chromosomal anomalies may be numerical or structural.

1. Numerical anomalies can result in either aneuploidy or polyploidy.

   i. Aneuploidy

   - is addition or loss of 1 or rarely 2 chromosomes.

ii. Polyploidy

- is the addition of complete haploid sets of chromosomes.

2. Structural anomalies are rearrangements of genetic material within or between chromosomes. These may be either genetically balanced or unbalanced. In balanced structural anomalies, there is no change in the amount of essential genetic material whereas in the unbalanced ones there is a gain or loss of essential chromosome segments.

1. Numerical anomalies

a. Aneuploidy

- is a chromosome number that is not an exact multiple of 23 (i.e. the normal haploid number). The most common forms of aneuploidy are trisomy & monosomy. Trisomy is the presence of 3 copies of a particular chromosome instead of the normal 2 copies. I.e. it is the presence of an extra chromosome. Monosomy is the presence of only one copy of a particular chromosome instead of the normal pair. I.e. it is the absence of a single chromosome.
- is caused most often by an addition or loss of 1 or 2 chromosomes. This change may result from anaphase lag or nondisjunction.

i. Anaphase lag

- During meiosis or mitosis, one chromosome lags behind & is left out of the cell nucleus. This results in one normal cell & one cell with monosomy.

ii. Nondisjunction

- is the failure of chromosomes to separate during meiosis or mitosis. The following figure illustrates meiotic nondisjunction.

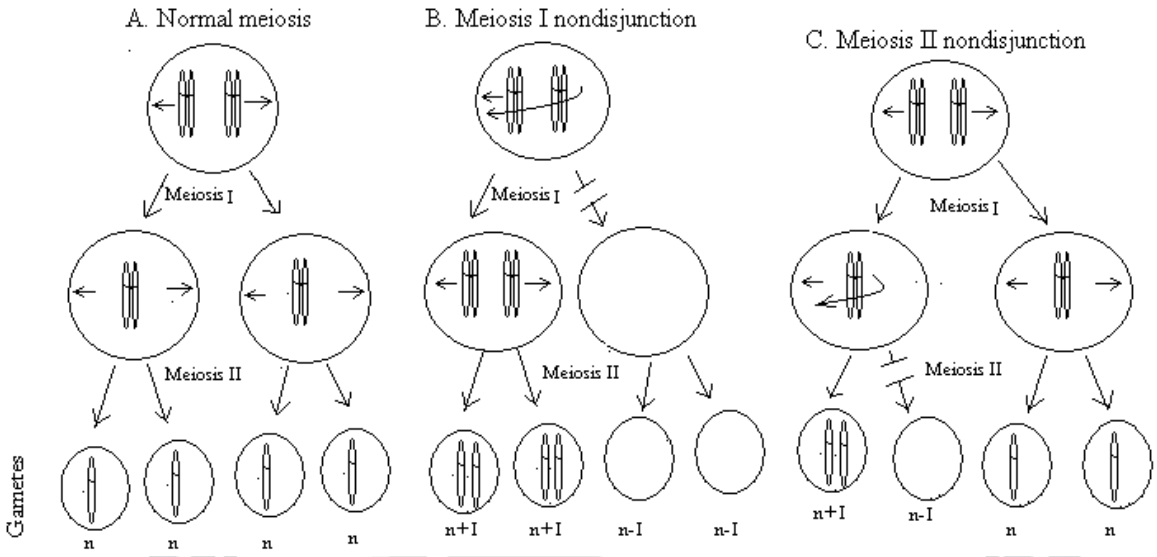


Fig.6.8. (A) Normal disjunction of a chromosome pair during meiosis I & II. (B) In meiosis I nondisjunction, the homologous chromosomes fail to segregate into the 2 daughter cells. (C) In meiosis II nondisjunction, the sister chromatids fail to segregate. The chromosome number in the gametes, which in humans is 23, is denoted by n. As shown in this figure both (B) & (C) produce gametes that are disomic or nullisomic for a specific chromosome. And fertilization of such gametes results in trisomic or monosomic zygotes (i.e. aneuploid zygotes). Meiotic nondisjunction is the most common cause of aneuploidy. It is responsible for disorders such as trisomy 21, the most common form of Down syndrome.

Nondisjunction can also occur in a mitotic division of somatic cells after the formation of the zygote. Mitotic nondisjunction is the failure of sister chromatids to segregate at anaphase (like the meiosis II error shown above). If mitotic nondisjunction occurs at an early stage of embryonic development, then clinically significant mosaicism may result. See the following figure.

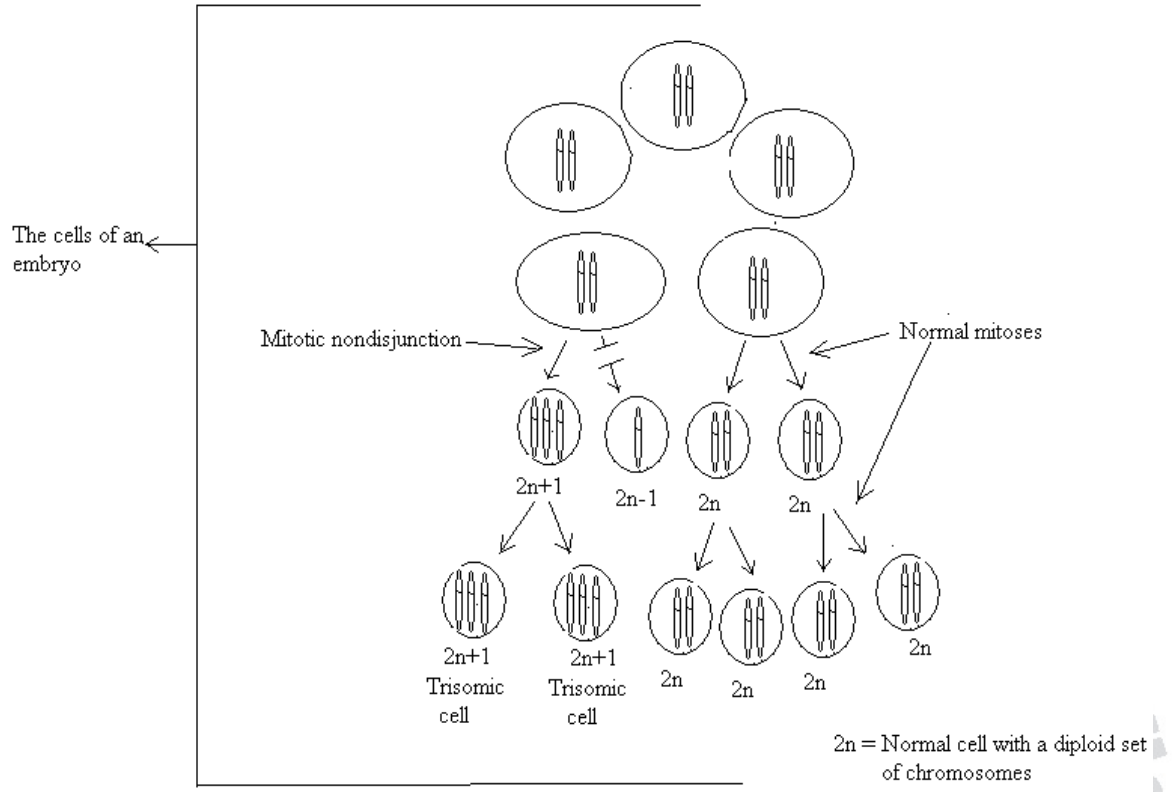


Fig.6.9. Mitotic nondisjunction at an early stage of development of an embryo.

The mitotic nondisjunction occurred in one of cells & resulted in a trisomic cell. All the descendants of this trisomic cell will also be trisomic. Also note that most of the cells undergo normal mitosis resulting in normal cells. Therefore, an individual formed from such an embryo will have 2 populations of cells – a trisomic cell population & a normal cell population. Such an individual is called a mosaic. And the clinical appearance of such an individual depends on the proportion of trisomic cells. Anyway, the clinical feature is less severe than that of an individual in whom all the cells are trisomic.

In general, monosomies & trisomies of the sex chromosomes are compatible with life & usually cause phenotypic abnormalities. But monosomies & trisomies of the autosomal chromosomes are not well tolerated. I.e. monosomies of autosomal chromosomes are lethal to the conceptus. And trisomies of all autosomal chromosomes except chromosomes 13, 18, & 21 cause abortion or early death. However, trisomies of the autosomal chromosomes, 13, 18, & 21 permit survival with phenotypic abnormalities.

b. Polyploidy

- is a chromosome number that is a multiple greater than 2 of the haploid number. Triploidy is 3x the haploid number (i.e.69 chromosomes).Tetraploidy is 4x.
- is rarely compatible with life & usually results in spontaneous abortion.

2. Structural anomalies

- result from breakage of chromosomes followed by loss or rearrangement of genetic material
- are of the following types (See Fig.9 below):

i. Deletion

- is loss of a portion of a chromosome.
- has the following subtypes (See Fig. 9):-

a. Terminal deletions arise from one break. The acentric fragments that are formed are lost at the next cell division. This is denoted by using the prefix 'del' before the notation for the site of the deletion. E.g. 46,XX, del (18)(p14) or it can also be denoted by a minus sign following the number of the chromosome & the sign for the chromosomal arm involved. E.g. 46,XX, 5p- (which indicates deletion of the short arm of chromosome 5)

b. Interstitial deletions arise from 2 breaks, loss of the interstitial acentric segment & fusion at the break sites.

c. Ring chromosomes arise from breaks on either side of the centromere & fusion at the breakpoints on the centric segment. Segments distal to the breaks are lost so that individuals with chromosome rings have deletions from both the long arm & short arm of the chromosome involved. May be denoted as for example 46,XX, r(15).

ii. Isochromosome formation

- results when one arm of a chromosome is lost & the remaining arm is duplicated, resulting in a chromosome consisting of 2 short arms only or 2 long arms only. The arm on one side of the centromere is a mirror image of the other. E.g. i(X)(q10) results in monosomy of the genes on the short arm of X & trisomy of the genes on the long arm of X. (See Fig.9)

iii. Inversion

- is reunion of a chromosome broken at 2 points, in which the internal segment is reinserted in an inverted position.
- are compatible with normal development.

iv. Translocation.

- is an exchange of chromosomal segments between 2 non-homologous chromosomes.
- is denoted by a "t" followed by the involved chromosomes in numerical order.

  E.g. the translocation form of Down's syndrome is designated as t(14q;21q).
- has 2 types (See Fig. 9):

a. Reciprocal (balanced translocation)

- is a break in 2 chromosomes leading to an exchange of chromosomal material between the two chromosomes. Since no genetic material is lost, balanced translocation is often clinically silent. But it can also cause disease as in the t(9,22) which causes chronic myelogenous leukaemia.

b. Robertsonian translocation

- is a variant in which the long arms of 2 acrocentric chromosomes are joined with a common centromere, & the short arms are lost.

Before going into the discussion of some of the chromosomal disorders, it is good to remember what mosaicism is.

Mosaicism

- is the presence of 2 or more cell lines with different karyotypes in a single individual. In a mosaic individual, a normal diploid cell commonly coexists with an abnormal cell line. The abnormal cell line may have a numerical or structural anomaly. A specific cell line may be represented in all tissues or may be confined to single or multiple tissues. The expression of the phenotype depends on the proportion & distribution of the abnormal cell line.
- is caused by mitotic errors in early development (i.e. in the fertilized ovum/embryo). (See above).

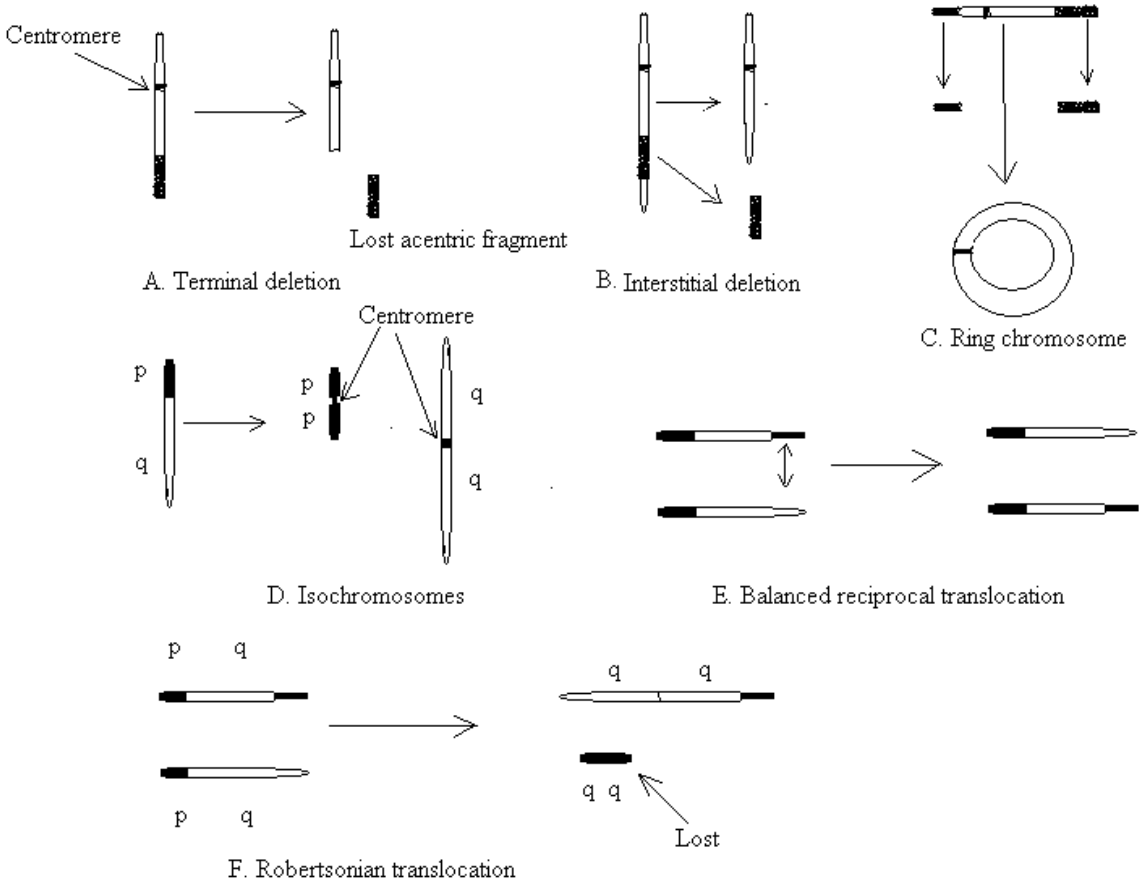


Fig. 6.10. Types of structural anomalies (structural rearrangements) of chromosomes.

C. Cytogenetic disorders involving autosomes

- include:
    - o Down syndrome
    - o Edward syndrome
    - o Patau syndrome
    - o Chromosome 22q11 deletion syndrome

Down syndrome

- is the most frequent chromosomal disorder.
- is caused by:-

1. Trisomy 21
    - accounts for 95% of cases & its incidence increases with maternal age.
    - is produced usually (i.e. in 95% of cases of trisomy) by maternal meiotic nondisjunction. When the cause is paternal nondisjunction, there is no relation to paternal age.

2. Translocation

- accounts for 4% of all cases of Down's syndrome.
- has no relation to maternal age.
- is caused by paternal meiotic robertsonian translocation between chromosome 21 & another chromosome. The fertilized ovum will have 3 chromosomes bearing the chromosome 21 material, the functional equivalent of trisomy 21.
- leads to a familial form of Down's syndrome, with a significant risk of the syndrome in subsequent children

3. Mosaicism

- accounts for 1% of cases.
- usually shows a mixture of cells with 46 & 47 chromosomes.
- results from mitotic nondisjunction of chromosome 21 during an early stage of embryogenesis.
- has milder symptoms depending on the proportion of the abnormal cells.
- is not influenced by maternal age.

Down syndrome

- has the following clinical features:
    - Severe mental retardation
    - Broad (flat) nasal bridge & oblique palpebral fissure. Because of these features the old name of this disease is mongolism.
    - Epicanthic folds
    - Wide-spaced eyes
    - Large protruding tongue
    - Small low-set ears
    - Bruschfield's spots (small white spots in the periphery of the iris).
    - Short broad hands with curvature of the 5^{th} finger, simian crease (a single palmar crease)
    - Unusually wide space between the 1^{st} & the 2^{nd} toes
    - Congenital heart disease (in about 40% of the cases).
    - A 10 – 20 fold increased risk of developing acute leukaemia.
    - Increased susceptibility to infection
    - In patients surviving to middle age, morphologic changes similar to Alzheimer's disease.

D. Cytogenetic disorders involving sex chromosomes

The following subtopics will be discussed below:

1. General features
2. Klinefelter syndrome
3. Turner syndrome
4. Disorders of sexual differentiation

1. General features

Sex chromosomal disorders have the following general features:

a. They generally induce subtle, chronic problems relating to sexual development & fertility.
b. They are often difficult to diagnose at birth & many are first recognized at the time of puberty.
c. The higher the number of the X chromosomes (both males & females), the higher the likelihood of mental retardation.
d. They are far more common than those related to autosomal aberrations.
e. They are better tolerated than autosomal disorders. I.e. extreme karyotype deviations in the sex chromosomes are compatible with life. This is due to the following 2 factors that are unique to the sex chromosomes:
    i. The scant genetic information carried by the Y chromosome &
    ii. The lyonization of the X chromosomes

i. The scant genetic information carried by the Y chromosome:

Most of the Y chromosome appears to be "junk DNA" i.e. repetitive sequences that are without function. But there are some essential genes on the Y chromosome such as the genes which determine the testes, spermatogenesis, etc…. The Y chromosome is both necessary & sufficient for male development. Regardless of the number of the X chromosomes, the presence of a single Y chromosome leads to the male sex.

ii. The lyonization of the X chromosomes (X chromosome inactivation)

In normal female somatic cells, there are 2 X chromosomes, but most of the genes on one of the X chromosomes are inactive. The process by which this occurs is called X chromosome inactivation or lyonization or Lyon's hypothesis.

a. The X chromosome with most of the genes turned off is called the inactive X chromosome. The other one is called the active X chromosome.

b. If a somatic cell contains more than one X chromosome, all but one are inactivated. E.g.1. In a 47, XXY cell, there is one active & one inactive X chromosome (the Y chromosome is irrelevant to the process of X inactivation). E.g. 2 in a 49, XXXXX cell, there is one active X chromosome & 4 inactive chromosomes

c. X inactivation occurs early in embryogenesis among all cells of the bastocyst at about the 16^{th} day of embryonic life.

d. The process of X inactivation is random in any single cell. Either the X chromosome inherited from the mother (called Xm) or the X chromosome inherited from the father (called Xp) may be inactivated with equal likelihood.

e. Once X inactivation occurs in an embryonic cell, the same X chromosome remains inactivated in all of the progeny of that cell. Females are mosaics. On average, half of the cells in a female have an inactive Xm & the other half of the cells have an inactive Xp. However, some tissues (& some women) may have substantially more cells with one or the other X chromosome active by chance.

f. X inactivation involves most, but not all, genes on the X chromosome. Some essential genes must be expressed in 2 copies from both X chromosomes for normal growth & development. For this reason, some essential genes on the X chromosome escape X-inactivation. So if one of these essential genes is absent (as occurs in Turner syndrome), it results in abnormal growth & development. Likewise, the presence of an extra X chromosome (as occurs in Klinefelter syndrome) leads to abnormal phenotype.

g. The inactive X-chromosome may be visible in an interphase cell as a condensed mass of chromatin called the Barr body (X chromatin). The maximum number of Barr bodies seen in a cell is equal to the number of inactivated X chromosomes (i.e. one less than the total number of X chromosomes in the cell). E.g. Normal females (46,XX) will have one Barr body, & individuals with 3 chromosomes (XXX) will have 2 Barr bodies, & those with 4 X chromosomes will have 3, & so on. Counting the number of Barr bodies in somatic cells (usually in smears of buccal mucosa) is the basis of the sex chromatin test for sex chromatin aneuplody. This test is no longer used in the Western countries because karyotyping is much more accurate.

h. X-chromosome inactivation produces dosage compensation. Females normally have 2 X-chromosomes & males have only one. And most of the genes on the X chromosome do not have homologues on the Y chromosome. Despite the fact that females have double doses of most X-linked genes in comparison to males, the amount of X –linked products is usually about the same in males & females. This dosage compensation is produced by X inactivation.

i. X inactivation has the following clinical implications:

1. A female who carries an X-linked recessive mutation on one of her 2 X chromosomes may express the mutant phenotype if most of her cells happen to have inactivated the X chromosome carrying the normal gene.

2. A female carrier of an X-linked recessive disease may not detectable by gene product assays (e.g. by the amount of protein or enzyme activity) if most of her cells happen to inactivate the X chromosome carrying the mutant gene.

3. Although monosomy (i.e. the presence of one instead of the normal 2 copies) for any autosome is lethal early in embryogenesis; monosomy for the X chromosome is relatively common in live born infants & produces a relatively mild phenotype called Turner syndrome.

4. Trisomy (i.e. the presence of 3 rather than the normal 2 copies) of the sex chromosomes produces a much less severe phenotype than trisomy for any of the autosomes. Trisomy of the sex chromosomes produces phenotypic changes because of the triple dosage of the essential genes on the X chromosomes (1 copy of these essential genes on the active X chromosome & 2 copies of the activated 'escapee' essential genes on the inactivated X chromosome). Points 3 & 4 above illustrate that aneuploidy of the sex chromosomes is better tolerated than the aneuploidy of the autosomes.

2. Klinefelter syndrome

- is a disorder that occurs when there are at least 2 X chromosomes & 1 or more Y chromosomes.
- is most often characterized by the karyotype 47, XXY. Variants include additional X chromosomes (e.g. XXXY) & rare mosaic forms. In the typical XXY form, a single Barr body is noted in the buccal smear preparations.
- is caused by parental meiotic nondisjunction & incidence increases with maternal age.
- is characterized by male hypogonadism & its secondary effects.
- shows atrophic testes with decreased spermatogenesis which leads to infertility & decreased testosterone production. In addition, it also shows increased plasma estradiol levels (by unknown mechanism). The ratio of estrogens & testosterone determines the degree of feminization.
- therefore, shows lack of secondary male sexual characteristics (i.e. no deep voice, no beard, no male distribution of pubic hair).

- also shows tall stature (because fusion of the epiphyses is delayed), & eunuchoid appearance with gynecomastia.
- is rarely associated with mental retardation, which is usually mild. The extent of retardation increases with increased number of X chromosomes.
- is usually undiagnosed before puberty.

3. Turner syndrome

- is a disorder that occurs when there is a complete or partial monosomy of the X chromosome.
- is associated with one of the following 3 types of karyotypic abnormalities:
  - 45,X karyotype (in which no Barr bodies are seen in the buccal smear)
  - mosaics (45, X cell plus one or more karyotypically normal or abnormal cell types)
  - structural abnormalities of the X chromosomes which result in partial monosomy of the X chromosome. E.g. deletion of one of the arms of the X chromosome.

This karyotypic heterogeneity associated with Turner's syndrome is responsible for significant variations in phenotype. E.g. 45, XO causes a severe phenotype whereas mosaics may have a normal appearance with only primary amenorrhea.

- is characterized by female hypogonadism & its secondary effects.
- shows:-
  - Replacement of the ovaries by fibrous streaks .
  - Decreased estrogen production & increased pituitary gonadotropins from loss of feedback inhibition.
  - Infantile genitalia & poor breast development & little pubic hair.
  - Short stature (rarely exceeding 150cm in height), webbed neck, shield-like chest with widely spaced nipples, & wide carrying angles of the arms.
  - Lymphedema of the extremities & neck.
  - Congenital heart disease (especially preductal coarctation of the aorta & bicuspid aortic valve).

4. Disorders of sexual differentiation (Sexual ambiguity)

- are said to be present when genetic sex, gonadal sex, or genital sex of an individual are discordant.

i. Definitions

The sex of an individual can be defined on many levels:-

1. Genetic sex

- is determined by the presence or absence of a Y chromosome. No matter how many X chromosomes are present, the presence of a single Y chromosome leads to testicular development & a genetic male. At least one Y chromosome is necessary & sufficient for male gender to manifest.

2. Gonadal sex

- is determined by the presence of ovaries or testes. The gene responsible for the development of the testes is localized to the Y chromosome.

3. Ductal sex

- depends on the presence of derivatives of the Mullerian or Wolffian ducts.

4. Phenotypic or genital sex

- is based on the appearance of the external genitalia.

Sexual ambiguity is present whenever there is discordance among these various criteria for determining sex.

ii. True hermaphrodite

- is very rare.
- has both ovaries & testicular tissue, with ambiguous external genitalia.
- may result from the fusion of 2 sperms (one X-carrying sperm & one Y-carrying sperm) with a binucleated ovum.

iii. Pseudohermaphrodite

- shows discordance between the phenotypic sex & gonadal sex.
- i.e. has gonads of only one sex, but the appearance of the external genitalia does not correspond to the gonads present. Thus, a male pseudohermaphrodite has testicular tissue but female-type genitalia. A female pseudohermaphrodite has a ovaries but male external genitalia (or the external genitalia are not clearly male).

1. Female pseudohermaphroditism

- is caused by exposure of the fetus to increased androgenic hormones during the early part of gestation as occurs in congenital adrenal hyperplasia, androgen-secreting ovarian or adrenal tumor in the mother, or hormones administered to the mother during pregnancy.

2. Male pseudohermaphroditism

- has a Y chromosome & only testes but the genital ducts or the external genitalia are either ambiguous or completely female.
- may be caused by tissue resistance to androgens (called testicular feminization), by defects in testosterone synthesis, or by estrogens administered to the mother during pregnancy.

V. Disorders with multifactorial inheritance

- are more common than mendelian disorders.
- result from the combined actions of environmental factors & 2 or more mutant genes having additive effects (i.e. the greater the number of inherited mutant genes, the more severe the phenotypic expression of the disease). The disease clinically manifests only when the combined influences of the genes & the environment cross a certain threshold.
- include such common diseases as:-
    - Diabetes mellitus,
    - Hypertension,
    - Ischemic heart disease,
    - Gout,
    - Schizophrenia,
    - Bipolar disorders,
    - Neural tube defects,
    - Cleft lip/ cleft palate,
    - Pyloric stenosis,
    - Congenital heart disease, etc….
- are characterized by the following features:-

1. The risk of expressing a multifactorial disorder partly depends on the number of inherited mutant genes.

   Hence, if a patient has more severe expression of the disease, then his relatives have a greater risk of expressing the disease (because they have a higher chance inheriting a

greater number of the mutant gene). In addition, the greater the number of affected relatives, the higher the risk for other relatives.

