Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

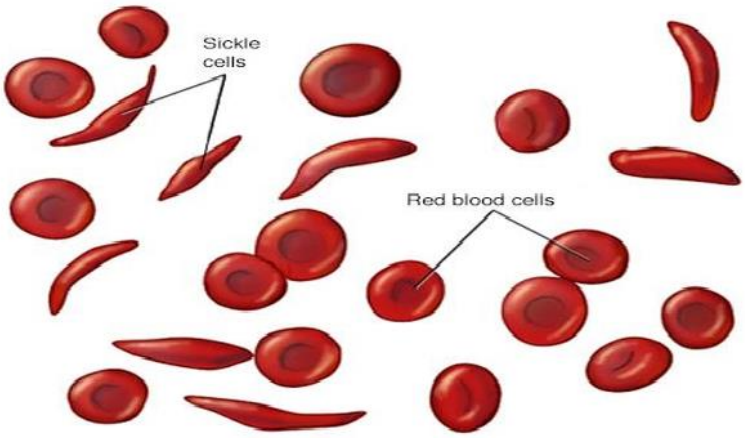
Module of General hematology

15 weeks

Third Level-First Semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for students of the second stage, Department of Renal Dialysis Technologies, in learning about hematology, methods of diagnosis, symptoms of blood diseases, and practical methods of diagnosis in laboratories.

Special Objectives of the Module

At the end of the module, the student is expected to be able to:

1. Definition of hematology.
2. Classification types of anemia.
3. Differentiate between types of anemia.
4. Distinguishes between hereditary and acquired blood diseases or due to other causes.
5. Recognizes the clinical symptoms and signs of diseases.
6. Compares between the necessary diagnostic methods for blood diseases.
7. Realizes the importance of laboratory tests in the process of diagnosing blood diseases.
8. Mastering practical methods for conducting the necessary tests to diagnose blood diseases.

Course components of College curriculum of Health and Medical Technical /Kirkuk, Medical Laboratory Department

Week	Details
1st	Introduction of haematology (definition, importance, general functions of blood), Blood composition, Blood plasma Components, Physical-chemical constants of blood,
2nd	Stem Cells, definition, type, functions of stem cell, Haemopoiesis (definition, Site of haemopoiesis, Bone marrow stroma, The regulation of hemaopoiesis, Haemopoietic growth factors.
3rd	Erythropoiesis, RBCs morphology, the RBCs' cell membrane, and RBCs' metabolism. Haemoglobin (structure, synthesis, and levels in blood and in erythrocytes).
4th	Homeostasis, Death, and Disposal of RBCs.
5th	Anaemia (definition, causes, Clinical features, Symptoms, Signs, laboratory findings, diagnosis). Classification of anaemia.
6th	Iron metabolism, iron deficiency anaemia.
7th	Megaloblastic anaemia (B12 metabolism, Absorption, Transport, Biochemical function, B12 deficiency anaemia). Folate metabolism (Absorption, transport, and function, Floated deficiency anaemia).
8th	Haemolytic anaemia (Normal red cell destruction) Classification, Hereditary hemolytic anemia, Acquired hemolytic anemia.

9th	Thalassemia (definitions, types, causes, and diagnosis).
10th	Sickle cell anaemia.
11th	Aplastic anaemia.
12th	Polycythaemia.
13th	Acute leukaemia (acute lymphocytic leukaemia, acute myeloid leukaemia) causes and diagnosis of each one. Chronic leukaemia (chronic lymphocytic leukemia, chronic myeloid leukaemia) causes and diagnosis of each one.
14th	Lymphoma (Hodgkin's lymphoma, causes, lab. Findings). Non-Hodgkin's lymphoma, causes, and lab. Findings.
15th	Bleeding disorders, Arterial thrombosis, venous thrombosis, and risk factors

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

5. Gillian Rozenberg. Microscopic Haematology a practical guide for the laboratory. Elsevier Australia. ACN 001 002 357 (a division of Reed International Books Australia Pty Ltd) Tower 1, 475 Victoria Avenue, Chatswood, NSW 2067 This edition © 2011 Elsevier Australia.

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

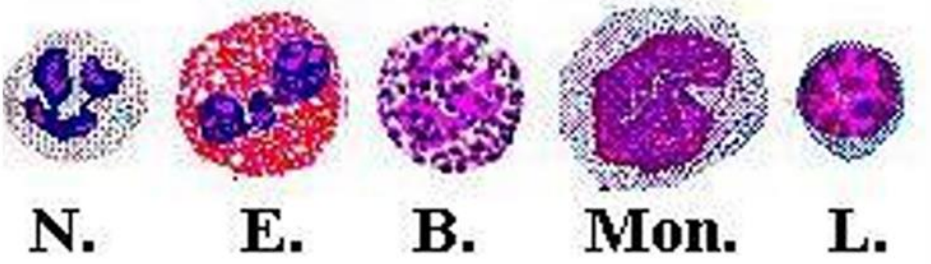
First - Week

Lecture Title: Introduction of haematology & Blood components

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for the students in identifying the concept of hematology, the components of blood, and the morphology and function of each compnent.

Special Objectives of the Module

1. Definition of hematology.
2. Definition of blood.
3. Enumeration of blood functions.
4. Differentiates between blood components.
5. Distinguishes between blood components in terms of morphology, function, and life span.

.....

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.
2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.
3. Learning Guide Series Hematology.
4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

Introduction

Thebranch of science concerned with the study of blood, blood-forming tissues, and the disorders associated with them is called hematology.

Hematology is the study of blood in health and in pathological conditions.

- ✓ Blood, lymph, and tissue fluid form the organism's internal environment.
- ✓Blood is the fluid constituent of the cardiovascular system, comprising both plasma and cellular components.**55%** of total blood volume is composed of plasma. Red blood cells, white blood cells, and platelets 45% form the cellular component.
- ✓Blood has many essential homeostatic functions including the transport of oxygen and carbon dioxide, nutrients and waste products, immune function, buffering, and hemostasis. Blood has such an important role in the maintenance of homeostasis.
- ✓Problems with blood composition or circulation can lead to downstream tissue malfunction. Blood also maintains homeostasis by acting as a medium for transferring heat to the skin and acting as a buffer system for bodily pH.
- ✓¬ Pre-test:--Blood consists of---------- and----------.-The decrease in blood production can lead to----------.

Blood

- ✓ Is a type of liquid connective tissue and is one of the most complex organ systems in the human body.
- ✓ The key parts that make up the hematological system are the blood, bone marrow, spleen, and lymph system.
- ✓ Blood is a vitally important fluid for the body.
- ✓ Itis thicker than water and feels a bit sticky.
- ✓ The temperature of blood in the body is **38°**C **(100.4°F)**, which is about one degree higher than body temperature.
- ✓ Blood makes up about 7-8 % of your body's weight.
- ✓ Blood consists of hematopoietic stem cells in the bone marrow.
- ✓ Blood is responsible for the most important functions of life, such as the transport of metabolic components, nutrients, hormones, gas exchange, immune defense, and coagulation.
- ✓ In the adult, blood consists of approximately 55% plasma (liquid component) and 45% formed elements including erythrocytes (red blood cells– RBC), Leukocytes (white blood cells– WBC), and thrombocytes (platelets- PL).

Functions of Blood

In our body, the blood performs the following functions:

1. Respiratory.

Blood transports oxygen from the lungs to the cells of the body and carbon dioxide from the

cells to the lungs.

2. Transport

- ✓Oxygen from lungs to body cells
- ✓Carbon dioxide from body cells to lungs.
- ✓Nutrients from GI tract to body cells
- ✓Nitrogenous wastes from body cells to kidneys

3. Excretion

Excretion of metabolic wastes to the kidney, lungs, and skin.

4. Regulation

- ✓Regulation of body temperature by distribution of body heat.
- ✓PH.
- ✓The concentrations of various substances (for example, glucose, sodium, potassium, etc.) in our body fluids.
- ✓The blood helps to keep certain things in the body in balance. For instance, it makes sure that the right body temperature is maintained.This is done through the liquid part of the blood (plasma), which can absorb or give off heat, and through the speed at which the blood is flowing:
- ✓When the blood vessels expand, the blood flows more slowly and this causes heat to be lost.
- ✓ When the temperature outside the body is low, the blood vessels can contract to reduce the amount of heat lost.
- ✓The pH value of the blood is kept at a level ideal for the body.
- ✓ The pH value tells us how acidic or alkaline a liquid is.
- ✓ A constant pH value is very important for things in the body to function properly.

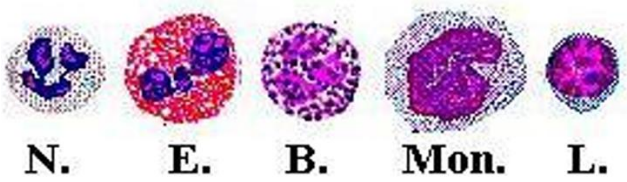
5. Protection

- ✓ Platelets and proteins "seal" vessel damage protection from foreign material and infections.
- ✓ Defense against infections **(WBCs, antibodies).**

PROPERTIES OF BLOOD	
Colour	Bright red in arteries & dark red in veins
Mass	8 % of the body mass
pH	Slightly alkaline (pH = 7.35 – 7.45)
Taste	Salty
Temperature	38° C (100.4° F)
Viscosity	3 – 4 times more viscous than water
Volume	5 – 6 litre

Composition of Blood

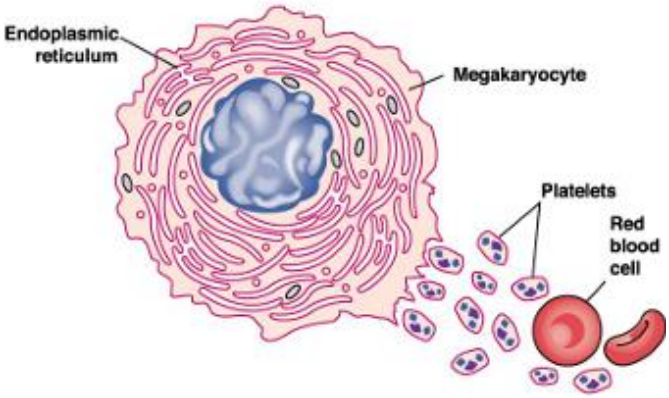
- ✓ Fluid medium i.e. the plasma.
- ✓ Formed Elements.

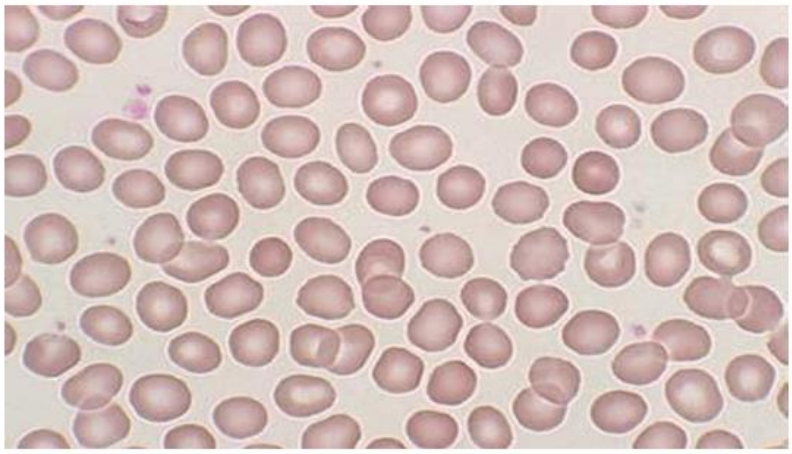


i. Red blood cells (erythrocytes).

ii. White blood cells (leukocytes).

iii. Platelets (thrombocytes).



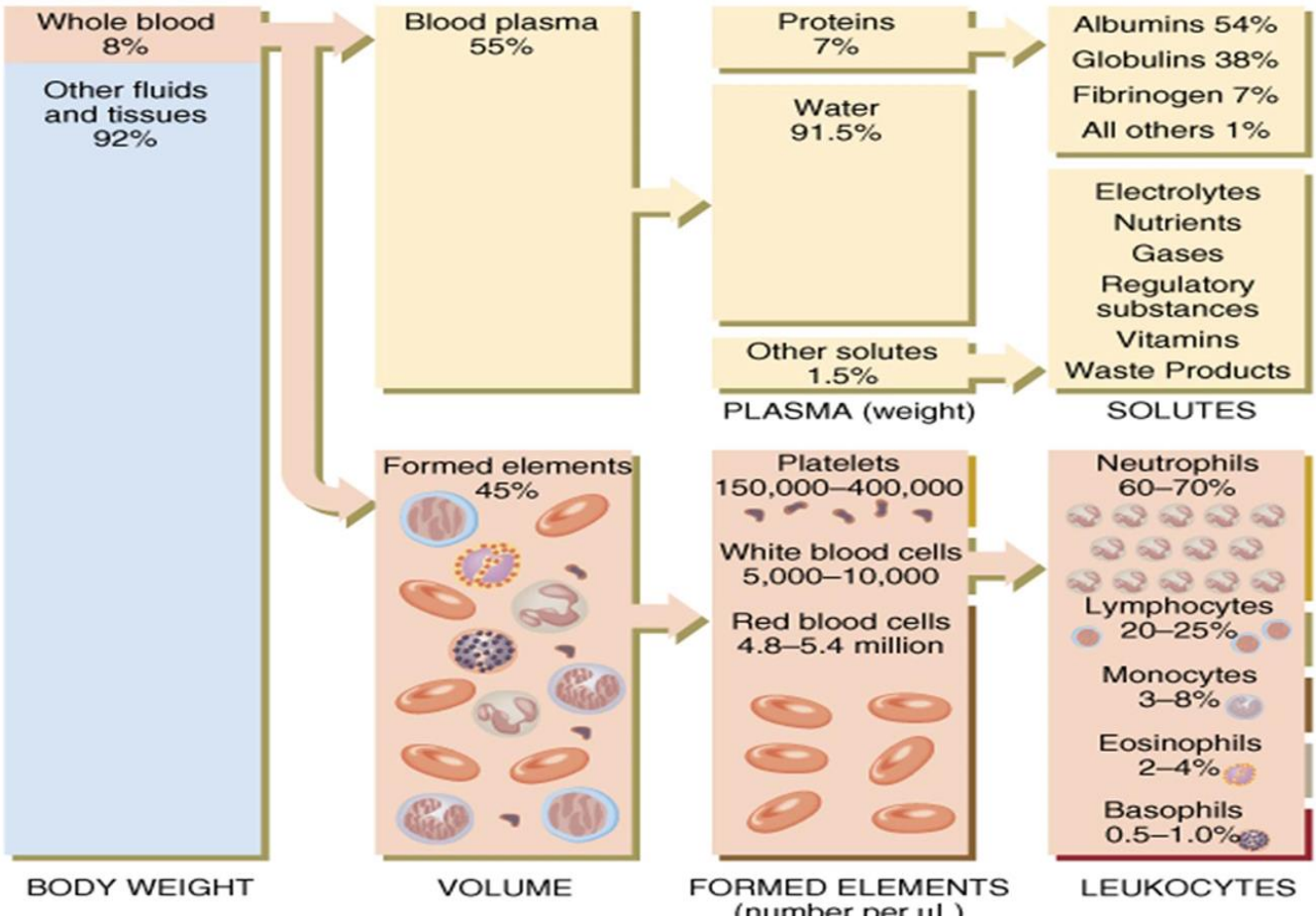


Mature red cells in the peripheral blood.

.....

Gross composition of Plasma and Blood Cells

Constituents	Plasma	Red blood cells
Water	91-95%	%65
Solid	8-9 %	35%
Protein	6-8 gm %	31-33%
Specific gravity	1.026	



Whole blood → No anticoagulant → Clot formation → Centrifuge

→ Clot + Clear yellowish fluid (Serum).

Whole blood → anticoagulant → Clot formation → Centrifuge

→ Clot → Clear yellowish fluid (Plasma).

Plasma without fibrinogen is called serum.

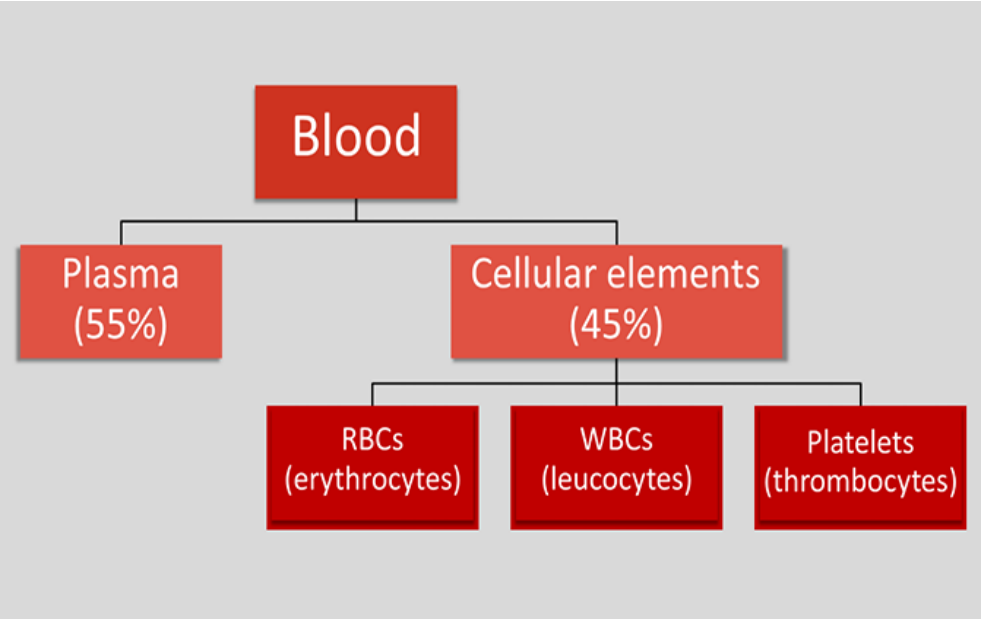
Serum = plasma - anticoagulant factors

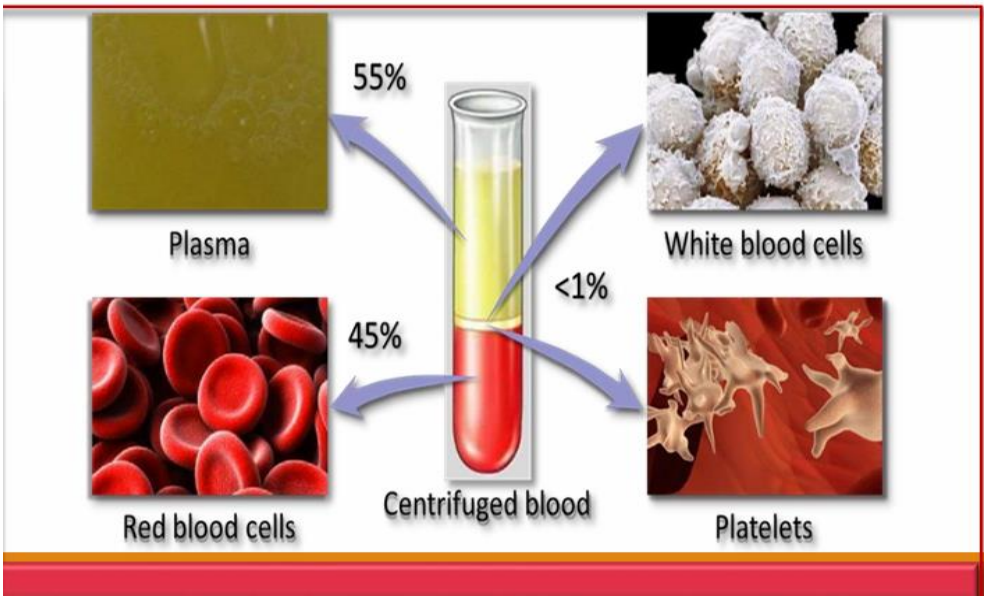
Separation of Components in a Centrifuge

1.Clear/yellowish Plasma.

2.Thin/whitish buffy coat Leukocytes and platelets.

3.Reddish mass Erythrocytes.



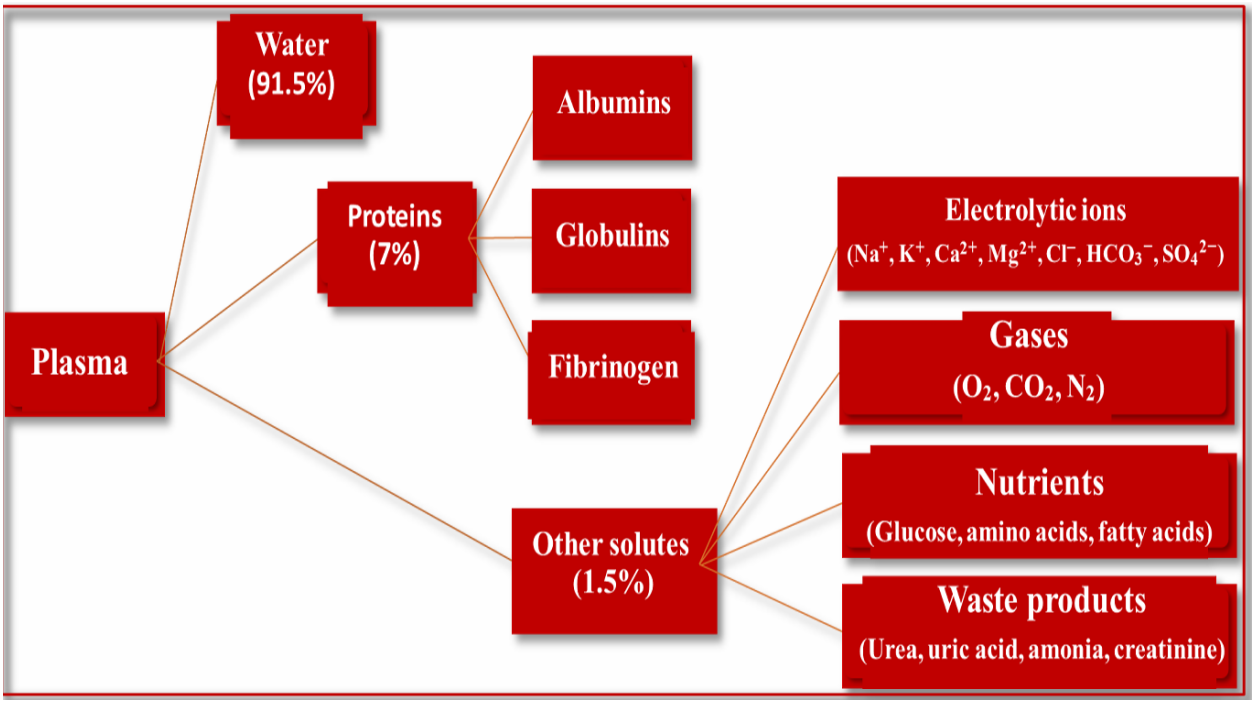


Plasma

✓ Plasma is apale yellow- colored liquid component of blood that holds the cellular Elements of blood in suspension.

Constituent	Function
Water	Absorbs, transports, and releases heat
Albumins	Osmotic balance
Globulins	Defense mechanism
Fibrinogen	Blood clotting
Electrolytic ions	pH buffering

✓ Constituents of plasma



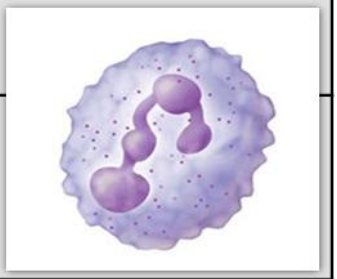
Red blood cells

- ✓ An increase in the number of **RBCs** is Known as polycythemia.
- ✓ A decrease in the number of **RBCs** is Known as erythropenia.
- ✓ Normal blood contains **13–15g of Hb** per **100** ml of blood.
- ✓ One RBC contains about **250** million Molecules of Hb.
- ✓ Each molecule of **Hb** carries four Molecules of oxygen.

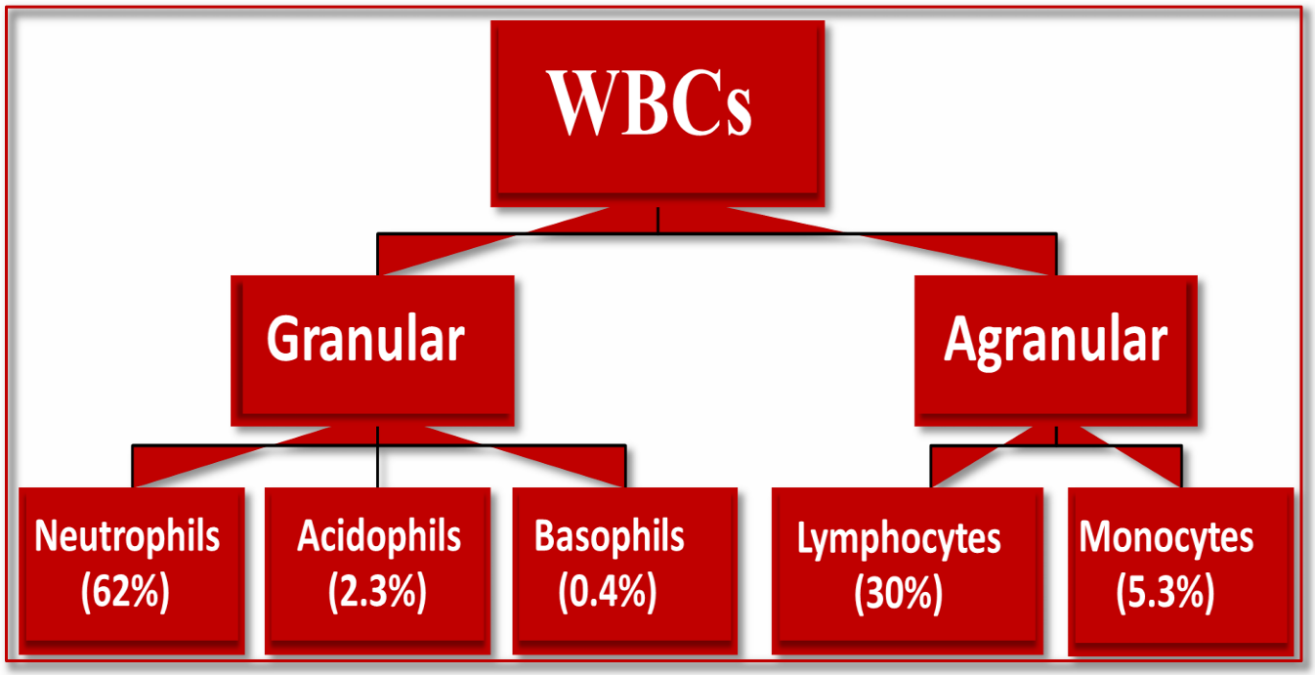
Shape	**Circular biconcave non-nucleated**
Size	**Diameter = 7 – 8µm Thickness = 2.5 µm**
Color	**Red (hemoglobin pigment)**
Count	**RBCs/µL Adult male = 5.4 million Adult female = 4.8 million RBCs/µL**
Life span	**120 days**

White blood cells

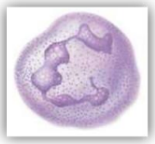
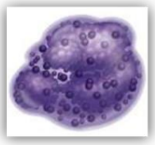
- Increase in the number of WBCs is known as leukocytosis.
- Decrease in the number of WBCs is known as leucopenia.
- Pathological increase in number of WBCs is known as leukemia(blood cancer).