2. The risk of recurrence of the disorder is the same for all first degree relatives of the affected individual & this is in the range of 2-7%. First-degree relatives are parents, siblings, & offspring. Hence, if parents have had one affected child, then risk that the next child will be affected is between 2 & 7%. Similarly, there is the same chance that one of the parents will be affected.
3. The concordance rate for identical twins (i.e. the probability that both identical twins will be affected) is 20 – 40% but this is much greater than the concordance rate for non-identical twins.
4. The risk of recurrence of the phenotypic abnormality in subsequent pregnancies depends on the outcome in previous pregnancies. When one child is affected, the chance that the next child will be affected is 7%. When 2 children are affected, then the chance that the next child will be affected increases to 9%.

VII. Single gene disorders with nonclassic inheritance

- are rare & are briefly mentioned here.
- can be classified into the following categories:
  - A. Diseases caused by mutations in mitochondrial genes.
    - E.g. Leber hereditary optic neuropathy
  - B. Diseases associated with genomic imprinting
    - E.g. Prader-Willi syndrome, Angelman syndrome
  - C. Diseases associated with gonadal mosaicism

    Gonadal mosaicism can explain unusual pedigrees seen in some autosomal dominant disorders such as osteogenesis imperfecta in which phenotypically normal parents have more than **one** affected children. This cannot be explained by new mutations. Instead, it can be explained by gonadal mosaicism
  - D. Disorders caused by triplet repeat mutations
    - E.g. **Fragile X syndrome**
- is the second most frequent cause of hereditary mental retardation next to Down syndrome.
- is clinically manifest in both males & females. In males, it is characterized by bilateral macro-orchidism (enlarged testes).

VIII. Review Exerecise

1. What is mutation? List the various types of mutations & discuss their effects by giving examples for each type.
2. What are the 4 major categories of genetic diseases?
3. Classify mendelian disorders based on their patterns of inheritance.
4. Explain the criteria, the pathogenesis, & give clinical examples for the 3 main mendelian patterns of inheritance.
5. Explain the general pathogenesis of mendelian disorders associated with enzyme defects.
6. Discuss the various types of numerical & structural chromosomal anomalies.
7. Describe meiotic & mitotic nondisjunction.
8. What is Down syndrome? Describe its causes & its clinical features.
9. What are the clinical consequences of X chromosome inactivation?
10. Describe the karyotypes & the clinical features of Turner syndrome Hypofunction of which organ can explain these clinical features?
11. Describe the cause, the karyotypes, & the clinical features of Klinefelter syndrome. Hypofunction of which organ can explain these clinical features?
12. W/ro Almaz is pregnant for the second time. Her first child, Abebe has disease X. W/ro Almaz has 2 brothers, Tesfaye & Fantu, & a sister Desta. Fantu & Desta are unmarried. Tesfaye is married to an unrelated woman called Tenagne, & has a 2 year old daughter, Mimi. W/ro Almaz's parents are Ato Kebede & W/ro Beletech. Beletech's sister who is called W/ro Kelemuwa is the mother of W/ro Almaz's husband, Ato Worku, who is 25 years old. There is no previous family history of disease X.
    a. Draw the pedigree using standard symbols.
    b. What is the pattern of transmission of the of disease X, & what is the risk of disease X for W/ro Almaz's next child?
    c. Which people in this pedigree are necessarily heterozygores?
13. What does the term "multifactorial inheritance" mean? Mention some common multifactorial disorders. Describe the 4 common general features of multifactorial disorders.

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CHAPTER SEVEN

IMMUNOPATHOLOGY

I. Learning objectives

At the end of the chapter, the student is expected to:

1. Learn mechanisms and examples of hypersensitivity reaction
2. Understand etiologic factors in autoimmune diseases
3. Have bird's eye view concept on immunodeficiency states

Before reading this chapter, the student is advised to review his/her immunology text or lecture note.

Disorders of the immune system are divided into three broad categories:

1. Hypersensitivity reactions (immunologically mediated tissue injury)
2. Autoimmune diseases
3. Immunodeficiency diseases

We will discuss these categories sequentially.

II. Hypersensitivity Reactions

The purpose of the immune response is to protect against invasion by foreign organisms, but they often lead to host tissue damage. An exaggerated immune response that results in tissue injury is broadly referred to as a hypersensitivity reaction.

Classification:

a. According to Gell and Comb's classification, hypersensitivity reactions can be divided into four types (type I, II, III, and IV) depending on the mechanism of immune recognition involved and on the inflammatory mediator system recruited.

b. Types – I II, and III reactions are dependent on the interaction of specific antibodies with the given antigen, whereas, in type IV reactions recognition is achieved by antigen receptors on T-cells.

1) Type I hypersensitivity (anaphylactic or immediate type) reaction

Definition: Type I hypersensitivity reaction may be defined as a rapidly developing Immunologic reaction occurring, within minutes after the combination of an antigen with antibody bound to mast cells or basophilic in individuals previously sensitized o the antigen. The reactions depend on the site of antigen exposure for example in skin – hive, upper respiratory tract – Hay fever, bronchial asthma and systemic reaction – anaphylactic syndrome

Pathogenesis:

- -Presentation of the antigen (allergen) to precursor of TH2cells by antigen presenting dendritic cells on epithelial surfaces
- Newly minted TH2 cells produce clusters of cytokines including IL-3,IL-4,IL-5 and GM-CSF
- The IL-4 is essential for activation of B cells to produce IgE and IL-3 and IL-5 are important for the survival of eosinophillic activation
- The IgE antibodies produced has a high affinity to attach to mast cells and basophiles
- A mast cell or basophil armed with cytophilic IgE antibodies is re-exposed to the specific allergen
- In this process multivalent antigens binds to more than one IgE molecules and cause cross-linkage with adjacent IgE antibody.
- This bridging of IgE molecules activates signal transduction pathways from cytoplasmic portion of IgE fc receptors. This signal initiates two parallel but independent processes. One leading to mast cell degranulation with discharge of preformed (primary) mediators and the other involving denovo synthesis and release of secondary mediators.
    (I) Mast cell degranulation discharge preformed granules such as primary mediators including biogenic amines, histamine, adenosine, eosinophlic and neutrophlic chemotactic factors, enzymes including proteases, and several acid hydrolases.
    (II) The other involved is de novo synthesis and release of secondary mediators such as arachidomic metabolites
        - Leukotriens C_4 & D_4 – most potent vasoactive and spasmogenic agents known by – highly chemotatic for neutrophiles, eosinophiles and monocytes
        - Prostaglandin D_2 – causes intense bronchospasm & increase mucus secretion
        - Platelet-activating factor release histamine, ↑ed vascular permeability
        - Cytokines—activation of inflammatory cells

- Thus, type I reactions have two well-defined phases.

a. **Initial phase (response**):

- Characterized by vasodilatation, vascular leakage, and depending on the location, smooth muscle spasm or glandular secretions.

b. **Late phase**

- As it is manifested for example in allergic rhinitis and bronchial asthma, more intense infiltration of eosinophiles, neutrophiles, basophilic, monocytes and CD4 + T cells are encountered and so does tissue destruction (epithelial mucosal cells).
- Mast cells and basophiles are central to the development of Type I reaction. Mast cells are bone marrow driven cells widely distributed in tissues around blood vessels, and sub epithelial sites where type I reaction occurs.

Morphology:

- Histamine and leukotriens are released rapidly from sensitized mast cells and are responsible for intense immediate reaction characterized by edema, mucous secretions and smooth muscles spasms.
- Others exemplified by leukotriens platelet activating factor (PAF), TNF-α and cytokines are responsible for the late phase response by recruiting additional leukocytes, basophilic neutrophiles and eosinophiles. These cells secrete other waves of mediators and thus, damage epithelial cells.
- Eosinophiles are particularly important in the late phase. The armamentarium of eosinophiles is as extensive as the mast cells.
- Prototype example of Morphologic features in type I reactions is exemplified by bronchial asthma with
  -Increased mucous glands with resultant mucous secretion
  -Hypertrophy of bronchial smooth muscles with attending brochoconstriction
  -Edema formations with inflammatory cells infiltrations peribronchially

2) Type II hypersensitivity reaction

Definition:Type II hypersensitivity is mediated by antibodies directed towards antigens present on the surface of exogenous antigens.

Three different antibody-dependent mechanisms are involved in this type of reaction

(i) Complement-dependent reaction

i. Direct lysis:

a) It is effected by complements activation, formation of membrane attack complex (C5 – 9). This membrane attack complex then disrupts cell membrane integrity by drilling a hole. In anucleated cells once and in nucleated cells many attacks of the complex are needed for cell lysis, because the latter ones have abilities to repair cell membrane injuries rapidly.
b) Opsoinization: By C3b, fragment of the complement to the cell surface enhances phagcytosis.

Examples include red blood cells, leukocytes and platelets disorders: Transfusion reaction; haemolytic anemia; Agranuloytosis; Thrombocytopenia; Certain drug reaction

ii. Antibody dependent cell - mediated cytotoxicity /ADCC/

- This type of antibody mediated Cell injury does not involve fixation of complements. The target cells coated with IgG antibodies are killed by a variety of nonsensitized cells that have Fc receptors.
- The non-sensitized cells included in ADCC are monocytes/large granular/ lympholytes / Natural killer cells, neutrophils and eosinophils.
- The cell lysis proceeds without phagocytosis. Example include graft rejection

iii. Antibody-mediated cellular dysfunction

- In some cases, antibodies directed against cell surface receptors impair or dysregulated function without causing cell injury or inflammation. For example: In Myasthenia Gravis, antibodies reactive with acetylcholine receptors in the motor end plates of skeletal muscles impair neuromuscular transmission and cause muscle weakness.
- The converse is noted in Graves disease where antibodies against the thyroid-stimulating hormone receptor on thyroid epithelial cells stimulate the cells to produce more thyroid hormones.

3) Type III hypersensetivity / immune complex-mediated

Type III hypersensitivity reaction is induced by antigen-antibody complex that produces tissue damage as a result of their capacity to activate the complement system. The antibodies involved in this reaction are IgG, IgM or IgA.

Sources of antigens include:

a. Exogenous origin Bacteria –streptococcus (infective endocarditis) Viruses –Hepatitis B virus (Polyarteritis nodosa) Fungi – Actinomycetes (farmer's lung) Parasites – plasmodium species (glomerulonephritis) Drugs – quinidin (hemolytic anemia) Foreign serum (serum sickness)

b. Endogeneous origin

Nuclear components (systemic lupus erythematosis) Immunoglobulins (rheumatoid arthritis) Tumour antigen (glomerulonephritis) Therefore, autoimmune diseases are hypersensitivity diseases in which the exaggerated immune response is directed against the self antigens as exemplified by the above three diseases.

The pathogenesis of systemic immune complex diseases has three phases:

a. Formation of Ag-Ab complex

- Introduction of an antigen into the circulation, then Production of specific antibodies by immuno-competent cells and subsequent antigen antibody formation

b. Deposition of immune complexes

- The mere formation of antigen-antibody complex in the circulation does not imply presence of disease.

Immune deposition depends on:

i) **Size of immune complexes**. Large complexes in great antibody excess are rapidly removed by mononuclear phagocytic system (MPS).Most pathogenic ones are of small or intermediate size / formed in slight Ag excess/

ii) **Functional status of MPS**: MPS clears circulating immune complexes however, its overload or dysfunction increase the persistence of immune complexes in circulation and resulting in tissue depositions.

Other factors for immune deposition include charge of immune complexes, valence of antigen, avidity of the antibody, affinity of the antigen to various tissue components, three-dimenstional /lattice/ structure of the complex, hemodynamic factors, etc.

Sites of immune complex deposition include:

Renal glomeruli, joints, skin, heart, serosal surfaces, & small blood vessels

c. Inflammatory reaction

- After immune complexes are deposited in tissues acute inflammatory reactions ensues and the damage is similar despite the nature and location of tissues. Due to this inflammatory phase two mechanisms operate

i) Activation of complement cascades:

- C-3b, the opsonizing, and -C-5 fragments, the chemotaxins are characterized by neutrophlic aggregation, phagocytosis of complexes and release of lysosomal enzymes that result in necrosis.-C3a, C5a – anaphylatoxins contribute to vascular permeability and contraction of smooth muscles that result in vasodilation and edema-C5-9 – membrane attack complexes formation leads to cell lysis (necrosis)

ii) Activation of neutrophiles and macrophages through their Fc receptors.Neutorphiles and macrophages can be activates by immune complexes even in absence of complements. With either scenario, phagocytosis of immune complexes is effected with subsequent release of chemical mediators at site of immune deposition and subsequent tissue necrosis.

Morphology of immune complex-mediated hypersensitivity reaction

- The morphologic consequences are dominated by acute necrotizing vasculitis with intense neutrophilic exudation permiting the entire arterial wall. Affected glomeruli are hyper cellular with proliferation of endothelial and mesengial cells accompanied by neutrophillic and mononuclear infiltration. Arthritis may also occur.

Classification of immune complex-mediated diseases:

Immune complex-mediated diseases can be categorized into systemic immune complexes diseases (e.g. serum sickness) and localized diseases (e.g.Arthus reaction).

Systemic immune-complex diseases:

- Acute forms: If the disease results from a single large exposure of antigen / ex: acute post-streptococal glomerulonephritis and acute serum sickness/ all lesion then tend to resolve owing to catabolism of the immune complexes.

Arthus reaction:

- The Arthur reaction is defined as a localized area of tissue necrosis resulting from an immune complex vasculitis usually elicited in the skin. Arthus reaction occurs at site of inoculation of an antigen and depends on the presence of precipitating antibody in the

circulation / with antibody excess/ that resulted in immune complex deposition. Inflammatory reaction develops over 4-8 hours and may progress to tissue necrosis as described above.

- **Chronic forms of systemic immune complex diseases** result from repeated or prolonged exposure of an antigen. Continuous antigen is necessary for the development of chronic immune complex disease. Excess ones are most likely to be deposited in vascular beds.

Clinical examples of systemic immune complex diseases:

Various types of glomerulonephritis
Rhematic fever
Various vasculitides
Quartan nephropathy
Systemic lupus erytomatosis
Rheumatoid arthritis

4) Type IV hypersensitivity (Cell-mediated) reaction

Definition: The cell-mediated type of hypersensitivity is initiated by specifically sensitized T-lymphocytes. It includes the classic delayed type hypersensitivity reactions initiated by CD4+Tcell and direct cell cytotoxicity mediated by CD8+Tcell. Typical variety of intracellular microbial agents including M. tuberculosis and so many viruses, fungi, as well as contact dermatitis and graft rejection are examples of type IV reactions

The two forms of type IV hypersensitivity are:

1. **Delayed type hypersensitivity:** this is typically seen in tuberculin reaction, which is produced by the intra-cutaneous injection of tuberculin, a protein lipopolysaccharide component of the tubercle bacilli.

Steps involved in type IV reaction include

a. First the individual is exposed to an antigen for example to the tubercle bacilli where surface monocytes or epidermal dendritic (Langhane's) cells engulf the bacilli and present it to naïve CD4+ T-cells through MHC type ll antigens found on surfaces of antigen presenting cells (APC),
b. The initial macrophage (APC) and lymphocytes interactions result in differentiation of CD4+TH type one cells

c. Some of these activated cells so formed enter into the circulation and remain in the memory pool of T cells for long period of time.
d. An intracutanous injection of the tuberculin for example to a person previously exposed individual to the tubercle bacilli , the memory TH1 cells interact with the antigen on the surface of APC and are activated with formation of granulomatous reactions

2. **T-cell mediated cytotoxicity**

In this variant of type IV reaction, sensitized CD8+T cells kill antigen-bearing cells. Such effector cells are called cytotoxic T lymphocytes (CTLs). CTLs are directed against cell surface of MHC type I antigens and it plays an important role in graft rejection and in resistance to viral infections. It is believed that many tumour-associated antigens are effected by CTLs. Two mechanisms by which CTLs cause T cell damage are:

- Preforin-Granzyme dependant killing where perforin drill a hole into the cell membrane with resultant osmotic lysis and granzyme activates apoptosis of the target cells
- FAS-FAS ligand dependant killing which induce apoptosis of the target cells

III. Immunologic Tolerance

Immunologic tolerance is a state in which an individual is incapable of developing an immune response to specific antigens. Self-tolerance refers to lack of responsiveness to an individual's antigens. Tolerance can be broadly classified into two groups: central and peripheral tolerance.

i) Central tolerance

- This refers to clonal deletion where immature clones of T and B-lymphocytes that bear receptors for self-antigens are eliminated from the immune system during development in central lymphoid organs. T cells that bear receptors from self-antigens undergo apoptosis within/ during the process of T-cell maturation.

ii) Peripheral tolerance

1. **Clonal deletion by activation–induced cell death**. The engagement of Fas by Fas ligand co-expressed on activated T-cells dampens the immune response by inducing apotosis of activated T-cells (Fas mediated apoptosis)
2. **Clonal anergy**: Activaton of Ag specific T-cell requires two signals
   a). Recognition of peptide Ag wtih self-MHC molecules

b). Co-stimulatory signals such as CD 28 must bind to their ligand called B7-1 and B7-2 on antigen presenting cells (APC) and if the Ag is presented by cell that do not bear CD 28 ligand /i.e B7-1 or B7-2/ a negative signal is delivered and the cell becomes anergic.

iii). Peripheral suppression by T- cell suppressor.

- There are some evidence that peripheral suppression of autoreactivity may be mediated in part by the regulated secretion of cytokines.
- The CD+ T cells of the TH2 type have been implicated in mediating self-tolerance by regulating the functions of pathogenic TH1 type cells. Cytokines produced by TH2 T cell can down regulate autoreactive CD+Th1 by elaborating IL-4, IL-10 and TGF-B.

When normal tolerance of the self antigens by the immune system fails, autoimmune diseases result.

IV. Autoimmune Diseases

- **Definition**: Autoimmunity implies that an immune response has been generated against self-antigens /Autoantigens/. Central to the concept of autoimmune diseases is a breakdown of the ability of the immune system to differentiate between self and non-self antigens. The presence of circulating autoantibodies does not necessarily indicate the presence of autoimmune disease. Thus, pathologic autoimmunity is characterized by
- the autoimmune response is not secondary to tissue injury but it has primary pathologic significance
- Absence of other well-defined cause of disease.

Mechanisms of autoimmune diseases

1. Genetic: Evidences include

- -Familial clustering of several diseases such as SLE, autoimmune hemolytic anemia, Hashimoto's thyroditis.
- -linkage of several autoimmune diseases such as Hashimotos thyroditis, pernicious anemia, Addion's disease, primary hypothyroidism, etc so-called Schmidt's syndrome.
- -Induction of autoimmune diseases with HLA especially class II antigens exemplified by HLA-B27

2. Immunologic:

- **Failure of peripheral tolerance**: Breakdown of T-cell anergy

  T-cell anergy may be broken if the APC can be induced to express co-stimulatory molecules such as B7-1 and to secrete cytokines such as IL-12 that stimulate the generation of TH 1 cells. This up regulation of co-stimulator molecule B7-1 has been noted in multiple sclerosis, Rheumatoid arthritis, psoriasis and Insulin dependant diabetes mellitus (IDD).

Failure of activation induced cell death defects in Fas – Fas ligand

- System in generating apoptosis may allow persistence and proliferation of auto reactive T- cells in peripheral tissues. No known disease is incremented but SLE suggested only on experimental basis.

Failure of T-cell – mediated suppression

- Loss of regulatory or suppressor T-cells can limit the function of auto reactive T and B cells and thus, can lead to autoimmunity. There is evidence that patients with SLE have a deficiency of T-suppressor cells activity that would result in hypergamaglobinmea and the production of autoantibodies.

Molecular mimicry (cross – reacting antigens).

- Some infections agents share epitopes with self-antigens. An immune response against such microbes may produce tissue-damaging reactions against the cross-reacting self-antigen.
- The classic example is streptococcal pharyngitis, in which antibodies are produced to the streptococcal M – protein and cross-react with M – protein of the sarcolemma of cardiac muscle to produce the acute rheumatic fever. Another example is the immunologic cross-reactivity between the glycoprotein D of the herpes simplex virus and certain bacterial antigens with acetylcholine receptor.

Polyclonal B-lymphocytic activation

- Tolerance in some cases is maintained by clonal anergy. Autoimmunity may occur if such self – reactive but anergic clones are stimulated by antigen-independent mechanisms.Several micro-organisms and their products are capable of polyclonal (i.e antigen nonspecific) activation of B –cells.Examples include Epestein-barr virus (in infections mononucleosis), gram-negative lipopolysaccharides (endotoxins).
- Among the T-cells activated by super-antigens, some may be reactive to self-antigens and thus, autoimmunity may result from arousal of such cells (Certain bacterial products can bind to and activate a large pool of CD4 + T-cells in an antigen independent manner. They do so by binding to class II MHC molecules on

APCs and the (beta) **B**. chains of the T-cell receptor (TCR) outside the antigen – binding groove. Because they stimulate all T-cells they are called superantigens)

Release of sequestrated antigens

- Regardless of the exact mechanism by which self-tolerance is achieved (clonal deletion or anergy), it is clear that induction of tolerance requires interaction between the antigen and the immune system. Thus, any self-antigen that is completely sequestrated during development is likely to be viewed as foreign if introduced into the circulation, and an immune response develops.
- Examples include the release of crystalline from the lens of the eye during cataract extraction, or antigens from the uveal tract due to trauma, is followed by autoimmune uveitis. Agglutinating antibodies to spermatozoa may be produced following testicular trauma or rupture of an epidermal retention cyst.
- The mere release of antigens is not sufficient to cause autoimmunity; the inflammation associated with the tissue injury is essential.

3. Microbial agents:

- Some bacteria, mycoplasm and viruses are implicated. Viruses and other microbes may share cross-reacting epitopes with self-antigens. Example: Cross-reaction between certain coxsackieviruses and islet cells antigen glutamic acid decarboxylase. Microbial infections with resultant tissue necrosis and inflammation can cause up regulation of co-stimulatory molecules on resting antigen-presenting cells in tissue, thus favouring a breakdown of T- cell anergy. The inflammatory response also facilitates presentation of cryptic antigens, and thus induces epitope spreading.

Classification of autoimmune diseases

The classification is based on the number of organs involved

- Organ specific autoimmune diseases affect a single organ or tissue including Hashimoto's thyroiditis, Graves disease, 1^{o} myxedema, Diabetes, chronic atropic gastritis, Myasthenia gravis
- Organ nonspecific autoimmune diseases affect many organs and tissues including
  - Systemic lupus erythematosus
  - Rheumatoid arthritis
  - Systemic sclerosis
  - Dermatomyositis
  - Polymyositis

- Polyarteritis Nodosa
- Sojourn's syndrome

Here, only SLE (a prototype of autoimmune diseases) is given as an illustration.

Systemic lupus erythematosis (SLE)

- Systemic lupus erythematosis is a classic prototype of non-organ specific autoimmune disease characterized by a bewildering array of autoantibodies particularly antinuclear antibodies (ANAS).
- It is a chronic remitting and relapsing often-febrile illness characterized principally by injury to the skin, joints, kidney and serosal membranes. Each and very part of the body may be affected. It is common among women of child bearing age and a female to male ratio of 9:1, pick on set 2^{nd} to 4^{th} decade

Etiology and pathogenesis

- The fundamental defect in SLE is a failure of regulatory mechanism that sustains self-tolerance.
- The cause of SLE remains unknown but it appears to be a complex disorder of multi-factorial origin resulting from interactions including:

1. Genetic factors

Increased familial risk, 24% concordance in monozygotic twins (1-3 in dizygotic) defects in early complement components (C_2 or C_4) etc.MHC regulates production of specific auto-antibodies

2. Hormonal factors

Estrogens confer increased risks (10 times more common in females than males) that accelerate during pregnancy and menses. Androgens however, confer decreased risk

3. Environmental factors

Drugs such as hydralazine, pencillin etc induce SLE – like illness) in which all acting in concert to cause activation of helper T-cells and B-cells that results in the secretion of several species of autoantibodies.

Ultraviolet rays

Emotional stress

Surgery

4. Immunologic factors

i) B cell hyperactivity with hypergammaglobulinemia

ii) Autoantibodies present with reactivity to DNA, RNA, or phospholipids thus, antinuclear anti bodies (ANA) are the ones that are directed against several nuclear antigen grouped into four categories

1. Antibodies to DNA
2. Antibodies to history
3. Antibodies to non-histone proteins bound to RNA
4. Antibodies to nucleolar antigens

- ANAs is virtually positive in every patient with SLE i.e sensitive but it is not specific (other autoimmunity positivity. Regardless of the exact sequence by which autoantibodies are formed, they are clearly the mediators of tissue injury. Type III hypersensitivity reaction is responsible for most visceral lesions. DNA – anti – DNA complexes can be detected in the glomeruli and small blood vessels. Autoantibodies against red cells, white cells and platelets mediate their effects via type II hypersensitivity.
- In tissues, nuclei of damaged cells react with ANAs, lose their chromatin pattern, and become homogenous to produce so – called lupus erythematous (LE) bodies or hematoxylin bodies. Related to this phenomenon is the LE cell, which is readily seen, in vitro.Basically, the LE cell may phagocyte leukocytes (neutrophiles or macrophages) that has engulfed the denatured nucleus of an injured cell.

Serum complement levels often decreased due to immune mediated compliment consumption

It is mostly IgG type, lesser extent of the IgM type

Clinicopathologic features

- Typically the patients young woman with a butterfly rash over the face
- Generally the course of the disease is varible and almost unpredictable
- ANAs can be found in virtually 100% of patients but, not specific
- The detection of antibodies against double stranded DNA and Sm antigen are virtually diagnostic of SLE
- Chronic discoid lupus erythmatosis is a disease where its skin involvement may mimic SLE.
- SLE may affect almost any organ system in variable combinations; this vast heterogenicity in clinical presentation requires a clinical index of suspicion followed by laboratory confirmation. Thus, criteria for the diagnosis of SLE are coined such as molar rash, photosensitivity, oral ulcer, arthritis, renal disorders, hematological disorder, immunologic disorder and antinuclear antibody.

- A person is said to have SLE in any four or more of the criteria are present serially or simultaneously.
- Morphologic changes in SLE are also extremely variable. The most characteristic lesions result from the deposition of immune complexes found in the blood vessels, kidneys, connective tissue and skin. Acute necrotizing vasculitis of small arteries and arterioles is characterized by fibrinoid necrosis.
- Kidney: 60 – 70% involvement by SLE. Anti–dsDNA (60-90%associated with nephritis

  WHO morphologic classification of the renal lesions of SLE:

  Class I Normal light etc microscopy

  II Mesengial glomerulonephritis

  III focal proliferative glomerulonephritis

  IV diffuse proliferative glomerulonephritis

  V membranous glomerulonephritis
- Skin: Acute lesions **"**butterfly" rash (50%) where histology shows liqufactive degeneration of the basal layer of the epidermis. Sunlight exposure incites or accentuates the erythema. Chronic lesion: Descoid (plaques with scales, scarring with central atrophy)
- Joints: Arthralgia or non erosive synovitis (unlike rheumatoid arthritis) with little deformity
- CNS: Neuropsycatric manifestations including seizures, (focal or generalized), psychosis and organic brain syndrome.
- Serositis - Acute, subacute or chronic inflammations of the serosal linings pluritis (30-60%), pericarditis (20-30%), peritonitis (60%)
- CVS - Myocarditis leading to arrhythmias, congestive heart failures etc. Non-bacterial varrucous endocarditis (Libbman sacks endocarditis) is a warty deposition of valvular walls. Accelerated coronary atherosclerosis with evidence of angina pectoris and myocardial infection.
- Spleen - is moderately enlarged with focal hyperplasia
- Lungs -Pleuritis and pleural effusion are the most common pulmonary manifestations.

V. Immunodeficiency Diseases

The term immunodeficiency covers a group of disorders of specific immune responses, neutrophil, macrophage and natural killer cells functions, as well as defects in the compliment system that lead to impaired resistance to microbial infections.

Classification – These diseases are crudely classified into primary and secondary types.

1) Primary immunodeficiency diseases (exceedingly rare)

- These disorders usually manifest in early childhood and are almost always genetically determined. Though, some overlap exists primary immunodeficiency diseases are further divided into:

Deficiencies of antibody (B – cells) immunity.

Eg. Infantile X-linked agammaglobinmea

Transient hypogammaglobulinmea of infancy

Deficiencies of cell mediated (T-cell) Immunity

T-cell deficiencies are difficult to trace as T-cells affects B – cell functions

Eg. Di George's syndrome:

Combined T-cell and B-cell deficiencies

Eg Severe combined immunodeficiency disease (SCID).

2) Secondary immunodeficiencies States

These immunodeficiency states may be acquired secondary to various disease processes or drug effects

- **Protein deficiency**

  Lack of protein leads to cell mediated immunity and hypocomplementamia

- **Hematologic malignancies**

  Leukemia and lymphomas where normal functioning cell replaced by neoplastic ones here both humeral and cell mediated immunity are impaired

- **Acute viral infection**

  Especially infectious mononucleosis and misels cause temporary impairment of cell-mediated immunity

- **Chronic renal failure**

  Probably due to toxic effects of accumulated metabolites that affects both B and T-cell functions

- **Iatrogenic**

  Steroids etc for organ transplants, cytotoxic drugs or radiotherapy for the treatment of malignancies.

- **Splenctomy**

  After staging operations of lymphomas or traumatic spleen rupture

  Splenectomy leads to a characteristic immunodeficiency in which the patient is susceptible to infections by phylogenic bacteria especially pneumococal pneumonia.

- **Acquired immunodeficiency syndrome (AIDS)**

  As a prototype example of secondary immunodeficiency states, AIDS is discussed in some detail below.

Acquired Immunodeficiency Syndrome (AIDS)

- AIDS is a retroviral disease characterized by profound immuno suppression that leads to opportunistic infections, secondary neoplasms and neurological manifestations.

Overview:

- **Prevalence**: Currently AIDS affects more than 40 million people all over the world and more than 90 % of the infections prevail in developing countries. Currently, the sub-Saharan Africa in general and South Africa, Ethiopia and Nigeria in particular shoulder the greatest burden of this pan endemic.
- **Age**: Mostly affected individuals are those aged between 15 and 49 years of age however, the epidemiology is quite different in children less than 13 years. Close to 2 % of all AIDS, occur in this age group presently where more than 90 % of this transmission results from transmission of the virus from the mother to the child.
- **Sex**: Women are more vulnerable than men:

  Receptive sexual partners-

  - Uterine, cervical and vaginal conditions that promote HIV transmission easily include cervical erosion, cervical ectopy, sexually transmitted diseases (STD), and cervical cancer.
  - STD often goes unnoticed due to inaccessible anatomic locations.
  - Menstruation: May make the transmission of HIV easier just before, during or after menstruation*.* It results in a large raw exposed area in the inner uterine lining to the virus
  - Those with very low socio economic backgrounds are vulnerable to sex trade (HIV is said to be" the holocaust of the poor").

- Age of earlier sexual contact where the very young female genital linings are vulnerable to easy lacerations.

Modes of transmission:

- **Sexual activities** 75% of all world-wide transmission is heterosexual transmission
- **Parenteral transmission** In intravenous drug abusers, hemophiliacs who received factor viii concentrates and random recipients of blood transfusion
- **Mother to child transmission**

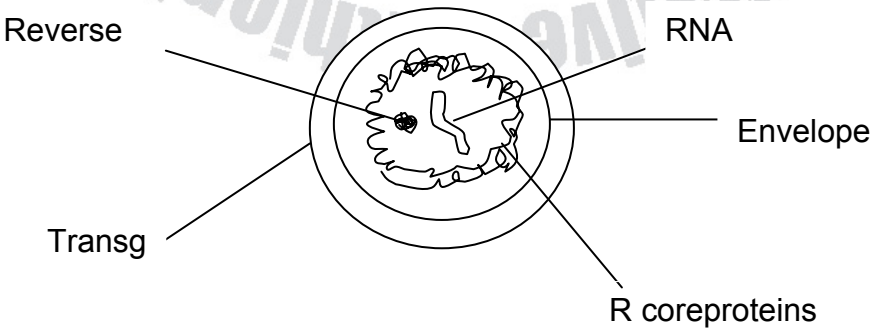
  About 25 –30% HIV, positive mother will transmit HIV to their infants.About 60% of this infection is transmitted during child- birth 25% during pregnancy and 15% during breast-feeding.
- **Needle Pricking** Accidental needle struck injury or exposure to non-intact skin to infected blood in laboratories accounts for about 0.3% risk of stereovision as compared to a 30% risk of accidental exposure to hepatitis B infected blood.

Etiology:

- HIV causes AIDS and HIV is a non-transforming retrovirus belonging to Lentivirus family. The retrovirus undergoes an unusual biologic process in which the genetic material in form of a single stranded RNA, can be converted to double stranded DNA by the effect of reverse transcriptase.
- Two type of HIV viruses

  HIV – 1 - USA, EUROPE, East & central Africa

  HIV - 2 - West Africa
- HIV genome consists of a single stranded RNA enclosed within a core of viral proteins. The core is in-turn enveloped by a phospholipids bilayer deprived from the host cell membrane. The Envelop contains glycoproteins such as gp 120 and GP 41, GP 24.



The viral core consists of two protein shells

- The outer contains the core protein P 18 and the inner core protein P 24. The lipid bilayer consists of the viral glycoprotein gp 41 while gp 120 protrudes into the environment. The RNA genome and the reverse transcriptase are contained within the inner shell.

  HIV – 1 proviral genome contains 3 genes -Gag – caspid protein P^{24}, matrix protein P^{17}, nuclocospial protein $P^{7/9}$

- Pol - revers trancriptase, protease integrase, ribonuclease
- Env – gp^{120} and gp^{41}

In addition to these standard genes, the HIV contains other genes including

TAT- Potent transactivator of viral transcription

NEF- Essential for viral replication

REV- regulator of structural gene expression

VIF- Requires for maturation of HIV virus and if also promotes infectivity of cell free virus

VEF- essential for efficient viral replication

VPU- Required for efficient viron budding

VPR Required for viral replication in non-dividing cells and causing viral replication by causing arrest of cycling cells in G_2

- On the basis of genetic analysis HIV- I can be divided into two groups designated as M (major) and 0 (outliers). Group M viruses are the most common viruses worldwide and subdivided into several subtypes or clades designated A -J.
- The HIV strains can be classified into two groups based on their absolute to infect macrophage and CD4 + T-cells. Macrophage tropic (M-tropic) stains can infect both monocytes/ macrophages and freshly isolated peripheral blood T-cells. T Tropic strains can infect only T- cells both freshly isolated and retained in culture. M – tropic strains use CCR5 co receptors whereas T – Tropic strains bind to CXCR4 co receptors. Ninty percent (90%) of HIV is transmitted by M – tropic strains, however over the course of infection T – Tropic **ciruses** gradually accumulate and these are virulent and cause final rapid phase of the disease progression.

Pathogenesis:

- Targets of HIV infections are: The immune system and Central nervous system
- Target cells are those having CD4 receptors include

  CD4 + T helper cells

Monocytes /macrophages

Tissue cells such as dendritic cells present in genital tracts and anorectal region

Certain brain cells (glial cells)

Some other cells as well

- CD_4 - Receptor molecule is a high affinity receptor for HIV. This explains for the selective tropism of the virus to aforementioned cells.
- Initial binding of gp 120 to CD4 molecule leads to conformational change for the new recognition site on gp120 for the co receptors CCR5 or CXCR4.
- The second conformational change in gp41 results in insertion of a fusion peptide into the cell membrane of the target T-cells or macrophages.
- -After fusion, the viral core containing the HIV genome enters the cytoplasm of the cell (internalization).

The life cycle of HIV virus after internalization, include

- **DNA Synthesis-** the uncoated viral RNA is copied into double stranded DNA by reverse transcriptase
- **Viral integration-t**he DNA derived from the viruses in integrated into host genome by the viral integrate enzyme, thereby producing the latest proviral form of HIV-I.
- **Viral replication-** viral RNA is reproduced by transcriptional activation of the integrated HIV provirus
- **Viral dissemination- t**o complete the life cycle, nascent viruses assembled in the cytoplasm and disseminate to other target cells after directly lysing the cell (direct cytopathic effect of the virus).
- The HIV virus after internalization assumes two forms of infectivity such as latent infection and productive infections. In latent infections, the virus may be lacked in tne cytoplasm (preintegration latency) or after being integrated into host DNA (Post integration latency). Hereafter the highlight of HIV productive infection is surfaced.

- This productive infection is predominantly occur in lymphoid tissues, within macrophages, dendritic cells and CD4+T CELLS.
- Viremia after 8 weeks of infection supervene
- Viremia is subsequently cleared by the development of an anti viral immune response effected by CD8+cytotoxic T cells
- This results in transient decrease in CD4+T cells and an apparent rise in CD8+T cells

- As viremia declines, the HIV disseminates into lymphoid tissues and undergoes clinical latency but not viral latency
- Finally the ebbs and flows of the CD4+T cells count with variable time results in AIDS.

Mechanisms of CD_4 + T cell loss (quantitative defects):

- Loss of immature precursors of CD_4 + T cells by direct infection of thymic progenitor cells or by infection of accessory cells that secrete cytokines essential for CD_4 + cells differentiation.
- Fusions of infected & uninfected cells with formation of syncythia (giant cells) develop ballooning and these cells usually die in few hours.
- Apoptosis of uninfected CD_4 + T cells by binding of soluble gp120 to CD_4 molecule.

Qualitative defects on other immune cells as a result of CD4+T helper/ inducer cells loss:

1. Defects of CD_4 + T cells:

Reduced antigen induced T – Cell proliferation

- Imbalance between the T-helper 1 (TH_1) and TH_2 responses favour humoral immune responses over cell mediated immunity
- Decreased lymphokine secretion

T-cell anergy (dysfunction) can also result from the binding of gp120 Ag-Ab complexes to CD_4 molecules impairing the antigen presentation by anti -gp 120 antibodies that cross-react with and bind to class II HLA molecule on antigen presenting cells.

Anergy also result from binding of HIV derived suferantigen to B-chains of T-cell receptors.

2. Defects of monocytes / macrophages

These cells are extremely important in the pathogenesis of HIV infection. Similar to T cells majority infected cells are found in tissues not in peripheral blood. A relatively high productive infection of macrophages (10 to 15%) is detected in certain tissues such as Brain and lungs.

1) Several aspects of HIV infection of macrophages include

   Hiv-1 can also infect and multiply in terminally differentiated non- dividing macrophages. This property of HIV-1 is dependant on HIV-1 VPR gene.The VPR protein allows nuclear targeting of the HIV pre integration complex through the nuclear pore.

2) Infected macrophages bud relatively small amounts of virus from the cell surface, however, they are quite resistant to the cytopathic effects of HIV.

3) Macrophages in all likelihood act as gatekeepers of infections. More than 90 % of HIV infection is transmitted by M- trophic strains. It has been suggested that macrophages or dendritic cells may be important in the pathogenesis of HIV diseases.

   Thus, HIV infection of macrophages has three important implications.

   I) Monocytes and macrophages represent a veritable virus factory and reservoir whose output remains largely protected from host defences.

   II) Second macrophages provide a safe vehicle for HIV transport to various parts of the body particularly to the central nervous system.

   III) In the late stages of HIV, infection when theCD4+T cells numbers decline greatly, macrophages may be the major sites of continued viral replication.

The following are some of the qualitative changes seen in macrophages -Poor capacity of present antigens to T-cells – (most important one)

- Macrophages are quite resistant to cytopathic effects of the HIV
- impaired microbicidal activity,
- decreased chemotaxis
- Decreased secretion of IL-I
- Increased spontaneous secretion of IL-I, TNF - α, IL – 6.

3. B-lymphocyte dysfunctions

- Hypergamaglobinemia and circulating immune complexes
- Inability to mount do novo antibody response to a new antigen
- Decreased immunoglobuline production in response to new antigen

4. Defets of natural killer cells

- Decreased killing of tumour cells
- CD8 + cytotoxic T-cells: decreased specific cytotoxicity, thus, delayed cell mediated immunity

How a latent HIV infection is transformed into productive infection?

- Antigen or mutagen-induced activation of T-cells is associated with transcription of genes encoding the cytokine IL-2 and its receptor (IL-2R) and after some steps induction of nuclear factor- KB (NF– KB) activates the transcription of HIV provirus DNA and leads ultimately to the production of virons and to cell lysis.
- Cellular activation, by antigens or cytokines (e.g.TNF-∞, IL-1, IL-2) induces cytoplasmic kinases that translocate NF-KB from the cytoplasm to the nucleous. In the nucleous, NF-KB binds to the enhancer sequence within the promoter region and similarly long terminal repeats (LTR) have KB sites. Thus, induction of NF-KB

activates the transcription of HIV proviral DNA and leads ultimately to the production of virons and to cell lysis.

- Furthermore, TNF-∞, also leads to transcriptional activation of HIV –m RNA by production of nuclear factors of that bind to KB enhancer elements of HIV.

There are also profound B – cell dysfunctions such as

- Hypergamagobinemea and circulating immune complexes are due to polyclonal B-cell activation of CMV, and EBV
- Gp^{41} can also promote B – cell growth and differentiation
- HIV infected cells induce increased production of IL –6 which favours activation of B
- – cells

Patients with AIDS are unable to mount antibody response to a new antigen. This could be due to lack of T-cell help. Impaired humeral immunity renders these patients prey to disseminate infections caused by encapsulated bacteria such as S. pneumonia and H. influenza both of which require antibodies for effective optimization. In totality loss of CD4 + T cells, the master cells, has ripple effect on virtually every other cell of the immune system.

Pathogenesis of central nervous system involvement:

- CNS is a major target of HIV infection. Macrophages and imbroglio are the predominant cell types in the brain that are infected with HIV. HIV is believed to be carried to CNS by infected monocytes, which are almost exclusively of M – tropic type. Neurons in CNS are not affected directly but indirectly by viral products and soluble factors produced by macrophages / microglia. Included among soluble factors are IL – I, TNF - α and IL – 6. In addition, nitric oxides also induce neuronal damage (according to most investigators).
- Direct damage of neurons by soluble HIV gp120 has also been postulated. According to some investigators these diverse soluble neurotoxins act by triggering excessive entry of Ca^{2x} into the neurons through their action on glutamate – activated ion – channel that regulate intracellular calcium.

Natural history of HIV infection:

Three phases reflecting the dynamics of virus – host interaction are recognized:

I. Early acute phase

High level of viral production, viremia and widespread seeding of lymphoid tissues. Its spread is controlled by immune system and the virus is mainly swept into the lymph nodes

The decline in HIV viral load usually coincides with the time of sero-conversion and the primary or early HIV infection. Acute phase occurs 4 – 8 weeks after acquiring the virus There may be a short (1 – 2 weeks) seroconversion illness which cause the following in about 50-70% individuals: fever, rash sore throat, muscle and joint pain and some lymph node swelling. The level of viral load in early acute phase of the disease is called the set point and anti retroviral therapy can reduce this set point thus, early detection especially in cases of needle stick injuries, rape and other known risky exercises can benefit from it.

II. The middle chronic phase

Relative containment of the viruses with a period of clinical latency (not viral latency). Patients develop asymptomatic infections. Persistent generalized lymph adenopathyes (PGL) develops and PGL is defined as palpable lymphodenopathy at two or more extra – inguinal sites, persisting for more than 3 months in persons infected with HIV. Minor opportunistic infections such as trush, herpes zoster etc.

III. The final, crisis phase

Characterized by breakdown of host defences with increased plasmal viral load and clinical disease. Typically the patient presents with

Prolonged fever > 1/12

Fatigue, weight loss and diarrhea

CD_4 + T cells < 500 /μl

After a variable period:

(i) Serious opportunistic infections, (Bacterial, Fungal, Viral

(ii) Secondary neoplasms: Kaposi sarcoma, Non Hodgkin's lymphoma, Cervical carcinoma

(iii) Clinical neurological diseases (AIDS defining disease)

Case definition of AIDS according to Centre for disease control (CDC)

Any HIV infected person with fewer than 200 CD_4 + T-cells /μl + is considered to have AIDS. HIV infection progress to AIDS within 7 to 10 years in most cases, however exceptions to this typical course also occur:

- Long term non progresses
- Those who stay symptomatic for 10 years or more with stable CD4 + T-cells count and low plasma viremia
- Rapid Progresses

- Groups of patients with the middle chronic phase is telescoped to 2-3 years after primary infection

Phases of HIV infections and corresponding CDC classification categories

Phase	**CDC categories**
Early acute	Group1 acute infection
Middle, chronic	group2 asymptomatic infection
Group3 PGL	
Final, crisis	Group4
	Subgroup A-constitutional diseases
	Subgroup B-neurological Diseases
	Subgroup C-Secondary infections
	Subgroup D-Secondary neoplasms
	Subgroup E-other Conditions

CDC=Center for Disease Control

The relationship between the immune status, the CD4+ T cell count, the lymphocyte count, and the presence of symptomatic disease:

Clinical condition	Cd4+T cells/m	lymphocytes counts/mm3
Well with no symptoms	More than500-600 cells /mm3	More than 2500
Minor symptoms	350-500cells/mm3	1000- 2500
Major symptoms and opportunistic diseases	200-300cells/mm3	500-1000
AIDS	Less than 200cells/mm3	Less than 500

VI. Exercises

1. Discuss the pathogenic mechanisms of each type of hypersensitivity reaction with examples.
2. Elaborate the mechanisms of autoimmune diseases
3. Discuss SLE.
4. List out conditions that lower immune resistance
5. Discuss the entry, the life cycle and genetic components of HIV virus
6. Elaborate the natural history of HIV infection and AIDS after internalization

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CHAPTER EIGHT
SELECTED TROPICAL DISEASES

I. Learning objectives:

At the end of this chapter, the student is expected to:

Explain the etiology, pathogenesis, morphologic, & some clinical features of typhoid fever, tuberculosis, leprosy, syphilis, malaria, leishmaniasis, schistosomiasis, & selected fungal.

II. Typhoid Fever

Definition: Typoid fever is an acute enteric disease caused by an obligate intracellular bacillus called Salmonella Typhi and this bacillus resides within mononuclear phagocytic cells of lymphoid tissues. The disease is unique humans and it is characterized by fever, splenomegaly and neutropenia.
Transmission: Feco-oral routes through contaminated foods

Carriers:

- convalescent carrier – for up to 6 months of infection
- Chronic fecal and chronic urinary carriers are associated with chronic cholecystitis and pyelonephritis respectively.
- S. mansoni and S. hematobium co-infections protract the course of typhoid fever.

Pathogenesis:

- Infection is by ingestion of the organism, (>10 to the power of 7) in 50% of cases penetrate the small intestine mucosa and reach the circulation with transient bactremia
- The bacilli are taken by the lymphatic to lymph nodes and they are engulfed by mononuclear phagocytic cells.
- After a period of multiplication in these phagocytic cells, the organisms rupture the cells and invade the blood stream via the thoracic duct. The liver, gallbladder, spleen, kidney and bone marrow become infected during this second bactermic phase, characterizing the clinical features of the diseases.

- The main pathological changes are found in the gastrointestinal tract particularly The Payer's patches, which are the sub mucosal lymphoid follicles in this tract. This invasion arises from the gall bladder. Payer's patches may show
  - Hyperplasia in first week
  - Necrosis in second week
  - Ulceration in third week
  - Healing in fourth week
- Typhoid ulcers are oval and are situated longitudinally along the long axis of the colon, which are in contra -distinction of tuberculous ulcers that are set transversally.

Diagnosis:

- Leukopenia 3000-4000/mm^3
- Blood culture - 1st week (70-90%)
- Fecal culture - 2nd – 3rd week best (75%)
- Urine culture - 2nd – 3rd week
- Serology 2nd week

Clinical course:

Typhoid fever is a protracted disease that is associated with

- Bactermia, fever and chills during the first week
- Widespread reticuloendothelial involvement with rash, abdominal pain and prostration in the second week and
- Ulceration of payer's patches with intestinal bleeding and shock during the third week

Complications may include:

- Intestinal perforation: 3 – 4% and it is responsible to 25% of the death
- Intestinal hemorrhage: 8% and usually seen between 14-21 days of illness
- Acute cholecystitis, etc

III. Acute Osteomyelitis

Definition: It is an inflammation of the bone and marrow (osteo- means bone and myelo – marrow), commonly in children and adolescents

Route: Hematogenous spread – most common in long and vertebral bones extension from contagious site- otitis media, dental caries

Direct implantation-compound fracture,

Etiology:

- All types of organisms possible; however, pyogenic organisms most notably Staphylococcus aurous represent 80 - 90% of pyogenic osteomylitis. Others include pseudomonas, Klebsiella, Salmonella in sickle cell anemic patients.

Sites:

- Any bone may be affected but the metaphysics of long bones (distal femur, proximal tibia and humorus) adjacent to actively growing epiphyses and the vertebral column are most often involved.

Pathogenesis:

- The location of the lesions within specific bones is influenced by the vascular circulation, which varies with age. In the neonate, the metaphysical vessels penetrate the growth plate resulting in frequent infection of the metaphysis, epiphysis or both.
- In children, localization of microorganisms in the metaphysics is typical.
- In adults, the epiphyscal growth plate is closed and the metaphysical vessels reunite with their epiphyses counterparts, which provide a route for bacteria to seed in the epiphysis and subchondral regions.
- The susceptibility of the metaphysis to acute osteomylitis is in part, explained by the dilated vascularature of the marrow spaces where sluggish blood flow provides an ideal site for multiplication of bacteria.
- Then acute inflammatory response with exudation follows with venous and arterial thrombosis. These reaction increases intravenous pressure with a resultant bone necrosis. Infection spreads rapidly through marrow spaces which perpetuates the Haversian systems of the metaphysical cortex, elevates the periosteum and forms a subperiosteal abscess in children and adolescents as opposed to adults periosteum that is adherent to the bone.
- Accession of both peri-osteal and endo-osteal vessels lead to segmental bone necrosis of some or all of the diaphysis, the portion of dead bone is known as a **sequestrum**. Small sequestra especially in children tend to be completely absorbed by osteoclastic activity. Large sequestra form a nidus for episodes of infection. In the presence of a sequestrum, the periosteal reactive woven or laminar bone may be deposited as a

sleeve of living tissue known as **involcrum**, around the segment of devitalized bone (sequestrum). The involcrum around sequestrum is usually irregular and perforated.

- In infants, acute ostemylitis may complicate acute arthritis through infrequent it also occurs in adults. The picture is different in children.
- The patient complains of fever, sever pain and tenderness aggravated by any movement, ESR elevated, leukocytosis
- Complications include septicemia, septic arthritis, alteration in growth rate, chronic osteomylitis

IV. Tuberculosis

Tuberculosis is a prototype example of granulomatius inflammation.
Tuberculosis infects one third of world populations and kills about three million people yearly and it is the single most important infectious disease.

Etiology: *Mycobacterium tuberculosis* and *Mycobacterium bovis* are the regular infecting rod shaped, acid fast and alcohol fast, strict aerobic, non-spore forming bacteria with a waxy coat. It has a slow generation time of 4-6 weeks to obtain a colony of mycobacterium tuberculosis. M. tuberculosis is transmitted by inhalation of infective droplets coughed or sneezed into the air by a patient with open tuberculosis, however, M. bovis is transmitted by milk from infected cows. Rarely, it transmits via breached skin surfaces and conjunctiva.
M. Avium and *M. intracellulare* cause disseminated infection in 15%-24% of patients with AIDS.

Pathogenicity of the bacillus is related to its cell wall components. Pathogenicty of tuberculosis is attributed to its cell wall component.

1. **Cord factor** which is a cell wall glycolipid component is aviable on virulent strains
2. **Lipoarabinomannan (LAM)**: It inhibits macrophage activation by interferonδ LAM induce macrophages to secrete TNF - α which causes fever, weight loss, and tissue damage and LAM also induce IL-10 which suppresses mycobacteria induced T-cell proliferation
3. **Complement activated** on the surface of mycobacteria may opsinize the organism and faclitate its uptake by macrophages complement receptor CR3 (mac-1 integrin) without triggering the respiratory burst necessary to kill the organisms.