Shape	Amoeboid nucleated 
Size	12 – 15 µm
Color	Colorless & translucent
Count	5000 – 10000 WBCs/µL
Life span	10 – 13 days

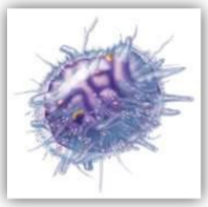
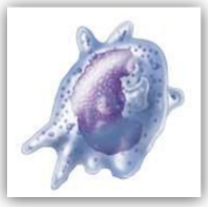
Types of WBCs



Granular WBCs

Type	Appearance	Features	Functions	Location produced
Neutrophils		• Nucleus with 3-4 lobes • Stain with neutral dye (hematoxylin)	Destroy bacteria by phagocytosis	Bone marrow
Acidophils (eosinophils)		• Nucleus with 2 lobes • Stain with acidic dye (eosin)	Combat the effect of histamine in allergic reactions	Bone marrow
Basophils		• Nucleus with indistinct lobes • Stain with basic dye (methylene blue)	Liberate heparin and histamine in allergic reactions to intensify inflammatory response	Bone marrow

Agranular WBCs

Type	Appearance	Features	Functions	Location produced
Lymphocyte		• Smallest of WBCs • Large round nucleus	Produce antibodies	Bone marrow, spleen, tonsils
Monocyte		• Largest of WBCs • Large kidney shaped nucleus	Ingest microorganisms	Bone marrow

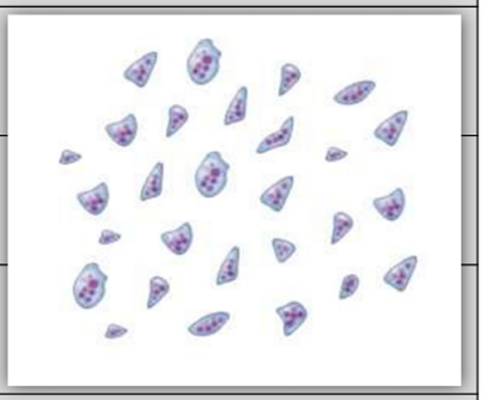
Mononuclear phagocyte cells

Stem cell in bone marrow → monocytes from bloodstream → macrophages in tissues or organs

- Connective tissue proper – Macrophages.
- Liver – Kupffer cells.
- Lungs – Dust cells (alveolar macrophages).
- Kidney – Mesangial cells.
- Thymus, spleen, lymph node – Different macrophages
- Nervous tissue, brain – Microglia
- Bone – Osteoclasts
- Skin – Langerhans cells

Platelets

Shape	**Circular biconvex non-nucleated**
Size	**2 – 4 µm**
Count	**1,50,000 – 4,00,000 platelets/µL**
Life span	**5 – 9 days**
Function	**Blood clotting**

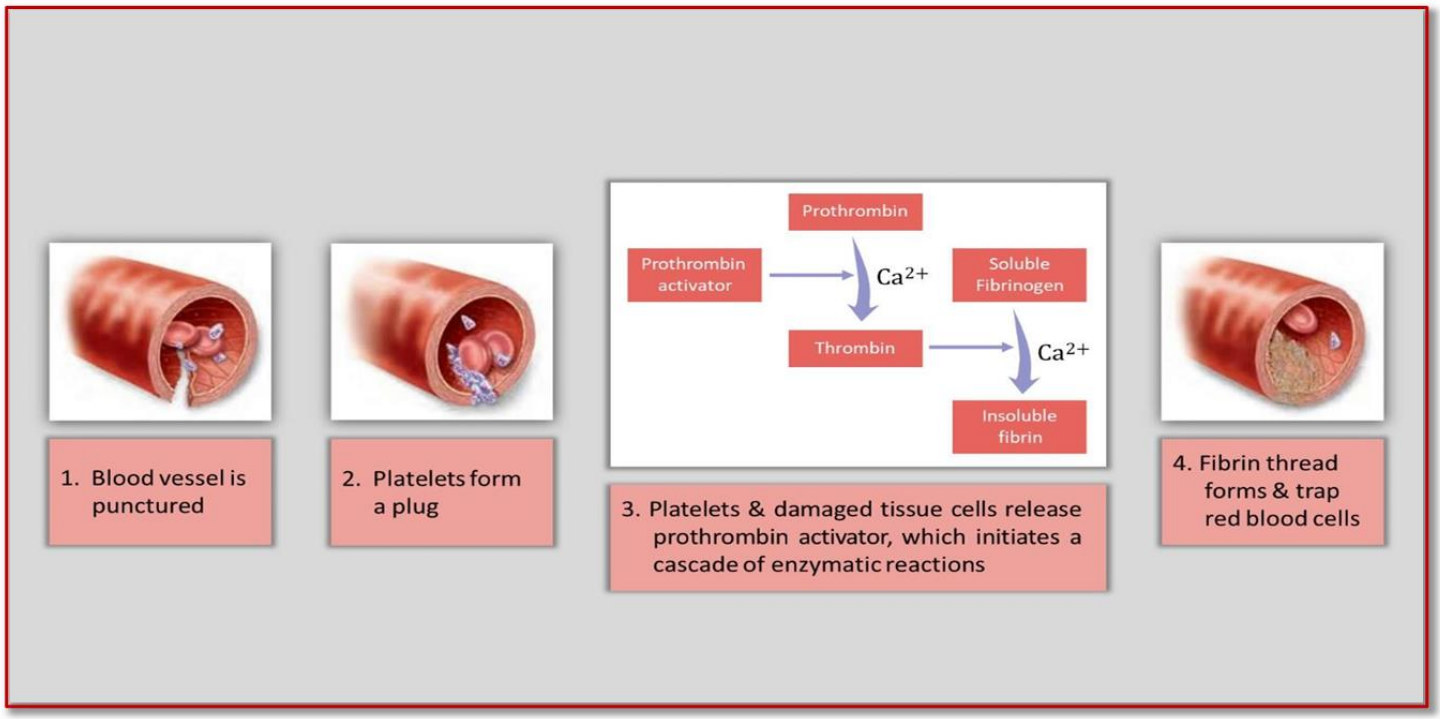


- Platelets are the fragments of large cells called **megakaryocytes** that remain in the bone marrow.
- An increase in the number of platelets is known as thrombocytosis.
- A decrease in the number of platelets is known as thrombocytopenia.

Blood clotting

- ✓ Blood clotting is the process in which blood loses its fluidity and becomes a jelly-like mass a few minutes after it is shed out.
- ✓ Asubstance that prevents the coagulation of blood is called an anticoagulant.
- ✓ Heparin is a natural anticoagulant present in the blood.
- ✓ If blood clots too easily, the result can be thrombosis– clotting in an undamaged blood vessel.
- ✓ If the blood takes too long to clot, hemorrhage can occur.

Process of blood clotting



Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

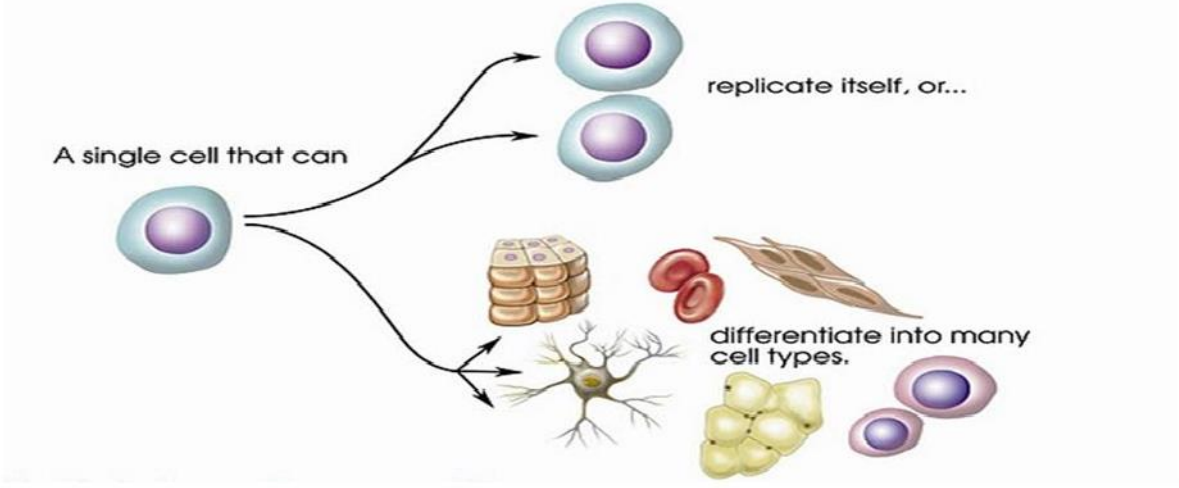
Second Week

Lecture Title: Stem Cells & Hematopoiesis

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Providing the necessary skills and developing knowledge for the students to recognize the concept of stem cells, their types, hematopoietic stem cells, and the process of blood formation.

Special Objectives of the Module

1. Definition of stem cells.

2. Definition of hematopoiesis.

3. Explain the factors that control the process of blood formation.

4. Distinguish between the paths of development and differentiation of blood cell types until they reach maturity.

5. Designs a scheme that shows the path of blood cell ripening, starting from the stem cell consisting of each type of blood cell until they reach the mature cell.

.....

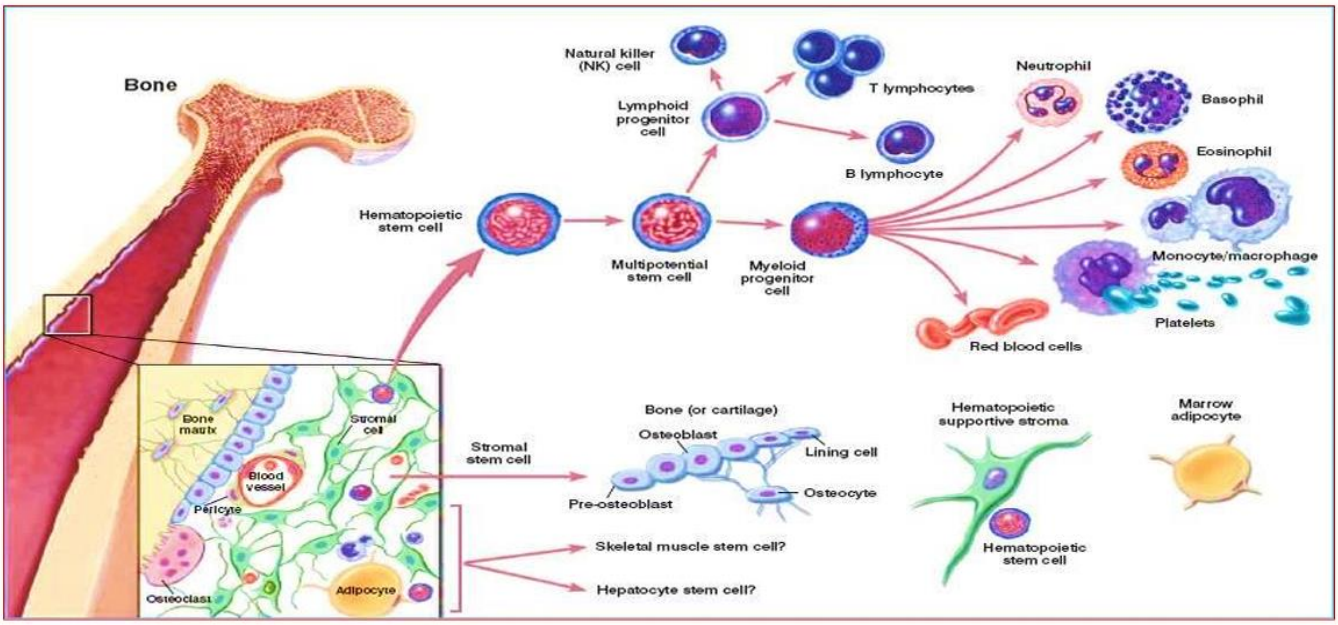
References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.



What Are Stem Cells

- ✓ Stem cells are the raw material from which all of the body's mature, differentiated cells are made.
- ✓ Stem cells give rise to brain cells, nerve cells, heart cells, pancreatic cells, etc.

Stem Cell – Definition

- ✓ A cell that has the ability to continuously divide and differentiate (develop) into various other kinds of cells/tissues.
- ✓ Stem cells are different from other cells of the body in that they have the ability to differentiate into another cell/tissue types.

- ✓ This ability allows them to replace cells that have died. With this ability, they have been used to replace defective cells/tissues in patients who have certain diseases or defects
- ✓ Stem cells are "mother" cells that give rise to all other cells in the body.
- ✓ Stem cells are cells found in all multicellular organisms that can
- ✓ undergo mitosis
- ✓ Theyserve as a repair system for the body.

Thereare two main types of stem cells:

- ❖ **Embryonic stem cells.**
- ❖ **Adult stem cells.**

Stem cells are different from other cells in the body in three ways:

- ✓ Theycan divide and renew themselves over a long time
- ✓ They are unspecialized, so they cannot do specific functions in the body
- ✓ They have the potential to become specialized cells, such as uscle cells, blood cells, and brain cells

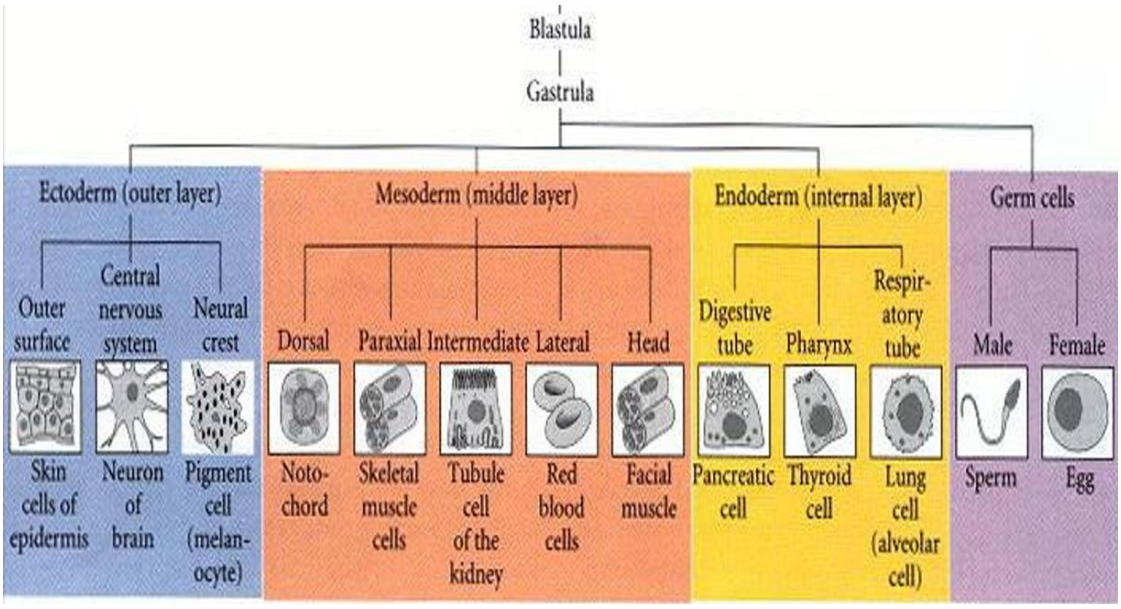
Embryogenesis and Differentiation

Specific regions of the embryo give rise to the specific organ systems

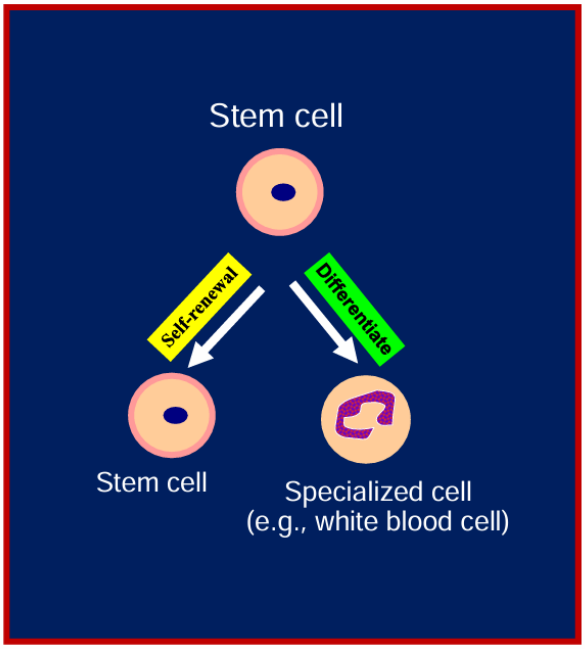
Ectoderm generates the outer layer of the embryo and produces the surface layer (epidermis) of the skin and forms the nerves.

Mesoderm becomes sandwiched between the ectoderm and endoderm and generates the blood, heart, kidney, gonads, bones, and connective tissues.

Endoderm becomes the innermost layer of the embryo and produces the digestive tube and its associated organs (including the lungs).

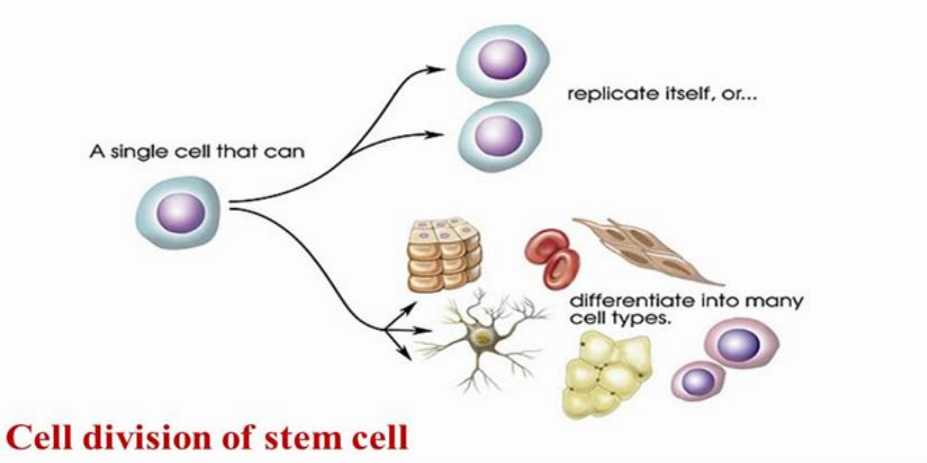


Stem Cell –are Dynamic



Special Characteristics of all Stem Cells

- Self-renewal (proliferation)- the ability of a stem cell to clone itself indefinitely by cell division.
- Relocation and Differentiation are abilities of stem cells to "migrate" to where they're needed in the body and specialize into a particular type of mature cell.



Cell division of stem cell

Major types of Stem Cells

- ✓ Embryonic (also called "pluripotent") stem cells are capable of developing into all the cell types of the body.
- ✓ Adult (also called "Multipotent") stem cells are less versatile
- ✓ and more difficult to identify, isolate, and purify.

……..

Stem cells can be classified into three broad categories, based on their ability to differentiate.

- ✓ **Totipotent stem cells** are found only in early embryos. Each cell can form a complete organism (e.g., identical twins).
- ✓ **Pluripotent stem cells** exist in the undifferentiated inner cell mass of the blastocyst and can form any of the over **200** different cell types found in the body.
- ✓ **Multipotent stem cells** are derived from fetal tissue, cord blood and adult stem cells. Although their ability to differentiate is more limited than pluripotent stem cells, they already have a track record of success in cell-based therapies

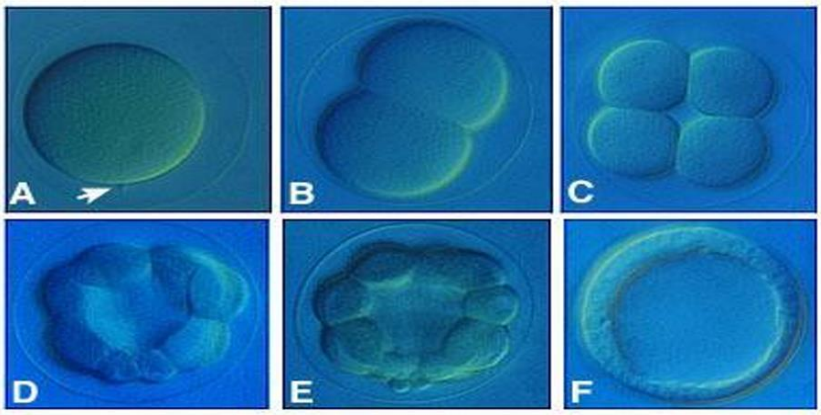
Here is a list of the sources of stem cells:

1. **Embryonic stem cells**- are harvested from the inner cell mass of the blastocyst four to five days after fertilization.
2. **Fetal stem cells-** are taken from the germ line tissues that will make up the gonads of aborted fetuses.
3. **Umbilical cord stem cells**- Umbilical cord blood contains stem cells similar to those found in bone marrow.
4. **Placenta derived stem cells**- up to ten times as many stem cells can be harvested from a placenta as from cord blood.
5. **Adult stem cells**- Many adult tissues contain stem cells that can be isolated.

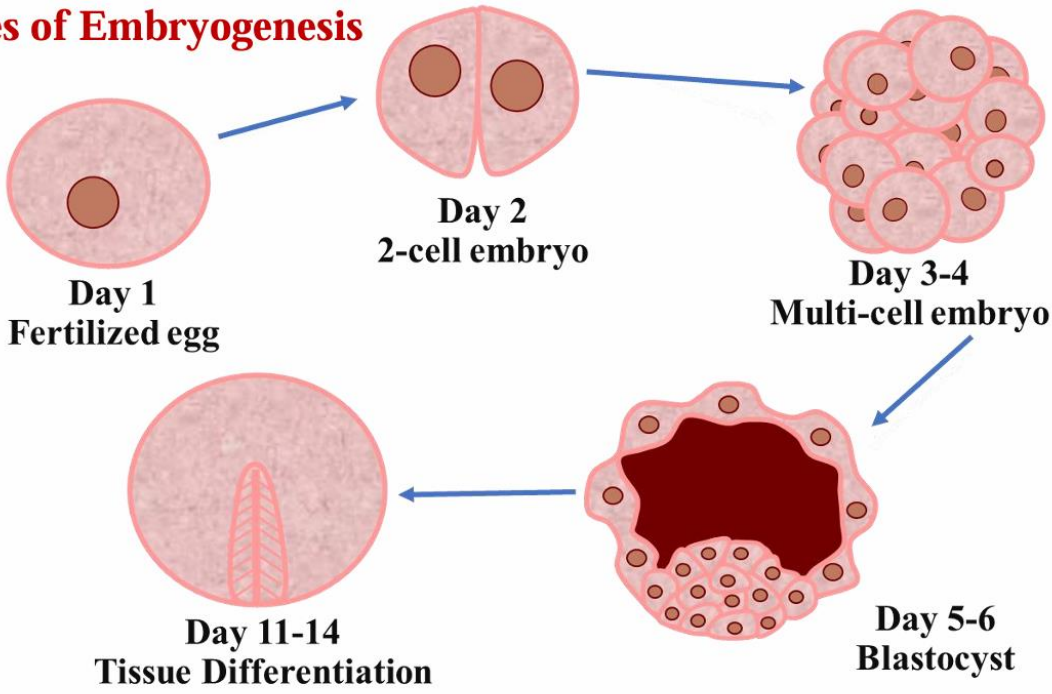
……

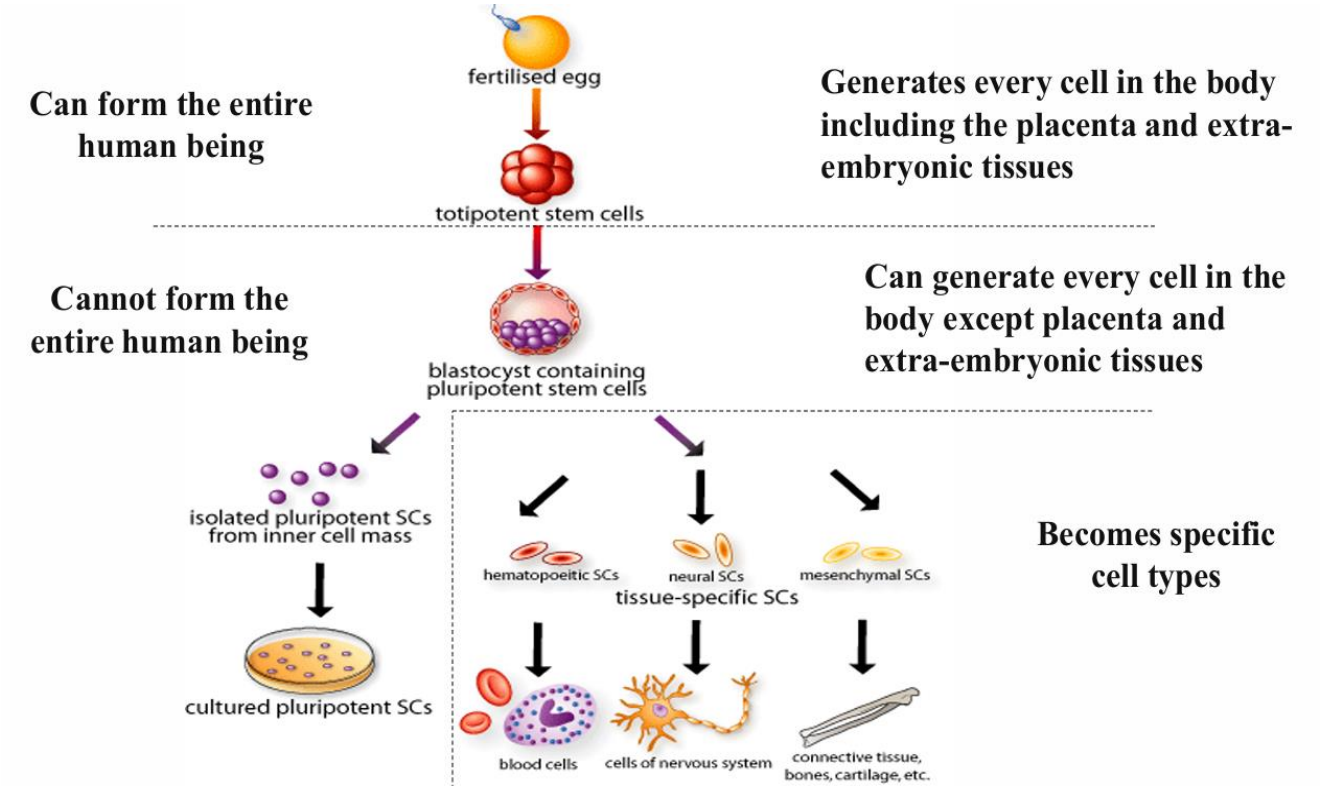
Embryonic stem (ES) cells

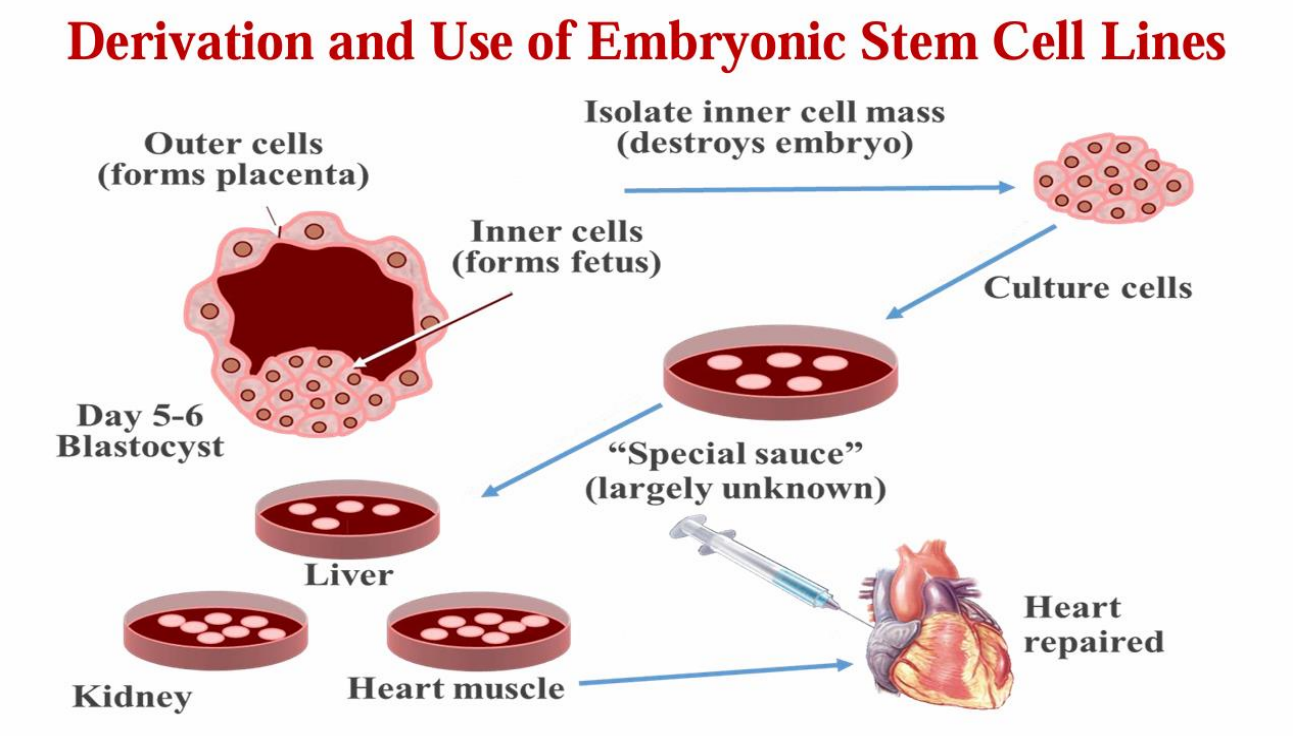
ES cells are found at the blastocyst stage, four to five days after the union of the sperm and egg, before the embryo implants in the terus.