4. *M. Tuberculosis* **heat shock protein** is similar to human heat shock protein and may have a role in autoimmune reactions induced by M. tuberculosis.

The bacillus resides in phagosome, which are not acidified in lysosomes. Inhibition of acidification has been associated with urase secreted by the mycobacteria.

Who are those more susceptible to develop tuberculosis?

- **Race:** North American Indians, black Africans and Asians are much more susceptible than others
- **Age**: Extremes of ages due to imperfect immune responses
- **Immunologic and other host factors** immunocompromized patients are more liable to develop tuberculosis. These include patients with steroid therapy or immunosuppressive drugs, HIV infection, diabetes mellitus, cirrhosis, malnutrition and damage of lung for example with silicosis etc.

Pathogenesis:

Primary infection: Primary phase of *M. tuberculosis* infection begins with inhalation of the mycobacteria most often in the lower segment of the lower and middle lobes and anterior segment of the lower lobe of the lung. First, the organisms are phagocytosed by alveolar macrophages and transported by these cells to hilar lymph nodes. Naïve macrophages are unable to kill the mycobacteria, thus they multiply and lyse these host cells, infect other macrophages and sometimes disseminate through blood to other parts of the lung and elsewhere in the body.

- After few weeks T-cell mediated immunity is demonstrable by PPD reaction first the CD4 T cells interaction with macrophages secrete interferon, which activate macrophages to kill intracellular mycobacteria through reactive nitrogen intermediates, including NO, NO_2, HNO_3.
- Second CD 8+ suppressor T-cells lyse macrophages infected with mycobacteria through a FAS -independent, granular dependent reaction and kill mycobacteria.
- Third CD4-CD8- (double negative) T cell lyse macrophages in a FAS dependant manner without killing mycobacteria. Lyses of these macrophages results in the formation of caseating granuloma and direct toxicity to the mycobacteria may contribute to the necrotic caseous centers.

The primary infection of sub-pleural lesion, the intervening macrophage reactions within accompanying lymphangitis and the hilar lymph nodes caseous lesions is called **primary complex (often called a Ghon focus)**.

Hence, fate of primary complex include

i). T-cell mediated immune response induces hypersensitivity to the organisms and controls 95% of primary infection. This is associated with progressive fibrosis and calcification of persistent caseous debris. Moreover, most bacilli die but few remain viable for years until the person's immune response fails.

However, if the infected person is immunologically immature, as in a young child or immunocompromized (eg. AIDS patients) the course of this primary infection is quite different. Such persons lack the capacity to coordinate integrated hypersensitivity and cell- mediated immune responses to the organism and thus often lack the capacity to contain the infection. Granulomas are poorly formed or not formed at all, and infection progresses at the primary site in the lung, the regional lymph nodes or at multiple sites of disseminations. This process produces progressive primary tuberculosis.

ii. Progressive **primary tuberculous pneumonia**: commonly seen in children less than five years of age but it ours in adults as well in those with suppressed or defective immunity.

iii. **Subpleural focus** may discharge bacilli or antigen into the pleural cavity resulting in the development of pleural effusion. It is common in adolescent infected with M. tuberculosis for the first time.

Hilar or mediastinal groups of lymph nodes enlargement with caseous necrosis that may result in:

a. Obstruction of the bronchus by the enlarged lymph nodes leading to lobar collapse.

b. The caseous hilar lymph node may penetrate the bronchial wall and resulting in rupture of the wall with pouring of caseous materials into the bronchus hence, tuberculosis broncho-pneumonia ensues.

iv. The caseous materials may be disseminated to other parts of the body via blood streams.

Miliary tuberculosis

It refers to disseminated sites that produce multiple, small yellow nodular lesions in several organs. The term miliary emphasizes the resemblance of the lesion to millet seeds. The lungs, lymph nodes, kidneys, adrenals, bone marow, spleen, menings and liver are common sites for miliary lesions.

v. Seeding of the bacilli in lungs, bones, kidneys, fallopian tubes, bladder, epididimis etc, that may persist in and their subsequent reactivation produces destructive, necrotizing granulmatious disease, sometimes known as **end organ tuberculosis.**

Others sites of primary tuberculosis infection

i. **Intestinal primary infection**

The primary complex is similar to that of the lungs the initial site may be in the gum with lymphatic spread of bacilli to the cervical lymph nodes the commonest location for the primary lesion is the illocaecal region with local mesenteric node involvement.

ii. **Lymph nodes**

Tuberculous lymph adenitis is the most common type of extra pulmonary tuberculosis that frequently involves the cervical groups of lymph nodes with enlargement, and subsequent periadenitis followed by matting and eventual ulcerations if left untreated.

iii. **Skin** is also involved in various forms of tuberculosis

Post -primary (secondary) tuberculosis

Conventionally the term post-primary tuberculosis is used for lung infections occurring 5 years or more after the primary infection. If an adult acquires TB for the first time, it presents as post primary not Primary manifestation. The commonest sites for post primary tuberculosis are the posterior or apical segment of the upper lobe and the superior segment of the lower lobe and their predilection for the anatomy location is due to good ventilation. Hilar lymph node enlargement is not usually recorded. Hypersensitivity reaction is well-developed and it thus, restricts the granulomatous reactions locally. Post primary Tuberculosis is characterized by cavitary and fibrosing lesions. Pulmonary and bronchial arteries around caseous cavities are occluded by endarteritis obliterans where the wall of the artery may weaken resulting in aneurysm formation (mycotic aneurism) that may occasionally rupture and cause hemoptosis.

Post primary (2^0) tuberculosis in endemic countries occurs due to re-infection or reactivation of previously residing bacilli. In non-endemic (uncommon) countries, reactivation phenomenon is more important.

- Infected sputa may be swallowed resulting in tuberculous ulcer in the larynx or small intestine
- Secondary amyloidosis is a common complication of chronic tuberculosis. Certain tissues are relatively resistant to tuberculous infection, so it is rare to find tubercles in the heart, skeletal muscle, thyrord and pancreas.

***M. tuberculosis* and *M. avium intracellulare* lesions in AIDS**

Mycobacteria infection in AIDS patients can take three forms depending on the degree of immunosuppression.

1. HIV infected individuals often have primary and secondary M. tuberculosis infection with the usual well-formed granulomas and acid fast my cobacteria are few in number and Often difficult to find under microscopy.
2. When HIV positive patient develop AIDS with moderate immunosuppression (less than 200 CD4+ heper T-cell /mm^3) which is characterized by failure of helper T-cells to elaborate lymphokines and the relative increase in the number of CD 8+ cytotoxic T-cells may also cause macrophage destruction in the *M. tuberculosis* lesions. This results in less well-formed granulomas, and more frequently necrotic material that contain more abundant acid-fast organisms histologically.

   Sputum is positive for acid-fast bacilli in 31%-82% of patients with AIDS. Extra pulmonary tuberculosis occurs in 70% of such patients involving lymph nodes, blood, CNS and bowel.
3. Opportunistic infection with *M. avium- interacellurare* occurs in severely immuno suppressed patients (less than 60 $CD4^+$ cells /mm^3. Most of these infections originate in the gastrointestinal tract. These infections are usually widely disseminated throughout the reticuloendothelial systems causing enlargement of involved lymph nodes, liver and spleen. The organisms are present in large numbers as many as 10^{10} organism per gram of tissue. Granulomas, lymphocytes and tissue destruction are rare.

Differences between primary and postprimary tuberculosis

	primary TB	**post primary TB**
mainly affected ages	children	adults
Hilar node involvements	Usual	uncommon
type IV reaction	less developed	more developed
tissue lesions	diffuse disease	localized disease
Frequency	Infrequent	Dominant (>80%)

Diagnosis of tuberculosis include:

Radiography

Culture

Zeihl Neelsen stain for Acid fast bacilli

Fine needle aspiration cytology

Exsional biopsy

V. Leprosy

Definiton: Leprosy or Hansen disease is a slowly progressive infection caused by *Mycobacterium leprae* affecting the skin and peripheral nerves and resulting mainly in deformity, paralysis and ulceration. Though *M. leprae* is in most part contained in the skin, the disease is believed to be transmitted from person to person through aerosols from lesions in upper respiratory tract.

Pathogenesis:

- The bacillus is acid fast, obligate intracellular organism that does not grow in culture and it grows best at 32-34 ^{0}C of the temperature of human skin.
- Like *M. tuberculosis, M leprae* secrets no toxins but its virulence is based on properties of its cell wall. The bacilli thus produce either potentially destructive granulomas or by interference with the metabolism of cells. The bacilli are taken by alveolar macrophages; disseminate through the blood but grows only in relatively cool tissues of the skin and extremities.
- Classification based on host immune responses. Leprosy is a bipolar disease. Two forms of the disease occur depending on whether the host mounts a T-cell mediated immune response (tuberculoid leprosy) or the host is anergic (lepromatous leprosy). The polar forms are relatively stable but the borderline forms (border line-tuberculoid, borderline-borderline, and borderline-lepromatous) are unstable without treatment. It may usually deteriorate to lepromatous leprosy. Patients with tuberculoid leprosy form granuloma with few surviving bacteria (paucibacillary disease). The 48 hour leporine skin test is strongly positive and this is effected largely by CD4 $^+$ type 1 helper T-cell that secretes IL-2 & interferon δ .
- In contrast, patients with lepromatous leprosy lack T-cell mediated immunity, and are anergic to lepromin and have diffuse lesions (globi) containing foamy macrophages, stuffed with large numbers of mycobacteria (multibacillary disease). Lepromatous leprosy lesions lack CD4$^+$ type I T-cell at their margins but in stead contain many CD8$^+$ suppressor T-cell in a diffuse pattern. The CD8$^+$ suppressor T-cell secrete IL-10, which inhibits helper-cells and may mediate the anergy seen in lepromatous leprosy. These CD 8+ suppressors T-cell also secrete IL-4, which induce antibody production by B-cell. Antibody production is not protective in lepromatous leprosy and rather the formation of antigen antibody complexes in lepromatous leprosy leads to erythema nodosum leprosum, a life threatening vasculitis, and glomerulonephrits

- Because of the diffuse parasite filled lesions lepromatous leprosy is more infectious than those with tuberculoid leprosy.

Table: **Differences between tuberculoid and lepomatous leprosy**

Tuberculoid leprosy	**Lepromatous leprosy**
Epitheoid granuloma without giant cell	Active macrophages, with every many bacilli (globi)
Dense zone of lymphocyte infiltration around granuloma	Scanty and diffuse
Nerves destroyed by granulomas	May show neuronal damage but not infiltration or cuffing
No clear sub-epidermal zone	Clear sub-epidermal zone
Bacilli in granuloma are not seen	Numerous bacilli 5+ or 6+
Few macules + plaques with well defined edges	Macules, papules, plaques and nodules present with vague edges
Lesions distributed asymmetrically	Lesions distributed symmetrically
hair loss	*no hair loss*
Lesions are anesthetic	Lesions are not anesthetic
Nerve thickening often singly and early	Nerve thickening is symmetrical and late (stocking & glove patterns)
First manifestation may be neural	First manifestation never neuronal
Lepromin test is strongly positive	Lepromin test is negative

Clinical course and complications

- Lepromatous leprosy involves primarily the shin, peripheral nerves, anterior eye, upper airways (down to larynx), testis, hands and feet. The vital organs and the central nervous system are rarely affected presumably because the core temperature is too high for the growth of M.leprae.

VI. Syphilis

Definition: Syphilis is a systemic infection caused by the spirochete *Treponema pallidium,* which is transmitted mainly by direct sexual intercourse (venereal syphilis) and less commonly via placenta (congenital syphilis) or by accidental inoculation from the infectious materials.

- *T. Pallidum* spirochetes cannot be cultured but are detected by silver stains, dark field examination and immunofluorescence technique.

Pathogenesis:

- The organism is delicate and susceptible to drying and does not survive long outside the body.
- The organism invades mucosa directly possibly aided by surface abrasions following intercourse with an infected person, a primary lesion, an ulcer known as the chancre, develops at the site of infection usually the external genetalia but also lips and anorectal region. Within hours, the *T. pallidum* pass to regional lymph nodes and gain access to systemic circulations. Thereafter, the disease is unpredictable. Its incubation period is about 3 weeks.
- Whatever the stage of the disease and location of the lesions the histologic hallmarks of syphilis are
    A. Obliterative endarteritis
    B. Plasma cell rich mononuclear cell infiltrates.
- The endarteritis is secondary to the binding of spirochetes to endothelial cells mediated by fibronectin molecules bound to the surface of the spirochetes. The mononuclear infiltrates are immunologic response.
- Host humeral and cellular immune responses may prevent the formation of chancre on subsequent infections with *T. pallidum* but are insufficient to clear the spirochetes.

Morphology: Syphilis is classified into three stages

Primary syphilis (chancre):

- Chancre appears as a hard, erythematous, firm; painless slightly elevated papule on nodule with regional lymph nodes enlargements. Common sites are Prepuce / scrotum in men-70%,Vulva or cervix in females -50%
- The chancre may last 3-12 weeks. Patients with primary syphilis who stayed for more than two week cannot be reinfected by a challenge.

Secondary syphilis:

- Almost any organ is involved (great mimickery). Widespread mucocutaneous lesions involving the oral cavity, plams of the hands and soles of the feet characterize it.
- There are also generalized lymphadenopathies mucosal patches (snail track ulcers) on the pharynx and genitalia, which is highly infectious.

- Condylomata lata: - which is papular lesions in moist areas such as axillae, perineum, vulva and scrotum, which are stuffed with abundant spirochetes.
- Follicular syphilitidis: - Small papulary lesion around hair follicules that cause loss of hair. Nummular syphilitidis:- It is coin-like lesions involving the face and perineum
- Generalized lymphadenopathy and the uncommon swelling of epithrochlear lymph nodes have long been associated with syphilis.
- Though, asymptomatic, if untreated, secondary syphilis can relapse (latent syphilis) and more episodes of relapses may show a more granulomatous histology in skin lesions and progress to the next stage.

Tertiary syphilis:

The three basic forms of tertiary syphilis are:

1. **Syphilitic gummas** - there are grey white rubbery masses of variable sizes. They occur in most organs but in skin, subcutaneous tissue, bone, Joints and testis. In the liver, scarring as a result of gummas may cause a distinctive hepatic lesion known as hepar lobatum.
   - Collapse of the bridge of the nose and palate can occur with perforation
   - Osteitis and periosteitis may lead to thickening and deformity of long bones such as the sabre tibia
   - Histologically, gummas look like a central coagulative necrosis characterized by peripheral granumatous responses. TheTrepanosomas are scanty in these gummas and difficult to demonstrate.

2**. Cardiovascular syphilis**

- This is most common manifestation of tertiary syphilis. The lesions include aortitis, aortic value regurgitation, aortic aneurysm, and coronary artery ostia stenosis. The proximal aorta affected shows a tree -barking appearance as a result of medial scarring and secondary atherosclerosis. Endartereritis and periaortitis of the vasa vasoum in the wall of the aorta, is responsible for aortic lesions and in time, this may dilate and form aneurysm and eventually rupture classically in the arch.

3. **Neurosyphilis:**

- occurs in about 10% of untreated patients. The neurosyphllis comprises of
  i. **Meningiovascular syphilis** – particularly in base of brain
  ii. **General PARESIS of insane** it affects the cerebral artery with grey matter with subsequent atrophy.

iii. **Tabes dorsalis** – Result of damage by the spirochetes to the sensory nerves in the dorsal roots resulting in locomotion ataxia, Charcots joint, lighting pain and absence of deep tendon reflexes

Congenital syphilis

- This infection is most severe when the mother's infection is recent. Treponemas do not invade the placental tissue or the fetus until the fifth month of gestation (since immunologic competence only commences then) syphilis causes late abortion, still birth or death soon after delivery or It may persist in latent forms to become apparent only during childhood or adult life.
- The out come of congenital syphilis depends on stage of maternal infection (i.e. the degree of maternal spirochataemia). In primary and secondary stages, the fetus is heavily infected and may die of hydrops in utero or shortly after birth. Liver and pancrease show diffuse fibrosis. The placentis is heavy, and pale with plasmacytic villitis. After maternal second stage, the effects of congenital syphilis are progressively less severe.
- Less dramatic visceral disease, papular lesions on skin and mucosae such as the nose snuffles, may be seen with Huchinton's teeth, and interstial keratitis.
- Children infected in utero who are sero -positive show no lesions until two or more years after birth are classified as having late congenital syphilis. The late congenital syphilis is distinctive for the triads: Interstial keratitis; Hutchinson teeth and Eight nerve deafness

VII. Malaria

Malaria is caused by the intracellular protozoan parasite called Plasmodium species and plasomodium Faliprium is the worldwide infections that affect 100 million people and kill 1 to 1.5 million people yearly. P.Falciparium and P.Vivax, P. ovale, and P.malarie represent 60%, 49 %, <1.0% and reported cases respectively in Ethiopia. P. falciparum cause high parasitmias, severe anemia, cerebral symptoms, and pulmonary edma and death.

Pathogenesis (P.Falciparum):

Infected humans produce gametocytes that mosquitoes acquire on feeding. Within these insects' body, the organism produces sporozites, which the mosquito transmits to human when it feeds

- Malarial sporozites after being released in the blood within minutes attach to a serum protein thrombosroridin and properidine located on the basolateral surface of hepatocytes. These sporozites multiply and release merozytes by rupturing liver cells.
- Once released, P. falciparum merozites bind by a parasite lactin like molecule to on the surface of red blood cells
- Within 2 to3 weeks of hepatic infection, merozites rupture from their host hepatocytes and invade erythrocytes establishing erytrocytic phase of malarial infection.
- The merozites feed on hemoglobin grow and reproduce within erythrocytes. Repeated cycles of parasitemia occur with subsequent ruptures of these cells with resultant clinical manifestations such as chills, fever etc.
- P. Vivax merozites however, bind by homologous lectin to the Duffy antigen on RBC so many cases who are Duffy negative are resistant to this infection.
- HLA –B53 associated resistance in some Africans is related to the ability of HLA –B53 to present the liver stage specific malarial antigen to cytotoxic T-cells, which then kill malarial, infected hepatocytes.
- Individuals with sickle cell trait are resistant to malaria because the red cells that are parasitized in these individuals are removed by the spleen.
- Most malarial parasites infect new RBC & some develop to sexual form called gametocytes and the mosquito when it takes this blood meal the cycle continues.

Morphology:

- Spleen enlarged upto 1000gm (normally 150grams) and this splenomegaly can be attributed to increased phagocytosis in splenic reticuloendothelial cells in chronic malaria. The parenchyma imparts grey or blue discolouration due to hemozoin.
- Liver kuffer cells are heavily laden with malarial pigments, parasites, and cellular debris. Pigmented phagocytes may be dispersed through out bone marrow, lymph nodes, subcutaneous tissues and lungs.
- Malignant cerebral malaria: Patients with cerebral malaria have increased amount of inter-cellular adhesion molecules (ICAM- 1). These patients manifest diffuse symmetric encephalopathy; brain vessels are plugged with parasitized red cells. There are ring hemorrhages related to local hypoxia. Cerebral involvement by *P. falciparum* causing 80% of childhood death is due to adhesion of the P. falciparum parasite to endothelial cells with in the brain.

Hypoglycemia- result from failure of hepatic gluconeogenesis & glucose consumption by the host and the parasite lactic acidosis -due to anaerobic glycolysis, non cardiogenic pulmonary edema, renal impairment, anemias etc

P. falciparum, the cause of malignant malaria produces much more aggressive and lethal disease than the other human malarias. This organism is distinguished from other malarial parasites in four aspects.

1) It has no secondary exoerythocytic (hepatic) stage
2) It parasitizes erythrocytes of any stage, causing marked parasitmia and anemia. In other types of malaria only subpopulations of erythrocytes are parasitized, and thus low level parasitemias and more modest anemias occur. P. Vivax and P>Ovale attack immature erythrocytes while P. malarie attack senescent cells.
3) There may be several parasites in single erythrocyte
4) P. falciparum alters the flow characteristics and adhesive qualities of infected erythrocytes so that they adhere to the endothelial cells of small blood vessels frequently produce severe ischemia. Infected red cells sequester inside the small blood vessels at the same time P. falciparum infected RBCS may also adhere to uninfected red cells to form rosettes. The process of cytoadhernce and rosetting are central to the pathogenesis of falcparum malaria in the other three " benign" malarias sequestrations does not occur and all stages of parasites' development are evident on peripheral blood smears.

   P. falciparum invades erythroytes of all ages and is associated with high level parasitemia whereas, P. vivax, P.ovale and P.malariae show predilection for either old red cells or reticuloytes & level of parasitmias seldom exceeds 2 percent.

Malaria in pregnancy

In pregnancy, malaria may be associated with hypoglycemia, fetal distress syndrome and low birth weight. Congenital malaria can occur rarely. P. falcparum malaria is an important cause of fetal death. Congenital malaria occurs in fewer than 5% of newborns.

Malaria in children

Most of the estimated 1-3 million persons who die of falciparum malaria each year are young African children. Convulsion, coma, hypoglycemia, metabolic acidosis and severe anemia are relatively common.

Transfusion malaria

Malaria can be transmitted by blood transfusion, needle -stick injury, sharing of needles by infected drug addicts, or organ transplants. The incubation period is short because there is no pre-erythrocytic stage of development**.**

Complications of malaria include:

Tropical splnomegaly syndrome (**Hyperreactive** malarial splenomegaly),

Burkitt's lymphoma and EBV infection

Quartan malarial nephropathy

Black water fever

Algid malaria

VIII. Leishmaniasis

Definition: Chronic inflammatory disease of skin, mucous membranes or viscera caused by obligate intracellular Kinetoplastid protozoal parasites (Leishmania species) transmitted through infected sand fly.

- Leishmanial infections like with other intracellular organism (including mycobactria, histoplasma, toxoplasma and tryprosoma) is exacerbated by AIDS.
- Different leishmanial parasites in new and old world appeared to show tropism related to temperature, because parasites that cause visceral disease grow at 37% in vitro whereas parasite that cause multiple diseases grow only at 34^0c.
- Leishmania are phagocytozed by macrophage and acidity within phagolysosome induces them to transform into amastigate from promatigate by losing flagella.
- Leismanial amastigotes are the only protozoal parasites that survive and reproduce in macrophage phagolysosomes, which have a PH of 4.5. Amastigotes are protected from the intravascular acid by a proton -transforming ATPase which maintains the intracellular parasite PH at 6.5
- Leismanial parasites have two glycocongugates, which appeared important in their virulence. The first is lipophosphoglycans that are glycolipids & bind C_{3b} and iC_{3b}. Organisms resist lysis by complement $C_{5\text{-}9}$ but are phagocytozed by macrophages through complement receptors CR1 and CR3
- Lipophosphoglycans may also protect the parasite within phagolysosomes by supplying oxygen radical and by inhibiting lysosmal enzymes.
- Like *M. laprae* severity of disease is determined by host immune response.

Parasites specific to CD+T-cells of TH^1 class may secrete interferon gamma which along with TNF-α secreted by other macrophages activates phagocytes to kill the parasites through toxic metabolites of oxygen or nitric acid (or both). In contrast, down regulation of the immune response that lead to anergy and progressive diseases may be caused by

parasite specific CD4+T cells of T helper class 2 that secrete IL-4 which inhibits macrophages activation by interferon gamma and inhibits secretion of TNF α .

Morphology:

- Visceral leishmaniasis (L.donovanni & L.chagasi) macrophages of RES are invaded so hepatosplenomegly, lymphadenopathy, pancytopenia, fever & weight loss, hyperpigmentation of the skin (kalazar, black fever) glomerulonephritis (mesangioproliferative) and in advanced cases amyloid deposits**.**

Cutaneous leishmaniasis

Localized single ulcer on exposed skin (slowly expanding and irregular borders, usually heals within 6 months by involution. The lesion is granulomatous.

Diffuse cutaneous leishmaniasis

Lesions of diffuse cutaneous leishmaniasis resembles lepromatous leprosy nodules. The lesions do not ulcerate but contain vast aggregates of foamy macrophages filled with leishmania. The patients are usually anergic not only to Leshmania but also to other skin antigens and the disease respond poorly to therapy.

IX.Schistosomiasis

It is the most important helmenthic disease infecting 200 million people & killing 250,000 annually.

Life cycle: Schistosomal larval, (cercaria) & penetrate human skin. Ghycocalyx that protect the organism from osmotic is shed but it activates complement by alternative pathway. Schistosoms migrate into peripheral vasculature transverse to the lung and little in the portal venous system where they develop into adult male and female schistosomes. Females produce hundreds of eggs per day around which granulmas and fibrosis form the major manifestation in schistosomiasis. Some schist some eggs are passed from the portal veins through the intestinal wall into the colonic lumen are shed with the feces and released into fresh water, form to miracidia that infect the snail to complete the life cycle.

Pathogenesis

1. S. mansoni eggs cause liver disease in multiple ways. The schistosome eggs are direct hepatotoxicity.

2. Carbohydrate antigens of the eggs induce macrophage accumulation and granulomas formation mediated by TNF only TH^1 and TH^2 helper cells.

   TH^2 helper T-cells are responsible for eosinophilla mastocytosis and high level of serum in human schistosomiasis, because these cells secrete IL-3 and IL-4, which stimulate mastocytosis and IL-5, which is the growth factor for eosinophils. Resistance to reinfection by schistosomes after treatment correlates with IgE levels whereas, eosinophile major basic proteins may destroy larvae schistsomula.

3. Eggs release factors that stimulate lymphocytes to secrete a lymphokine that stimulate fibroblast proliferation and portal fibrosis the exuberant fibrosis which is out of proportion to the injury caused by the eggs and granucoma, occurs in 5% of persons infected with schistosomes and cause severe portal hypertension esophageal varicoses and ascites - the hallmark of severe schistosomiasis.

Morphology:

White granulomas scattered in the liver and gut. The center of the granuloma is the schistosome eggs. The granuloma degenerate overtime and undergo fibrosis and calcification. The liver is darken by regurgitated pigments from the schistosome gut which like malaria pigment are iron negative and accumulate in kuffer cells and splenic macrophages.

Severe infection (s. mansoni & S. Japanicum)

Colonic pseudopolyps

Liver surface is bumpy and its cut section shows granuloma and wide spreading fibrous portal enlargement without distortion of the intervening parenchyma.

Portal fibrosis (PIPE-stem fibrosis) many of these portal triads lack a vein lumen causing perisinusoidal portal hypertension and severe congestive splenomegaly, esophageal varices. Schistome eggs diverted to the lungs through portal collateral may produce granulomatous pulmonary arteritis with intimal hyperplasia progressive arterial obstruction and ultimately heart failure (cor pulmonale).

Patients with hepatosplenic Schistosomiasis have also increased frequency of mesangioproliferative glomerulonephritis or membranous glomerulonepritis in which

glomeruli contain deposits of immunoglobulins and compliments but rarely schstosomal antigens.

S. hamatobium infection

Massive egg depositions and early granuloma formation that when erode the vasculature (hamaturia). Latter the granuomas calcify and develop a sandy appearance and in severe cases, it causes concentric rim on the wall of the bladder forming calcified bladder on x- rays films.

When the urinary inflammation involves the ureteral orifices, it causes obstructive hydronephrosis and chronic pylonehphritis. Urinary schistosomiasis is also associated with squamous cell carcinoma of the bladder that is commonly seen in Egypt.

X. Fungal Infections

There are 100,000 known fungi and only few infect humans mostly opportunistically. Only few are involved in human diseases because most fungi are destroyed by cell-mediated immune responses however, humoral immunity plays little or no role.

Predisposing factors for fungal infections include:

- Corticosteroid administration, acquired or congenital immunodeficiency states, defects in neutrophillic and macrophage functions
- Fungal infections are divided into superficial and deep fungal infections (mycosis). Here are few examples of systemic fungal infections

1. Candidiasis (Moniliasis)

   Normally found in mouth, skin and gastrointestinal tracts. It is the most common fugal infection mostly caused by (C. albicans). It affects locally the skin, nail and mucous membranes and it grows best in warm, moist surface and cause vaginitis, diaper rash & oral trush. Systemic candidiasis widespread in persons with depressed immune responses including lymphohemopiotic malignancy, immunosuppressive therapies and broad-spectrum antibiotic usage as well as patients with dialysis, cardiac surgery, IV drug abusers.

Pathogenesis:

Candida has molecules on its surface that mediates its adherence to tissues including

1) A homologue to human CR3 integrin
2) A lectin that binds sugars on epithelial cells

3) Mannose containing protein that binds to lectin like molecule on epithelial cells

Finally, the transition of yeast to hyphal forms is important to fungal virulence because the hyphae appear to spear their way out of cells, which engulf them.

Morphology:

- **Oral trush & vaginitis** are superficial lesions characterized by white patches (or fluffy membrane)
- **Cutaneous eczematous lesion**: Seen in moist area such as between fingers, & toes and in inguinal areas, inflamammary folds and ano-genital regions. These lesions may contain acute and chronic inflammations with micro abscesses but in their chronic states granulomatous inflammations may develop.
- **Invasive candidiasis**: seen in Immunosuppression. Many organs may be involved for examples include kidney with micro abscesses in 90%, and right side candidal endocarditis. The brain, liver subcutis etc may be involved with micro abscesses.

2. Cryptococcosis

Cryptococcos neoformans is encapsulated yeast. It causes menigioencephalitis in normal individuals but more frequently in patients with AIDS, leukemias, lymphomas, SLE, Hodgkin's lymphomas and transplant recipients and those on steroid therapy.

Pathogenesis:

- Found in soil and droppings of birds (peogons): Three factors associated with virulence
  1) Capsular polysaccharides
  2) Resistant to killing by alveolar macrophages
  3) Production of phenol oxidase, which consumes host epinephrine oxidase system. This enzyme consumes host epinephrines in the synthesis of fungal melanin thus, preventing the fungus from epinephrine oxidase system

C. neoformans affect brain because of CSF lacks the alternative pathway complement components that binds to carbohydrate capsule and facilitates phagocytosis and killing by Polymorphonuclear leukocytes.

Morphology:

Lung is the primary site of localization with minor or asymptomatic presentation; here solitary granulomatous lesions may appear.

The major pathologic changes are in the CNS involving meninges, cortical grey matter and basal ganglia. The tissue response to C. neoformans is extremely variable. In immunosupressed patients, the organisms may evoke no inflammatory reactions so; gelatinous masses of fungi grow in the meninges or in small cysts within the grey matter (soap bubble lesion)

3. Aspergillosis

Aspargillus is a ubiquitous mold that causes allergies in otherwise healthy persons and serious sinusitis, pneumonia and fungemia in neutropenic persons. Aspargillus form fruiting bodies.

Pathogenesis:

Aspargillus species have three toxins:

- Aflatoxin: Aspargillus species may grow on surfaces of peanuts and may be a major cause of cancer in Africa.
- Resrictocin and mitogilin: They inhibit protein synthesis by degrading MRNA
- Mitogilin: It also induce IgE production so may be associated with allergic Alveolitis by inducing type III & IV reactions, allergic bronchopulmonary aspargillosis which often-in asthmatic that eventually leads to COLD.

Morphology:

Colonizing Aspargilosis (Aspargiloma): It implies growth of fungus in pulmonary cavity with minimal or no invasion of the tissues. The cavity usually result from the pre-existing tuberculosis, bronchiactasis, old infracts and abscesses,

Invasive Aspargilosis It is an opportunistic infection confined to immunosupressed and debilitated hosts. Common sites of disseminations include the heart valves, brain and kidneys.

The Aspargilus Species have a tendency to invade blood vessels and thus, areas of hemorrhages and infarction are usually superimposed on necrotizing inflammatory reactions

4. Histoplasmosis

- The causative organism H. capsulatum is recovered from dust particles of soil, bird or bat droppings contain small spores (micro conidia).
- Histoplasmosis and Coccidiomycosis resemble pulmonary tuberculosis and both are causedby fungi that are thermally dimorphic (hyphae and yeast forms)

- **Natural history of histoplasmosis include.**

1) Self limited with subsequent coin lesions on X-ray films
2) Chronic progressive secondary lung disease in lung apices
3) Localized lesion in extra pulmonary site including mediastinum, adrenals, liver and meninges
4) Widely disseminated disease especially in immunocompromised individuals

Histopasma yeasts are phagocytosed by unstamulated macrophages and multiply in phagosomes and lyse host cells. Histopasma infection is controlled by T helper cells. Subsequently secreted interferon gamma activates macrophages to kill intracellular yeasts. Tumour necrotizing factor alpha (TNF-α) is also secreted to kill histoplasma. Lacking cellular immunity, patients with AIDS are susceptible to disseminated disease.

Morphology:

Granulomatous inflammation with areas of solidifications that may liquefy subsequently. The lesion may undergo fibrosis spontaneously or with drug therapy in the lungs.
Fulminant disseminated histoplasmosis is seen in immunocompromized individuals where immune granulomas are not formed and mononuclear phagocytes are stuffed with numerous fungi throughout the body.

XI. Viral Infections

Mechanisms of viral injury:

Viruses damage host cells by entering the cell and replicating at the host's expense.
Viral tropism -in part caused by the binding of specific viral surface proteins to particular host cell surface receptor proteins.
The second major cause of viral tropism is the ability of the virus to replicate inside some cells but not in others. For example, JC papovavirus, which causes leukoencephalopathy is restricted to oligoderdroglia, in the CNS.

- Once attached the entire viron or a portion containing the genome and the essential polymerase penetrate into the cell cytoplasm in one of the three ways
    1) Translocation of the entire virus across the plasma membrane
    2) Fusion of viral envelop with the cell membrane or

3) Receptor -mediated endocytosis of the virus and fusion with endosomal membranes

Within the cell, the virus uncoats separating its genome from its structural component and losing its infectivity. Viruses also use host system for viral synthesis.

- Newly synthesized viral genome and capsid proteins are then assembled into progeny virons in the nucleus or cytoplasm and are released directly (unencapsulated viruses) or bud through the plasma membrane (encapsulated viruses)
- Viral infection can be abortive with incomplete replicative cycle
- Latent in which the virus (eg herpes zoster) persists in a cryptic state within the dorsal root ganglia and then present with painful shingles
- Or persistent in which virons are synthesized continuously with or without altered cell function (eg. Hepatitis B)
- **Viruses kill host cell in a number of ways**
- Viruses infect host cell DNA ,RNA or protein synthesis, ex. Poliovirus
- Viral proteins insert into the host cells plasma membrane and directly damage its integrity or promote cell fusion ex HIV ,measles herpes viruses (direct cytotopathic effect).
- Viruses replicate effiently and lyse host cell ex yellow fever virus in liver and neurons by poliovirus.
- Viral proteins on the surface of the host cell are recognized by the immune system, and the host cytotoxic lymphocytes then attack the virus-infected cells ex hepatitis B virus infection, and respiratory synaytial virus.
- Viruses damage cells involved in host antimicrobial defense leading to secondary infection for example viral damaged respiratory epithelium allows subsequent bacterial pneumonias and HIV depletes CD4+ T cell predisposing to opportunistic infection.
- Viral killing of one cell type causes the death of other cells that depend on them, Example poliovirus cause motor neuron injury and atrophy of distal skeletal muscle.
- Slow virus infection cause in severe progressive disease after a long latency period for example sub acute pan encephalitis caused by measles virus.
- Some viruses induce cellular proliferation and transformation example HBV, HPV, EBV which result in neoplasm.

XII. Exercise

Describe the etiology, pathogenesis, morphologic changes and clinical effects of each of the above mentioned diseases.

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CHAPTER NINE

NEOPLASIA

I. Learning objectives:

At the end of this chapter, students are expected to:

1. Differentiate neoplastic lesions from non-neoplastic ones.
2. Contrast benign from malignant tumours.
3. Describe methods and mechanisms of metastasis.
4. List the etiologic factors in carcinogenesis.
5. Understand clinical effects of neoplasms
6. Know the diagnostic modalities for cancers

II. Definition amd Nomenclature

Literally, neoplasia means new growth and technically, it is defined as abnormal mass of tissues the growth of which exceeds and persists in the same excessive manner after cessation of the stimulus, evoking the transformation.

Nomenclature: Neoplasms are named based upon two factors

- on the histologic types : mesenchymal and epithelial
- on behavioral patterns : benign and malignant neoplasms

Thus, the suffix -oma denotes a benign neoplasm. Benign mesenchymal neoplasms originating from muscle, bone, fat, blood vessel nerve, fibrous tissue and cartilages are named as Rhabdomyoma, osteoma, lipoma, hemangioma, neuroma, fibroma and chondroma respectively. Benign epithelial neoplasms are classified on the basis of cell of origin for example adenoma is the term for benign epithelial neoplasm that form glandular pattern or on basis of microscopic or macroscopic patterns for example visible finger like or warty projection from epithelial surface are referred to as papillomas.

This nomenclature has, however, some exceptions

(I) Nonneoplastic misnomers hematoma, granuloma, hamartoma

(II) Malgnant misnomers melanoma, lymphoma, seminoma, glioma, hepatoma.

Malignant neoplasm nomenclature essentially follows the same scheme used for benign neoplasm with certain additions. Malignant neoplasms arising from mesenchymal tissues are called sarcomas (Greed sar =fleshy). Thus, it is a fleshy tumour. These neoplasms are named as fibrosarcoma, liposarcoma, osteosarcoma, hemangiosarcoma etc.

Malignant neoplasms of epithelial cell origin derived from any of the three germ layers are called carcinomas.

Eg. Ectodermal origin: skin (epidermis squamous cell carcinoma, basal cell carcinoma)Mesodermal origin: renal tubules (renal cell carcinoma).Endodermal origin: linings of the gastrointestinal tract (colonic carcinoma) Carcinomas can be furtherly classified those producing glandular microscopic pictures are called Aden carcinomas and those producing recognizable squamous cells are designated as squamous cell carcinoma etc furthermore, when possible the carcinoma can be specified by naming the origin of the tumour such as renal cell adenocarcinoma etc

Tumors that arise from more than tissue components:

- Teratomas contain representative of parenchyma cells of more than one germ layer, usually all three layers. They arise from totipotential cells and so are principally encountered in ovary and testis.
- Mixed tumors containing both epithelial and mesenchymal components Examples include pleomorphic adenoma and fibroadenoma

III. Characteristics of Benign and Malignant Neoplasms

The difference in characteristics of these neoplasms can be conveniently discussed under the following headings:

1. Differentiation & anaplasia
2. Rate of growth
3. Local invasion
4. Metastasis

1. Differentiation and anaplasia

- Differentiation refers to the extent to which parenchymal cells resemble comparable normal cells both morphologically and functionally. Thus, well-differentiated tumours

cells resemble mature normal cells of tissue of origin. Poorly differentiated or undifferentiated tumours have primitive appearing, unspecialized cells. In general, benign neoplasms are well differentiated. Malignant neoplasms in contrast, range from well differentiated, moderately differentiated to poorly differentiate types. Malignant neoplasm composed of undifferentiated cells are said to be anaplastic, literally anaplasia means to form backward.

- Morphology of anaplastic cell includes large Pleomorphic; hyperchromatic nucleus with high nuclear cytoplasmic ratio 1:1(normally 1:4 to 1:6). The cell usually reveals large nucleoli with high and often abnormal mitoses. Tumour giant cells and frequent loss of polarity of epithelial arrangements are encountered.
- On functional differentiation, the well differentiated the neoplasm, the more completely it retains the functional capabilities found in its normal counterparts thus, endocrine tumours produce hormone (ex. Thyroid, adrenal) so also, well differentiated squamous cell carcinoma and well differentiated hepatocellular carcinomas produce keratine and bile respectively.
- However, highly anaplastic or undifferentiated cells of what cell tissue of origin come to resemble each other functionally and morphologically more than the normal cells which they have arisen this is called chemical convergence.

2. Rate of growth

- Most benign tumours grow slowly whereas; most malignant tumours grow rapidly sometimes, at erratic pace. Some benign tumours for example uterine leiomyoma increase in size during pregnancy due to probably steroidal effects (estrogen) and regress in menopause. In general, the growth rate of neoplasms correlate with their level of differentiation and thus, most malignant neoplasms grow more rapidly than do benign neoplasms. On occasions, cancers have been observed to decrease in size and even spontaneously disappear. Examples include renal cell carcinoma, malignant melanoma, choriocarcinoma.

3. Local invasion

- Nearly all benign neoplasms grow as cohesive expansile masses that remains localized to their site of origin and do not have the capacity to invade or metastasize to distant sites, as do malignant neoplasms.
- Rims of fibrous capsules encapsulate most benign neoplasms. However, hemangiomas and neurofibromas are exceptions. Thus, such encapsulations tend to contain the

benign neoplasms as a discrete, rapidly palpable and easily movable mass that can easily surgically enucleated.

- The growth of malignant neoplasms is accompanied by progressive infiltration, invasion and destruction of the surrounding tissue. Generally, they are poorly demarcated from the surrounding normal tissue (and a well-defined cleavage plane is lacking).
- Next to the development of metastasis, invasiveness is the most reliable feature that differentiates malignant from benign neoplasms.
- Even though, malignant neoplasms can invade all tissues in the body, connective tissues are the favoured invasive path for most malignant neoplasms, due to the elaboration of some enzymes such as type IV collagnases & plasmin, which is specific to collagen of basement membrane. Several matrix-degrading enzymes including glycosidase may be associated with tumour invasion.
- Arteries are much more resistant to invasion than are veins and lymphatic channels due to its increased elastic fibers contents and its thickened wall. Densely compact collagens such as membranous tendons, and joint capsules. Cartilage is probably the most resistant of all tissues to invasions and this is may be due to the biologic stability and slow turnover of cartilage.

Sequential steps in mechanisms of tumor invasion & metastasis:

a. Carcinoma in-situ
b. Malignant cell surface receptors bind to basement membrane components (ex laminin).
c. Malignant cell disrupt and invade basement membrane by releasing collagenase type IV and other protease.
d. Invasion of the extracellular matrix
e. Detachment
f. Embolization
g. Survival in the circulation
h. Arrest
i. Extravasation
j. Evasion of host defense
k. Progressive growth
l. Metastasis

Most carcinomas begin as localized growth confined to the epithelium in which they arise. As long as this early cancers do not penetrate the basement membrane on which the epithelium rests such tumours are called carcinoma in-situ.

In those situations in which cancers arise from cell that are not confined by a basement membrane, such as connective tissue cells, lymphoid elements and hepatocytes, an in-situ stage is not defined.

4. Metastasis

- It is defined as a transfer of malignant cells from one site to another not directly connected with it (as it is described in the above steps).
- Metastasis is the most reliable sign of malignancy. The invasiveness of cancers permits them to penetrate in to the blood vessel, lymphatic and body cavities providing the opportunity for spread.
- Most malignant neoplasm metastasies except few such as gliomas in the central nervous system, basal cell carcinoma (Rodent ulcer) in the skin and dermatofibrosarcoma in soft tissues.
- Organs least favoured for metastatic spread include striated muscles and spleen.
- Since the pattern of metastasis is unpredictable, no judgment can be made about the possibility of metastasis from pathologic examination of the primary tumour.
- Approximately 30% of newly diagnosed patients with solid tumours (excluding skin cancers other than melanoma) present with metastasis in the studied populations.

Pathways of spread:

Dissemination of malignant neoplasm may occur through one of the following pathways.

1. Seeding of body cavities and surfaces (transcoelomic spread)

- This seeding may occur wherever a malignant neoplasm penetrates into a natural “open field”. Most often involved is the peritoneal cavity, but any other cavities such as pleural, pericardial, sub-arachnoid and joint spaces-may be affected.
- Particular examples are krukenberg tumour that is a classical example of mucin producing signet ring adenocarcinomas arising from gastrointestinal tract, pancreas, breast, and gall bladder may spread to one or both ovaries and the peritoneal cavities.
- The other example is pseudomyxoma peritoni which are mucus secreting adrocarcinoma arising either from ovary or appendix. These carcinomas fill the peritoneal cavity with a

gelatinous soft, translucent neoplastic mass. It can also be associated with primaries in the gallbladder and pancreas.

2. Lymphatic spread

- Lymphatic route is the most common pathway for the initial dissemination of carcinomas
- The pattern of lymph node involvement follows the natural routes of drainage. Lymph nodes involvement in cancers is in direct proportion to the number of tumour cell reaching the nodes.
- Due to numerous inter connections between vascular and lymphatic channels the emphasis that used to be given, lymphatic spread for carcinomas and vascular spread for sarcomas is misreading.
- Metastasis to lymph nodes first lodge in the marginal sinus and then extends throughout the node. The cut surface of this enlarged lymph node usually resembles that of the primary tumour in colour and consistency. The best examples of lymphatic spread of malignant neoplasm can be exemplified by breast carcinoma.
- -Skip metastasis may occur when local lymph nodes may be by- passed and occasionally found in lymph node distant from the site of the primary malignant neoplasm. Skip metastasis happen to occur because of venous lymphatic anastomoses or because inflammation or radiation has obliterated the lymphatic channels for example abdominal cancer (gastric cancer) may be initially signaled by supra clavicular (sentinel node).
- A clinical presence of enlarged lymph node is not necessarily synonymous with a metastasis. Conversely, the absence of tumour cells in reseated lymph nodes does not guarantee that there is no underlying cancer.

3. Hematogenous spread

- Typical for all sarcomas and certain carcinomas- the spread appears to be selective with seed and soil phenomenon. Lung & liver are common sites of metastasis because they receive the systemic and venous out flow respectively. Other major sites of hematogenous spread include brain and bones.
- In the circulation, tumour cells form emboli by aggregation and by adhering to circulating leukocytes particularly platelets. The site where tumour cell emboli lodge and produce secondary growth is influenced by
    - Vascular (and lymphatic) drainage from the site of the primary tumour
    - Interaction of tumour cells with organ specific receptors
    - The microenvironment of the organ or site, example a tissue rich in protease inhibitors might be resistant to penetration by tumour cells.

IV. Cancer Epidemiology

- The only certain way to avoid cancer is not to be born, to live is to incur the risk.
- Thus, In USA one in five deaths is due to cancers. Over the years cancer incidence increased in males while it slightly decreased in females (due to largely screening Procedures-cervical, breast etc.). In the studied populations the most common cancer in males is broncogenic carcinoma while breast carcinoma in females.
- Most cancers in adults occur in those over 55 years of age.
- Children under 15 years of age however, are susceptible to acute leukemia, central nervous system tumours, neuroblastoma, wilm's tumour, retinoblastoma, rhabdo myosarcoma and etc. Acute leukemias and neoplasms of the central nervous system accounts for about 60% of the deaths.

Geographic factors (geographic pathology):

Specific differences in incidence rates of cancers are seen worldwide.For example,

Stomach carcinoma	- Japan
Lung cancer	- USA
Skin cancer	- New zeland & Australia
Liver cancer	- Ethiopia

Environmental factors (occupational hazards) include:

Asbestos-----Lung cancer, mesothelioma,esophagus and, stomach carcinomas;
Vinyl chloride----Angiosarcoma of liver
Benzene ---Leukemias
Cigarette smoking-----Brochogenic carcinomas
Venereal infection (HPV)--Cervical carcinoma

Premalignant disorders

a) Heredity premalignant disorders

Inherited predisposition to cancer is categorized in to three groups:

i. Inherited cancer syndromes (Autosomal dominant) with strong familial history include

- Familial retinoblastomas usually bilateral, and a second cancer risk particularly osteogenic sarcoma. Oncosupressor gene is the basis for this carcinogenesis

- Familial adenomatous polyps of the colon. virtually all cases are fatal to develop carcinoma of the colon by the age of 50.

ii. Familial cancers:

Evidence of familial clustering of cancer are documented

E.g. Breast, ovarian, colonic, and brain cancers

iii. Autosmal recessive syndromes of defective DNA repair Characterized by chromosomal or DNA instability syndrome such as xeroderma pigmentosium, Ataxia telaangietasia, Bloom syndrome and Fanconi anemia

B) Acquired preneoplastic disorders

- **Regenerative, hyperplasic and dysplastic proliferations are fertile soil for the origin of malignant neoplasms.**

  Endometrial hyperplasia - endometrial carcinoma
  Cervical dysplasia - cervical cancer
  Bronchial dysplasia - bronchogenic carcinoma
  Regenerative nodules - liver cancer

- **Certain non-neoplastic disorders may predispose to cancers**.

  Chronic atrophic gastritis - gastric cancer
  Solar keratosis of skin - skin cancer
  Chronic ulcerative colitis - colonic cancer
  Leukoplakia of the
  oral cavity, vulva and penis - squamous cell carcinoma

- **Certain types of benign neoplasms**

  Large cumulative experiences indicate that most benign neoplasms do not become malignant. However, some benign neoplasms can constitute premalignant conditions. For example:

  Villous colonic adenoma - Colonic cancer

V. Molecular Basis of Cancer (Carcinogenesis)

Basic principles of carcinogenesis:

The fundamental principles in carcinogenesis include

1) Non-lethal genetic damage lies at the heart of carcinogenesis. Such genetic damage (mutation) may be acquired by the action of environmental agents such as chemicals, radiation or viruses or it may be inherited in the germ line.

2) The three classes of normal regulatory genes are:

i) **The growth promoting proto-oncogenes**

Activation of proto-oncogenes activation gives rise to oncogenes (cancer causing genes).Proto

- oncogenes are activated by
- Point mutation
- Chromosomal rearrangements ranslocation Inversion
- Gene amplification

ii) **Cancer suppressor genes (anti oncogenes**)

- Its physiologic role is to regulate cell growth however, the inactivation of cancer suppressor genes is the key event in cancer genesis
- Examples of tumour suppressor genes include-Rb, P53, APC and NF-1&2 genes

iii) **Genes that regulate apoptosis**

- Genes that prevent or induce programmed cell death are also important variables in the cancer equation. These genes include bcl-2 that inhibits apoptosis whereas, others such as bax. Bad, and bcl-x5 favour programmed cell death. Genes that regulate apoptosis may be dominant as are proto-oncogenes or may behave as cancer suppressor genes (recessive in nature)

iv) **Genes that regulate DNA repair**

- Inability to DNA repair can predispose to mutations in the genome and hence, to neoplastic transformations

3) Carcinogenesis is a multifactorial process

- at both the phenotypic and genotypic levels**.**

Types of carcinogenesis:

A large number of agents cause genetic damages and induce neoplastic transformation of cells. They fall into the following three categories:

a) Chemical carcinogenesis
b) Radiation carcinogenesis
c) Viral carcinogenesis

A) Chemical carcinogenesis

An enormous variety of chemicals may induce tumours and this was exemplified by Sir Percival Pott's observation in the last century that astutely related the increased incidence of scrotal skin cancer in chimney sweeps to chronic exposure to soot.

Steps involved in chemical carcinogenesis

- appropriate dose of a chemical carcinogenic agents to a cell results in the formation of initiation –promotion sequence
- Initiation causes permanent DNA damage (mutation) which, is rapid and irreversible. However, initiation alone is not sufficient for tumour formation and thus, promoters can induce tumours in initiated cells, but they are non-tumourogenic by themselves. Furthermore, tumours do not result when a promoting agent applied before, the initiating agent.
- In contrast to the effects of initiators, the cellular changes resulting from the application of promoters do not affect DNA directly and are reversible.
- Promoters render cells susceptible to additional mutations by causing cellular proliferation. Examples of promoters include phorbol ester, hormones, phenols and drugs.

Chemical carcinogenic agents fall into two categories

1. **Directly acting compound**
   - These are ultimate carcinogens and have one property in common:
   - They are highly reactive electrophiles (have electron deficient atoms) that can react with nucleophilic (electron-rich) sites in the cell. This reaction is non-enzymatic and result in the formation of covalent adducts (addition products) between the chemical carcinogen and a nucleotide in DNA.

- Electrophilic reactions may attack several electron-rich sites in the target cells including DNA, RNA, and proteins.
- Only a few alkylating and acylating agents are directly acting carcinogens

2. **Indirect acting compounds (or pro-carcinogens)**
   - Requires metabolic conversion in vivo to produce ultimate carcinogens capable of transforming cells.
   - Most known carcinogens are metabolized by cytochrome p-450 dependent mono-oxygenase.
   - Examples of this group include polycyclic and heterocyclic aromatic hydocarbones, and aromatic amines etc.…
   - These chemical carcinogens lead to mutations in cells by affecting the functions of oncogenes, onco-suppressor genes and genes that regulate apoptosis.