Stages of Embryogenesis

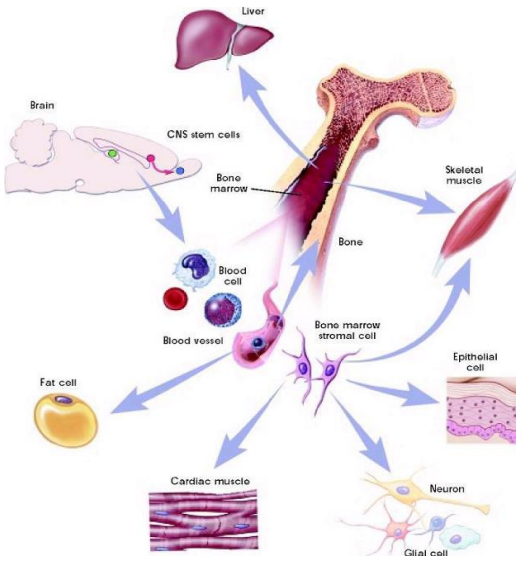






Adult multipotent stem cells

- ✓ Found in adult tissue.
- ✓ Can self-renew many times.
- ✓ Are multipotent–they can differentiate to become only the typesofcells inthe tissue theycomefrom.

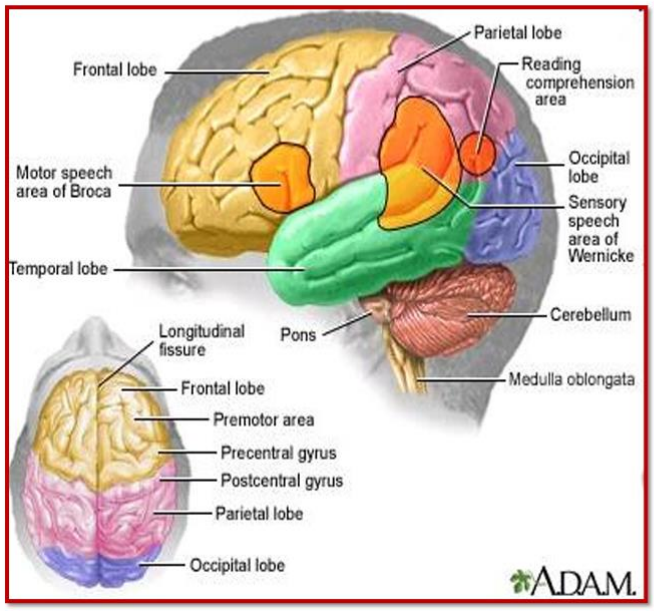


What's So Special About Stem Cells?

- ✓ Theyhave the potential to replace cell tissue that has been damaged or destroyed by severe illnesses.
- ✓ Theycan replicate themselves over and over for a very long time.
- ✓ Understanding how stem cells develop into healthy and diseased cells will assist the search for cures of many disease.

Potential uses of stem cells

- *Alzheimer's disease*
- **Parkinson's disease**
- *Spinal cord injury*
- **Heart disease**
- *Severe burns*
- **Diabetes**
- **arthritis.**



Hematopoiesis

Hemapoiesis (Hematopoiesis)

Hemo = Hemato = Blood

Poiesis = Production

Definition and Sites

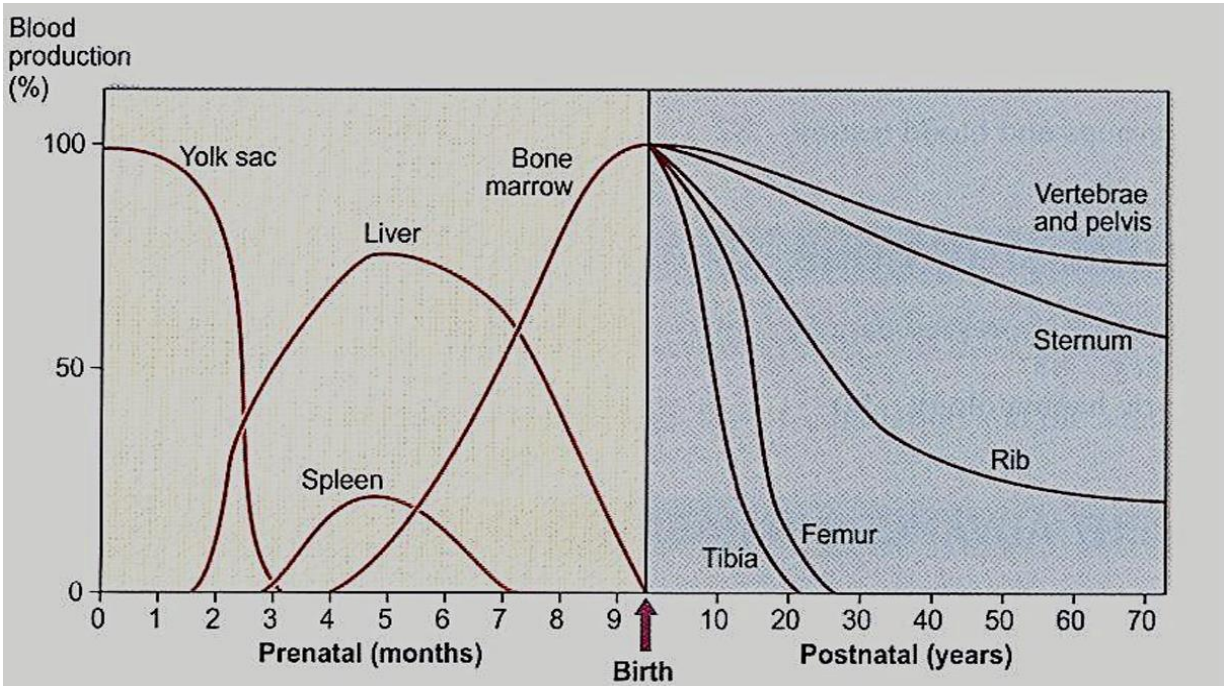
Haematopoiesis or haemopoiesis is the formation of blood cells. Haemopoietic organs are bone marrow, thymus, lymph nodes, spleen and liver.

- Hematopoiesis is the formation of cellular blood components during embryonic development and throughout adulthood to produce and replenish the blood system.
- The study of hematopoiesis can help scientists and clinicians better understand blood diseases and cancer.
- Depending on the body's needs, hematopoiesis starts with an nspecialized stem cell localized in the bone marrow called a Hematopoietic Stem Cells (HSCs).
- Hematopoiesis is a well-organized process that starts with the HSCs that develop into myeloid and lymphoid progenitor cells.

- Myeloid progenitor cells continue their development to produce erythrocytes, platelets (after fragmentation of megakaryocytes), neutrophils, monocytes, basophils, and eosinophils.
- On the other hand, lymphoid progenitor cells develop into B and T lymphocytes, natural killer (NK) cells, and dendritic cells.
- Therefore, a very complex process occurs in the bone marrow to produce at least **10** blood cell types daily and the numbers of these cells are kept within relatively narrow ranges by hematopoiesis, with the ability to increase production, if needed, in conditions that require increased demand
- Despite being from the same precursor stem cell, each type of blood cell has a different path to hematopoiesis.

The Site and Stages of Hematopoiesis

- ✓ Hematopoiesis is a continuous process that begins during embryonic life and continues through fetal, neonatal, and adult life.
- ✓ In the first few weeks of gestation, the process of hematopoiesis takes place in the embryonic yolk sac, where nucleated primitive erythrocytes are produced..
- ✓ In the fetus, at week **5** of gestation until **6–7** months of fetal life, the HSCs and progenitors travel from the yolk sac to the liver and spleen.
- ✓ Theliver and spleen are the major haemopoietic organs and continue to produce blood cells until about **2** weeks after birth.
- ✓ Moreover, the placenta becomes a major contributor to hematopoiesis, starting in fetal life and continuing until birth.
- ✓ At **4** to **5** months of gestational age, hematopoiesis takes place in the bone marrow (medullary hematopoiesis), which continues after birth and throughout life. With advance into adulthood, hematopoiesis slowly becomes restricted to the skull, vertebrae, pelvic bones, tibia, rib ,femur and end of long bones.



In medullary insufficiency conditions, such as in patients with thalassemia and myelofibrosis, hematopoiesis can revert to their original sites, including the liver and spleen. This is termed

extramedullary hematopoiesis.

The developing cells are situated outside the bone marrow sinuses; mature cells are released into the sinus spaces, the marrow microcirculation and so into the general circulation.

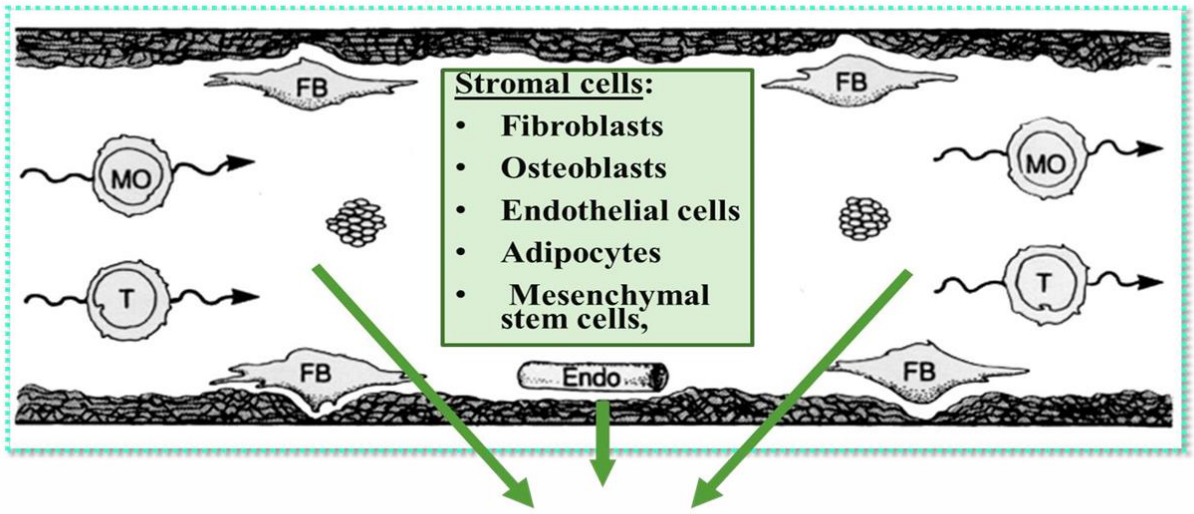
Sites of hematopoiesis

Fetus	**0–2 months (yolk sac)**
	2–7 months (liver, spleen)
	5–9 months (bone marrow)
Infants	**Bone marrow (practically all bones)**
Adults	**Vertebrae, ribs, sternum, skull,**
	sacrum and pelvis, femur

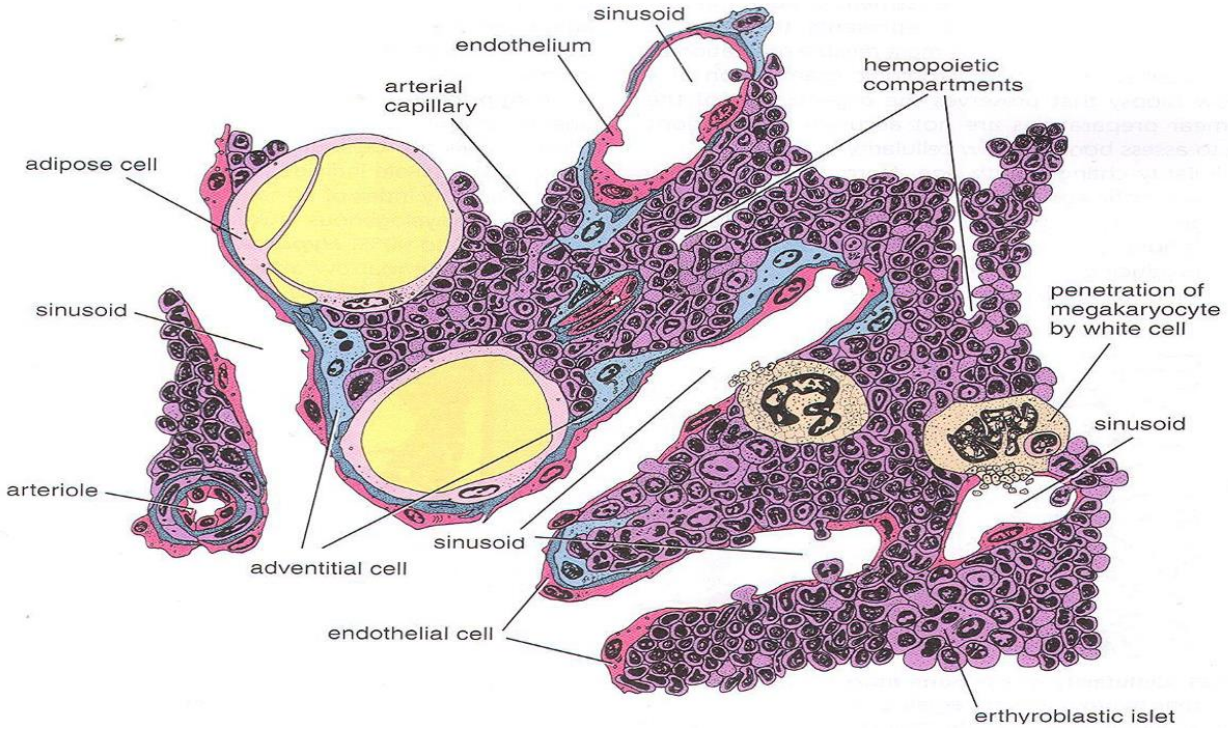
Structure of the Bone marrow stroma

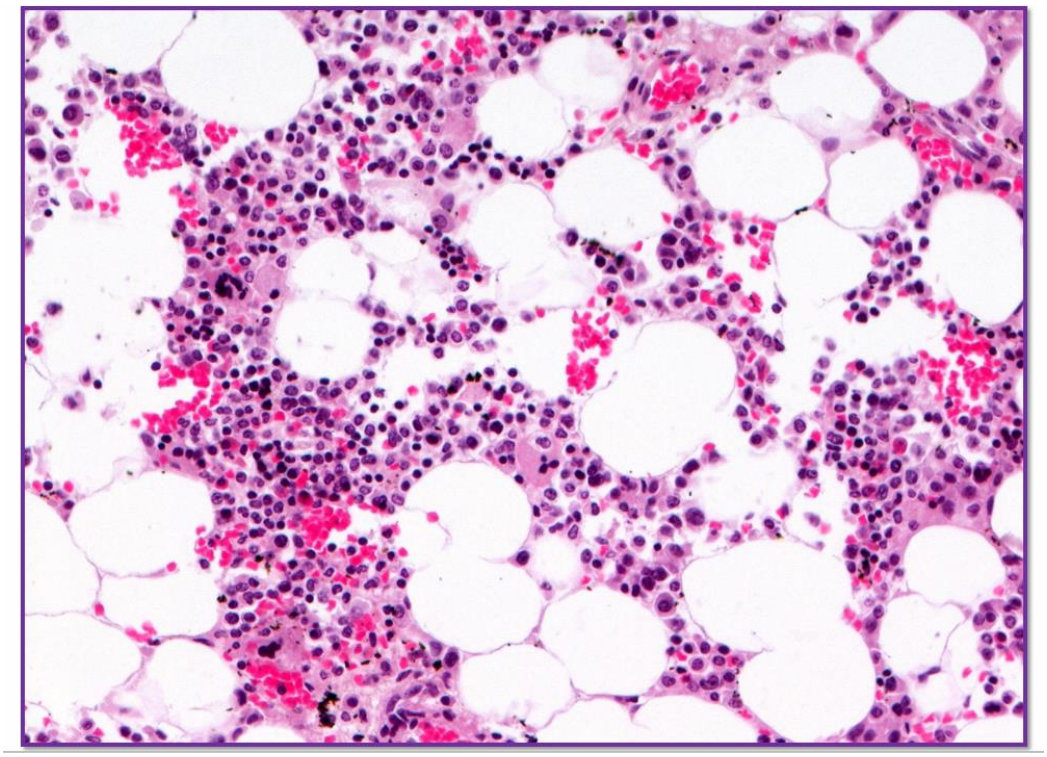
- ✓ The bone marrow forms a suitable environment for stem cell survival, self-renewal and formation of differentiated progenitor cells. It is composed of stromal cells and a microvascular network .
- ✓ The stromal cells include mesenchymal stem cells, adipocytes, fibroblasts, osteoblasts, endothelial cells.
- ✓ Sinusoids are wide, numerous, fenestrated blood capillaries and, macrophages, Hematopoietic stem cells and all stages in the maturation of blood elements and, secrete extracellular molecules such as collagen, glycoproteins (fibronectin and thrombospondin) and glycosaminoglycan to form an extracellular matrix.
- ✓ In addition, stromal cells secrete several growth factors necessary for stem cell survival.

Hematopoietic Microenvironment



Growth Factors





Pluripotential hematopoietic stem cells

- ✓ Undifferentiated stem cells can give rise to all the different types of specialized blood elements
- ✓ Differentiated to form progenitor cells in two different ways:
- ❖ **Multipotential lymphoid stem cells**
- ❖ **Multipotential myeloid stem cells**

Cellularity of the bone marrow

Is one of the most important factors in evaluating the function of bone marrow.

The ratio of hematopoietic cells to adipocytes

- ✓ Normocellular bone marrow 50% : 50%
- ✓ Hypocellular bone marrow–a small number of blood-forming cells can be found (after chemotherapy, aplastic anemia,
- ✓ Hypercellular bone marrow–tumors originating from hematopoietic cells

Hemopoietic Growth Factors

- ❖ Growth factors are glycoprotein hormones, a group of molecules secreted by the cells to induce cell growth and proliferation via target receptors on the cell surface.
- ❖ Some of these growth factors are made in the bone marrow by the stromal cells.
- ❖ The well-known hematopoietic growth factors include; stem cell factor (SCF, cytokines such as interleukin-3 (IL-3) and IL-6, thrombopoietin (TPO), granulocyte-macrophage colony-stimulating factor (GM-CSF), erythropoietin (EPO), and macrophage colony-stimulating factor (M-CSF)

There are two types of growth factors regulating hematopoiesis.

- ✓ The first group of regulatory factories controls the whole process through the modulation of early progenitors and these include SCF, IL-3, G-CSF, IL-11, IL-6, and IL-1.
- ✓ The second group acts exclusively on specific lineages and these include EPO, TPO, G-CSF, M-CSF, and IL-5, and they are needed for the production of RBCs, platelets, and WBCs.
- ✓ Cytokines also play a major role in the regulation of the hematopoiesis process.
- ✓ Cytokines are a family of proteins that produce positive and negative effects on cellular activities such as apoptosis, proliferation, and differentiation through a specific receptor and activate numerous signaling pathways.
- ✓ Examples of cytokines mediating hematopoiesis include; IL-1, IL 3, IL-5, IL-6, IL-7, ...).

Haemopoietic growth factors

Act on stromal cells

IL-1

TNF

Act on pluripotential stem cells

SCF

FLT3-L

VEGF

Haemopoietic growth factors

Act on multipotential

progenitor cells

IL-3

GM-CSF

IL-6

G-CSF

Thrombopoietin

Haemopoietic growth factors

Act on committed

progenitor cells

G-CSF*

M-CSF

IL-5 (eosinophil-CSF)

Erythropoietin

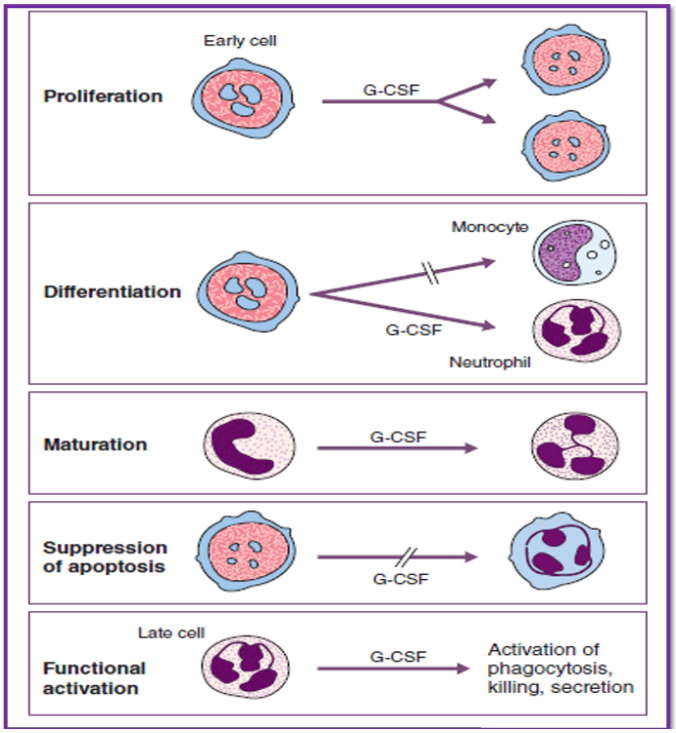
Thrombopoietin

CSF, colony-stimulating factor; FLT3-L, FLT3 ligand; G-CSF, granulocyte colonystimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

General characteristics of myeloid and lymphoid growth factors.

- ✓ Glycoproteins that act at very low concentrations
- ✓ Act hierarchically
- ✓ Usually produced by many cell types
- ✓ Usually affects more than one lineage
- ✓ Usually active on stem/progenitor cells and on differentiated cells
- ✓ Usually, show synergistic or additive interactions with other growth factors
- ✓ Multiple actions: proliferation, differentiation, maturation,
- ✓ functional activation, prevention of apoptosis of progenitor cells

- ➢ Growth factors may stimulate proliferation of early bone marrow cells.
- ➢ Direct differentiation to one or another cell type.
- ➢ Stimulate cell maturation.
- ➢ Suppress apoptosis.
- ➢ Affect the function of mature non-dividing cells.



Regulation of haemopoiesis

Erythropoitin (EPO, Kidneys)

Stimulates RBCs formation.

Thrombopoietin (TPO, Liver)

Stimulates platelet formation.

Cytokines (bone marrow, WBCs, fibroblasts, Endothelial cells)

Stimulate hemopoiesis, WBCs, colony- stimulating factors & interleukins.

……..

Components of Haemopoiesis

- Cells
- Bone marrow stroma
- Growth factors

Cells

- Stem cells
- Progenitor cells
- Mature cells

Hematopoiesis

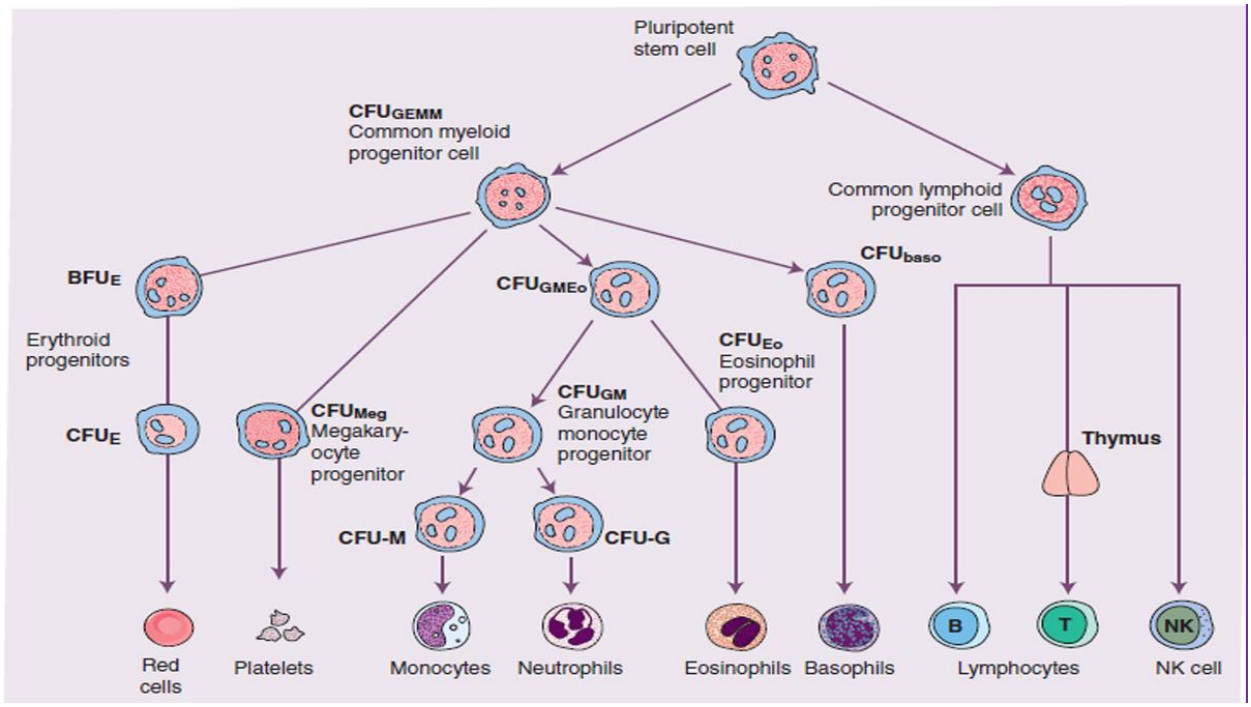
- ✓ Myeloid and lymphoid cells originate separately but from common progenitor cells.
- ✓ Erythrocytes, all leukocytes, macrophages, mast cells, and megakaryocytes originate from pluripotential stem cells.
- ✓ pluripotential hemopoietic stem cell, capable of differentiation into myeloid stem cells and lymphoid stem cells.
- ✓ Myeloid and lymphoid stem cells in turn give rise to committed cell units called colony - forming units (CFU) leading to specific series that produce mature cells.
- ✓ Neutrophils, monocytes and macrophages have a common progenitor, the colony forming unit-granulocyte-macrophage (CFU GM).
- ✓ Erythrocytes come from two progenitors

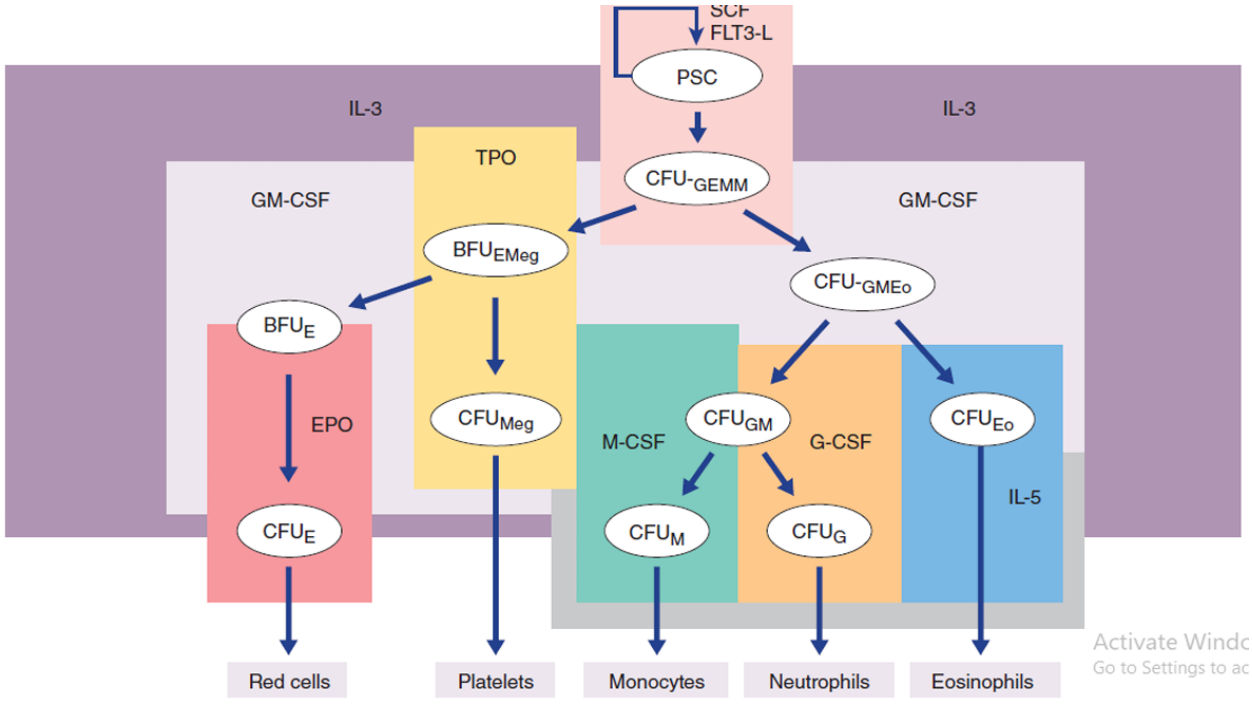
1. Burst forming unit– erythroid (BFU-E)

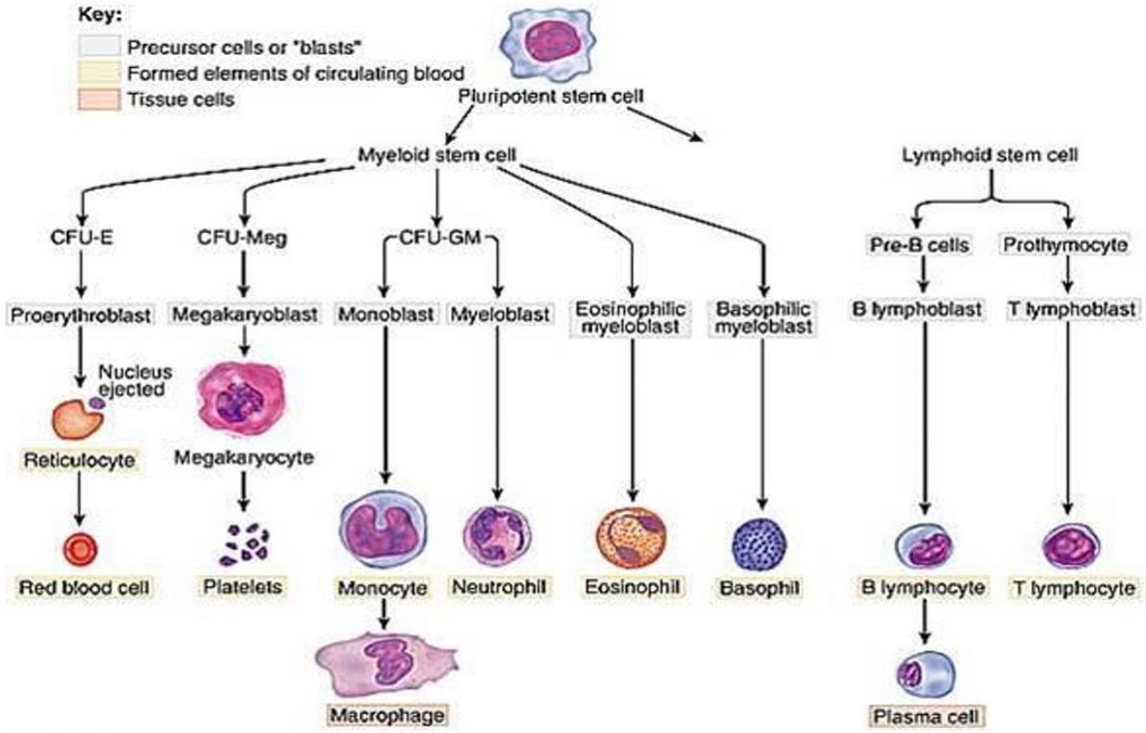
2. Colony forming unit-erythroid (CFU-E).