B) Radiation carcinogenesis

Radiant energy whether in form of ultraviolet (UV) sun light or ionizing electromagnetic (X rays and gamma (δ) rays) and particulates (α,β, protons and neutrons) radiation can transform and induce neoplasm in both humans and experimental animals.

Two types of radiation injuries are recognized:

i) Ultraviolet rays (UV light)
- UV rays are examples of non-ionizing radiation that cause vibration and rotation of atoms in biologic molecules
- UV rays induce an increased incidence of squamous cell carcinoma, basal cell carcinoma and possibly malignant melanoma of skin.
- Risk factors for developing UV rays related disorders depend on
  - Type of UV rays – UV type B
  - Intensity of exposure
  - Quality of light absorbing “protective mantle” of melanin in the skin
    Ex. Australians (queen's land etc.)
- UV rays’ effects on cell nucleus are:
  -The carcinogenesis of UV type B rays is attributable to its formation of pyrimidine dimmers in DNA
  - However, UV rays can also cause inhibition of cell division, inactivation of enzymes, Induction of mutation and sufficient dose kill cells.

As with other carcinogens, UVB also cause mutations on oncogenes and tumour suppressor genes mutant forms of P53 and ras genes have been detected.

ii) Ionizing radiation

- Ionizing radiations are of short wave lengh and high frequency which can ionize biologic target molecules and eject electrons
- Electromagnetic and particulate radiations in forms of theureptic, occupational or atomic bomb incidents can be carcinogenic
- Occupational hazards include:

Many of the poineers in the development of roentegen rays develop skin cancers.Miners for radioactive elements---lung cancer

- Therapeutic irradiations have been documented to be carcinogenic. Thyroid cancer may result from childhood & infancy irradiation (9%), and by the same taken radiation therapy for spondylitis may lead to a possible acute leukemia year later.
- In atomic bonds dropped in Hiroshima and Nagasaki initially principal cancers were acute and chronic mylogenous leukemias after a latent of about 7 years solid tumours such as breast, colon, thyroid and lung cancers) increased in incidence.
- In humans, there is a hierarchy of vulnerability of radiation-induced cancers. Most frequent are the leukemia except CLL, which almost never follow radiation injury. Cancer of the thyroid follows closely but only in the young. In intermediate category are cancers of the breast, lungs, and salivary glands
- In contrast, skin, bone and gastrointestinal tract are relatively resistant to radiation-induced neoplasia.

C) Viral and bacterial carcinogenesis

Large groups of DNA and RNA viruses have proved to be oncogenic and there is an association between infections by the bacterium Helicobacter Pylori and gastric lymphoma.

i) DNA oncogenic viruses

This group includes:

Human Papilloma Virus (HPV)

Epstein Barr Virus (EBV)

Hepatitis B Virus (HBV)

General feature of the oncogenic DNA virus

a) Transforming DNA virus form stable associations with the host cell genome. The integrated virus is unable to complete its replicative cycle because the viral genes essential for completion of replication are interrupted during integration of viral DNA (E1/E2)

b) Those viral genes that are transcribed early (early genes) in the viral life cycle are expressed in the transformed cells.

Human papilloma Virus (HPV)

- HPV definitely causes benign squamous papilloma (warts) (type 1,2,3,4, 7). It also implicated in the genesis of squamous cell carcinomas of cervix and anogenital region (types 16,18 and also 31,33,35,and 51 found in 85% SCC). It is also linked to the causation of oral and laryngeal cancers.
- The HPV DNA integration into host cell is random (viral DNA is found in different locations), however, the pattern of integration, is clonal (that is the site of integration is identical within all cells of a given cancer).

Epstein – Barr virus (EBV)

- Member of the herpes family has been implicated in the pathogenesis of four tumours. The African form of Burkitt's lymphoma, B- cell lymphomas in immuno suppressed individuals particularly in those with some cases of Hodgkin's disease and Naso pharyngeal carcinoma.
- EBV infects epithelial cell of the oro pharynx and B- lymphocytes. The infection of B- cell is latent and the latently infected B-cell is immortalized. Several viral genes dysregulate the normal proliferative and survival signals in latently infects cells for example the latent membrane protein –1 (LMP-1) prevents apoptosis of B- cells by up regulating the expression of bc1-2 and it activates growth promoting pathways. Thus, LMP-1 can induce both cell growth and cell survival. Similarly the EBV- encoded EBNA- gene transactivates several host genes including cyclin D and members of the src family EBNA- 2 also activates the transcription of LMP- 1. Thus, it seems that several *viral* genes collaborate to render B- cells immortal.
- The Association between African Burkett Lymphoma and EBV is quite strong.
  - More than 90% of African tumours carry the EBV genome
  - One hundred percent of the patients have elevated antibody titres against viral capsid antigens

- Serum antibody titres against viral capsid antigens are correlated with the risk of developing the tumour.Several other observations suggested that additional factors must be involved. In Africa poorly understood co-factor (ex chromic malaria), favor sustained proliferation of B- cells immortalized by EBV. The actively dividing B- cells are at increased risk of mutations (t- 8; 14) translocation that juxta - pose C- myc with one of Immuno- globuline gene loci.

Hepatitis B- virus (HBV)

- Strong epidemiologic association prevails between HBV and hepato cellular Carcinoma. HBV genome, however, does not encode any oncoproteins, and there is no consistent pattern of integration in the vicinity of any known protomcogeres
- The effects of HBV is indirect and possibly multi factorial.

i) By causing chronic liver cell injury and accompanying regenerative hyperplasic

ii) HBV encodes a regulatory element called HBx protein, which disrupts normal growth control of infected liver cells by transcriptional activation of several growth promoting genes such as insulin like growth factor II

iii) HBV binds to P53 and appears to interfere with its growth suppressing activities. Although not a DNA virus hepatitis virus is also strongly linked to the pathogenesis of hepato cellular carcinoma as evidenced by epidemiologic studies.

ii. RNA oncogenic viruses

- Although the study of animal retroviruses has provided spectacular insights to molecular basis of cancer , only one retrovirus is firmly implicated in the causation of cancer and it is Human T cells leukemia/ lymphoma virus type 1 (HTLV-1) .
- Similar to HIV virus. HTLV-1 has tropism for CD4+T cells > Human infection requires transmission of infected T cells through sexual intercourse, blood products, or breast feedings. Leukemia develops after a 20 or 30 years of latency in about 1% of patients. HTLV-1 is also associated tropical spastic Para paresis.

iii. Helicobacter pylori

- There is an association between gastric infections with helicobacter pylori as a cause of gastric lymphoma. The stronger link is with B cell lymphoma of stomach. Treatment of H. pylori with antibiotics results in regression of the lymphoma in most cases. The lymphoma arise from the mucosa associated lymphoid tissues (MALT) hence, they sometimes are called MALTOMAS. The lymphoid cells reside in the marginal zones of lymphoid follicles and hence alternatively named as mantle zone lymphoma.

VI. Clinical Features of Tumors

Neoplasms are essentially parasites. In fact, benign lesions are more common than cancers. Although cancer evaluation may suggest one or the other, the only unequivocal benign mass is the excised and histopathologically diagnosed one.

Effects of tumour on the host:

Both benigin and malignant neoplasms may cause problems because of

1. location and impingement on adjcent structures
2. functional activities such as hormone synthesis
3. bleeding and secondary infection when they ulcerate through adjacent natural surfaces
4. initiation of acute symptoms caused by either rupture or infarction local and hormonal effects
   - For example pituitary adenoma being located in critical location can cause serious endocrinopathies
   - Analogously cancers arising with or metastatic to an endocrine organ may cause an endocrine in suffiecency by destroying the gland.
   - Neoplasms in the gut (both bening and malignant may cause obstruction as they enlarge
   - Benign neoplasms more commonly of endocrine origin may produce manifestations by elaboration of hormones. For example a benign B- cell adenoma of pancreatic islets less than 1 cm in diameter may produce sufficient insulin to cause fatal hypoglycemia
   - The erosive destructive growth of cancers or expansile pressure on benign tumour of any natural surface may cause ulceration secondary infection and bleeding.

5. Cancer cachexia

- Cachexia is a progressive loss of body fat and lean body mass accompanied by profound weakness, anorexia and anemia .The origin of cancer cachexia are obscure
- Clinically anorexia is a common problem in patients with cancer. Reduced food intake has been related to abnormalities in taste and central control of appetite. In patents with cancer, calorie expenditure often remains high and basal metabolic rate is increased despite reduced food intake.
- The basis of metabolic abnormalities is not fully understood; however, TNF produced by macrophages and possibly by some tumour cells is the mediator of the wasting syndrome that accompanies cancer. Other cytokines such as IL-1 and IFN α synergize with TNFα –
- A protein-mobilizing factor has been isolated from the serum of animals and humans with cancer cachexia

6. Paraneoplastic syndromes

- Paraneoplastic syndrome is an aggregate of symptom complexes in cancer - bearing patients that can not readily be explained either by the local or distant spread of the tumour or by the elaboration of hormones indigenous to the tissue from which the tumour arose
- Paraneoplastic syndrome occurs in about 10% of patients with malignant disease
- Despite its infrequency, the syndrome is important for three reasons:
  1. They be the earliest manifestation of an occult neoplasm
  2. In affected patients, they may represent significant clinical problems and may even be lethal.
  3. They may mimic metastatic disease and, therefore, confound treatment

Classification of paraneoplastic syndromes

Cushing syndrome	Small lung ca, pancreatic ca	ACTH and ACTH like Substances
IADH Secretion	Small cell lung Ca	ADH or atrial natriuretic hormones
Hypercalcimia	SCC of lungs, breast ca, renal ca, Adult T-cell leukemia /lymphoma, ovarian cancers	Parathyroid hormone related peptide TGF -, TNF IL-1
Hypoglycemia	Fibrosarcoma other sarcomas HCC	Insulin or insulin like substances
Carcinoid syndrome	Bronchial adenoma, pancreatic ca, gastric ca	Serotonins, bradykinins ?histamine
Polycythemia	Renal ca, cerebellar Hemangioma, liver cancer	Erythropoietin
Dermatologic disorders such as acanthosiss nigricans, dermatomyositis	Gastric, lung & uterine cancers Bronchographie ca, breast ca	? immunologic ? immunologic
Vascular and hematologic		
Venous thrombosis	Pancreatic and bronchogenic cas	Mucin that activate clotting
Nonbacterial thrombotic endocarditis	Advanced cancers	Hypercoagulobilty

Hypercalcimia is probably the most common paraneoplastic syndrome and among endocrinopathies Cushing syndrome is the most common variety of paraneoplastic effect.

Grading and staging of cancers

- Grading denotes the level of differentiation whereas, staging expresses the extent of tumour spread and forcast the clinical gravity of cancers
- Grading of a cancer is based on the degree of differentiation of tumour cells and the number of mitoses within the tumour and presumably correlates to aggressive character of the neoplasm
- Cancers are classified into grades I to IV with increasing anaplasia. Criteria for individual grades vary with each form of neoplasm
- The staging of cancers is based on the size of primary lesions, its extent of spread to regional lymph nodes and the presence or absence of blood born metastases
- Two major staging systems are currently in use are Union internationale contre cancer (UICC) which utilizes the so- called TNM system T for primary tumour N for regional lymph node involvement and m for metastasis

The TNM staging varies for each specific form of cancer but there are general principles:

- With increasing size, the primary lesion is characterized as T to T4 to is added to indicate as in - situ lesion.
- No for no nodal involvement whereas, N1 -N3 wound denote involvement of an increasing number and range of nodes
- Mo signifies no distant metastasis whereas M1 or sometimes M_2 indicates the presence of blood born metastasis

The American joint committee (AJC) employs a somewhat different nomenclature and divides all cancers into stages to IV incorporating within each of these stages the size of the primary lesion as well as the presence of nodal spread and the distant metastasis

- The staging of neoplastic disease has assumed great importance in the selection of the best form of therapy for the patient. Indeed, staging has proved to be of greater clinical value than grading.

VII. Laboratory Diagnosis of Cancer

Every year approach to laboratory diagnosis of cancer becomes more complex more sophisticated and more specialized with time.

Histologic and cytologic methods:

- The laboratory diagnoses of most cancers is not difficult however, border line cases in no man's land where wise men trade cautiously pose the most difficulties
- Clinicians tend to underestimate the important contributions they make in the diagnosis of neoplasms. Clinical data are invaluable for optimal pathologic diagnosis for example radiation changes in the skin or mucosa can be similar to cancer and similarly section taken from a healing fracure can mimic remarkably an osteosarcoma.
- The laboratory sample to be diagnosed need to be adequate, representative and well preserved

Several sampling approaches are available:

1. Excisional or incisional biopsy
2. Cytologic smears:
   Fine needle aspiration
   PAP smear
   Fluid cytology
3. Advanced techniques
   Immunocytochemistry
   Flow cytometry
   Tumour markers

Excisional biopsy

- Selection of an appropriate site for biopsy of a large mass requires awareness that the margins may not be representative and the center largely necrotic .analogously disseminated lymphoma involving inguinal lymph nodes that drain large part of the body often have reactive changes that may mask neoplastic involement.
- Appropriate preservation of specimens is obvious thus, formalin for routine fixation glutaraldehide for electron microscopy prompt refrigration to permit optimal hormone by receptor analysis

- Requesting, “quick frozen section" diagnosis is sometimes desirable for determining (for example in breast carcinoma) for evaluating the margins of an excised cancer to ascertain that the entire neoplasm has been removed.

Fine needle aspiration

- The procedure involves aspirating cell and attendant fluid with a small needle followed by cytologic examination of the stained smear
- This method is used most commonly for the assessment of readily palpable lesions such as breasts, thyroid and lymph nodes etc.

Cytologic (PAP) smear

- This method is widely used for the discovery of carcinoma of the cervix, it also detect cervical cancers at an in situ stage, and other suspected malignancies such as endometrial, and bronchogenic carcinomas, bladder and prostatic tumours and gastric carcinoma.
- It is also used for the identification of tumour cell in abdominal, pleural joint and cerebrospinal fluids

Tumour markers

- Tumour markers are biochemical indicators of the presence of a tumour. They include cell surface antigens, cytoplasmic proteins, enzymes and hormones.
- Tumour markers can not be construed as primary modalites for the diagnosis of cancer and thus, act as supportive laboratory tests.
- A host of tumour markers have been described and new only appear every year

Selected Tumor Markers

Markers	**Associated cancers**
- Hormones	
Human gonadothrphic hormone (HCG)	Throphoblastic tumours, non seminomatous testicular tumours
Catecholamine	Medulary thyroid carcinoma (ca)
Cathecolamines & metabolites	Pheochromocytoma & related tumours
Ectopic hormones	Paraneoplastic syndromes
On co fetal antigens	
fetoprotein	Hepatic ca, non seminomatous germ cell tumours of tests
CEA	Ca s of colon, pancrease, lung, stomach and Breast
Isoenzymes	
Prostatic acid phosphatase	Prostate cancer
Non specific enolase	Small cell cancer of lung, neuroblastoma
Specific proteins	
Immunoglobulins	Multiple myloma and other gammopathres
Prostatic specific antigens	Prostate cancer
Mucins and other glycoproteins	
CA-125	Ovarian cancer
CA-19-9	Colon cancer, pancreatic cancer
CA15-3	Breast cancer

New advanced techniques are being constantly added to the tools of the surgical pathologists, which include:

1. Immunocytochemistry

The availability of specific monoclonal antibodies has greatly facilitates the identification of cell products and surface markers. Some examples of utility of immunocyto chemistry in the diagnosis of malignant neoplasms are

- Categorization of undifferentiated malignant tumours here intermediate filaments are important. Keratin for carcinomas, desmin for neoplasm's of muscle origin
- Categorization of leukemias / lymphomas
- Determination of site of origin of metastatic tumours
- Detection of molecules that have prognostic or therapeutic significance: Detection of hormone (estrogen /progesterone) receptors in breast cancer cells is of prognostic and therapeutic value because these cancers are susceptible to anti-estrogen therapy .Protein products of oncogen such as C- erb B_2 in breast cancer are prognosis
- Prognosis of malignant neoplasms
- Detection of minimal residual disease
- Diagnosis of hereditary predisposition cancer

2 Flow cytometry

- identification of cell surface antigens by flow cytometory is widely used in the classification of leukemias and lymphomas Follow cytometry is used for detection of aneuplody which is also associated with poorer prognosis in early stage breast cancer carcinomas of the urinary bladder lung cancer colorectal cancer, and prostate cancer

VIII. Exercises

1. Define neoplasia.

2. Discuss the differences between benigin and malignant neoplasms.

3. What are the etiologic factors in carcinogenesis?

4. Discuss the clinical features of neoplasms.

5. How is the diagnosis of cancer made in the laboratory?

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CHAPTER TEN
METABOLIC DISEASES

I. Learning objectives

By the time the student is through with this lecture note he/she should be able to:

1. Define diabetes mellitus.
2. Know the classification of DM and the basis for the classification.
3. List the criteria for the diagnosis of DM.
4. Describe the pathogenesis of type 1 and type 2 DM.
5. Describe the pathologic lesion seen in the pancreas of a patient with type 1 and type 2 MD.
6. Tell the clinical manifestation of DM.
7. Know the complications of DM

II. Introduction

Human beings are subjected to a variety of metabolic diseases, as we are a complex set of structures that function through quite a varied and intertwined metabolic processes. Most metabolic diseases have genetic basis while some are acquired in life or need the complex interplay between nature and nurture for their existence. Genetic diseases either follow a single gene disorder or a polygenic basis with multifactorial disorders.

A. Metabolic diseases with a single gene disorder

These metabolic diseases follow a Mendelian type of inheritance i.e. they follow an autosomal dominant disorder (e.g., Familial hypercholesterolemia, Acute intermittent porphyria); an autosomal recessive disorder (e.g. Phenylketonuria, Galactosemia, Glycogen storage diseases); and x-linked recessive disorders (e.g. Phosphorylase kinase deficiency, GSD). These are all rare Biochemical genetic diseases and they are beyond the scope of this lecture note.

B. Metabolic disease with a polygenic disorder

These metabolic diseases have multifactorial modes of inheritance.

They are caused by the additive effects of two or more genes of small effect but conditioned by environmental, non-genetic influences. Example in these groups of metabolic diseases

includes Diabetes mellitus and Gout. These two groups of metabolic diseases are further discussed below.

III. Diabetes Mellitus

The definition, classificatioin, epidemiology, pathogenesis, morphology, clinical featutres, diagnostic criteria, & complications of diabetes mellitus will be discussed next in this sequence.

1. Definition

DM represents a heterogeneous group of disorders that have hyperglycemia as a common feature.
It can also be defined as a chronic metabolic abnormality of CHO, Fat, and Protein.

2. Classification

Although all forms of DM are characterized by hyperglycemia the pathogenic mechanism by which hyperglycemia arises differ widely.

Recent classification of DM is based on the pathogenic mechanism that led to the development of the diabetic syndrome rather than age of onset and type of therapy.

As to the new classification there are four types of diabetes of which the first two are the major types.

A. Type 1 DM

further classified as type1A and type1B.

- Type 1A DM results from autoimmune beta cell destruction which results in absolute insulin deficiency
- Type 1B (idiopathic group) is also characterized by insulin deficiency and a tendency to develop ketosis (will be discussed later). The cause that led to insulin deficiency is not known

B. Type 2 DM

- It is a heterogeneous group of disorders usually characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production.

C. Other specific types of DM

Other causes for DM include specific genetic defects in insulin secretion or action, metabolic abnormalities that impair insulin secretion, conditions that impair glucose tolerance, exocrine disease of the pancreas that lead to destruction of beta cell, several endocrinopathies that can lead to DM as a result of excessive secretion of hormones that antagonize the action of insulin.

D. Gestational diabetes

N.B- the previously used terms, NIDDM to represent type 2 DM and IDDM to represent type 1 DM, are obsolete.

3. Epidemiology

- The prevalence of DM has increased dramatically in the world due to the increasing urbanization and consequent life style changes.
- There is considerable variation in DM prevalence among different ethnic groups world wide due to both genetic and environmental factors.
- Type 2DM accounts to 80% of all cases and type 1DM accounts to 5-10% of all cases of diabetes.
- The incidence of DM is similar in men and women throughout most age ranges but is slightly greater in men> 60 years of age.

4. Pathogenesis

A. Type 1A DM

- there is an autoimmune destruction of beta cells of the pancreas
- **It is at least after 80% of the beta cells are destroyed that a metabolic abnormality appears in IDDM.**
- The pathogenesis begins with a genetic susceptibility and some environmental factors initiates the autoimmune process in such susceptible individuals.

Genetic factors/ evidences

1. Less than 20% of Type 1 diabetics have a parent or sibling with the disease. This can be sited as evidence that genetic factors are involved in the pathogenesis of the disease.

2. In studies of identical (monozygotic) twins in which one or both were diabetic, both members of the pair were affected in approximately half of the cases [i.e. there is a 50% concordance rate]

    - N.B these 50 % concordance rate shows that environmental factors contribute to the development of the disease on a heritable predisposition

3. Type 1 DM is believed to be a polygenic disorder. Additional evidence came from studies of genes that code for antigens of the major Histocompatibilty complex. In patients with type 1 diabetes, 95% express either HLA- DR3 or HLA – DR4, or both, compared with 40% of the general population who exhibit the above MHC genes.

The above three genetic factors/ evidences show that there is a genetic factor that is/ are important for the susceptibly to the disease and environmental factors are required to the development of an autoimmune reaction on these susceptible individuals.

Autoimmunity

- This is a development of auto antibodies to self antigens.
- Environmental factors are essential for the development of the autoimmune process but the exact mode of their action is not clear (see the proposed mode of action below in the discussion of environmental factors).
- Evidences for autoimmune involvement in the destruction of B cells to wards the development of Type 1A DM are as follows.

1. Circulating auto- antibodies against components of the beta cells and against insulin were demonstrated in the large majority of all newly diagnosed children with diabetes.
2. The destruction of beta cells by an immune response is also evidenced by the presence of mononuclear cell infiltrates in the pancreatic tissue of a patient with type 1 diabetes

Environmental factors

As mentioned earlier, the fact that a significant proportion of monozygotic twins remain discordant for Diabetes suggests that non-genetic factors are required for development of diabetes.

- The environmental factor in many cases that is responsible for the initiative of an autoimmune reaction is believed to be a viral infection of the pancreas.
- Coxsakievirus, Rubella virus, Infectious mononucleosis, Mumps and other viruses are incremented as viral causes
- Presumably, viral infections of the pancreas could induce diabetes by two mechanisms. Direct inflammatory disruption of islets(which is a rare cause of DM) or induction of an autoimmune response by exposing crypting B cell antigens.
- It has also been suggested that exposure to proteins contained in cow's milk may be an environmental trigger for type1 diabetes. This is because milk proteins provide specific peptides that share antigenic sites (molecular mimicry) with human B cell surface proteins there by eliciting the production of auto reactive antibodies.

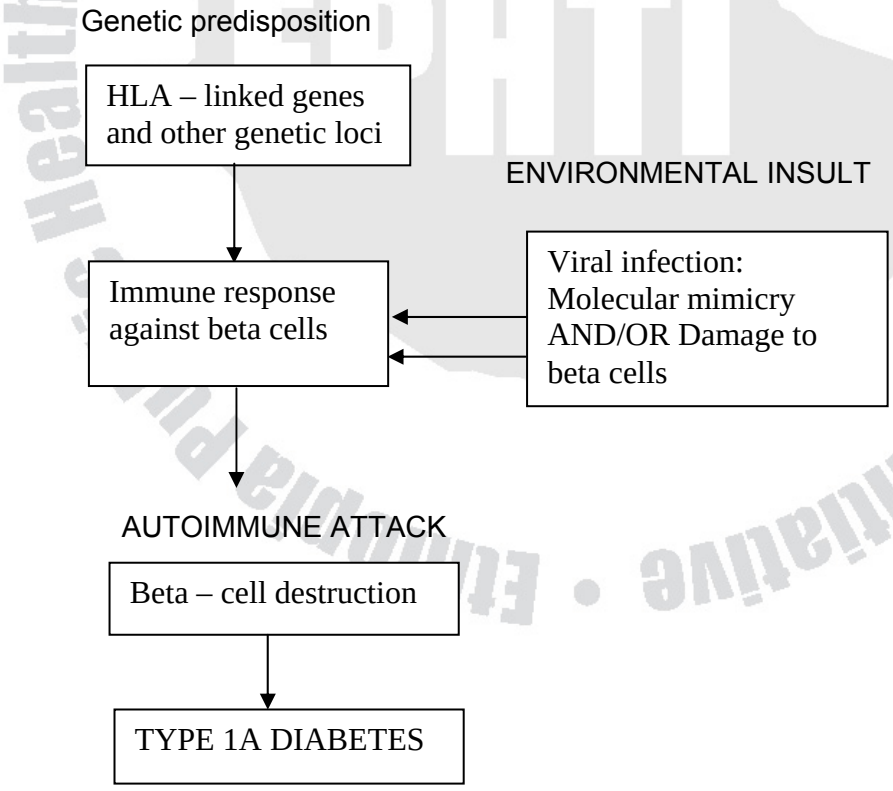

Figure 10-1- Suggested pathogenic mechanism of type I DM

B. Type 2 DM

Pathogenesis

- Is also a polygenic disorder
- Central to the development of type 2 DM are insulin resistance and abnormal insulin secretion. Many believe that peripheral insulin resistance precedes the latter

Genetic factors

- 60% of patients have either a parent or sibling with this disease
- >80% concordance rate in monozygotic twins
- The above figures suggests that type 2 DM has strong genetic basis than type 1 DM
- No association with MHC class genes is found
- Despite the defect in insulin secretion, many patients with type 2 diabetes have increased insulin concentration in the blood in an attempt to overcome the peripheral insulin resistance. The hyperinsulinemia inturn results in decreased insulin receptors peripherally in the muscle and adipose tissue.

Environmental factor

The major environmental factor is obesity, which augments the genetically determined insulin resistance of type 2 DM

Pathophysiology

Type 2 DM is characterized by three pathophysiologic abnormalities: impaired insulin secretion, peripheral insulin resistance, and excessive hepatic glucose production.

Insulin resistance.