BFU-Eis progenitor of CFU-E

- ✓ Progenitor for megakaryocytes is colony forming unit megakaryocyte (CFU-M or CFU-Meg)
- ✓ Progenitors for eosinophils, basophils and mast cells are colony forming unit-eosinophil (CFU-EOS) and colony forming unit basophil (CFU-BAS).






Key:
Precursor cells or "blasts"
Formed elements of circulating blood
Tissue cells
Pluripotent stem cell
Myeloid stem cell
Lymphoid stem cell
CFU-E
CFU-Meg
CFU-GM
Pre-B cells
Prothymocyte
Proerythroblast
Megakaryoblast
Monoblast
Myeloblast
Eosinophilic myeloblast
Basophilic myeloblast
B lymphoblast
T lymphoblast
Nucleus ejected
Reticulocyte
Megakaryocyte
Red blood cell
Platelets
Monocyte
Neutrophil
Eosinophil
Basophil
B lymphocyte
T lymphocyte
Macrophage
Plasma cell

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

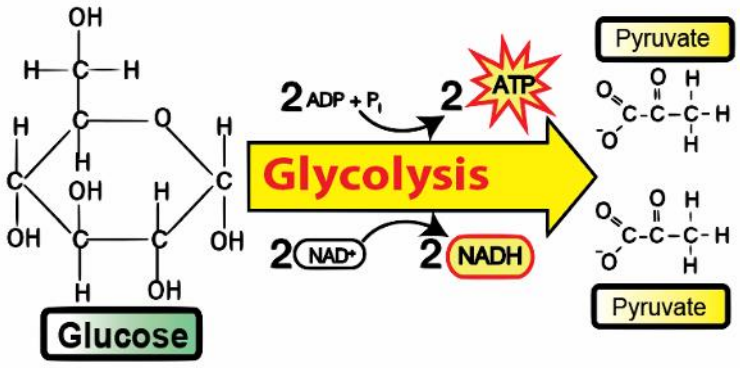
Third Week

Lecture Title: Erythropoiesis, RBCs morphology, the RBCs' cell membrane, and RBCs' metabolism.

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for the students to identify the concept of erythropoiesis, morphology, the RBCs' cell membrane, and RBCs' metabolism. Hemoglobin (structure, synthesis, and levels in blood and in erythrocytes).

Special Objectives of the Module

1. Definition of erythropoiesis.

2. Understand the components of the red blood cell membrane.

3. Explaining the methods of metabolism of red blood cells.

4. Distinguish between the structure, synthesis, function, and types of hemoglobin of hemoglobin.

.....

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

Erythrocytes

- ✓ Red blood cells, or erythrocytes, are the most abundant type of blood cell.
- ✓ Approximately 2.4 million new erythrocytes are produced per second.
- ✓ Approximately a quarter of the cells in the human body are red blood cells.
- ✓ The average number is about 5 million per cubic milliliter in males, 4.5 million per cubic milliliter in females.
- ✓ Diameter 7.5 micrometers = normocyte.
- ✓ Biconcave disc-shape (very large surface area, ideal for exchange of gases).
- ✓ Soft and elastic –can change its shape as it passes through small capillaries.

Erythrocytes –Structure

- ✓ In humans, mature red blood cells are oval biconcave disks and they are flexible.
- ✓ They lack a cell nucleus and most organelles, in order to accommodate maximum space for hemoglobin.

Erythrocytes

- ✓ The cells develop in the bone marrow and circulate for about **100–120** days in the body before their components are recycled by macrophages.
- ✓ Human red blood cells take on average **20** seconds to complete one cycle of circulation.

RBCs - Functions

- ✓ The major function of these cells is the transport of aemoglobin, which in turn carries oxygen from the lungs to the tissues
- ✓ Red blood cells contain carbonic anhydrase, which catalyzes the reaction between carbon dioxide and water, which has significance in transporting carbon dioxide (CO2) from tissues to the lungs.
  - ❖ Hemoglobin + O_2 = **Oxyhemoglobin**
  - ❖ Hemoglobin + CO_2 = **Karbaminohemoglobin**
- ✓ The haemoglobin is an excellent acid-base buffer
- ✓ Maintenance of acid-base balance.
- ✓ Blood group determination.

……

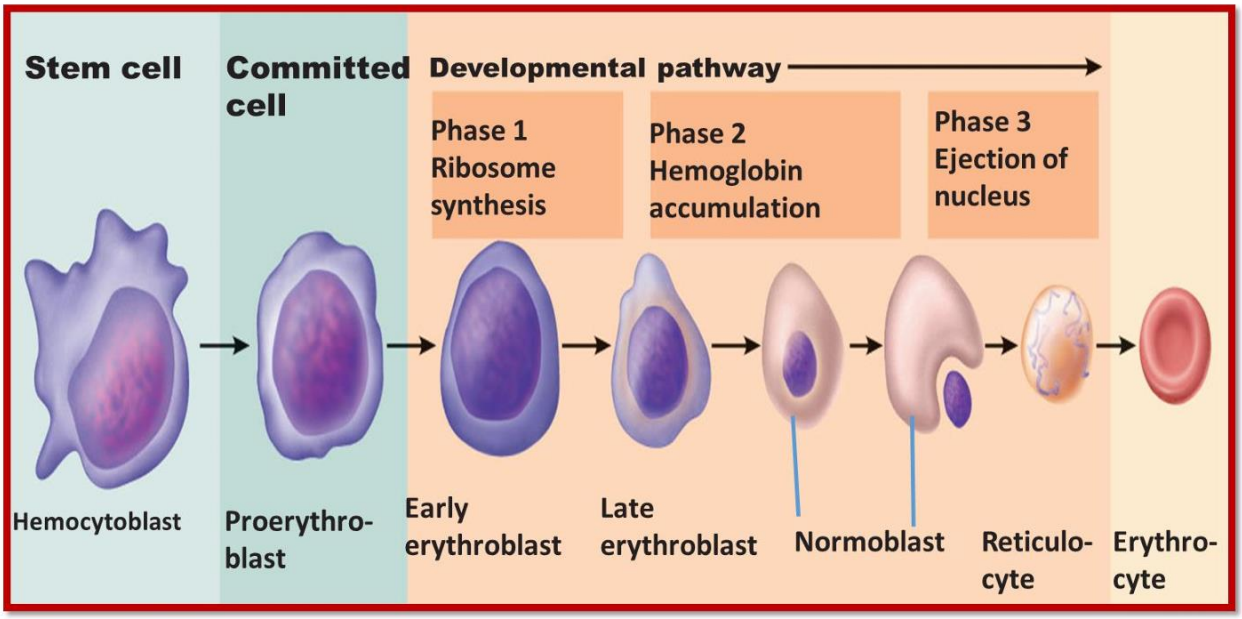
Erythropoiesis

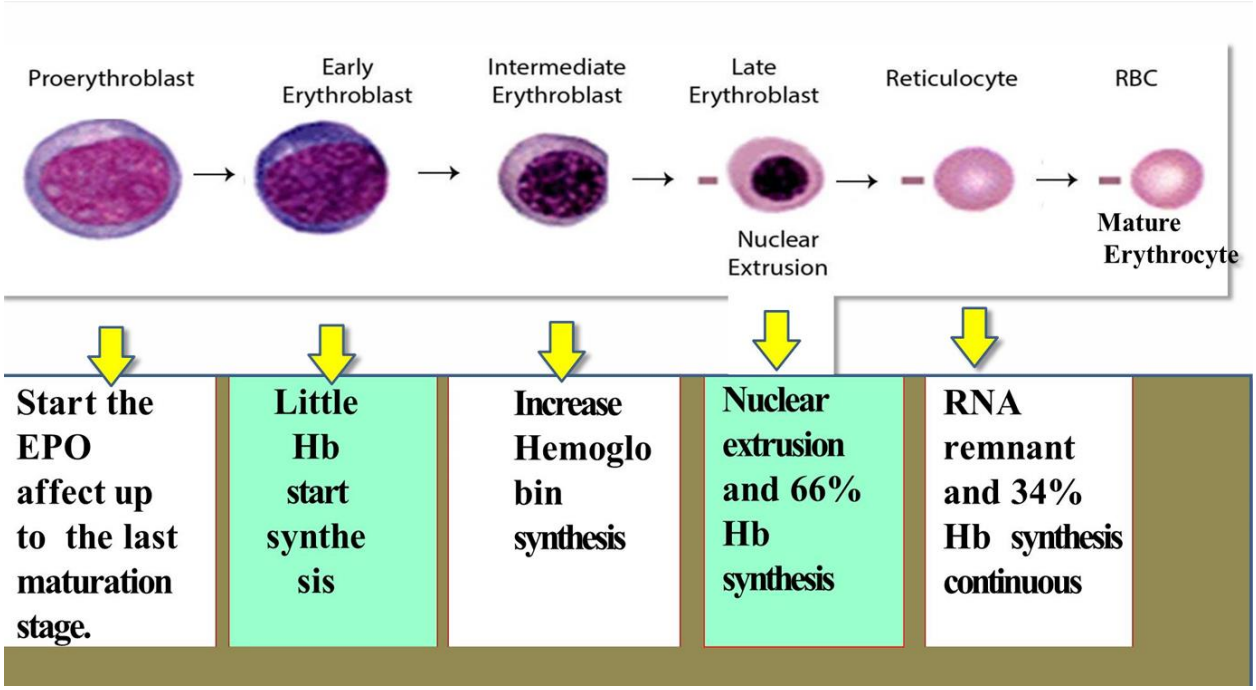
- ✓ Erythropoiesis is the process by which red blood cells (erythrocytes) are produced.
- ✓ It is stimulated by decreased O_2 in circulation, which is detected by the kidneys, hormone erythropoietin.

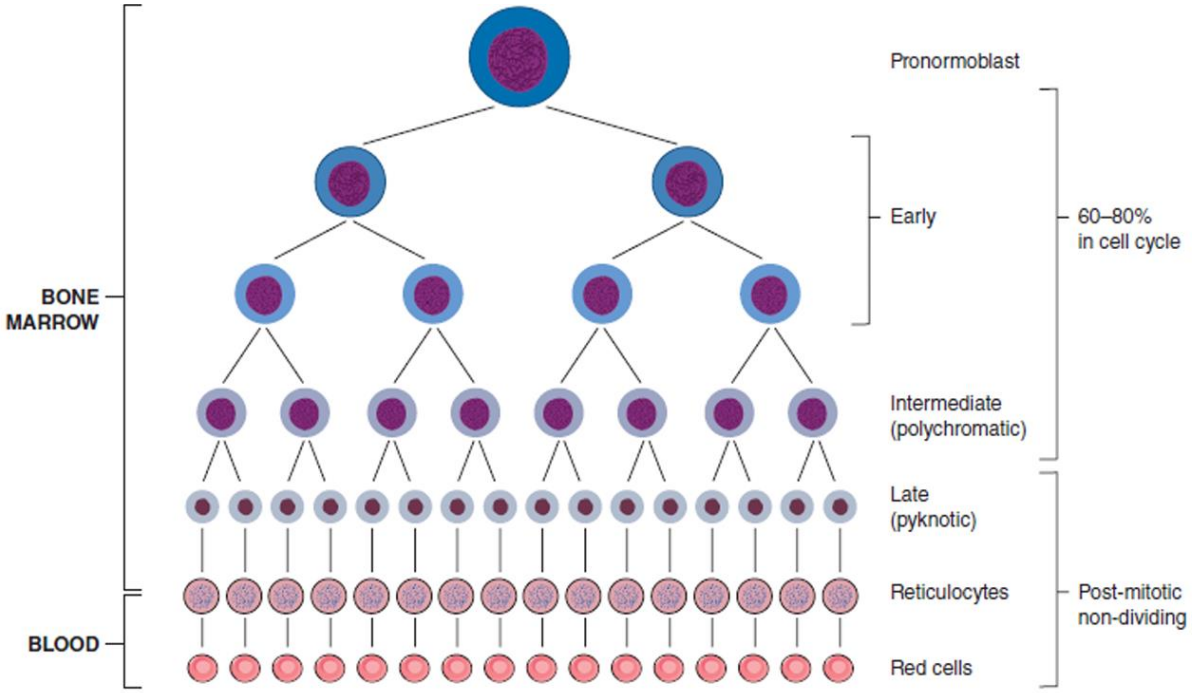
Erythrocyte Maturation

- ✓ Erythrocytes consist about **30%** of all hematopooietic cells in the bone marrow.
- ✓ **Production Site:** only in the bone marrow completed the RBC maturation.
- ✓ **Erythrocyte Maturation**
- ✓ Target of RBC production are to:
- ✓ Synthesis Hemoglobin (Hb)
- ✓ **Enzymes such as**
- ✓ Glucose 6 dehydrogenase phosphate (G6PD)
- ✓ pyruvate kinase (PK)
- ❖ The whole process lasts about **8-10** days.
- ❖ Through this process, erythrocytes are continuously
- ❖ produced in the red bone marrow of large bones, at a rate of
- ❖ about **2.4** million per second in a healthy adult.

Erythropoiesis







- Pronormoblast only has nucleoli.
- Pronormoblast,
- Early normoblast
- Intermediate normoblast

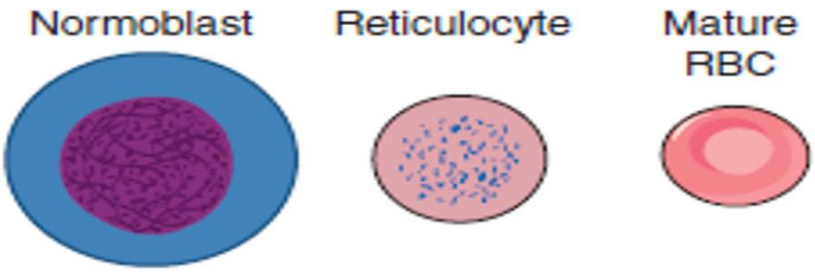
Are all capable of cell division.

- Late normoblast,
- Reticulocyte
- Mature RBC

Are not capable of cell division

Maturation effects on the Erythrocytes production

1. Synthesis of hemoglobin.
2. Synthesis of the main enzymes (G6PD and PK).
3. Loss of the nucleus in the late normoblast step.



Nuclear DNA	Yes	No	No
RNA in cytoplasm	Yes	Yes	No
In marrow	Yes	Yes	Yes
In blood	No	Yes	Yes

Healthy bone marrow

- ✓ Healthy bone marrow is essential for the production of erythrocytes.
- ✓ The bone marrow is destroyed by x ray, atomic radiation, and drugs as chloramphenicol and by malignant tumors leading to aplastic anemia.

Healthy liver

The liver is essential for erythropoiesis because it is the site of :

- ❖Formation of the globin portion of hemoglobin.
- ❖Formation of **10 %** of erythropoietin hormone.
- ❖Storage of iron and vitamin **B12**.

Hormones

- ❖Erythropoietin hormone
- ❖ Thyroid hormone stimulates general metabolism
- ❖Androgens stimulate erythropoietin secretion.

Erythropoietin

- ❖Erythropoiesis is regulated by the hormone rythropoietin.
- ❖ Erythropoietin is a heavily glycosylated polypeptide,consist of 193 amino-acids.
- ❖Gene responsible: located on the chromosome7.
- ❖ Normally, 90% of the hormone is produced in the peritubular interstitial cells of the kidney and 10% in the liver .

- ✓ Activity: stimulate and regulate the bone marrow to produce erythrocytes
- ✓ There are no preformed stores and the stimulus to rythropoietin production is the oxygen (O_2) tension in the tissues of the kidney.

Erythropoietin production increases in:

- ✓ Anemia.
- ✓ When hemoglobin for some metabolic or structural reason is
- ✓ unable to give up O2 normally when atmospheric O2 is low.
- ✓ When a defective cardiac or pulmonary function.
- ✓ Damage to the renal circulation affects O2 delivery to the
- ✓ kidney.

Diet

The following are essential for adequate erythropoiesis:

1. Proteins
2. Minerals:

- Iron
- Copper and cobalt as Co-factors for formation of Hb.
- Cobalt is a part of vitamin **B12.**

3.Vitamins: Vitamin C. Vitamin B12. Folic acid. Vitamin B6.

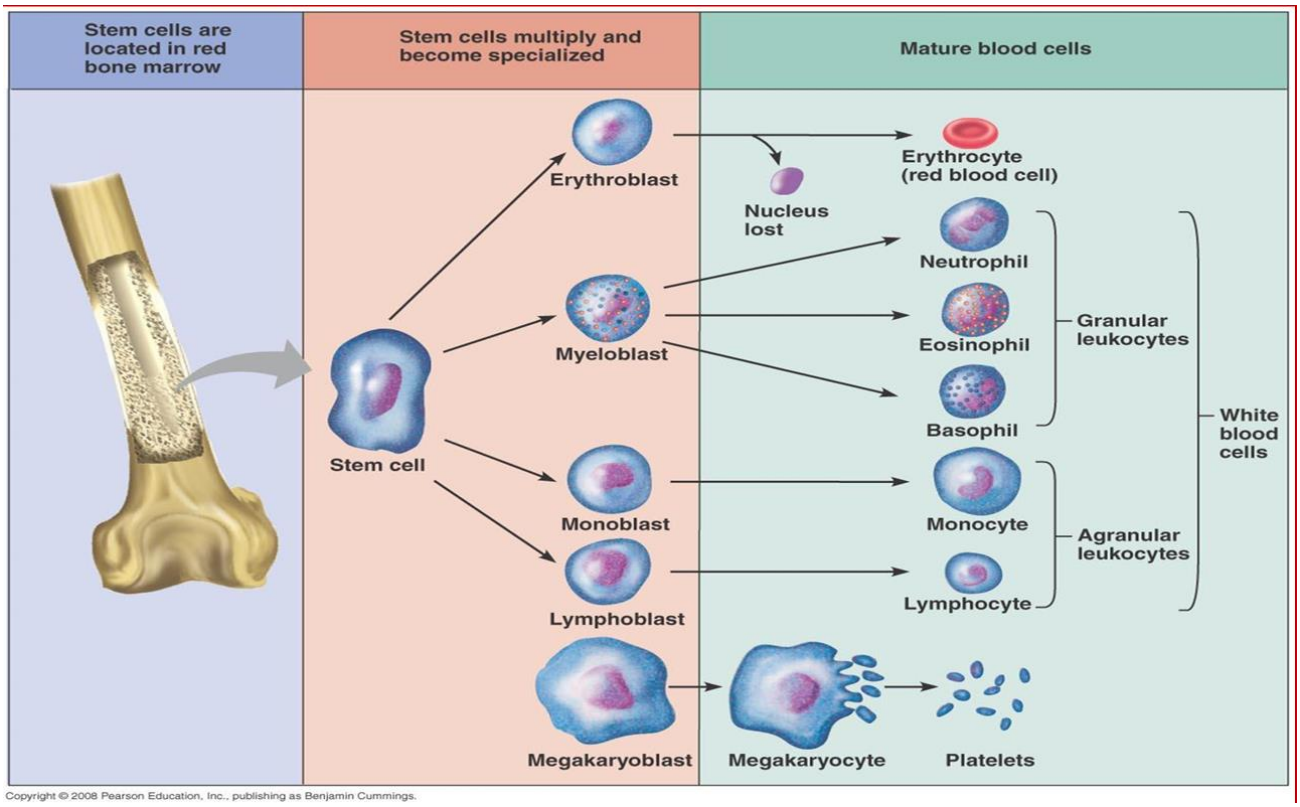
.....

Red Blood Cells

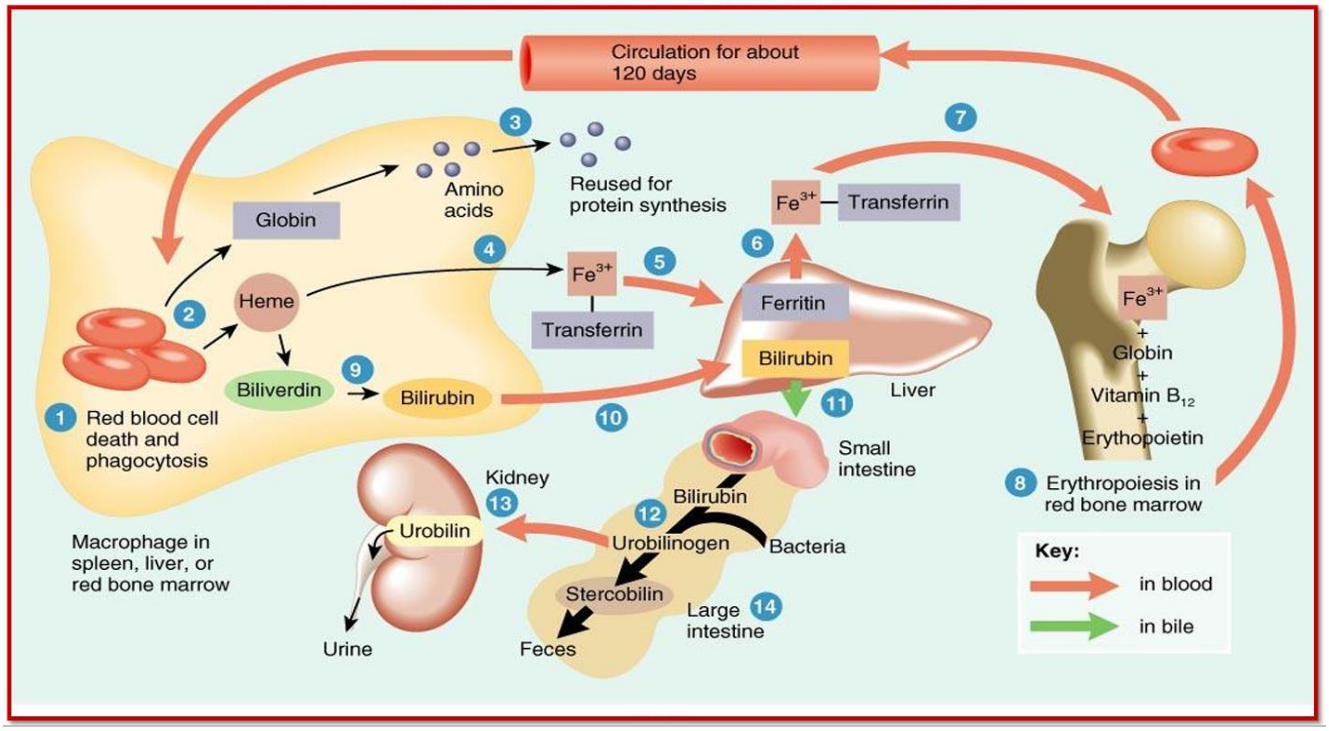
- **According to size :**
  - Normocytes - Normal sized RBCs.
  - Microcytes - Small sized RBCs.
  - Macrocytes - Large sized RBCs.

- **According to color:**
  - Normochromia - Normal colored RBCs.
  - Hyperchromia - Darker, due to increased hemoglobin.
  - Hypochromia - Paler, due to decreased hemoglobin.

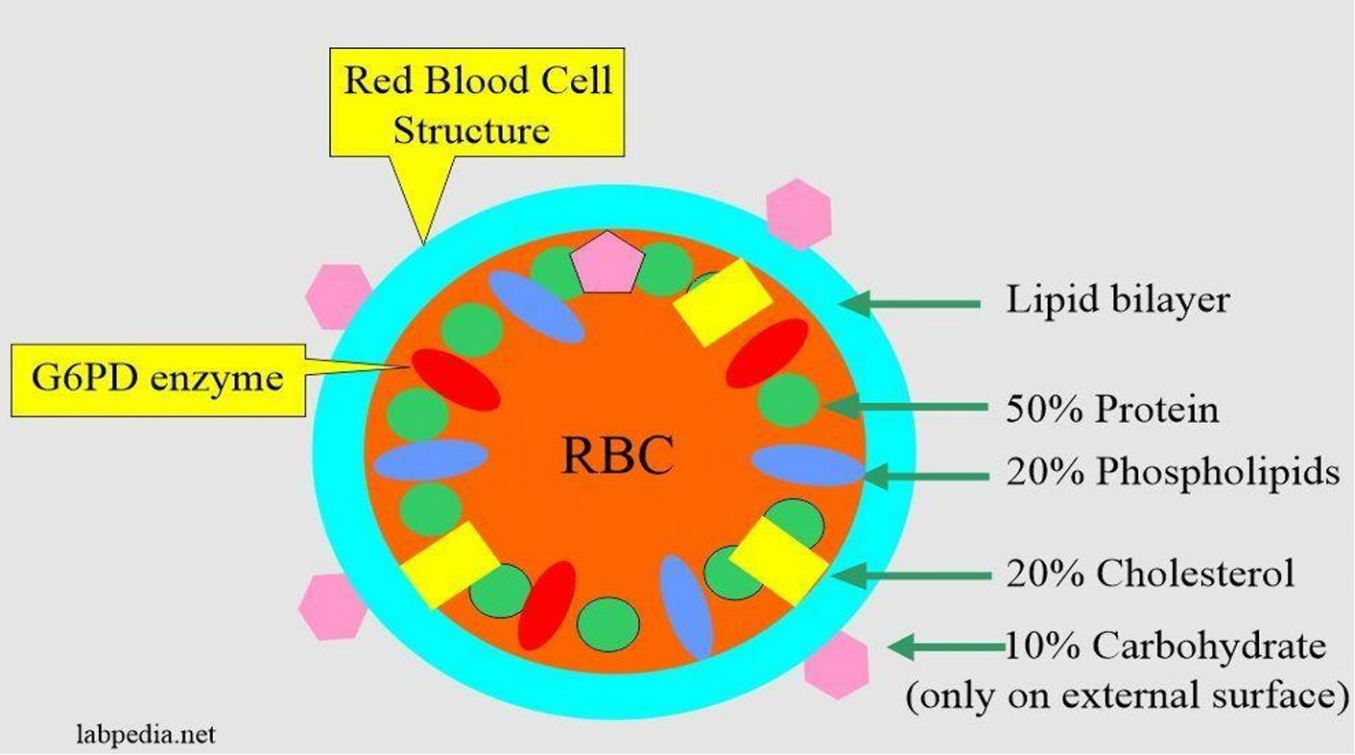
- **They are determined by measuring the :**
  - Mean corpuscular hemoglobin (MCH).
  - Mean corpuscular hemoglobin concentration (MCHC).



Formation & Destruction of RBCs



- ✓A single journey round the body takes **20** seconds and its total journey throughout its **120-day** lifespan has been estimated to be 480 km **(300 miles).**
- ✓To fulfil these functions, the cell is a flexible biconcave disc with an ability to generate energy as adenosine triphosphate (ATP) by the anaerobic glycolytic (Embden–Meyerhof) pathway and to generate reducing power as nicotinamide adenine dinucleotide (NADH) by this pathway and as reduced nicotinamide adenine dinucleotide phosphate (NADPH) by the hexose monophosphate shunt

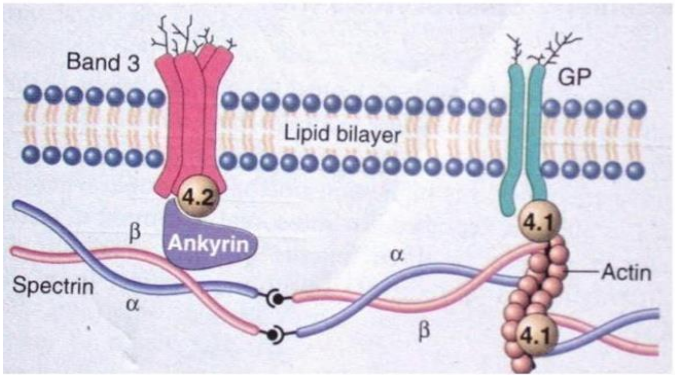


Red cell membrane

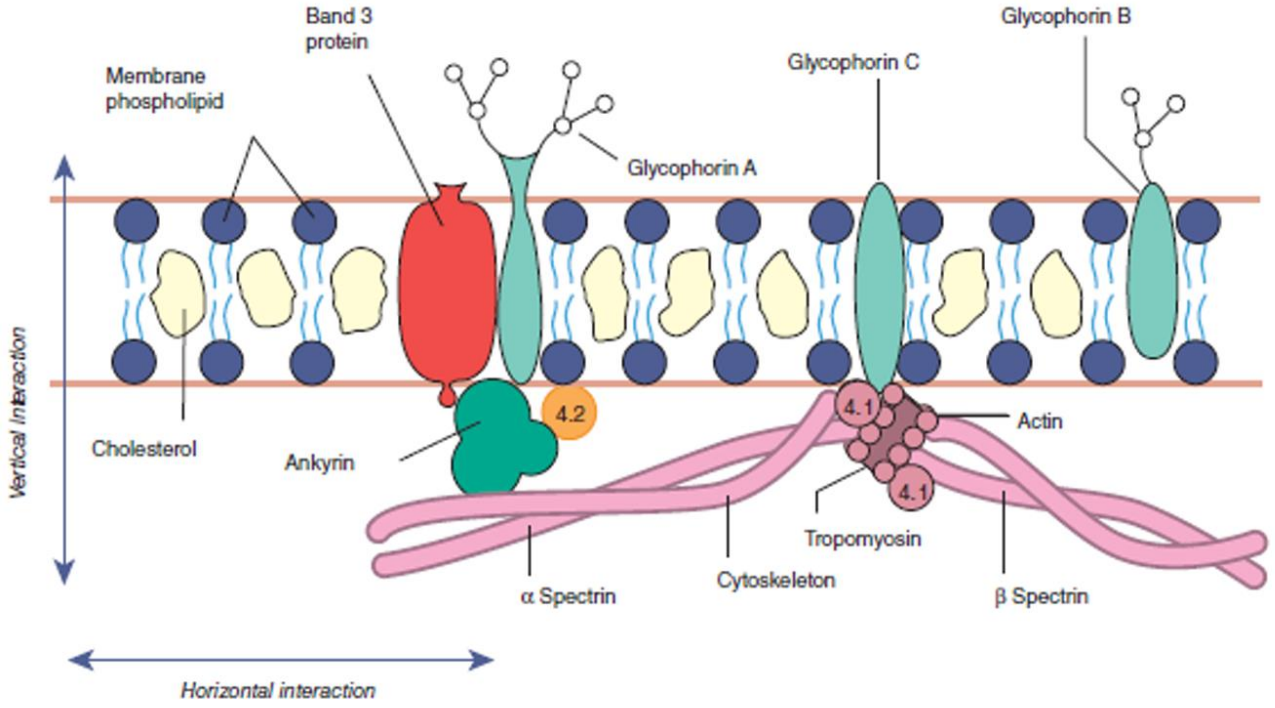
- ✓ The red cell membrane comprises a lipid bilayer, integral membrane proteins and a membrane skeleton.
- ✓ Approximately **50%** of the membrane is protein, **20%** phospholipids, **20%** cholesterol molecules and up to **10%** is carbohydrate.
- ✓ Carbohydrates occur only on the external surface while proteins are either peripheral or integral, penetrating the lipid bilayer.
- ✓ Several red cell proteins have been numbered according to their mobility on polyacrylamide gel electrophoresis (PAGE), e.g. band 3, proteins 4.1, 4.2.
- ✓ Several red cell proteins have been numbered according to their mobility on polyacrylamide gel electrophoresis (PAGE).

Proteins are either peripheral or integral, that include:

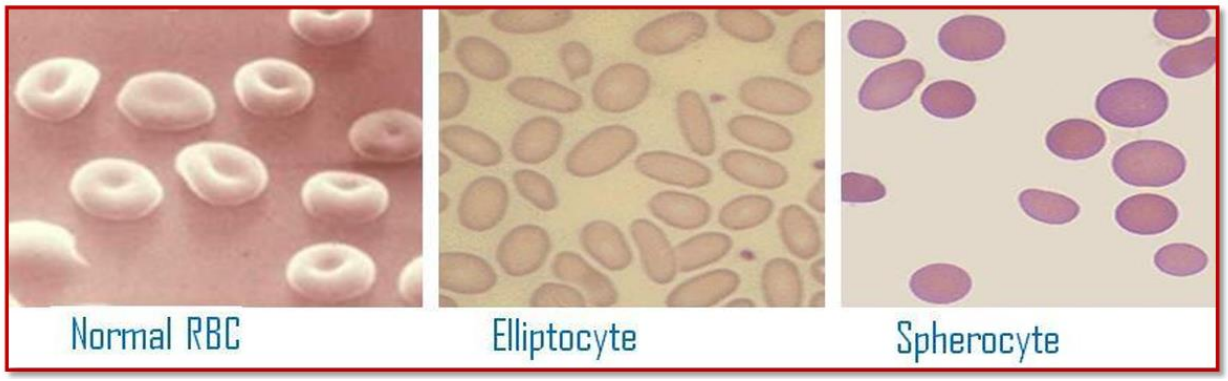
- ❖ α and β spectrin (most abundant).
- ❖ Ankyrin.
- ❖ Actin.
- ❖ Protein band 4.1 and 4.2.
- ❖ Band 3 protein.



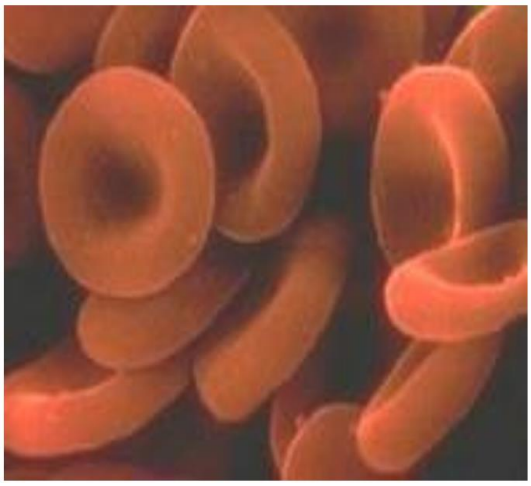
- The membrane skeleton is formed by structural proteins that include α and β spectrin, ankyrin, protein 4.1 and actin.
- These proteins form a horizontal lattice on the internal side of the red cell membrane and are important in maintaining the biconcave shape.

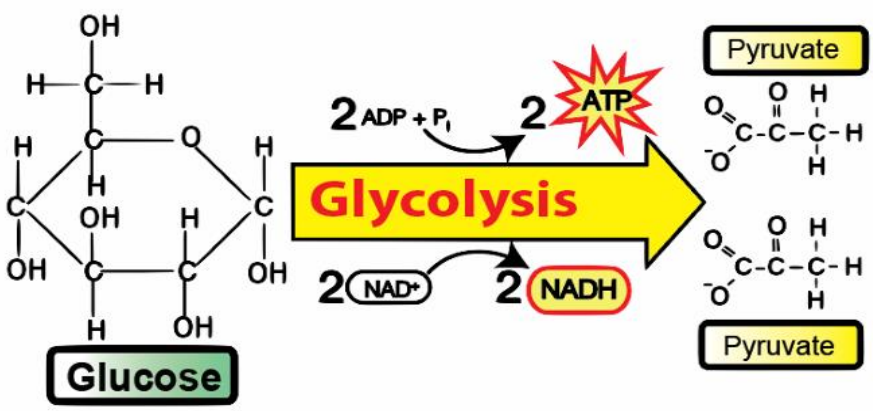


Defects of the membrane proteins explain some of the abnormalities of the shape of the red cell membrane (e.g. hereditary spherocytosis and elliptocytosis) , while alterations in lipid composition because of congenital or acquired abnormalities in plasma cholesterol or phospholipid may be associated with other membrane abnormalities.



Red cell metabolism





Red cell metabolism

1. Embden–Meyerhof pathway.
2. Hexose Monophosphate Shunt (HMS) or

   Pentose Phosphate Pathway (PPP)

1. Embden–Meyerhof pathway

- ✓ In this series of biochemical reactions, glucose that enters the red cell from plasma by the facilitated transfer is metabolized to lactate.
- ✓ For each molecule of glucose used, two molecules of ATP and thus two high-energy phosphate bonds are generated.
- ✓ This ATP provides energy for the maintenance of red cell volume, shape and flexibility.

The Red Cell Metabolism

RBC has no nuclear or mitochondrial organs for metabolizing fatty or amino acids.

RBC get the energy from the breakdown of glucose from the following enzymes:

- ❖ G6PD enzyme.
- ❖ Pyruvate kinase enzyme (PK).
- ❖ **Glycolysis:** This is the glycolytic pathway common to all cells of the human body whereby glucose is metabolized to lactate.

NOTE

- ✓ Nicotinamide adenine dinucleotide (NAD) is a coenzyme found in all living cells, exists in two forms, an oxidized and reduced form
- ✓ Abbreviated as NAD+ and NADH respectively

➢ Energy Forms

ATP ⟹ 3 PHOSPHATE

ADP ⟹ 2 PHOSPHATE

AMP ⟹ ONE PHOSPHATE

The Embden–Meyerhof pathway also generates NADH, which is needed by the enzyme methemoglobin reductase to reduce functionally dead methemoglobin containing ferric iron (produced by oxidation of approximately 3% of hemoglobin each day) to functionally active, reduced hemoglobin containing ferrous ions.

- ✓ The Luebering–Rapoport shunt, or side arm, of this pathway generates 2,3-diphosphoglycerate (2,3-DPG) important in the regulation of hemoglobin's oxygen affinity.
- ✓ Use the G_6PD enzyme as source of glucose
- ✓ It is anaerobic
- ✓ Provide **90%** of the cellular energy (ATP)
- ✓ Provide also NADH (Protect the Hb iron to stay in the form Fe^2)
- ✓ Provide 2,3 DPG (2,3 diphosphoglycerate): by The Luebering–Rapoport shunt

By The Luebering–Rapoport shunt

Only 15–25% of glucose passing through the glycolytic pathway enters this shunt;

Target: to generate 2,3 DPG (2,3 diphosphoglycerate):

Importance: regulate the oxygen affinity (regulate the loading and unloading of oxygen)

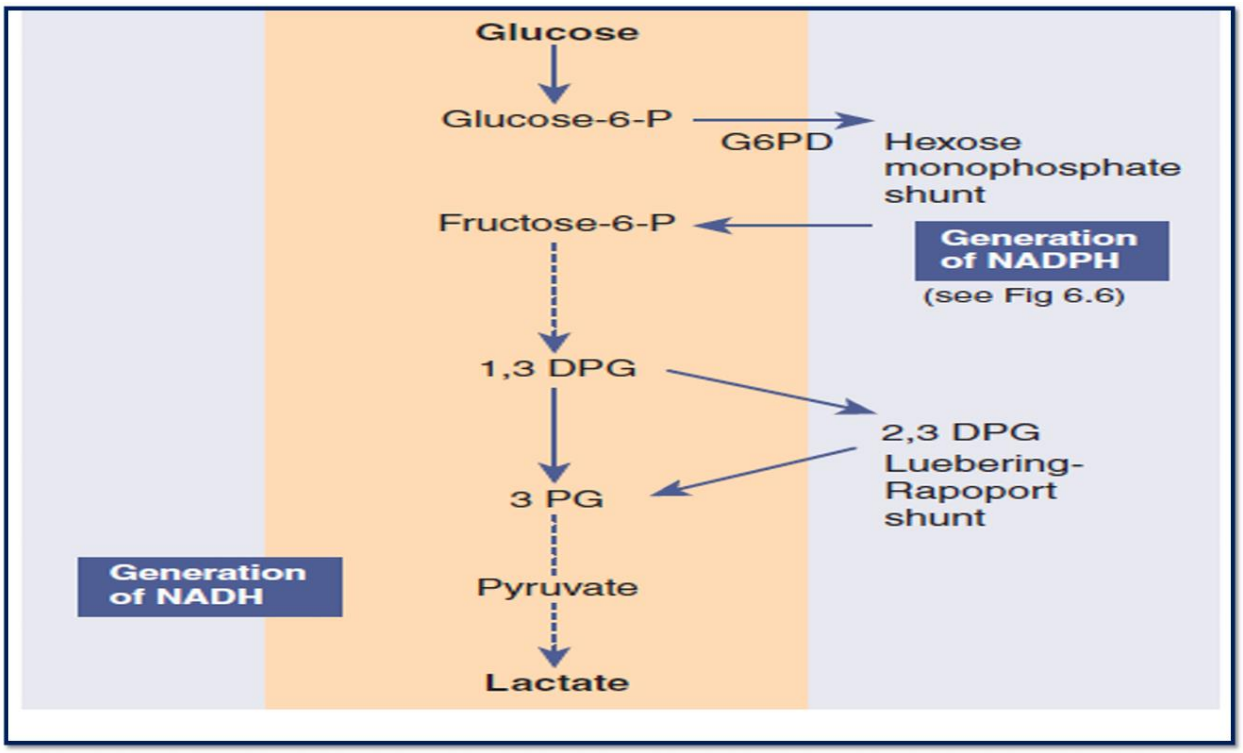
No ATP is produced.

- ✓ In this pathway the NAD reduction to NADH.
- ✓ It is anerobic pathway

Importance of NADH

- ✓ Protect the Hb iron to stay in the form Fe^2.
- ✓ So, for oxygen can be delivered to the tissues, is dependent on the reduction of NAD to NADH
- ✓ If this enzyme is absent, produce that called methemoglobin

What is methemoglobin? Fe^2 converted to Fe^3 inside the RBC, the Result the RBC cannot carry oxygen to the tissues



Metabolic pathways ATP is important

1. ATP is important to support oxygen transport and membrane flexibility.
2. Protect the membrane from damage
3. Regulate the movement of electrolytes
4. Keep hemoglobin in the reduced (Fe2) state)
5. Produce 2,3 DPG enzyme (The importance of 2,3 DPG enzyme: oxygen loading and unloading)

2. Hexose Monophosphate Shunt (HMS) or Pentose Phosphate Pathway (PPP)

Approximately 10% of glycolysis occurs by this oxidative

Use :glucose-6-phosphate –G6P

Pathway Produce **NADPH**

No ATP production

It is aerobic NADPH:

source : from glycolysis enter HMP (Hexose monophosphate pathway)

Main function to remove the toxicity from RBCs (antioxidant).

……..…….

Summary

RBCs metabolism:

RBCs able to produce energy that required it as:

ATP:

Source: From anaerobic glycolysis (Embden Meyerhof pathway).

Main function to maintain RBCs shape, volume, flexibility.

NADH: Source: Also from anaerobic glycolysis - Embden Meyerhof, The methemoglobin reductase pathway Main function to maintain the Hb iron to stay in the form Fe^{2}

2,3 DPG (2,3 diphosphoglycerate):

Source: From Embden Meyerhof pathway By The Luebering–Rapoport

shunt.

Main function Regulation of Hb-oxygen affinity.

NADPH:

Source: 5% from glycolysis enter HMP (Hexose monophosphate pathway).

- ✓ Main function to remove the toxicity from RBCs (antioxidant).
- ✓ NADPH is generated and is linked with glutathione which maintains sulfhydryl (SH) groups intact in the cell, including those in hemoglobin and the red cell membrane.
- ✓ In one of the most common inherited abnormalities of red cells, glucose-6-phosphate dehydrogenase (G6PD) deficiency, the red cells are extremely susceptible to oxidant stress

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

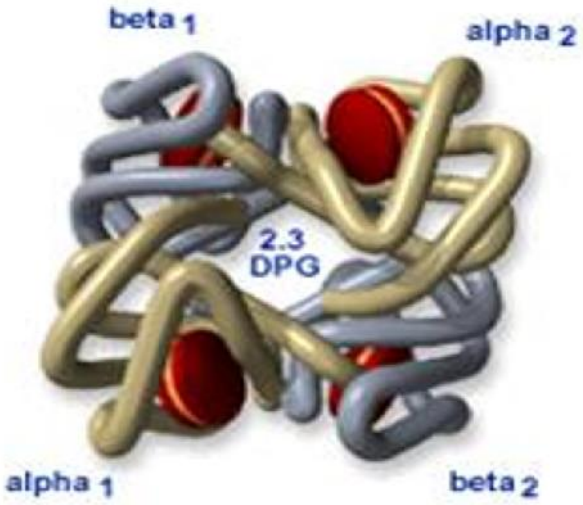
Fourth Week

Lecture Title: Haemoglobin (structure, synthesis, and levels in blood and in erythrocytes).

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for the students to identify the hemoglobin (structure, synthesis, and levels in blood and in erythrocytes).

Special Objectives of the Module

1. Definition of hemoglobin.

2. Distinguish between the structure, synthesis, function, and types of hemoglobin of hemoglobin.

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

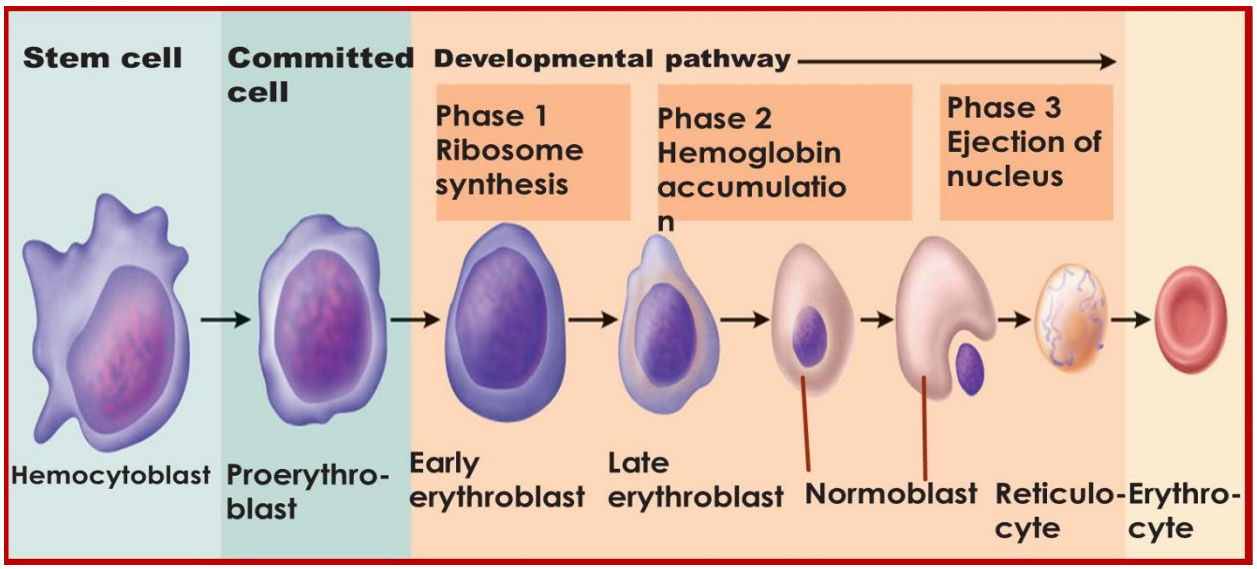
2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

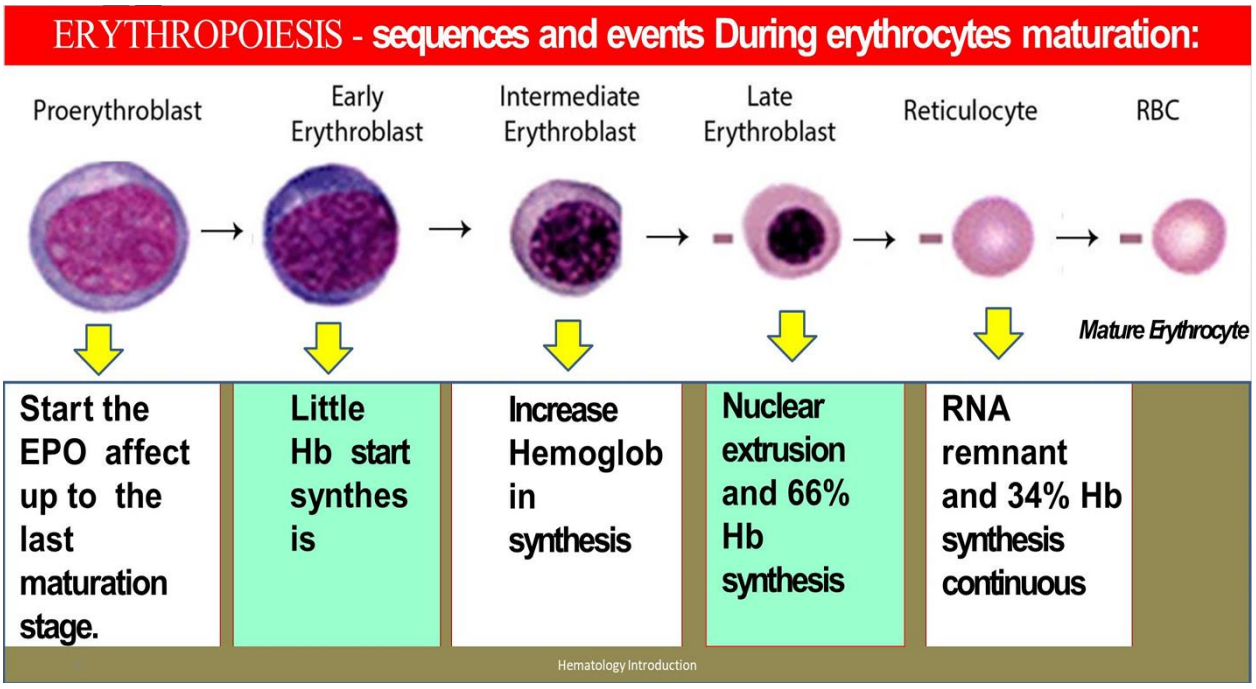
3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

- ✓ **Hemoglobin** is a highly specialized intracellular erythrocyte protein responsible for transporting oxygen from the lungs to tissue and facilitating carbon dioxide transport from the tissue to the lungs.
- ✓ Each gram of hemoglobin can carry 1.34 mL of oxygen.
- ✓ Hemoglobin occupies approximately **33%** of the volume of the erythrocyte and accounts for **90%** of the cell's dry weight.
- ✓ Each cell contains between **28 and 34** pg. of hemoglobin.
- ✓ This concentration is measured by cell analyzers and reported as mean corpuscular hemoglobin (MCH).
- ✓ In anemic states, the erythrocyte can contain less hemoglobin (decreased MCH), and/or the individual can have fewer erythrocytes present, both of which decrease the blood's oxygen-carrying capacity.
- ✓ The erythrocyte's membrane and its metabolic pathways are responsible for protecting and maintaining the hemoglobin molecule in its functional state.
- ✓ Abnormalities in the membrane that alter its permeability or alterations of the cell's enzyme systems can lead to changes in the structure and/or function of the hemoglobin molecule and affect the capacity of this protein to deliver oxygen.
- ✓ Although a small amount of hemoglobin is synthesized as early as the pronormoblast stage, the most hemoglobin synthesized in the developing normoblasts occurs at the polychromatophilic normoblast stage.
- ✓ In total, **75–80%** of the cell's hemoglobin is made before the extrusion of the nucleus.
- ✓ Because the reticulocyte does not have a nucleus, it cannot make new RNA for protein synthesis.
- ✓ However, residual RNA and mitochondria in the reticulocyte enable the cell to make the remaining **20–25%** of the cell's hemoglobin.

Erythropoiesis



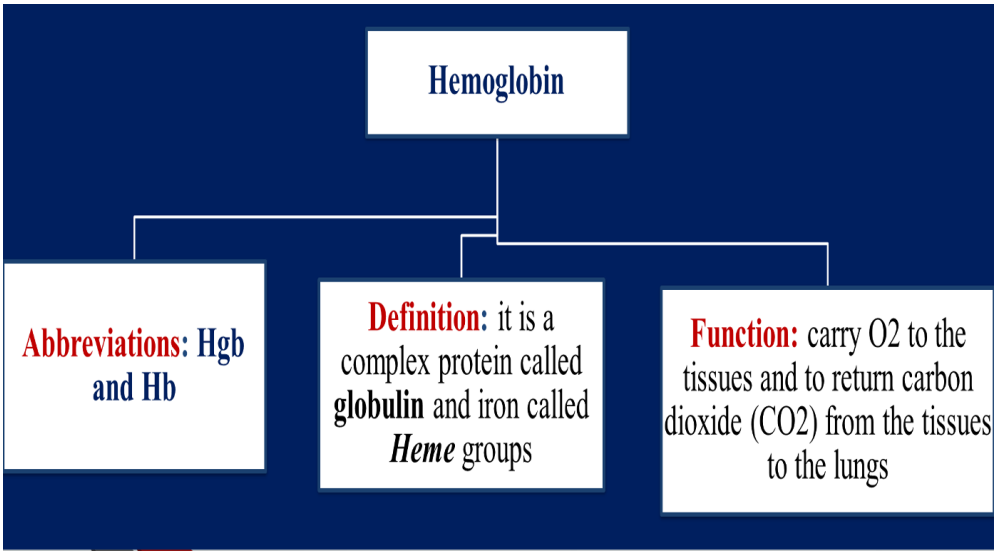


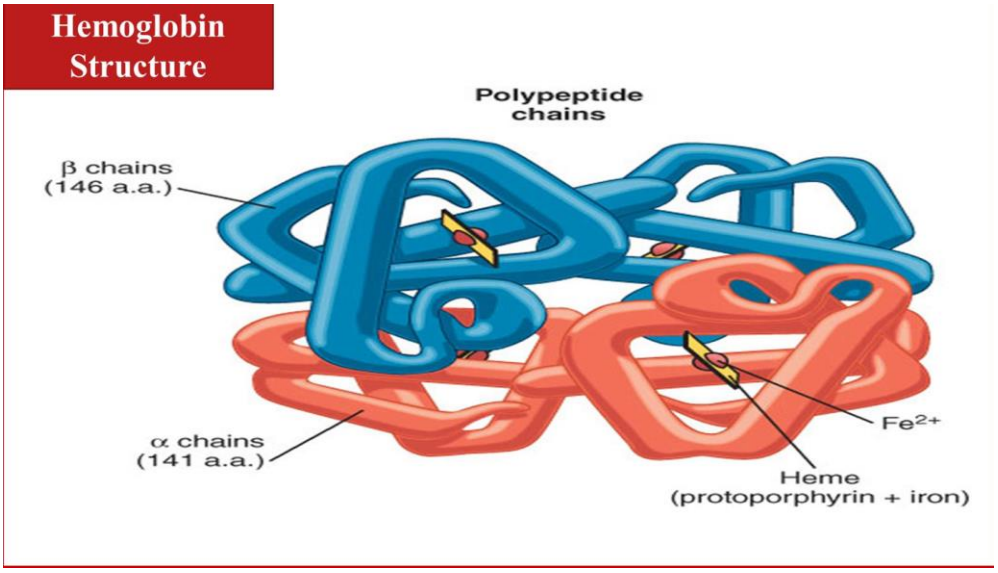
✓ The mature erythrocyte contains no nucleus, ribosomes, or mitochondria and is unable to synthesize new protein

Hemoglobin concentration in the body is the result of a fine balance between the production and destruction of erythrocytes

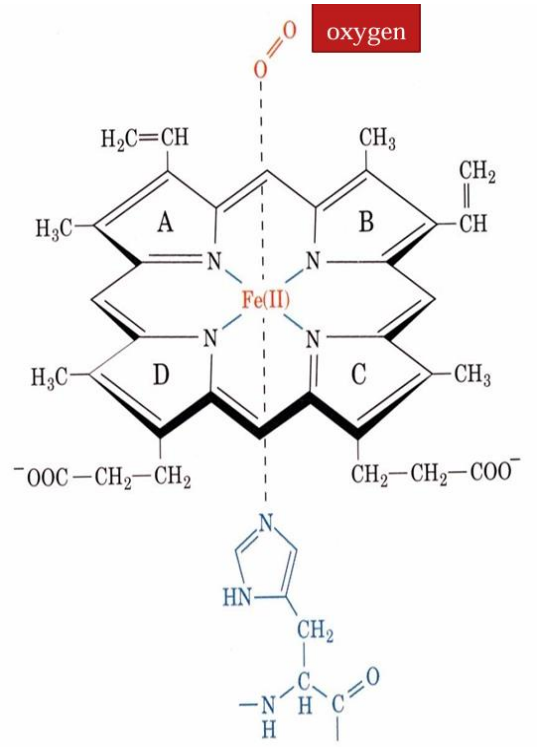
Content : It is composed of the protein globin (a polypeptide), and the heme.