- Is a prominent feature of type 2 DM
- Is caused by decreased efficiency of insulin to act on peripheral tissue especially liver and skeletal muscles.
- The precise mechanism of insulin resistance is unknown but a post receptor defect is believed to play the major role.
- Insulin resistance leads to:
  A. Decreased peripheral utilization of glucose. So increased blood glucose
  B. Increased hepatic glucose production

Impaired insulin secretion

- The reason for the impairment of insulin secretion is not clear
- Genetic defect, increased hyperglycemia ("glucose" toxicity), increased free fatty acid level ("lipotoxicity")- all are suggested as a cause or factors which worsen beta cell failure to secrete insulin

Increased hepatic glucose production

- Insulin promotes storage of glucose as hepatic glycogen and suppresses gluconeogenesis.
- Unopposed action of insulin counter regulatory hormones results in increased hepatic glucose production.

5. Pathology (Morphology)

Type 1

- The characteristic lesions in the pancreases of children, who suffer from type1 DM, predominant lymphocytes infiltration, are seen in the islets accompanied by few macrophages. The B cell mass is significantly decreased.

Type 2

- No decrease is the number of beta cells and there is no morphologic lesion of these cells.
- Amyloid deposition is seen specially in patients older than 60 years of age

In some patients fibrosis of the islets is also seen

6. Clinical manifestations

- Symptoms are due to hyperglycemia

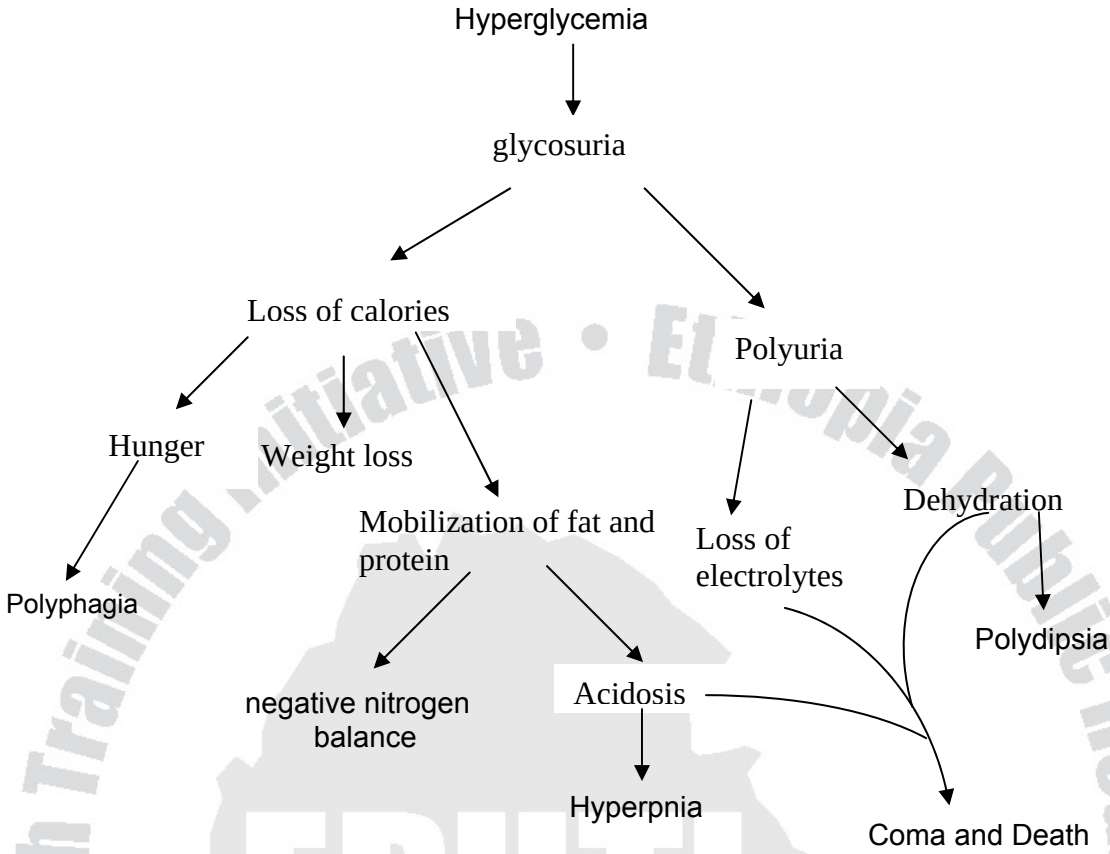

Figure 10-2- The clinical consequense and complication of DM

- Patients may present with classical symptoms of diabetes

Polyuria, polydipsia, polyphagia with weight loss or they may present with DKA.

7. Diagnosis

Is based on the criteria set by Consensus panels of experts from the National Diabetes Data Group

- Symptoms of DM plus random blood glucose concentration of ≥ 200mg/dl Or
- Fasting plasma glucose ≥ 126mg/dl. Or
- Two hour plasma glucose ≥ 200mg/dl during an oral glucose test (i.e. after an oral dose of 75g glucose dissolved in water)

*In the absence of unequivocal hyperglycemic and acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day.

8. Complications of Diabetes

A. Acute complications

i. Hypoglycemia

Common in Type1 patients.

Causes:

May be caused by missing meals or doing unexpected exercise after taking insulin doses.
In diabetic patients with autonomic neuropathy, there could be hypoglycemic unawareness. This is so because the patient doesn't have symptoms of hypoglycemia, and won't take appropriate measure.

Symptoms:

Symptoms of hypoglycemia include sweating, nervousness, tremor, and hunger if it is not corrected in time central nervous system symptoms ensue like confusion, abnormal loss of consciousness or convulsions.

ii. Diabetic ketoacidosis.

Mostly a complication of Type1DM

- Insulin deficiency coupled with Glucagon excess results in Accelerated ketogenesis (ketone body production) ------ DKA.
- Insulin deficiency results in activated lipolysis and so increased free fatty acid concentration in the plasma
- As a result body fat is metabolized as a source of energy. This oxidation produces ketone bodies (acetoacetic acid and Beta hydroxybutyric acid), which are released into the blood and lead to metabolic acidosis.
- Inadequate levels of plasma insulin for a variety of reasons can precipitate DKA. See the list below

Precipitating events:

Inadequate insulin administration
Infection (pneumonia/UTI/gastroenteritis/sepsis … etc.)

Infarction (cerebral, coronary, mesenteric)
Drugs

- Clinically, ketoacidosis begins with anorexia, nausea and vomiting , coupled with Polyuria . If condition is not treated it may go into altered consciousness and coma.

iii. Non-ketotic Hyperosmolar state

- Is usually a complication of Type 2 because there is enough insulin to prevent ketosis
- Patient present with profound dehydration resulting from a sustained hyperglycemic diuresis and finally goes to a comatose state

B. Late complications of Diabetes

Mechanisms of development of diabetic late complications:

Long-term hyperglycemia is essential for the development of diabetic late complications. Many mechanisms linking hyperglycemia to the complications of long-standing diabetes have been explored. Currently two such mechanisms are important.

1. Non-enzymatic glycosylation.

- Non-enzymatic binding of glucose (glycosylation) to cellular proteins. This leads to formation of advanced glycosylation End product (AGEs) which cross link proteins (e.g.. collagen, extra cellular proteins), promote glomerular dysfunctions, induce endothelial dysfunctions, and alter extra cellular matrix composition and structure.
  - AGEs have been shown to accelerate atherosclerosis.

  Increased Glycosylated low-density lipoproteins (LDL), which do not readily bind to the LDL receptor in the liver, thereby making LDL cholesterol available to the arterial wall.

2. Hyperglycemia leads to increased intracellular glucose, which is then metabolized by aldose reductase to sorbitol, a polyol, and eventually to fructose. These changes have several untoward effects. The accumulated sorbitol and fructose lead to increased intracellular osmolarity and influx of water, and eventually, to osmotic cell injury. In the lens, osmotically imbibed water causes swelling and opacity → cataract formation.**Sorbitol accumulation** also impairs ion pumps and is believed to promote injury of schwann cells

and pericytes of retinal capillaries, with resultant peripheral neuropathy and retinal microaneurysms.

Diabetic macrovascular disease:

- Atherosclerosis

⇨ The extent and severity of atherosclerotic lesions in large and medium sized arteries are increased in long standing diabetes, and their development tends to be accelerated.

Atherosclerotic lesions in large blood vessels lead to vascular insufficiency and an ultimate production of ischemia in the organs supplied by the injured vessels.

E.g. Myocardial infarction, Brain infarction (resulting in stroke), gangrene of the toes and feet.

Diabetic microvascular disease

Increased thickening of the basement membrane in small vessels leads to the following chronic complication:

Diabetic Retinopathy

- DM is the leading cause of blindness in the developed world at ages>20.
- Blindness is primarily the result of progressive diabetic retinopathy and significant macular edema

It is classified into two

1. Non – proliferative retinopathy

- Is characterized by retinal vascular micro aneurysms, blot hemorrhages, and cotton wool spots

2. Proliferative retinopathy

- As the vascular abnormalities tends to be severe, new blood vessels start to proliferate in the retina

Diabetic Nephropathy

- It is the leading cause of ESRD (End stage renal disease) in the developed world.

- It starts with microalbuminuria (30-300 mg/d in a 24 hr urine collection) and progresses to overt proteinuria (> 300mg/d)

Three important lesions are in a patient with diabetic nephropathy.

Thickening of glomerular basement membrane which results in glumerulosclerosis, renal arteriosclerosis as part of the systemic
Involvement of blood vessels, and pylonephritis.

Diabetic Neuropathy

- It occurs in approximately 50% of individuals with DM
- It may manifest as polyneuropathy, mononeuropathy, and /or autonomic neuropathy

a. Polyneuropathy

- The most common form of diabetic neuropathy is distal symmetric polyneuropathy. It most frequently presents with distal sensory loss,

Hyperesthesia, paraesthesia and pain also occur.

b. Mononeuropathy

- This is less common than polyneuropathy in DM and presents with pain and motor weakness in the distribution of a single nerve.
- Involvement of 3rd cranial nerve is most common sometimes-cranial nerves IV. VI or VII are affected.

c. Autonomic neuropathy

- DM related autonomic neuropathy can involve multiple systems, including: the cardiovascular, GI, genitourinary, and metabolic systems
- It may also result in hypoglycemic unawareness due to reduction in counter regulatory hormone release.

Summary

- DM comprises of a heterogeneous group of disorders in which the common denominator is hyperglycemia. It is also characterized by long-term complication affecting the eyes, kidneys, Nerves and blood vessels.
- The current revised classification of DM is based on etiologic and pathologic process that the hyperglycemia results.
- There are two broad categories of DM types 1 and Type 2 DM

- Types 1A DM is caused mainly by an autoimmune destruction of the B- cells of the pancreases that ultimately leads to absolute insulin deficiency.
- Type 2 DM is characterized primarily by peripheral insulin resistance. It also results from impaired insulin secretion, and increased glucose production. The precise mechanism of the above disorders in Type 2 DM is not clear but polygenic disorders are believed to contribute to development of the said disorders.
- DM clinically manifest as polydipsia, Polyuria, polyphagia, metabolic acidosis and weight loss which are a direct or indirect consequence of hyperglycemia (increased blood glucose level)
- Destruction of a significant amount of pancreatic B- cell population and infiltration of the pancreas by mononuclear cell is the typical pathologic lesion seen is patients with Type 1 DM.
- In contrast to Type 1 DM patients with Type 2 DM do not have a decrease in the number of beta cells and there is no morphologic lesion of the cells.
- Due to a macro vascular and micro vascular abnormality patients with DM are subjected to chronic complications of the eyes, kidneys, heart, Nerves, brain and other organs.
- Due to acute metabolic decompensation that may result from absolute or relative insulin deficiency, patients with DM are subjected to acute metabolic complication like DKA, which is more common in Type 1 DM and non-ketotic hyperosmolar state, which is commonly seen in Type 2 DM.

IV. Gout

Represents a heterogeneous group of diseases in which the common denominator is an increased serum uric acid level and the deposition of sodium urate crystals in joints, soft tissue around joints and kidneys.

Pathogenesis of hyperuricemia:

- Uric acid is the end product of the catabolism of purines, derived either from the diet or synthesized de novo.
- Uric acid is eliminated from the body mostly through urine
- Normal values of uric acid in the blood is 7.0 mg/dl in men and 6.0 mg/dl in women
- Hyperuricemia can result from over production of uric acid, decreased urinary excretion of uric acid or a combination of both.

Classification:

1. Primary (idiopathic) Gout
   - In this category the causes that result in hyperuricemia are unknown,

Most cases (75-90%) of so- called idiopathic Gout result from an as yet unexplained impairment of uric acid excretion by the kidney.

- In minority of the cases, though the causes are unknown there is an over production of uric acid.

2. Secondary
   - In this category the causes that result in Hyperuricemia are known

   a. Conditions that result in over production of uric acid
      - Most common cause of overproduction of uric acid is increased turn over of nucleic acids, as seen in leukemia and Lymphomas and after chemotherapy for cancer.
      - Accelerated ATP degradation for various reasons results in over production of uric acid

   ⇨ Some genetic factors are also incriminated for over production of uric acid

   b. Conditions that result in Decreased urinary excretion of uric acid

   - The most common cause of decreased urinary excretion of uric acid is chronic renal diseases that lead to renal failure. In renal failure the clearance of uric acid is decreased, and with a fall in the rate of Glomerular filtrates, hyperuricemia ensues.

   ⇨ Other factors are also incriminated as a cause of decreased urinary excretion of uric acid.

Pathology (Morphology):

- When a sodium urate crystal precipitates from super saturated body fluids, they absorb fibronectin, complement, and number of other proteins of their surfaces. In phagocytizing those protein coated crystals, Neutrophiles release inflammatory mediators resulting in local inflammatory reaction
- Uric acid crystals may be found intracellularly in leukocytes of the synovial fluid. Extra cellular soft tissue deposits of these crystals (tophi), are surrounded by foreign body giant cells and an associated inflammatory response of mononuclear cells. These granuloma like areas are found in the cartilages and in any soft tissue around the joints.

- Macroscopically, it appears as chalky, white deposits on the surfaces of extra-articular structures and soft tissues around joints.
- Uric acid crystals also deposit in the kidney.

Clinical features:

There are four steps in the clinical course of gout:

1. Asymptomatic hyperuricemia

   Precedes clinically evident gout by many years
2. Acute gouty arthritis

   Initially there is a monoarticular involvement and later in the course of the disease, poly articular involvement with fever is common.
3. Intercritical period
   - This is Asymptomatic interval between attacks
4. Tophaceous Gout
   - Develops in the untreated patient in the form of tophi in the cartilage, synovial membrane, tendons and soft tissue.
   - Thophus is a chalky; yellow white deposit of monosodium urate crystals. Classic locations are on the ear, heads, olecranon bursa, and in the Achilles tendon.

Urate stones

- Constitute10 percent of all kidney stones

Diagnosis: The presence of long needle- shaped crystals that are –vely bisfrinegent under polarized light is diagnostic of gout.

Summary

- Gout represents a heterogeneous group of diseases where there is an increased serum uric acid revel and the depositions of sodium urate crystals in joints and soft tissues around joints and kidneys.
- Hyperuricemia can result form over production of uric acid, decreased urinary excretion of uric acid or a combination of both.
- Gout is classified as primary, in cases where the caused that resulting hyperuricemia are unknown. Where the causes are identified, it is classified as secondary.
- The deposition of sodium urate crystals in joints results in elaboration of inflammatory mediators form neutrophiles, which results in inflammation of the joint or the soft tissue involved.

V. Exercises

Say true or false:

1. Diabetes is a disturbance of carbohydrate metabolism that does not affect the metabolism of lipids and proteins
2. If one monozygotic twin has type 1 diabetes, the other one has or will develop that disease in at least 50% of cases.
3. A family history of diabetes is more common in patients affected by type 1diabetes than type 2.
4. There is a ß-cell morphologic abnormalities in patients with type 2 DM.
5. Pathologic changes in patients with DM in any organ, depends on the duration of hyperglycemia.

Multiple choice questions:

1. Type 1 DM is characterized by

A. Mostly occurs below age 20
B. Has an abrupt onset
C. Low levels of insulin in the blood
D. All

2. One of the following is not a micro vascular complication of DM

A. Stroke
B. Retinopathy
C. Nephropathy
D. Neuropathy

3. DKA is more common is

A. Type 1 DM
B. Type 2 DM
C. Gestational DM
D. A&B

4. One of the following is diagnostic for DM

A. A random blood glucose e level 200mg /dl with profound hyperglycemia
B. A fasting glucose level of 110 mg /dl
C. A RBS level of 126 mg /dl
D. None

5. One of the following is false statement

A. Type 1 DM is more common than type 2NKHS is more common in type 2
B. Polyuria and polydipsia are due to the hyperglycemic state
C. None

Short answer questions: Define gout.

1. What is secondary gout? List the causes that result in hyperuricemia in secondary gout.
2. What are the clinical features of gout?
3. What is a urate nephropathy?

References:

1. Pathology 2nd edition, Emanuel Rubin John L. Farber
2. Harrison's Principles of Internal Medicine. 15th edition
3. Robbins Basic Pathology 7th edition.

CHAPTER ELEVEN
ENVIRONMENTAL DISEASES

I. Learning objectives

After reading this chapter the student is expected to:

1. Define environmental diseases
2. Know the health impacts of air pollution
3. Define pneumoconiosis
4. Describe the pathogenesis and types of pneumoconiosis
5. List the Health impacts of Tobacco smoking
6. Describe the effects of acute alcohol intoxication and chronic alcoholism
7. Describe the causes and types of physical injuries

II. Introduction

Environmental diseases include those caused by exposure to harmful substances in the environment, in a sense that it encompasses all nutritional, infectious, chemical and physical in origin.

Environmental diseases are surprisingly common. They can originate from occupational exposures, polluted ambient air, chemicals taken to the body through the lung or GIT for several reasons, or from noxious physical agents that come in contact to the body. International labor organization has estimated that work related injuries and illnesses kill 1.1 million people per year globally. Environmental diseases constitute an enormous burden financially and in disability and suffering. With this overview of the nature and magnitude of these diseases we will concentrate on the more important once.

III. Air pollution

Air is most of the time, loaded with potential causes of diseases. Agents from the air like microorganisms contaminating food and water, chemical and particulate pollutants found in the air are common causes of diseases. It is important to see air pollution dividing it into an outdoor and indoor.

A-Out door air pollution

The ambient air in industrialized countries is highly contaminated with gaseous and particulate pollutants. In undeveloped countries like ours air is less polluted. Air pollution is severe in big cities like, London, Mexico city and Losangeles. In addition air may be locally spoiled in the vicinities of heavy industries.

There are six major pollutants, which collectively produce the well-known smog making some big cities difficult to live in.

To emphasize on some important points:

- Ozone is the most important pollutant in that it is produced in large amounts and has serious health consequences. It is highly reactive producing free radicals, which injures airways by virtue of release of inflammatory mediators. When healthy individuals are exposed, they experience mild respiratory symptoms, but its effects are exaggerated in people already having asthma and emphysema.
- Particulates are harmful when their diameters are less than 10μm whatever their composition may be. Larger particles are filtered out in the nares or mucocilliary system along the airways. The size of smaller particles helps them to reach into airspaces (alveoli) where they are phagocytosed by macrophages and neutrophils. Inflammatory mediator released from these cells are the once which result in the damage.
- Even if single pollutants are able to cause lung function impairments, the results are move sever when pollutants are in a combination. It is clear that pollutants are often time available in the air combined.
- Pollutants have wide range of consequences in different organs but the lungs are the usual targets

Here is a table listing the six important pollutants, their origins and their potential consequences.

Table 11.1 Major outdoor air pollutants

	Origin (s)	**Consequences**
Ozone (o3)	Interaction of oxygen with various pollutants: oxide of nitrogen, sulfur and hydrocarbons.	Highly reactive, oxidizes polyunsaturated lipids that become irritants and induce release of inflammatory mediators affecting all airways
Nitrogen dioxide	Combustion of fossil fuels like coal, gasoline and wood	Dissolves in secretion in airways to form nitric & nitrous acids which irritates & damage linings of air ways
Sulfur dioxide	Combustion of fossils such as coal, gasoline, & wood	Yields sulfuricacid and bisulfites & sulfites which irritate and damage linings of airways, together with nitric acid contributes to acid rains
Carbon monoxide	Incomplete combustion of gasoline, oil, wood & natural gas	Combines with hemoglobin to displace oxyhemoglin & thus induce systemic asphyxia
Particulates	Great variety of finely divided pollutants may include asbestos, plaster dust, lead, ash hydrocarbon residue and other industrial nuclear wastes	Major contributor to smog & a major cause of respiratory diseases.

B-Indoor air pollution

Indoor air pollution is a major problem in undeveloped countries like Ethiopia where people cook inside living rooms. Here, large number of family members dwell in single rooms where cooking activities are also undertaken .So wood smoke produced in large quantities is accumulated to affect the health of adults and children. It contains oxides of nitrogen and carbon particulates which are irritants predisposing children to repeated lung infection. Tobacco smoke is the commonest pollutant in the house of people living in developed countries but additional offenders are listed in the table below

Table 11.2 Health Effects of indoor air pollutants

Pollutant	Population at risk	Effects
Carbon monoxide	Adults & children	Acute poisoning
Nitrogen dioxide	Children	Increased respiratory infection
Wood smoke	Children & Females	Increased respiratory infection Irritators
Formaldehyde	Adults & Children	Eye & nose irritant Asthma
Asbestos fibers	Maintenance & abatement workers	Lung cancer, mesothe lioma
Bioaerosoles	Adults & Children	Allergic rhinitis, asthma

IV. Industrial Exposures

Industrial workers are exposed to a wide range of organic and inorganic substances, which have different kinds of consequences on their health. Diseases can range from mere irritation of mucosa of airways due to organic fumes to lung cancer due to inorganic dusts and leukemia due to prolonged exposure to benzene and uranium. Pneumoconiosis is a typical example of the conditions which are brought by industrial exposures.

PNEUMOCONIOSES

Pneumoconioses are a group of non-neoplastic pulmonary disease, which are due to inhalation of organic and inorganic particulates. The mineral dust pneumoconiosis, which is due to coal dust, asbestos, silcon and beryllium, almost always occur from exposure in work places.

Pathogenesis

Pneumoconioses is a result of lung reactions towards offending inhaled substances. The reaction depends on the size, shape, solubility and reactivity of the particles. Particles greater than 10μm are not harmful because they are filtered out before reaching distal

airways. When they are less than 1μm in diameter they tend to move in and out of alveoli like gases so that they will not deposit and result in an injury.

Silica, asbestos & beryllium are more reactive than coal dust bringing about fibrotic reaction, while coal dust has to be deposited in huge amounts if it has to result in reaction because it is relatively inert. Most inhaled dust is removed out through the ciliary movement after being trapped in the mucus linings. When particles reach the alveoli they are in gulfed by macrophages. The more reactive particles activate macrophages to release fibrogenic factors, toxic factors and proinflammatory factors. The cumulative effect becomes lung injury and fibrosis. The important mediators released by macrophages are grouped in to three: -

1. Free radicals: reactive oxygen and reactive nitrogen species that induce lipid peroxidation and tissue damage
2. chemotactic factors: leukotriene B_4 (LTB_4) interleukin 8 (IL-8) IL-6 ,and TNF which recruit and activate inflammatory cells and which in turn release damaging oxidants (free radicals)
3. Fibrogenic cytokines: IL-1, TNF, fibronectine, platelet derived growth factor (PDGF), and insulin like growth factor (IG F-1), which recruit fibroblasts.

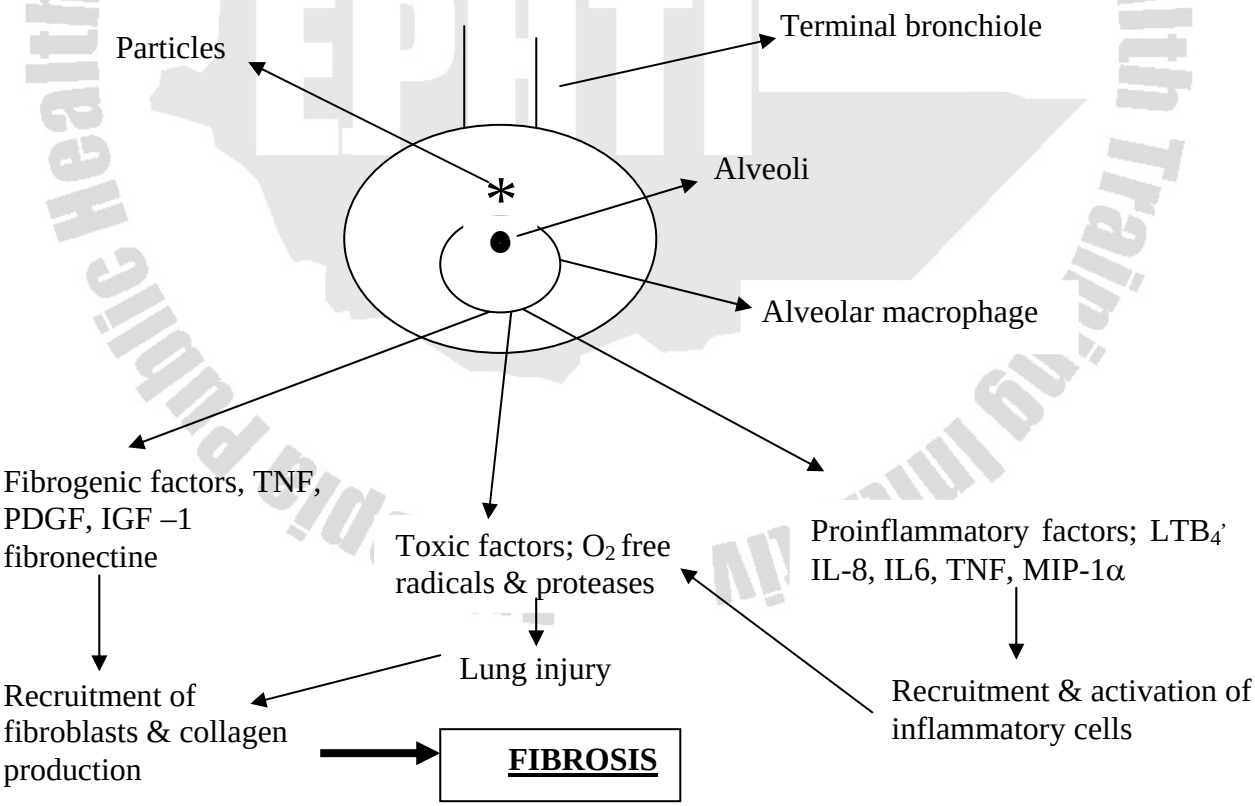

Figure 11.1 Pathogenesis of pneumoconiosis

Pneumoconiosis can be classified according to the substance incriminated.