Structure : the molecule. Hemoglobin has the ability to combine with oxygen due to the four iron atoms associated with each heme group within.





- ❖ Each subunit of hemoglobin contains a heme binds one molecule of oxygen
- ❖ The iron must be in the Fe(II) form or reduced form.

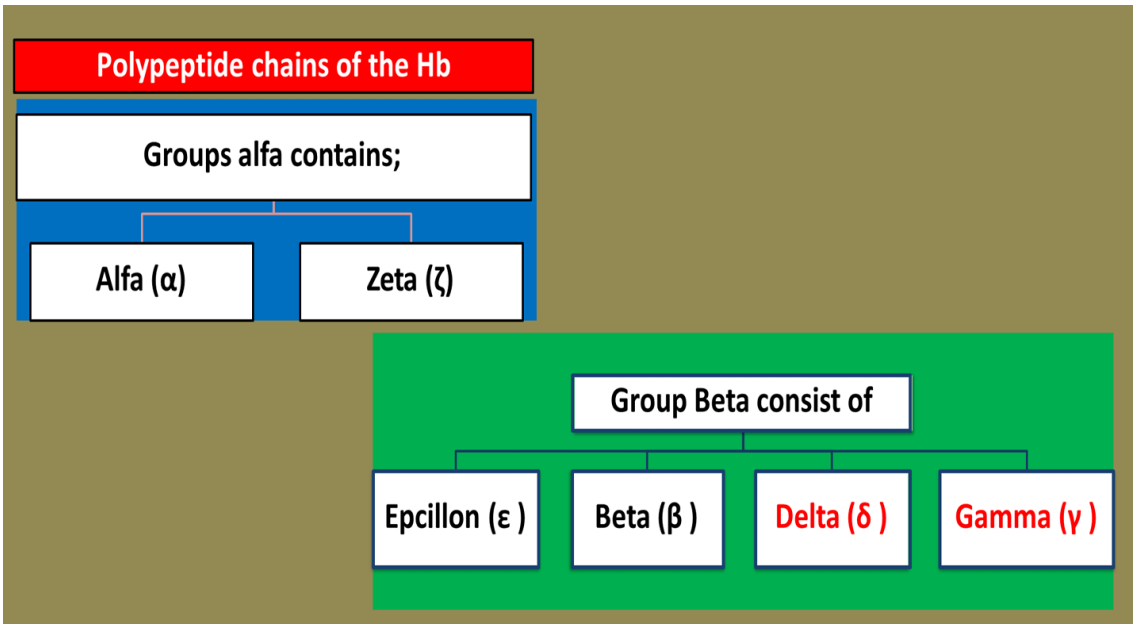


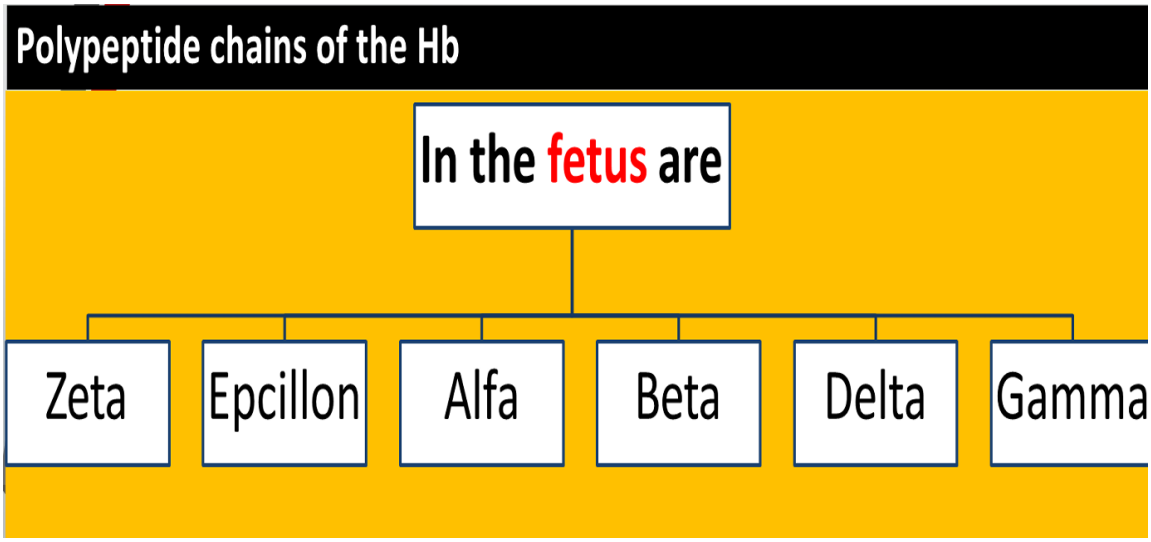
Hemoglobin structure

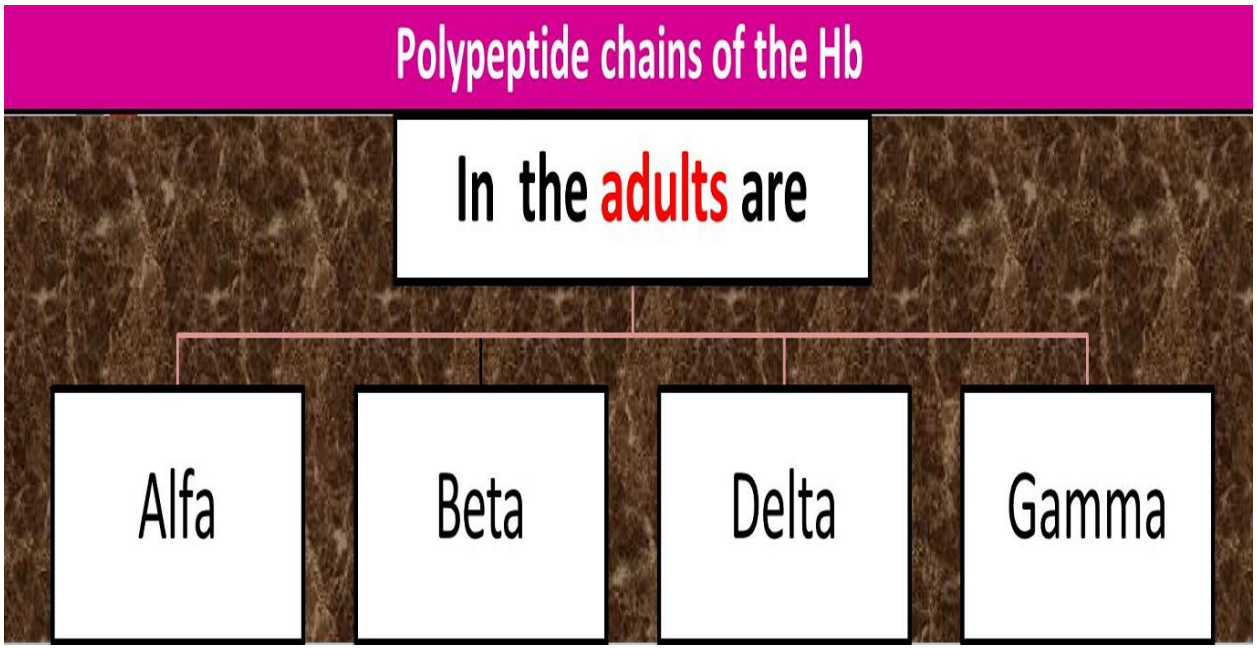
- ✓ Structurally, hemoglobin is a tetramer protein that consists of two pairs of identical Alpha (α) and Beta (β) chains.
- ✓ In addition, Alpha (α)- like globin called Zeta (ζ) and Epsilon (ε). Gamma (γ) is β- like goblins.
- ✓ Eachglobin chain is attached to one heme molecule. Delta (δ),
- ✓ Thenumber of amino acids in the α chain is 141 amino acids, and the β chain has 146 amino acids.

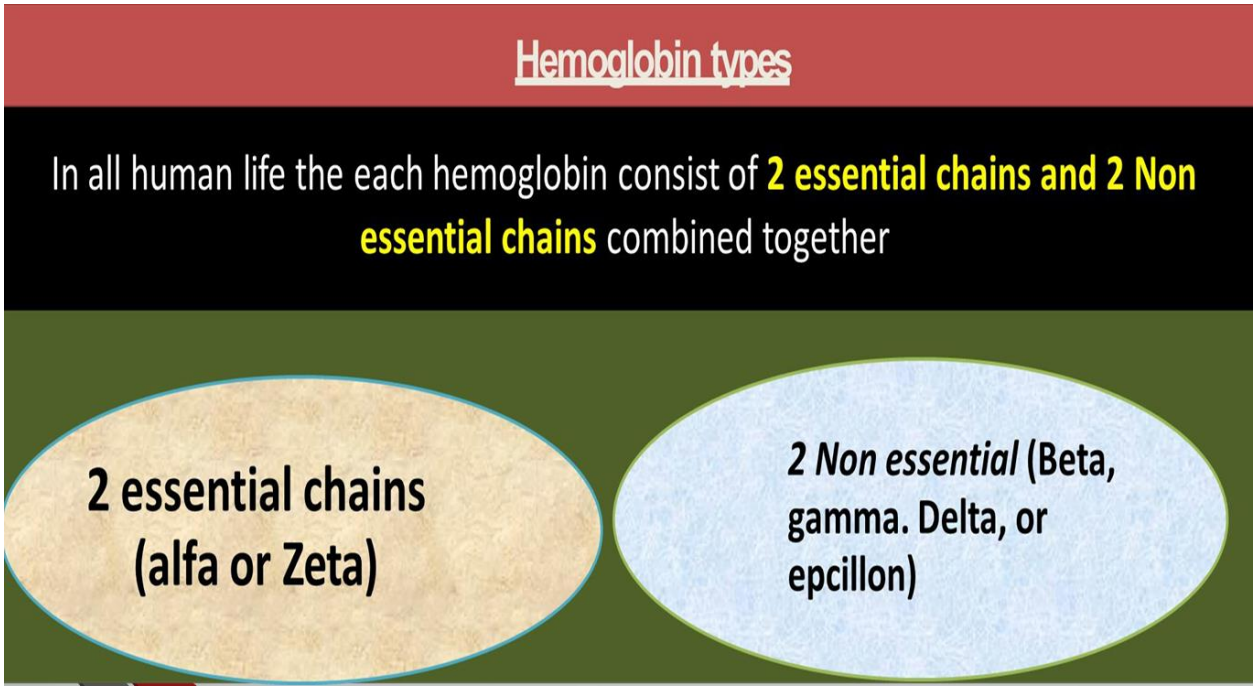
Under normal conditions

Hemoglobin tetramers are composed of two types of chains, 2a-like {Alpha (a) , Zeta (z)} and 2b- like {(Beta (b), Gamma (γ), and Delta (δ), Epsilon (ε)}.



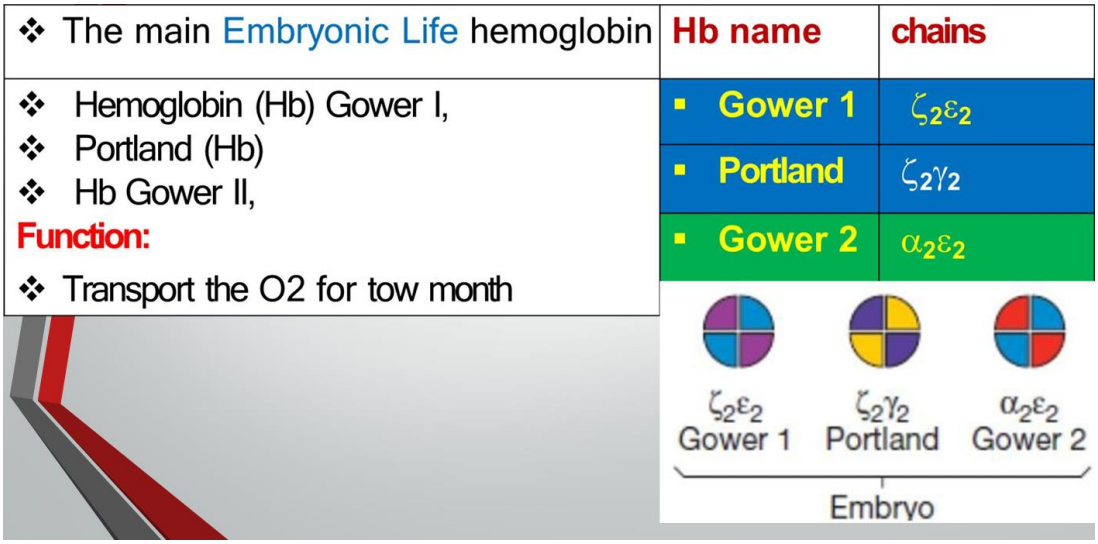



Polypeptide chains of the Hb
In the adults are
Alfa
Beta
Delta
Gamma


Hemoglobin types
In all human life the each hemoglobin consist of 2 essential chains and 2 Non essential chains combined together
2 essential chains (alfa or Zeta)
2 Non essential (Beta, gamma. Delta, or epcillon)

Development of Hemoglobin Synthesized Stages of Human Life

- ✓ The synthesis of hemoglobin starts early in the yolk sac in the 3rd week of gestation. At this stage hemoglobin tetramer is called Hb Gower1 (z2 e2), which is composed of 2 a like (2z) and 2 b like (2e) chains. After that, the synthesis of two other embryonic hemoglobin takes place: Hb Gower2 (a2 e2) and Hb Portland (z2 g2).
- ✓ Then, erythropoiesis starts in the liver and spleen, at 10 to 11 weeks of gestation, at which the Hb Gower1, Gower2, and Hb Portland decline.



The main fetal life Hb	Hb name	chains
❖ Hb F predominates during fetal life ❖ It is the main Hb from the 3rd to the 9th month of the fetal life	HbF	$\alpha_2\gamma_2$
❖ During this period HbA1 start developed and reach to 28% of all hemoglobin	HbA1	$\alpha_2\beta_2$
❖ HbA2 start produced and it is constantly 2% in all huma life	HbA2	$\alpha_2\delta_2$

Newborn Hb	%
▪ HbF	70
▪ HbA	28
▪ HbA2	2

HbF → Gradually converted into HbA during the first year of the life

The β-globin gene is expressed at a low level in early fetal life, but the main switch to adult haemoglobin occurs 3-6 months after birth when synthesis of the γ chain is replaced by β chains.

Adult-From 1 year up to end of the life	%
▪ HbA	97
▪ Hba2	2-3
▪ HbF	1-2

✓ Fetal hemoglobin (HbF: a2 g2) becomes predominant throughout the fetus.

✓ Different types of hemoglobin are synthesized during the developmental stages of human life.

Hemoglobin Type	Structure	Developmental Stage
Hb Gower 1	ζ2 ε2	Embryo
Hb Gower 2	α2 ε2	Embryo
Hb Portland	ζ2 γ2	Embryo
HbF	α2 γ2	Fetal and adult
HbA2	α2 δ2	Adult
HbA	α2 β2	Adult

- ✓ Normally, Hb functions to transport oxygen from the lungs to the tissues and Co2 from the tissues to the lungs.
- ✓ In order to achieve this gaseous exchange, they contain the specialized protein hemoglobin.
- ✓ Each molecule of normal adult (Hb A) (the dominant hemoglobin in α2β2, each with its own heme group. blood after the age of 6 months) consists of four polypeptide chains,
- ✓ Normal adult blood also contains small quantities of two other hemoglobins: Hb F and Hb A2, these also contain α chains, but with γ and δ chains, instead of β

Hemoglobin Synthesis

Normal adult blood contains three types of hemoglobin.

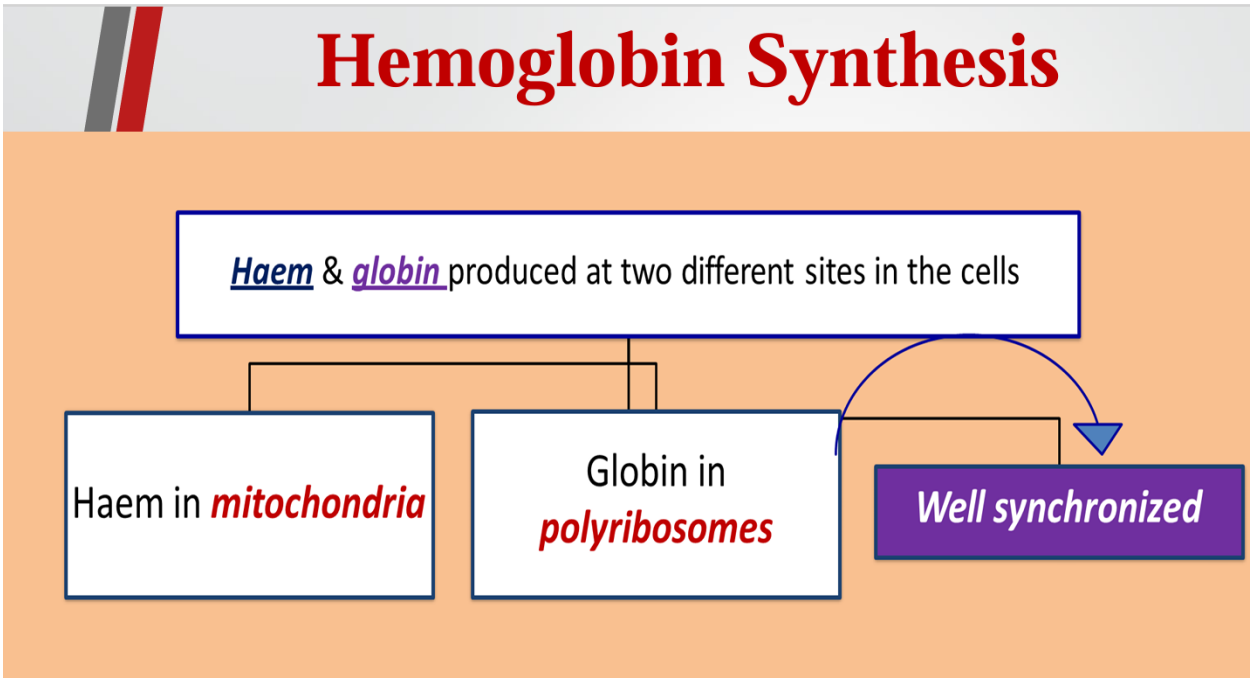
- ✓ The major component is hemoglobin A with the molecular structure α2β2.
- ✓ The minor hemoglobins contain, γ (fetal Hb or Hb F), δ (Hb A2) globin chains instead of β chains.
- ✓ When the production of the first nucleated RBCs starts from stem and progenitor cells in the fetal liver, the main β-like globin produced gamma-globin.
- ✓ The γ- globin chains unite with adult α-globin chains to form a remaining gestation. stable tetramer HbF. This will be kept as the main Hb for the
- ✓ Theadult b globin chains start to replace the g- and d globin chain after birth, causing a switch from HbF (a2 g2) and HbA2 (a2 d2) to the adult hemoglobin HbA(a2 b2) synthesis.
- ✓ This process takes place at about the time of birth and ends 6 months later.
- ✓ After shifting from fetal to adult hemoglobin, 97-98% of the hemoglobin is HbA, while HbA2 (a2 d2) accounts for approximately 2%. A very few amount (≈1%) of HbF is found in adult blood

Normal hemoglobin in adult blood

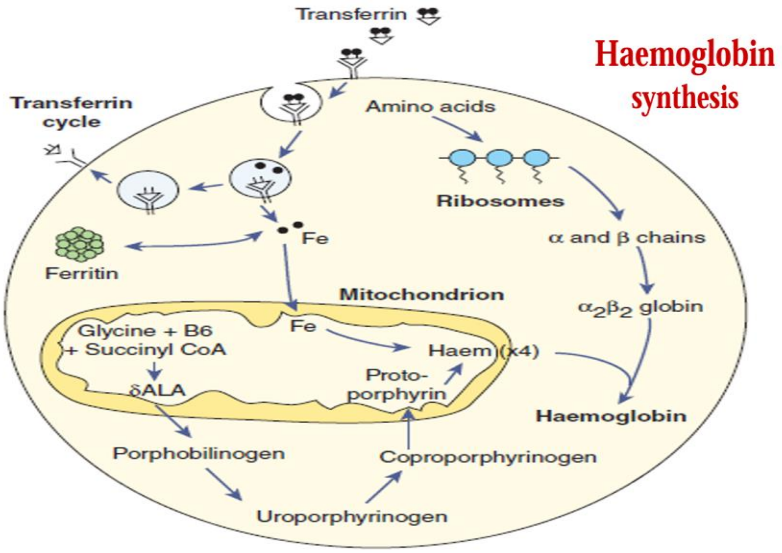
	Hb A	Hb F	Hb A 2
Structure	α 2 β 2	α 2 γ 2	α 2 δ 2
Normal (%)	96 – 98	0.5 – 0.8	1.5 – 3.2

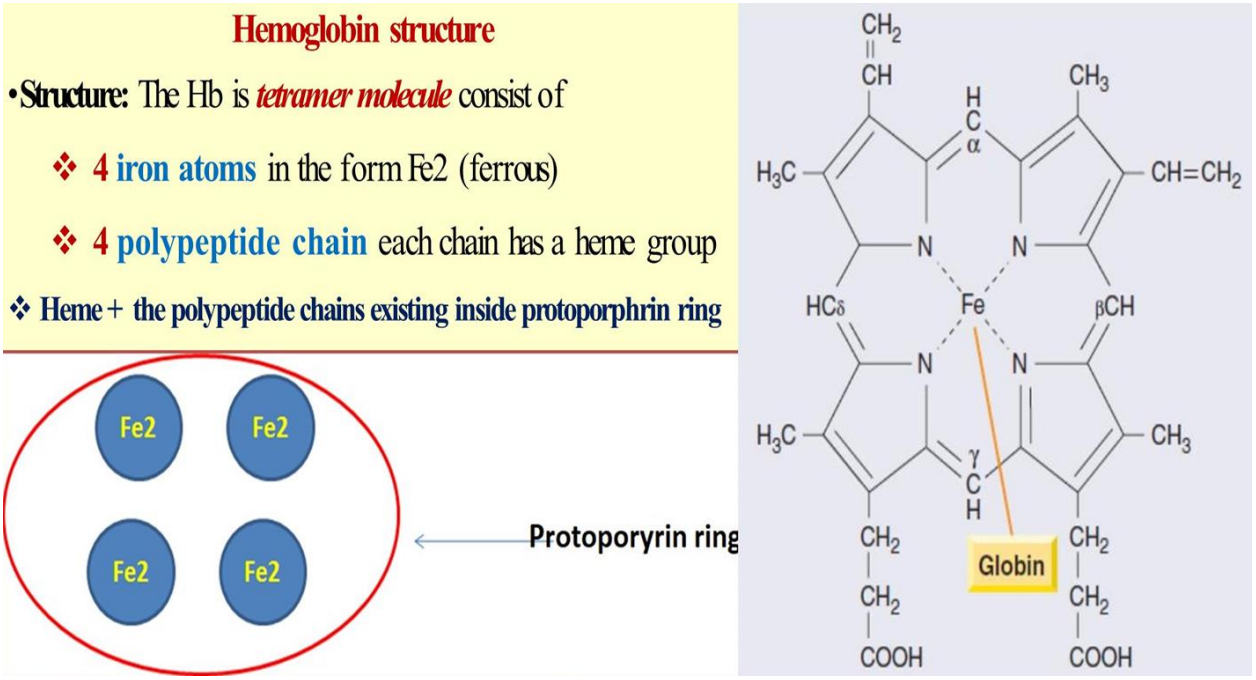
Switch from fetal to adult haemoglobin

The β-globin gene is expressed at a low level in early fetal life, but the main switch to adult hemoglobin occurs 3–6 months after birth when the synthesis of the γ chain is replaced by β chains.



- **Heme synthesis** begins in the mitochondria with the condensation of glycine and succinyl coenzyme A (CoA) to form 5- aminolevulinic acid (ALA). This reaction occurs in the presence of the cofactor pyridoxal phosphate and the enzyme 5-aminolevulinate synthase (ALAS).
- This first reaction is the rate-limiting step in the synthesis of heme and occurs only when the cell has an adequate supply of iron.
- Synthesis continues through a series of steps in the cytoplasm, eventually forming Coproporphyrinogen then reenters the mitochondria and is further modified to form the protoporphyrin ring coproporphyrinogen.
- The final step, also occurring in the mitochondria, is the chelation of iron with protoporphyrin catalyzed by ferrochelatase to form heme. Heme then leaves the mitochondria to combine with a globin chain in the cytoplasm.





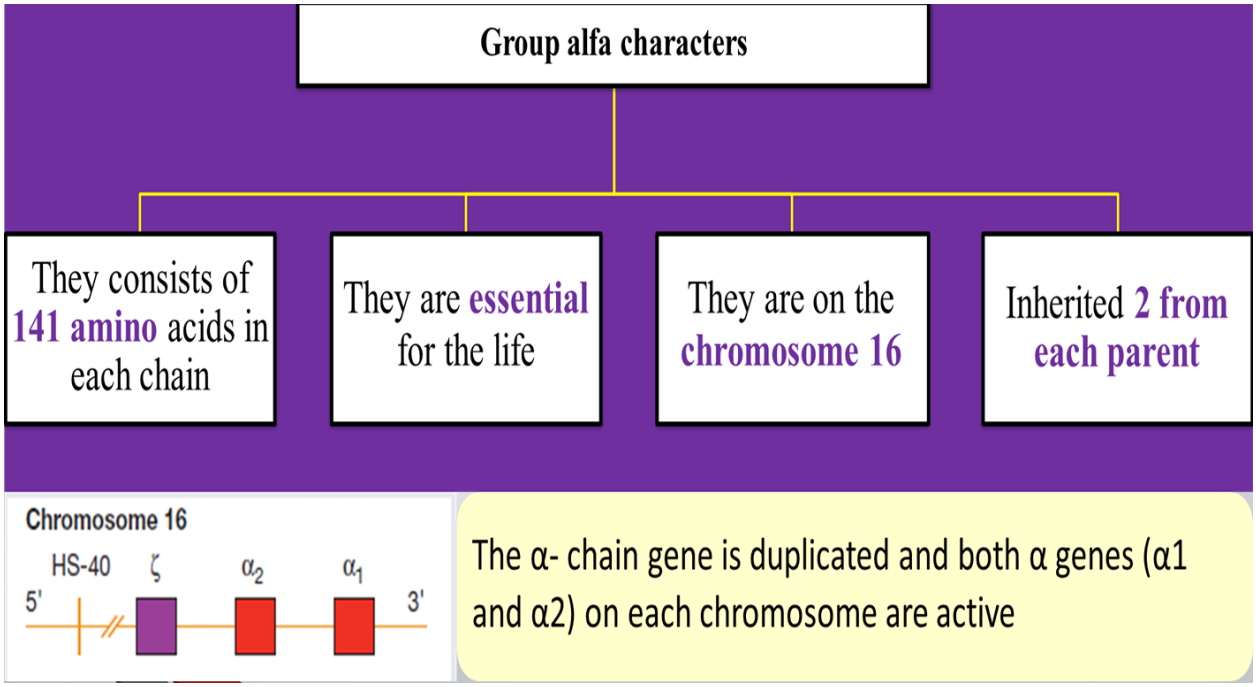
Globin Chain Synthesis

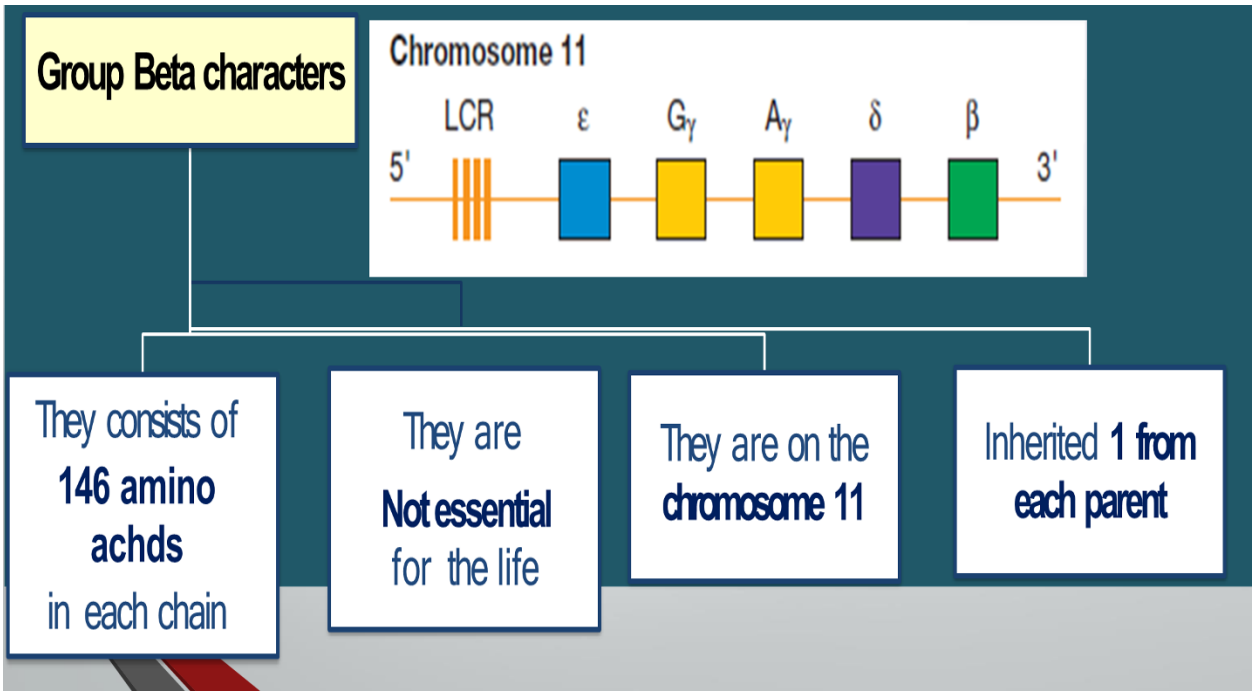
❖ Globin chain synthesis is directed by genes in two clusters on chromosomes 11 and 16.

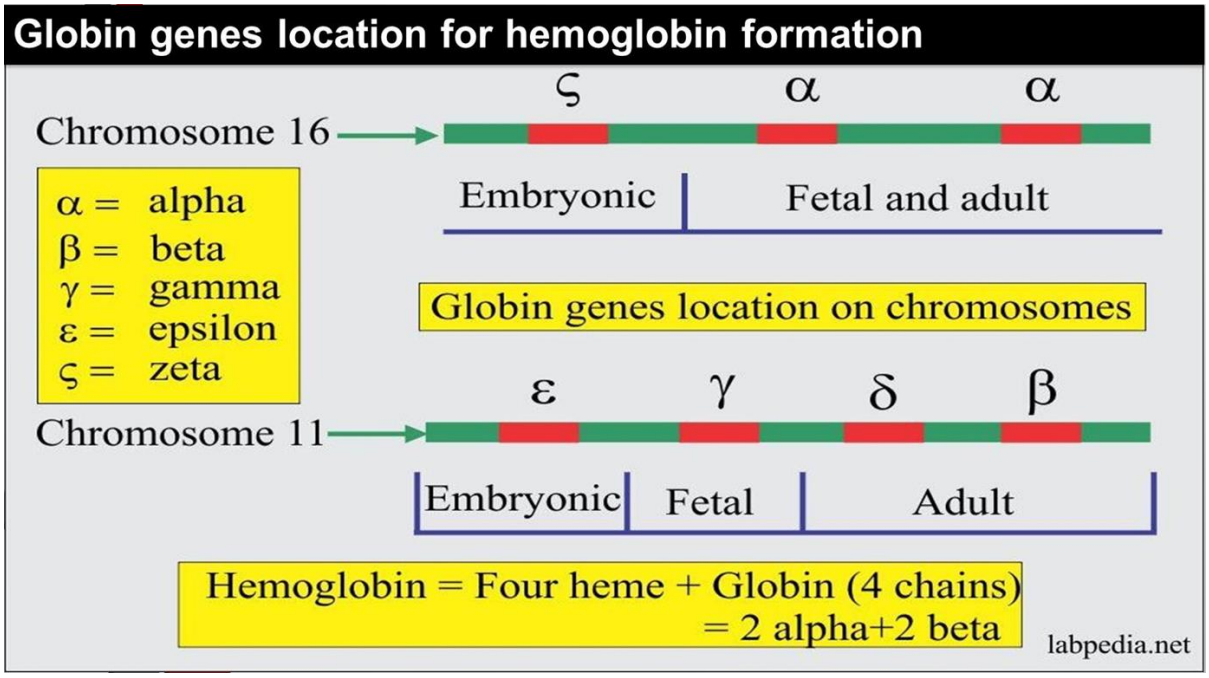
❖ These genes produce the seven different types of globin chains: zeta, alpha, epsilon, gamma, delta, and beta (α, ζ, ε,γ,δ, β).

❖ Two are found only in embryonic hemoglobins (ζ, ε).

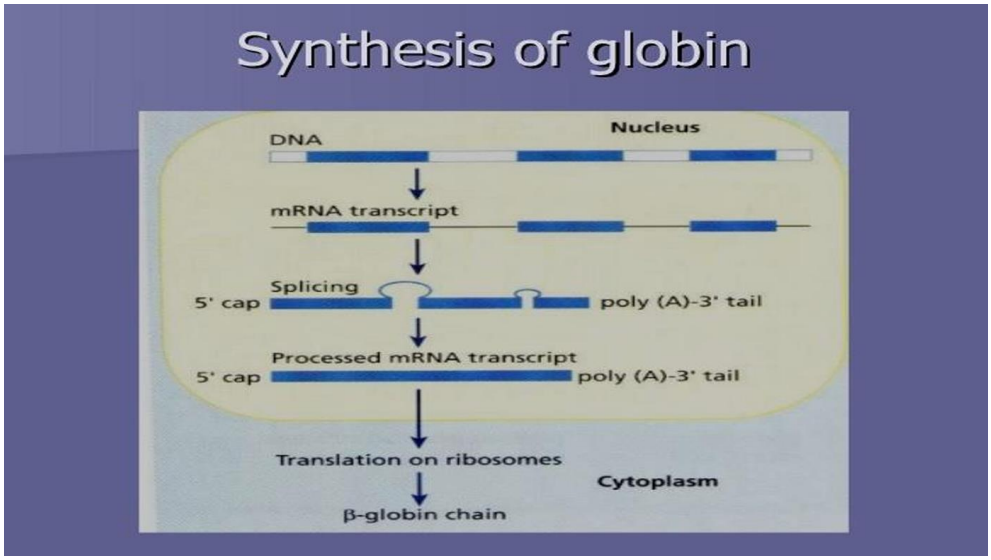
❖ The ζ -chain is synthesized very early in embryonic development, but after 8–12 weeks, ζ -chain synthesis is replaced by α - chain synthesis.


Group alfa characters
They consists of 141 amino acids in each chain
They are essential for the life
They are on the chromosome 16
Inherited 2 from each parent
Chromosome 16
HS-40
ζ
α2
α1
5'
3'
The α- chain gene is duplicated and both α genes (α1 and α2) on each chromosome are active


Group Beta characters
Chromosome 11
LCR
ε
Gγ
Aγ
δ
β
5'
3'
They consists of 146 amino achds in each chain
They are Not essential for the life
They are on the chromosome 11
Inherited 1 from each parent



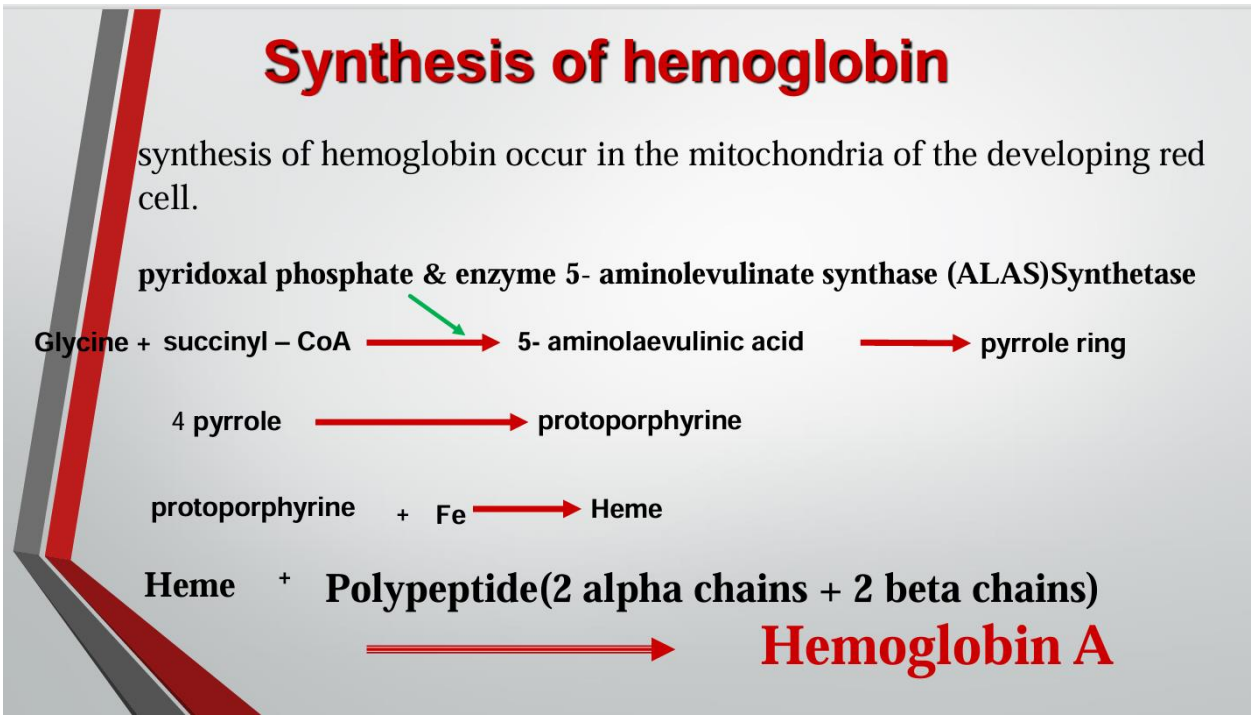
- The synthesis of globin peptide chains occurs on polyribosomes in the cytoplasm.
- Globin chains are released from the polyribosomes and mitochondria.



Hemoglobin synthesis is regulated by several mechanisms including:

- Activity and concentration of the erythroid enzyme 5 minolevulinate synthase (ALAS2)
- The activity of porphobilinogen deaminase (PBGD)
- Concentration of iron
- Regulation of globin chain synthesis

……



Globin Chains

- Alpha Globin
  - 141 amino acids
  - Coded for on Chromosome 16
  - Found in normal adult hemoglobin, A1 and A2
- Beta Globin
  - 146 amino acids
  - Coded for on Chromosome 11, found in Hemoglobin A1

Delta Globin

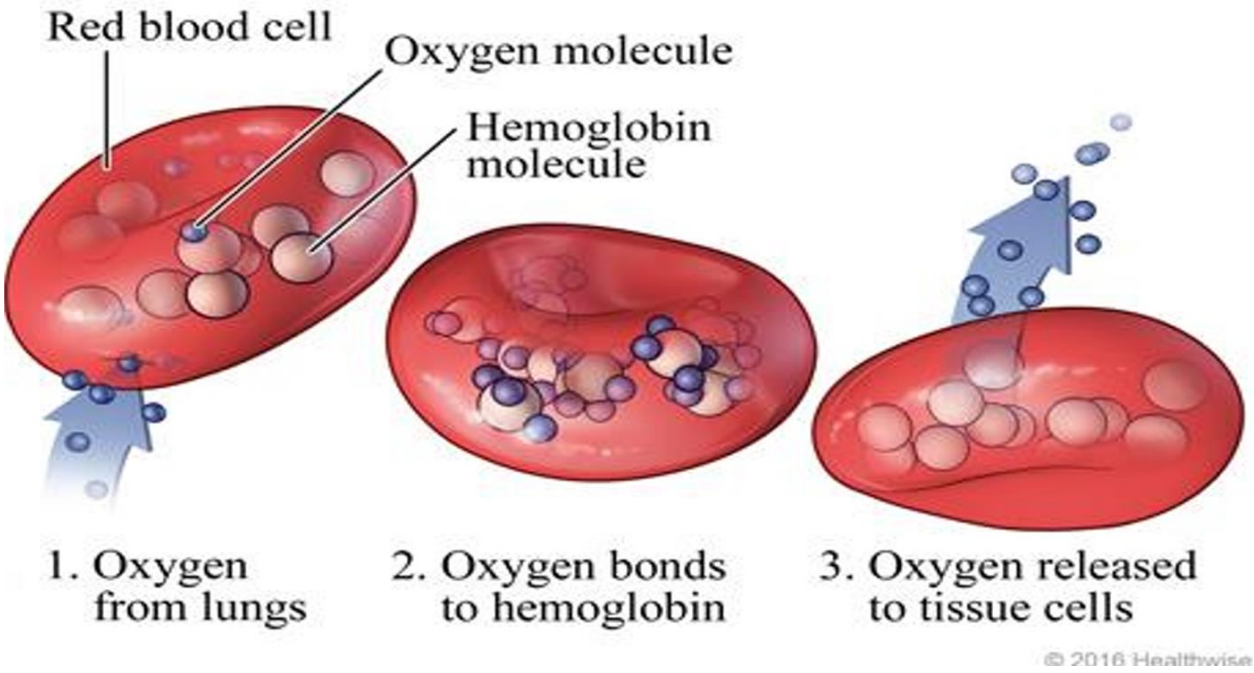
- Found in Hemoglobin A2--small amounts in all adults

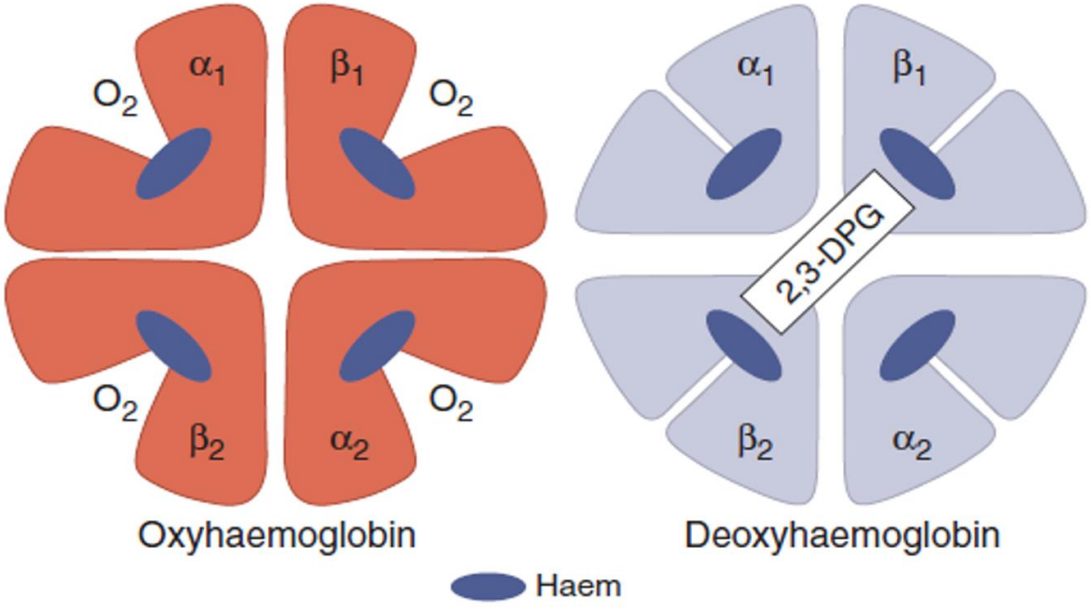
- Gamma Globin
  - Found in Fetal Hemoglobin

Hemoglobin Function

- The red cells in systemic arterial blood carry O2 from the lungs to the tissues and return in venous blood with CO2 to the lungs.
- As the hemoglobin molecule loads and unloads O2 the individual globin chains move on each other.
- The α1β1 and α2β2 contacts stabilize the molecule.
- When O2 is unloaded the β chains are pulled apart, permitting entry of the metabolite 2,3-diphosphoglycerate (2,3-DPG) resulting in a lower affinity of the molecule for O2.

- ✓ This movement is responsible for the sigmoid form of the emoglobin O_2 dissociation curve.
- ✓ The P50 (i.e. the partial pressure of O_2 at which hemoglobin is half saturated with O2) of normal blood is 26.6 mmHg.

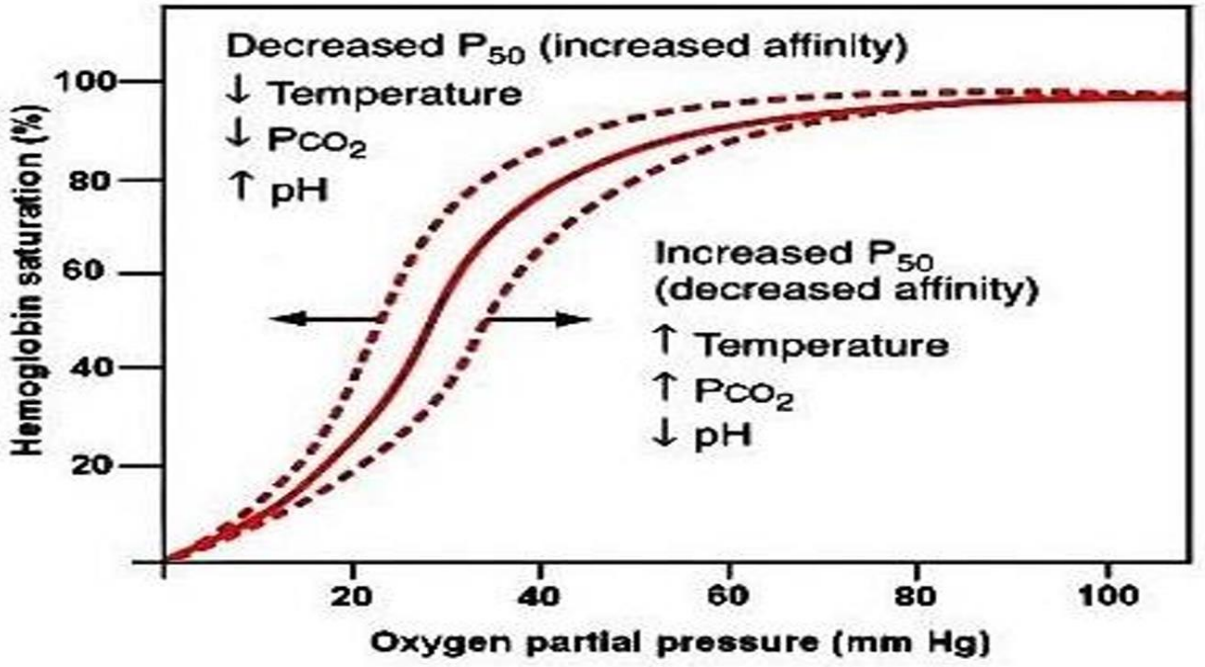




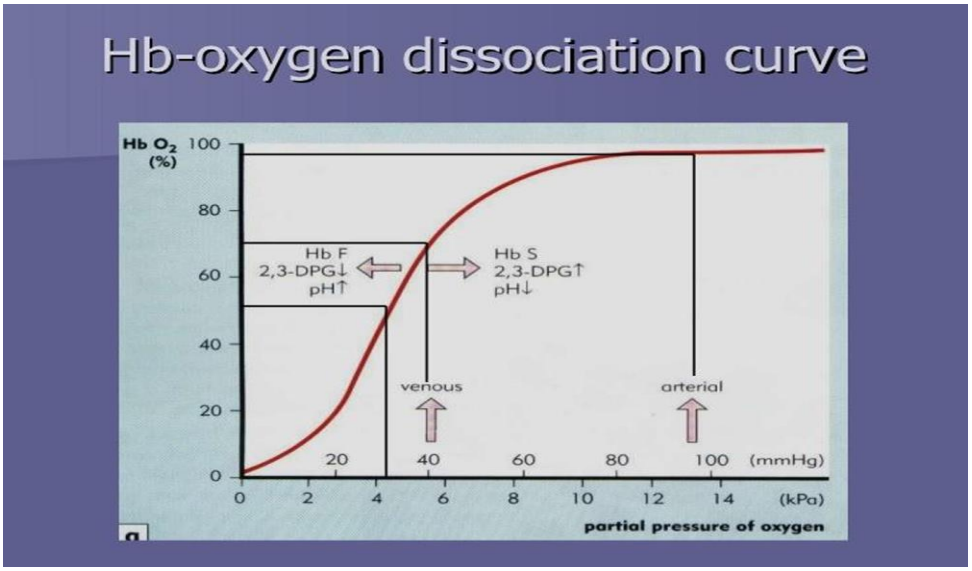
- ✓ If hemoglobin oxygen saturation is plotted versus the partial pressure of oxygen (PO_2), a sigmoid-shaped (S-shaped) curve results.
- ✓ This is referred to as the oxygen dissociation curve (ODC)
- ✓ The normal position of the curve depends on the concentration of 2,3-DPG, H+ ions, and CO_2 in the red cell and on the structure of the hemoglobin molecule.
- ✓ High concentrations of 2,3-DPG, H+, or CO_2, and the presence of given up more easily). sickle hemoglobin (Hb S), shift the curve to the right (oxygen is given up more easily).
- ✓ Whereas fetal hemoglobin (Hb F) – which is unable to bind 2,3-DPG – and certain rare abnormal hemoglobin's associated with polycythemia shift the curve to the left because they give up O_2 less readily than normal.
- ✓ The ease with which hemoglobin binds and releases oxygen is known as oxygen affinity.
- ✓ Hemoglobin affinity for oxygen determines the proportion of oxygen released to the tissues or loaded onto the cell at a given oxygen pressure (PO_2).
- ✓ Increased oxygen affinity means that the hemoglobin has a high affinity for oxygen, will bind oxygen more avidly, and does not readily give it up.
- ✓ Decreased oxygen affinity means the hemoglobin has a low affinity for oxygen and releases its oxygen more readily.
- ✓ With an increased affinity for O2, the curve shifts to the left (i.e. the P50 falls).

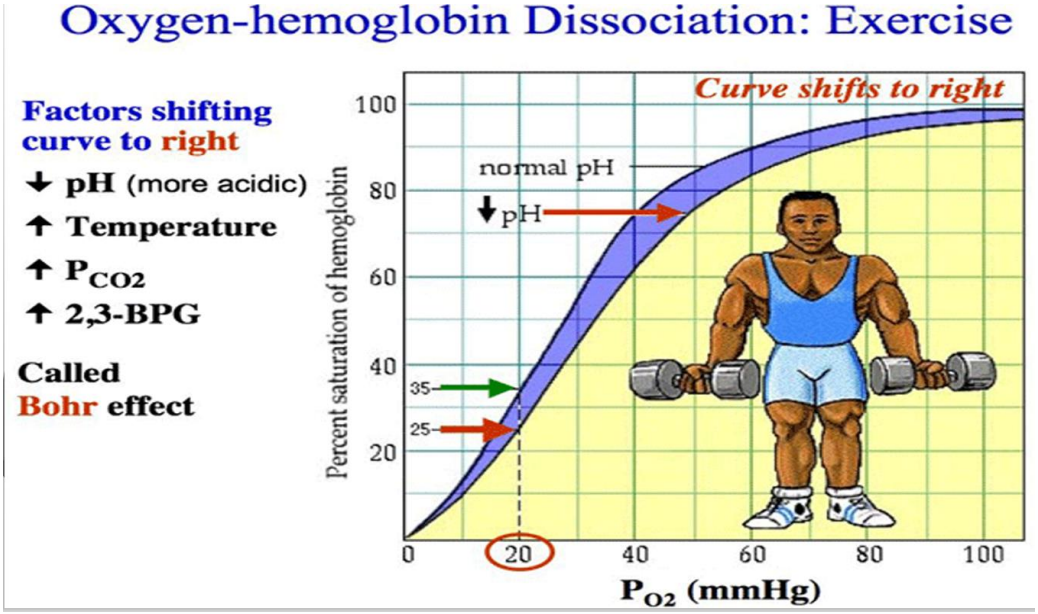
- ✓ While with decreased affinity for O2, the curve shifts to the right (i.e. the P50 rises).
- ✓ Normally, in vivo, O2 exchange operates between 95% saturation (arterial blood) with a mean arterial O2 tension of 95 mmHg and 70% saturation (venous blood) with a mean venous O2 tension of 40 mmHg

……..

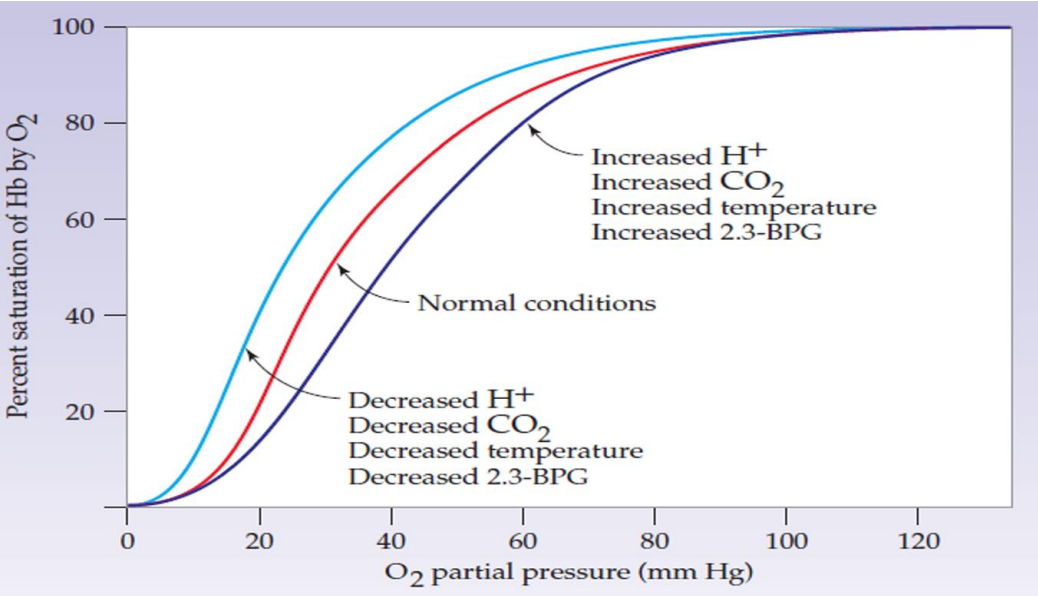


OXYGEN DISSOCIATION CURVE


Hb-oxygen dissociation curve
Hb O2 (%)
100
80
60
40
20
0
Hb F
2,3-DPG↓
pH↑
Hb S
2,3-DPG↑
pH↓
venous
arterial
20
40
60
80
100
(mmHg)
0
2
4
6
8
10
12
14
(kPa)
partial pressure of oxygen


Oxygen-hemoglobin Dissociation: Exercise
Factors shifting curve to right
↓ pH (more acidic)
↑ Temperature
↑ PCO2
↑ 2,3-BPG
Called Bohr effect
Curve shifts to right
normal pH
↓pH
Percent saturation of hemoglobin
100
80
60
40
20
35
25
0
20
40
60
80
100
PO2 (mmHg)

- The ODC shows that the oxygen saturation of hemoglobin drops from **100%** in the arteries to **75%** in the veins.
- This indicates that hemoglobin gives up about **25%** of its oxygen to the tissues.
- When the curve is shifted to the right, the P50 is increased, indicating that the oxygen affinity has decreased. This results in the release of more oxygen to the tissues
- When the curve is shifted to the left, the P50 is decreased, indicating that oxygen affinity has increased. In this case, less oxygen is released to the tissues



Hb-oxygen dissociation curve

- Right shift (easy oxygen delivery)
  - High 2,3-DPG
  - High H^+
  - High CO_2
  - HbS
- Left shift (give up oxygen less readily)
  - Low 2,3-DPG
  - HbF

Hemoglobin

Oxygen transport protein of red blood cells.