A- Coal workers Pneumoconiosis _Due to coal dust
B- Silicosis _Due to silica
C- Asbestosis _Due to asbestos
D- Berylliosis _Due to beryllium

Coal workers pneumoconiosis

Since earlier times of industrialization it has been noticed that coal miners were drying of "black lung" complicated by tuberculosis. Coal dust mainly contains carbon but has a variety of trace metals inorganic mineral and crystalline silica. Anthracite (hard) coal contains significantly more quartz than bituminous (soft) coal. Anthracite (hard) coal is more frequently associated with lesions in the lungs; hence the name pulmonary anthracosis is coined for coal induced pulmonary lesions.

The disease has three distinct pathological entities:

i. Anthracosis:- Where pigments are accumulated without cellular reaction and symptoms
ii. Simple coal workers pneumoconiosis With minimal cellular reaction and little or no pulmonary dysfunction
iii. Progressive massive fibrosis:- With extensive fibrosis and compromised pulmonary function

Morphology

- Pulmonary arthracosis - Macrophages in the alveoli and interstitium are found laden with carbon pigments. These macrophages are also seen along the lymphatics including pleural lymphatics or lymphoid tissue along bronchi and lung hilus.
- Simple Coal workers pneumoconiosis (CWP) characterized by coal macules and coal nodules. Coal macules constitutes of carbon-laden macrophages aggregated, coal nodule is when the macule additionally contains collagen fibers.
- Complicated CWP - progressive Massive fibrosis (PMF) - occurring in the background of CWP after many years by coalescence of coal nodules. It is characterized by coal nodules intermingled with collagen fibers with central necrosis, size ranging from 2cm to 10cm.

Clinical course

Pulmonary anthracosis and simple CWP result in no abnormalities in lung functions.When it progress to progressive massive fibrosis in minority of cases it results in pulmonary hypertension and corpulmonale. Progression from simple CWP to PMF has been linked to amount and duration of exposure to coal dust. Smoking also has been shown to have the same effects. Sometimes of course the progression does not need factors mentioned above.

Asbestos Related Disease

Asbestos is a generic name that embraces the silicate minerals that occur as long, thin fibers. Asbestosis refers to the pneumoconiosis that results from the inhalation of asbestos fibers

Pathogenesis

Asbestos fibers are thin and long so that they can reach the bifurcations of bronchioles and alveoli. There, they are engulfed by macrophages to induce the cascade of inflammatory process, which finally result in interstitial pulmonary fibrosis.

Pathology

1-Asbestosis:-is an interstitial fibrosis of the lung. At early stages, fibrosis is in and around the alveoli and terminal bronchioles. when disease progresses, gross examination of the lungs show gray streaks of fibrous tissue, which accentuate the interlobular septa, together with diffuse thickening of the visceral pleura. The asbestos body is the most diagnostic structure seen under the microscope, consisting of asbestos fiber beaded with aggregates of iron along its length.

2-Pleural plagues: - after a period of many years the inhalation of asbestos fibers will result in the appearance of plaques on the partial pleura.

They are 2 to 3 mm thick, and microscopically they are densely collagenous and hyalinized and sometimes calcified.

3-Mesothelioma:-a clear cut relationship between asbestos exposure and a maligment mesothelioma is now firmly established.

4-Other malignancies like lung and bladder cancers can also result from asbestos exposure.

V. Tobacco Smoking

Considering the globe, the adverse effects of tobacco smoking out number all the effects of other pollutants. It is considered as one of the most important preventable causes of death in the United States. In our society also even though its health impacts are not so pronounced it still has series health damage.

Tobacco smoking affects not only those who are actively smoking but it also has an adverse consequence on the health of those who are by the vicinity of the smoker. These individuals are termed as passive Smokers.

Active Smoking and disease

The cigarette smoke that is taken through the mouth into the lung has several types of chemicals that have diverse & serious effects on our health. The composition depends on the type of tobacco, length of the cigarette, and presence and effectiveness of filter tips.Usually present are (1) Carcinogens whose effects have been verified in lower animals (e.g.polycyclic hydrocarbons, betanaphthylamine, nitrosamines). (2) Cell irritants and toxins (e.g. Ammonia, formaldehyde, and oxides of nitrogen). (3) Carbon monoxide, and (4) nicotine, which has various effects on the sympathetic nervous system, blood pressure heart late and the like.

The more common adverse health effects of tobacco are lung cancer, coronary heart disease, COPD (Chronic bronchitis, emphysema), and systemic atherosclerosis.And the less common effects are peptic ulcer, Cancer that can originate from larynx, esophagus, pancreas, bladder & kidneys.

Cigarette smoking also causes COPD, which are chronic bronchitis and Emphysema, which have a tremendous health impact. Systemic arteriosclerosis and other forms of cancer are also diseases caused by cigarette smoking, which collectively contribute to many deaths. In general smoking is the single most important cause of cancer mortality in the United States.

Fetuses are also adversely affected by maternal smoking. Several studies have shown that maternal smoking could cause low birth weight, prematurity, still birth and infant mortality. Moreover other complications of pregnancy like abruptio placentae, placenta previa, and premature rupture of membranes have been found to be caused by maternal smoking.

The risk of mortality is dose dependent. The number of pack years i.e. number of packs per day times number of years is directly related to mortality rate.The more pack years of smoking the higher the risk of mortality.Coronary heart disease causes most of the deaths

when it comes to effects of cigarette smoking. Lung cancer closely follows causing a huge number of deaths.

Involuntary smoke exposure (Passive Smoking)

The effect of passive smoking has been identified during the last few decades. Its effect comes when non-smoking people inspire the ambient air, which is polluted by cigarette smoke. The health impact depends on the volume of the air in the room, number of active smokers, rate of air exchange and duration of exposure. Data from different countries show that the risks of lung cancer increase by 1.5 due to passive smoking. There is also increased risk of cardiovascular diseases specially MI, and high incidence of lower respiratory tract diseases in infants & children of smoking parents. Children & infants of smoking mothers will have an obvious intense exposure and hence retardation of physical and intellectual growth is likely to occur.

Benefits of cessation or reducing exposure to cigarette smoke

When a person stops smoking the risks of diseases and subsequent death start to decline The risk to reach to that of non-smoking people may take 20 years of smoke-free period.The amount of cigarettes smoked daily, and duration of smoking determines the rate of decrease of risks. The relative risk of lung cancer and laryngeal cancer start to decline after 1 to 2 smoke free years. However considering lung cancer former smokers will have slightly higher risk than non-smokers even after 30 years of smoke-free years.

When it comes to coronary diseases the decline of risk is rapid and it can level with those of non-smokers after 5 to 20 years. Once COPD has been developed quitting does not have any significant effect in reversing the situation.

VI. Chemical & Drug injury

Injuries due to chemicals can be from therapeutic agents and nontherapeutic agents.

A. Adverse drug reactions

Injuries due to therapeutic agents are known as adverse drug reactions. Adverse drug reactions are any response to a drug that is noxious and unintended and that occurs at doses used in humans for prophylaxis, diagnosis, or therapy. ADRs are only rarely due to physician failures like inadequately monitored use or over use of drugs.

ADRs can be divided into two categories.

1. Exaggeration of the intended pharmacologic effect which are largely predictable effects
2. An unpredictable response unrelated to the drugs primary action.

The 1st one-encompasses all adverse reactions, which result from use of powerful drugs used to treat potentially fatal diseases like cancer. For example use of large dose daunorubicin or doxorubicin to treat some forms of cancer may cause cardiotoxicity.

In the 2nd group the ADRs are unpredictable and they vary from individual to individual taking a specific drug. The reactions is called idiosyncrasy and is due to an abnormal immunologic response to the drug or un predictable cytotoxicity caused by the drug. Possible examples will be extensive hepatic necrosis, which develops after intake of therapeutic dose of acetaminophen, or anaphylaxis, which develop after therapeutic dose of penicillin.

B. Non-therapeutic agents

Ethyl alcohol

A large percentage of our population is a social drinker and still a significant number of individuals are alcoholic or alcohol dependent. Alcohol has an obvious acute effect but has also an effect of a long-standing use of alcohol on organs and tissues.

Alcohol Metabolism: -

About 2 to 10% of the ethanol consumed is excreted directly through the breath, urine or sweat. The amount exhaled is directly proportional to the blood level and hence is used by legal enforcement agencies. After ingestion, a small amount of ethanol is directly metabolized by gastric mucosa alcohol dehydrogenase. The rest is rapidly absorbed from stomach & intestines, once in the liver alcohol is metabolized by three pathways in the liver cells. The 1st involves hepatic alcohol dehydrogenase, yielding acetaldehyde, which is then converted to acetate by aldehydedehydrogenase. In this process NAD is converted to NADH. The 2nd involves the hepatic microsomal P-450 system. It too yields acetaldehyde, oxidisable to acetate. The 3rd pathway, which is less frequently utilized, involves peroxysomal catalase. All the three pathways yield acetaldehyde, which can be metabolized to acetate, and the 1st two produce more NADH from NAD.

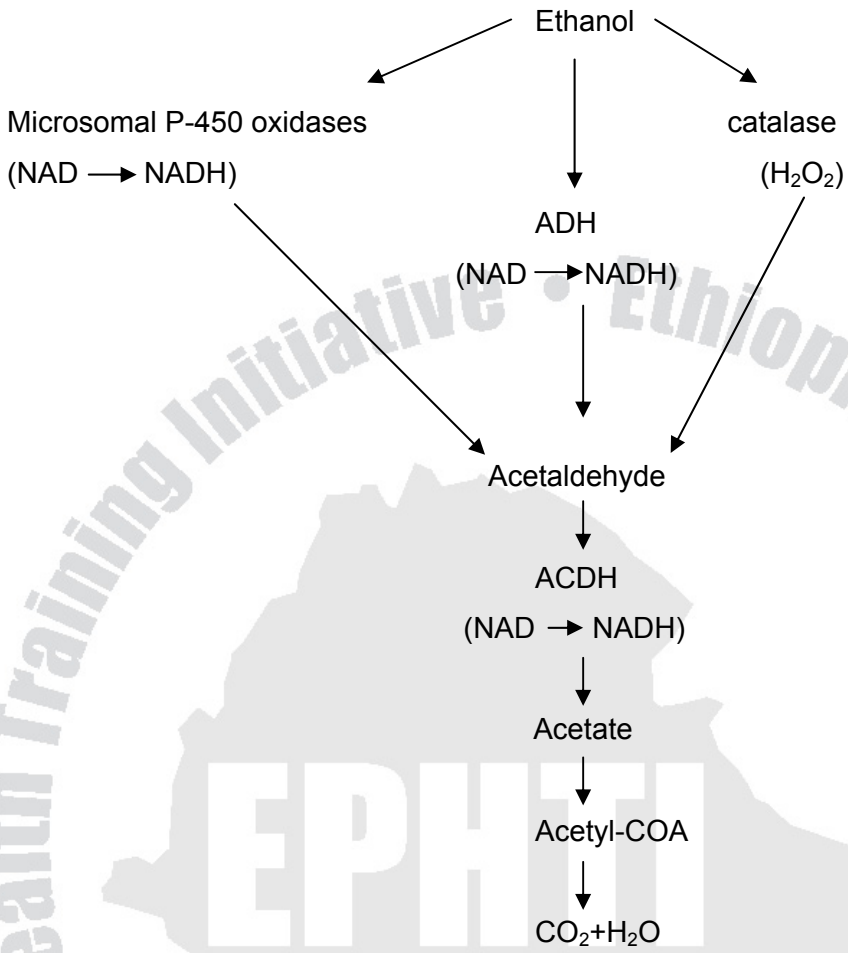

Fig 11.4 Metabolism of alcohol: the major (rate limiting) pathway is via ADH.

Acute Alcoholism

Acute alcoholism exerts its effects mainly on the CNS even though; the stomach and liver can also have reversible damages. Alcohol has depressant effect on the CNS.It depresses the inhibitory control centers thereby releasing excitatory pathways that accounts for the wide spread belief that alcohol is a stimulant. The extent of CNS depression depends on the alcohol blood levels. The cortex affected first, the limbic system, cerebellum and finally brainstem are affected as the blood level increases. Coma and total respiratory arrest become likely at 300 to 400 mg/dl levels. Not with a clearly understood mechanism, acute alcoholism has also "black out" effects that are episodes of forgetting what has happened during drinking.

Chronic alcoholism

The increase NADH:NAD ratio, which is created by alcohol metabolism, may be responsible for the metabolic consequences of chronic alcoholism. Chronic alcoholism produces morphologic changes in almost all organs and tissues.

Hepatic changes: these are the most common consequences of chronic alcoholism.These changes are namely fatty change, acute hepatitis and alcoholic cirrhosis. These are discussed in adequate detail in the chapter that deals with liver diseases. Fatty changes can occur with in a few days of even modest alcohol consumption. Cells are distended with fat accumulation, which can be mobilized when the exposure to alcohol is discontinued. Alcoholic hepatitis can occur with episodes of heavy drinking and may or may not be preceded by fat accumulation and may or may not be followed by cirrhosis which is the end stage of fatty changes that occur in chronic alcoholism.

Central nervous system changes

1-Wernicke's encephalopathy

The commonest CNs change is wernicke's encephalopathy.This is due to a thiamine deficiency that occurs during chronic alcoholism.The chronic alcoholic subsists with an inadequate dietary intake and alcohol itself impairs intestinal absorption of thiamine. This condition may occur in non-chronic alcoholics who become thiamine deficient for various reasons.

Wernicke's enephalopathy occur in a subset of alcoholics, probably in those who have an inherited or acquired abnormality of a thiamine dependent transketolase (enzyme involved in cerebral glucose & energy metabolism) reducing its affinity for thiamine.

Clinically it is characterized by ataxia, global confusion, ophtalmoplegia and often nystagmus. The underlying morphology includes foci of symmetric discoloration and sometimes softening with congestion, & punctate hemorrhage in the paraventricular region of the thalamus & Hypothalamus, in the mammillary bodies about the aqueduct in the midbrain, in the floor of the fourth ventricle and in the anterior cerebellum. The neurons may be relatively spared in the early stages but eventually reveal degenerative changes and eventually cell death.

2- Korsakoff's syndrome

After individuals, with Wernicke's encephalopathy, are treated with thiamine, they show a profound memory loss, which does not improve with thiamine treatment. This condition is termed as korsakoff's syndrome. There are no specific morphologic changes other than seen in wernicke's encephalopiathy but this does not show any improvement with thiamine treatment. Hence it is believed that korsakoff's syndrome is caused by direct neurotoxicity of ethanol compounded by a lack of thiamine.

3- Cerebellar ataxia

Cerebellar degeneration, related to loss of purkinge's cell in the cerebellar cortex, is a well-documented cerebellar change found in a minority of chronic alcoholics. These are supposed to be due to thiamine deficiency as well, rather than ethanol direct toxicity. Cortical atrophy is also a potential consequence but many studies didn't reveal any reduction in size of the cortex in chronic alcoholics.

Peripheral Nerves

The peripheral nerves suffer a demylinating polyneuropathy, occasionally mononeuropathy that is fairly common in chronic alcoholics who are malnourished. The basis is thought to be thiamine deficiency rather than ethanol toxicity.

Cardiovascular system

A moderate intake of alcohol tends to increase the level of HDL and hence protects from atheroma formation and coronary heart disease. However heavy consumption, which causes liver cell injury, will decrease the level of HDL and contributes to atherosclerosis & coronary heart disease. On the other hand a direct ethanol injury to myocardium will result in cardiomyopathy, which is discussed in the chapter that deals with heart diseases.

Miscellaneous changes:

Chronic alcohol intake has a tendency to produce hypertension even though in low doses alcohol (ethanol) tends to reduce blood pressure. Chronic alcoholics suffer higher incidence of acute & chronic pancreatitis and regressive changes in skeletal muscle referred as alcoholic myopathy. During pregnancy a condition known as fetal alcohol syndrome may

take place in infants whose mothers have been taking alcohol even as low as two to three drinks per day. The fetues can have microcephally, mental retardation, facial mal formation & cardiac defects at times. Increased risks of cancer of pharynx, larynx, esophagus, stomach, & possibly rectum & lung have also been encountered in chronic alcoholics.

VII. Physical injuries

These are mainly classified in to four groups: injuries due to mechanical forces, changes in temperature, changes in atmospheric presence, and electromagnetic energy.

A-Injuries due to mechanical forces

Injuries due to mechanical forces are (1) soft tissue injuries, (2) bone injuries, (3) head injuries. Injuries to the bones & head are considered else where. Here we deal with soft tissue injuries, which are divided accordingly to their depth.

Abrasion: this type of injury represents the most superficial type of skin injury, which involves the epidermal layer. It occurs when superficial epidermal cells are turnoff by friction or a glancing blow. There is no perforation of the skin & hence regeneration occurs with out scarring.

Laceration versus incision

Laceration is an injury over the skin which is an irregular tear produced by overstretching. Depending on the tearing force it can be linear or satellite. The margins of a laceration are frequently hemorrhagic & traumatized and there will be bridging stands of tissues like blood vessels or fibrous tissues at the base. An incision is made by a sharp cutting object like knife. The margins are relatively clean and there are no bridging fibrous strands or tissues. An incision, in contrast to laceration, will be approximated by sutures to heal leaving no or little scar. laceration can take place to deeper tissues or organs without apparent superficial injury for example when a fast moving vehicle collides with and object the liver of a driver, not using safety belts, can lacerate when his body impacts on the steering wheel.

Contusion

This is an injury that is cause by a blunt force that injurs small blood vessels & causes intestinal bleeding usually with out a breach on the superficial tissue the bleeding will be evident if the contusion is on a superficial tissue but if it is in deeper structures like skeletal

muscles the bleeding will be evident after several hours or may remain obscured excepts the swelling & pain that is felt at the area over the contusion.

Gunshot wounds

Looking at the gunshot wounds give a very detailed story as to whether the shot is from a distance or, near by, or from a rifle or a handgun. It also tells the direction from which the bullets came & other important information for a forensic pathologist. The character of a gunshot wound at entry & exit and the extent of injury depend on the type of gun used, caliber of bullet, the type of ammunition, the distance of the firearm from the body, etc.

Entry wounds in general are smaller than exit wounds. With a shot from close range, the entry wound has a gray – black discoloration produced by the heat, smoke and unburned powder. There are also peripheral stippling of discrete, larger particles formed by the unburned powder, When the shot distance increases a beat only the stippling are present and at greater distances no gray black discoloration or stippling are present rather a wound smaller in size from the bullet and with narrow enclosing rim of abrasion is present.

Cutaneous exit wounds are generally more irregular than the entry wounds due to the wobbling or trajectory motion of the bullet. In high velocity riffle bullets the exit wounds are larger and there are no stippling or dark discolorations. Large caliber, light velocity bullets cause extensive injury around the traversing wound due to the mass, velocity and motion of the bullet. Small caliber low velocity bullets cause a limited amount of injury to surrounding tissue.

In general, it suffices to say that gun shot wounds tell a story to the experienced individual.

B-Injuries related to changes in temperature

Human beings are homoeothermic and their internal temperature must be maintained between 30^0C and 43^0C. Even these limits can be tolerated for a brief time. Abnormally high and low temperatures are injurious to the body and their damage are different and have to be discussed separately.

A. Injuries due to abnormally high temprature

These can be brought by flame, boiled water or steam, electricity and etc. It involves mainly children and young adults and is very common in our society.

Cutaneous Burns

The severity of the burn effect depends on:

- % Of total body surface involved
- Depth of the burn
- Possible presence of internal injuries form inhalation of hot gases & fumes
- Promptness and efficacy of the post burn therapy

Burn can involve the most superficial part of the skin or it can go even deeper to internal organs. Terms like 'partial thickness' and 'full- thickness' burns are applied to describe the degree of burn injury. In partial thickness, the dermis with its skin appendages is preserved. Epidermis can be fully or partially devitalized and it continues to provide a cover to the burned area. Such burns are characterized by blistering, protinacious fluid exudation from dilated and injured small blood vessels. Inflammatory reaction and regeneration of the epidermis from preserved appendages of dermis are also common features. The epidermal cells may exhibit deranged membrane permeability, with nuclear and cellular swelling or may show clean pyknosis and granular coagulation of cytoplasm.

Full thickness burn implies total distraction of the entire epidermis extending into the dermis and even more deeply at times. Regeneration from dermal appendages is scarce and hence healing will result in scarring unless skin grafting is performed. With the epidermis burnt out the dermal collagen may take the appearance of a homogenous gel. The cytologic changes described in partial thickness burn may be seen in deeper structures and the inflammatory reaction seen in the partial thickness burn is greater here.

The systemic effects are feared more than the local injury. Neurogenic shock can prevail due to the pain and this can be followed by hypovolemic shock when the individual looses fluid from the burned area. Dreadful infection can develop because of a wide area, which is open to infection and due to a media favorable for proliferation of microorganism. The wound infections can progress to regional thrombophllbitis, infective endocarditis, pneumonia, cellulitis, and sepsis.

B. Injuries due to abnormally low temperature

The effects of hypothermia depended on whether there is whole body exposure or exposure only of parts. Death may result when the whole body is exposed, with out inducing apparent necrosis of cells or tissues.This is because of the slowing of metabolic process, particularly

in the brain and medullary centers, when parts of the body are exposed, local changes result depending on the types of exposure to low temperature

Local reactions

Injury to cells and tissues occur in two ways

1. Direct effect of low temperature on the cells
2. Indirect effects due to circulatory changes

Circulatory changes will be in two ways: slow temperature drop that will result in vasoconstriction and increased permeability leading to edematous changes as in 'trench foot', sudden sharp drop that will result in vasoconstriction and increased viscosity of the blood leading to ischemia and degenerative changes.Edematous changes in trench foot could be followed later by atrophy and fibrosis.

C. Injuries related to changes in atmospheric pressure

1. High altitude illness

This is encountered in mountain climbers in atmospheres encountered at altitudes above 4000m. The lower oxygen tension produces progressive mental obtundation and may be accompanied by poorly understood increased capillary permeability with systemic and, in particular pulmonary edema.

2. Air or Gas Embolism

This may occur as a complication of scuba diving, mechanical positive- pressure ventilatory support, and hyperbaric oxygen therapy. In all these occasions there is an abnormal increase in intra-alveolar air or gas pressure, leading to tearing of tissue with entrance of air into the interstitium and small blood vessels. Pulmonary, mediastinal, and subcutaneous emphysema may result. The coalescence of numerous small air or gas emboli that gain access to the arterial circulation may lead acutely to stroke- like syndrome or a myocardial ischemic episode.

D-Electrical Injuries

The passage of an electric current through the body:-

- May pass without effect
- May cause sudden death by disruption of neural regulatory impulse producing, for example, cardiac arrest

- Or may cause thermal injury to organs exposed to electric current

Although all tissues of the body are conductors, their resistance to flow varies inversely to their water content. Dry skin is particularly resistant, but when skin is wet or immersed in water resistance is greatly decreased. Thus, an electric current may cause only a surface burn of dry skin but, when transmitted through wet skin, may cause death by disruption of regulatory pathways.

Summary

Environmental pathology deals with diseases that are brought by exposure to harmful substances in the environment.

The ambient air could be polluted by potential causes of diseases. Out door air of industrialized cities is highly polluted with six major pollutants, which affect the health of inhabitants. In the developing world indoor air pollution could risk the health of families.

Industrial exposures pose a huge burden to the health of peoples. Organic fumes and particulates taken into the lung cause several types of Neoplastic and non-Neoplastic diseases. Pneumoconioses are a group of non neoplastic lung diseases caused by inhalation of organic and inorganic particulates. Coal dust, asbestos, silicon and beryllium are mineral dusts which cause most of the pneumoconiosis. People affected by different types of pneumoconiosis go through more or less, same kind of steps in to severe forms when exposure continues. In coal workers pneumoconiosis the patient will first have a non-symptomatic blackening seen along the lymphatics and lymphnodes which mark coal laden macrophages. This can progress to simple coal workers pneumoconiosis (CWP) characterized by fibrous macules and nodules. only few can go to the serious form called progressive massive fibrosis which has deleterious impact on pulmonary and cardiac function.

Smoking is the single most important pollutant, which affect the health of millions of individuals. Both active and passive smoking have serious health consequences resulting in lung cancer, coronary heart disease, COPD, systemic at hero sclerosis etc. Abstinence has a positive impact in progressively lowering the risks imposed by the previous years of smoking.

Alcohol, even though taken in small amounts have a health promoting effect, when taken in more amounts it will have short term and long term un healthy impacts. Alcohol is a CNS depressant capable of resulting in respiratory arrest and coma in large amounts. The liver is

mainly hit hard by its deleterious effects. Central and peripheral nerves systems, as well as cardiovascular systems are also its targets.

When dealing with environmental diseases injuries caused by physical forces have to be thought about. These could be caused by mechanical forces, extreme high or low temperatures, atmospheric pressure changes or electromagnetic energy.

VIII. Exercise

1. What could be causes of environmental diseases?
2. What is the pathogenesis of pneumoconiosis?
3. Describe the three morphologic appearances of coal workers pneumoconiosis.
4. How does progressive massive fibrosis result?
5. Mention four important adverse health impacts of smoking.
6. The pathogenesity of mineral dusts does not depend on
    a. Size of particles
    b. Reactivity of particles
    c. Duration of exposure
    d. The amount inhaled at a time
    e. None
7. Which one of the following can't be a result of coal workers pneumoconiosis
    a. 'Black lung'
    b. Progressive massive fibrosis
    c. Cor pulmonale
    d. Lung cancer
8. Which of t he following mineral is associated with mesothelioma
    a. Beryllium
    b. Charcoal
    c. Asbestos
    d. Silca
9. Which one of the following is the most common cause of mortality among cigarette smokers
    a. Lung cancer
    b. Coronary heart disease
    c. COPD
    d. Stroke

10. Which one of the following is true

a lcohol is a stimulant
b. Alcoholic fatty changes are irreversible
c. Wernicke's encephalopathy is an indirect effect of alcoholism
d. The liver takes all the responsibility of alcohol metabolism

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