Myoglobin

Oxygen storage protein of skeletal muscles.

The rate of O2 diffusion from capillaries to tissue is slow because of the solubility of oxygen.

- Myoglobin facilitates rapidly respiring muscle tissue
- Myoglobin increases the solubility of oxygen.
- Myoglobin facilitates oxygen diffusion.

Methemoglobin

- When Fe(II) goes to Fe(III), oxidized, it produces methemoglobin which is brown.
- The brown color of dry blood and old meat is due to methemoglobin
- Butchers use ascorbic acid to reduce methemoglobin to make the meat look fresh.
- There is an enzyme methemoglobin reductase that converts methemoglobin to regular hemoglobin.

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

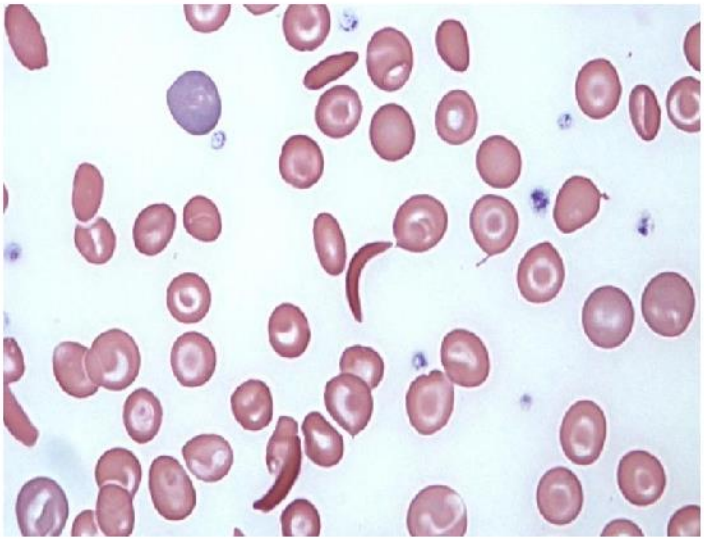
Fifth Week

Lecture Title: Anaemia & Classification of anaemia.

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for the students in identifying the concept of anemia, and classification of anemia.

Special Objectives of the Module

1. Definition of anemia.

2. Classification of anemia.

3. Differentiates between the types of anemia, according to the morphology of red blood cells and for other reasons.

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

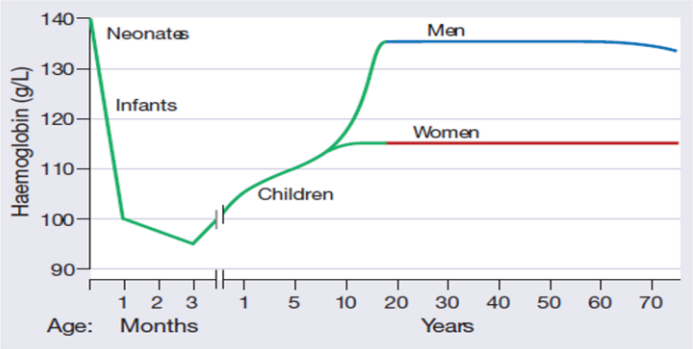
3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

Anemia

Anemia is reduced Haemoglobin concentration in blood than the amount appropriate for that age, sex, race and physiological status.

- Although normal values can vary between laboratories, typical values would be less than 135 g/L in adult males and less than 115 g/L in adult females.
- From the age of 2 years to puberty, less than 110 g/L indicates anemia.
- As newborn infants have a high hemoglobin level, 140 g/L is taken as the lower limit at birth



The lower limit of normal blood haemoglobin concentration in men, women and children of various ages.

symptoms and signs

- If the patient does have symptoms these are usually shortness of breath , lethargy , palpitation particularly on exertion, weakness , and headaches .
- In older subjects, symptoms of cardiac failure , pectoris or intermittent claudication present. Visual disturbances , or confusion because of retinal hemorrhages.

Signs

These may be divided into general and specific.

General signs

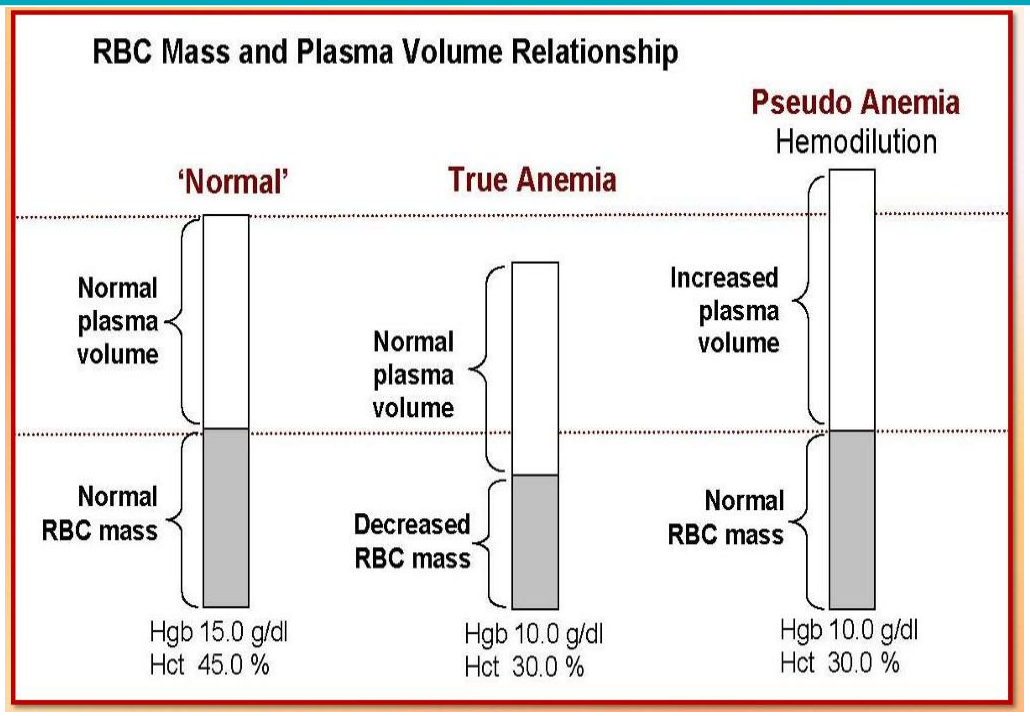
- ✓ Include pallor of mucous membranes or nail beds, which occurs if the hemoglobin level is less than 70 g/L.
- ✓ A hyperdynamic circulation may be present with tachycardia, a bounding pulse, cardiomegaly, and a systolic
- ✓ flow murmur, especially at the apex. Particularly in the elderly, features of congestive heart failure may be present.

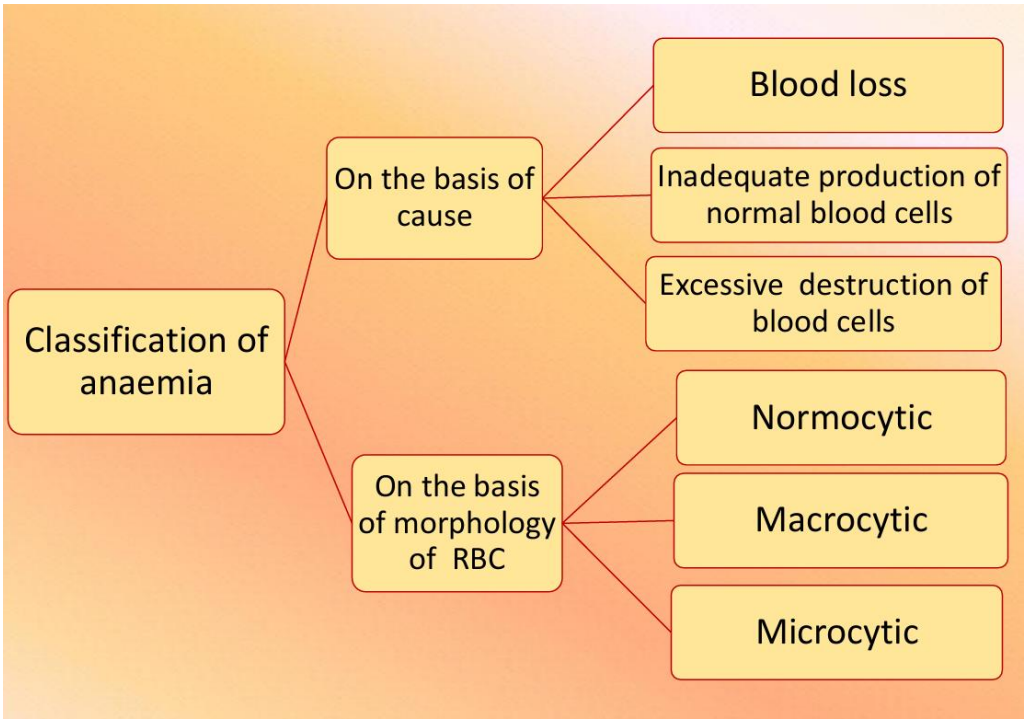
Specific signs

- ✓ Are associated with particular types of anemia, e.g. koilonychia (spoon nails) with iron deficiency, jaundice with hemolytic or megaloblastic anemias, leg ulcers with sickle cell and other hemolytic anemias, bone deformities with thalassemia major.
- ✓ The association of features of anemia with excess infections or spontaneous bruising suggests that neutropenia or thrombocytopenia may be present, possibly as a result of bone marrow failure.

Normal ranges of Hb

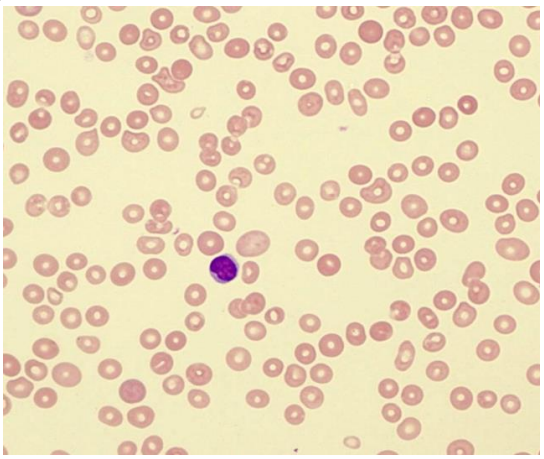
- ➢ Men: Hb 13.5 -17.5 g/dL
- ➢ Women: Hb 11.5-16 g/dL
- ➢ Infants : Hb 14 – 20 g/dL

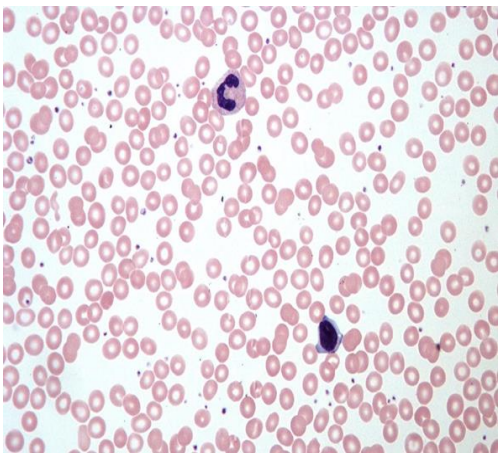

RBC Mass and Plasma Volume Relationship
'Normal'
True Anemia
Pseudo Anemia
Hemodilution
Normal plasma volume
Normal RBC mass
Hgb 15.0 g/dl
Hct 45.0 %
Normal plasma volume
Decreased RBC mass
Hgb 10.0 g/dl
Hct 30.0 %
Increased plasma volume
Normal RBC mass
Hgb 10.0 g/dl
Hct 30.0 %


Classification of anaemia
On the basis of cause
Blood loss
Inadequate production of normal blood cells
Excessive destruction of blood cells
On the basis of morphology of RBC
Normocytic
Macrocytic
Microcytic

Normocytic Normochromic Anaemia

- ✓ The primary cause - reduction of the number of RBCs. Eg: Endocrine disorders (hypopituitarism, hypothyroidism and hypoadrenalism).
- ✓ Hematological disorders(aplastic anemia ,hemolytic anemias)
- ✓ Acute blood loss
- ✓ Anemia of chronic diseases
- ✓





Normocytic **Normal**

Normocytic anaemia can be presented with elevation of reticulocyte count or a reduction of reticulocyte count.

Elivated reticulocyte count

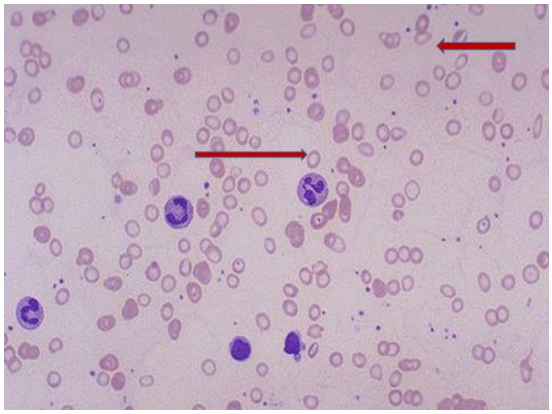
- Blood loss anaemia
- Haemolytic anaemia

Normal or low reticulocyte count

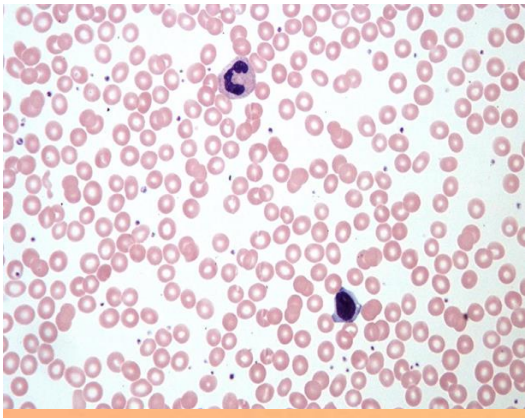
- Bone marrow disorders(Aplastic anaemia)
- Chronic disease
- Kidney disease

Microcytic Anaemia

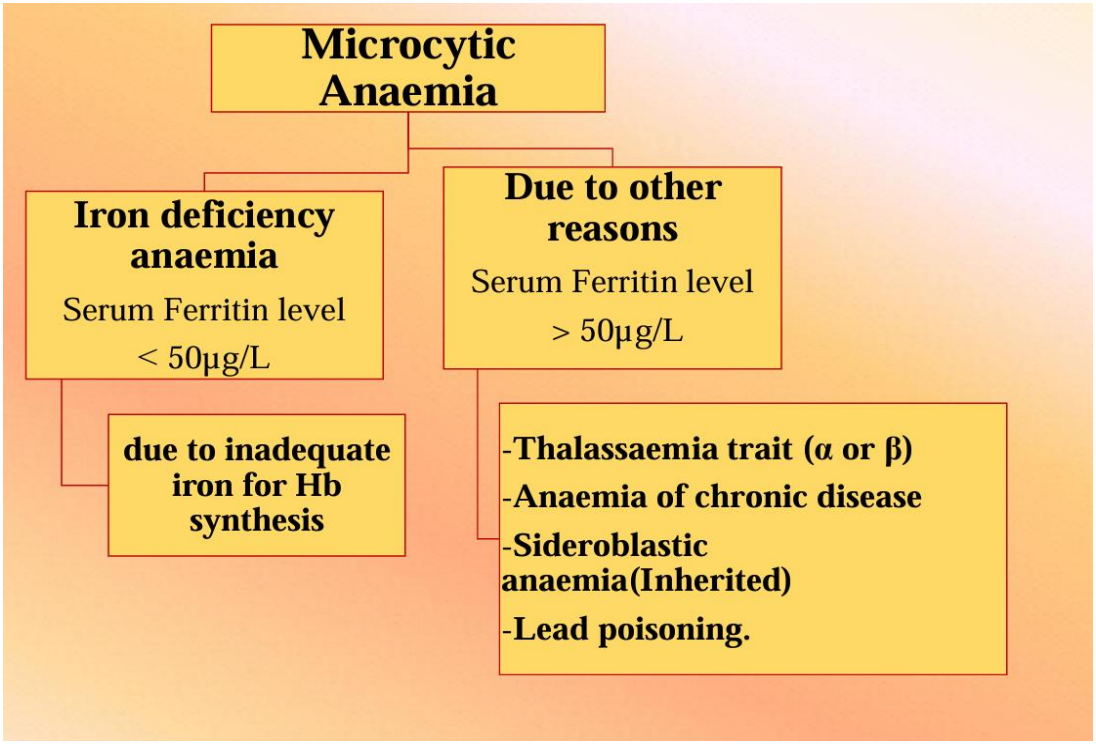
- Many RBCs smaller than normal (MCV<80fL)
- The RBCs are usually hypochromic (MCH<27pg)
- Increased zone of central pallor
- Cells are various in shape & size



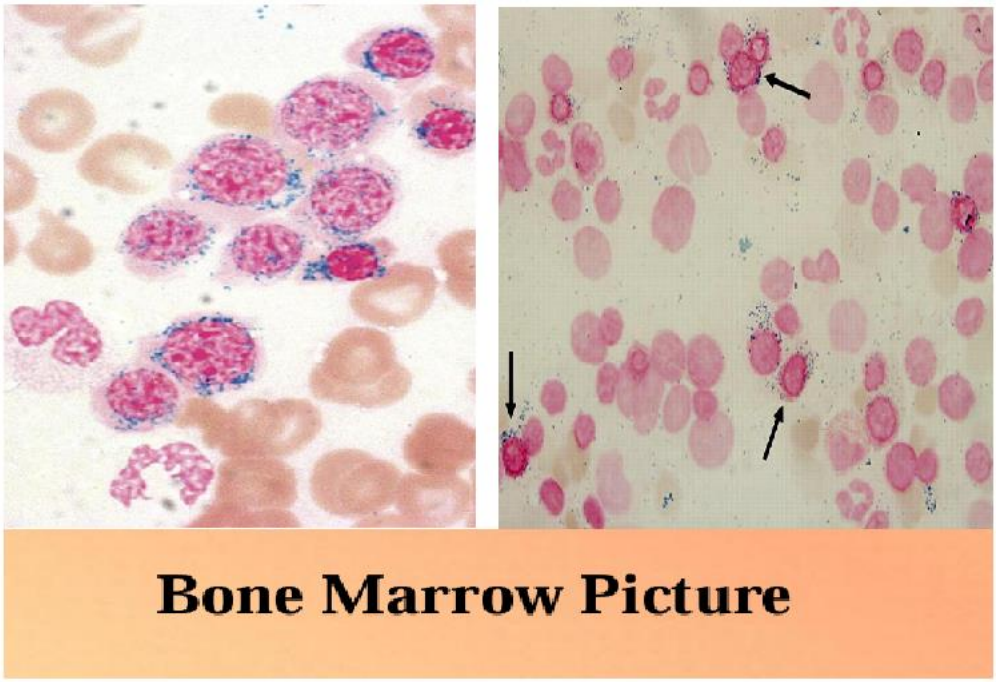
Microcytic



Normal



Sideroblastic anaemia



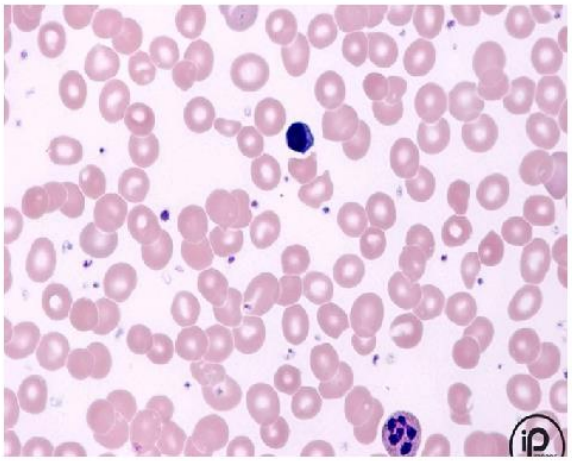
Bone Marrow Picture

Macrocytic Anaemia

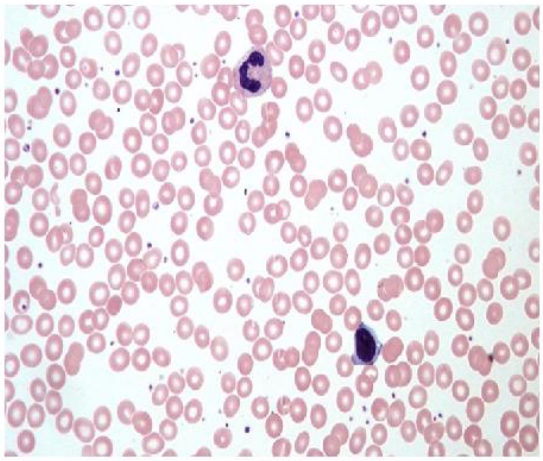
- The average size of RBCs are larger than
- normal(>100fL)
- {MCHC is normal or high}

Can be divided in to 2 types

- Megaloblastic anaemia
- Non megaloblastic anaemia



Macrocytic



Normal

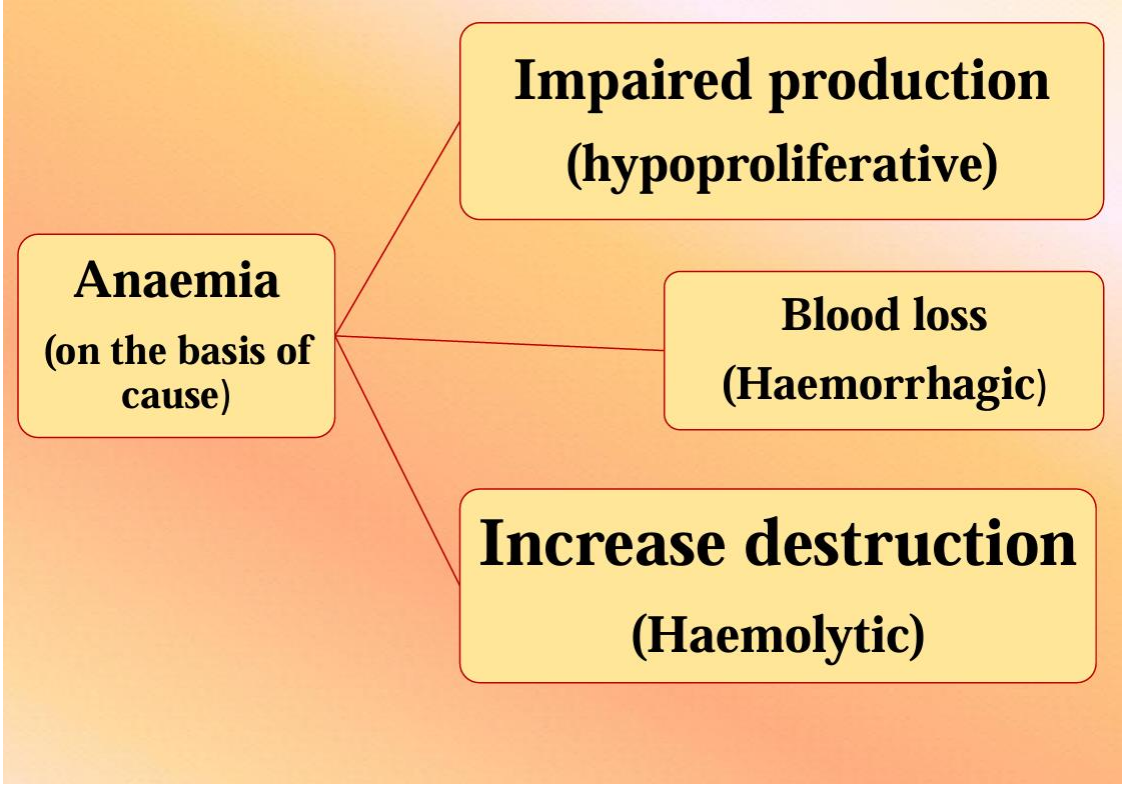
Macrocytic Anaemia

A. Megaloblastic Anaemia

- Vitamin B12 deficiency
- Folate deficiency
- Abnormal metabolism of folate and vit B12

B. Non megaloblastic anaemia

- Liver disease
- Alcoholism
- Post splenoctomy
- Neonatal macrocytosis
- **Stress erythropoiesis**

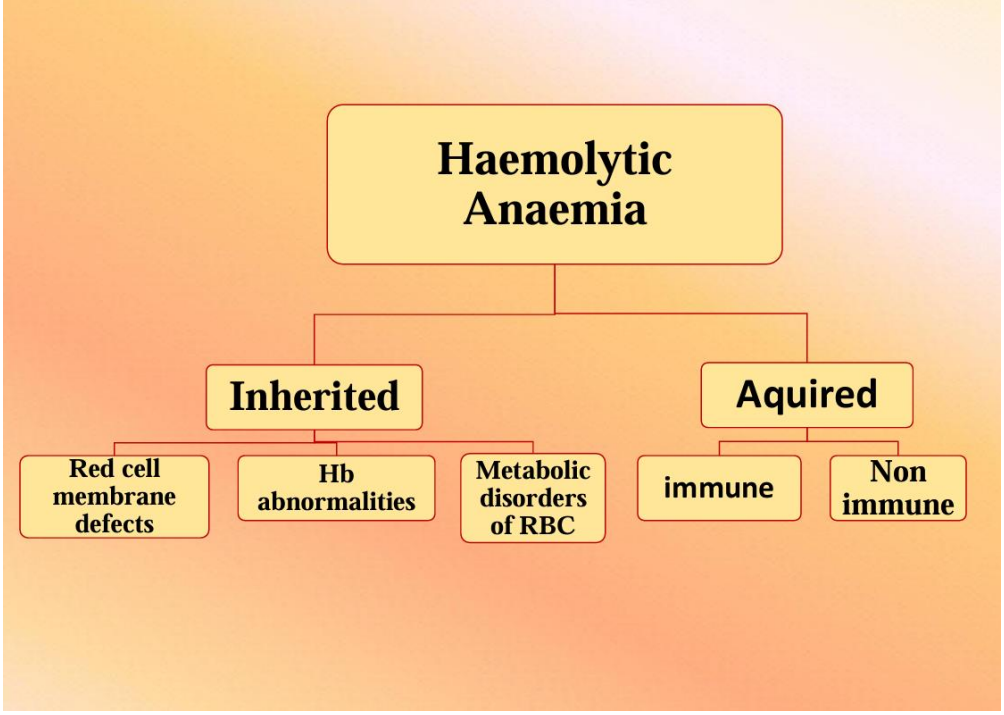


Reduced RBC Production

- Stem cell defects - Aplastic anaemia
- Nutritional deficiency - Iron deficiency anaemia
- Erythropoietin deficiency - Chronic renal faliure
- Hormone deficiency - Hypothyroidism
- Inhibitory effects of Cytokines - Chronic diseases
- Unsuitable microenvironment - Secondary deposits

Increased Loss

- (Anaemia due to haemorrhage)
- Acute blood loss
- Chronic blood loss

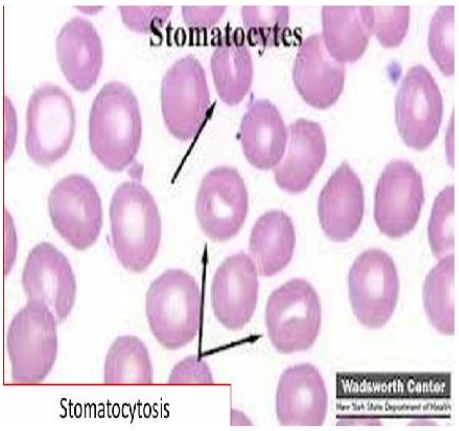


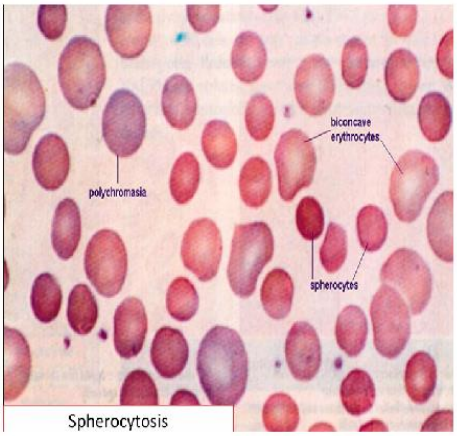
Inherited haemolytic anaemia

1)Red cell membrane defects

Eg:

- Hereditary spherocytosis
- Hereditary Elliptocytosis
- Hereditary Stomatocytosis



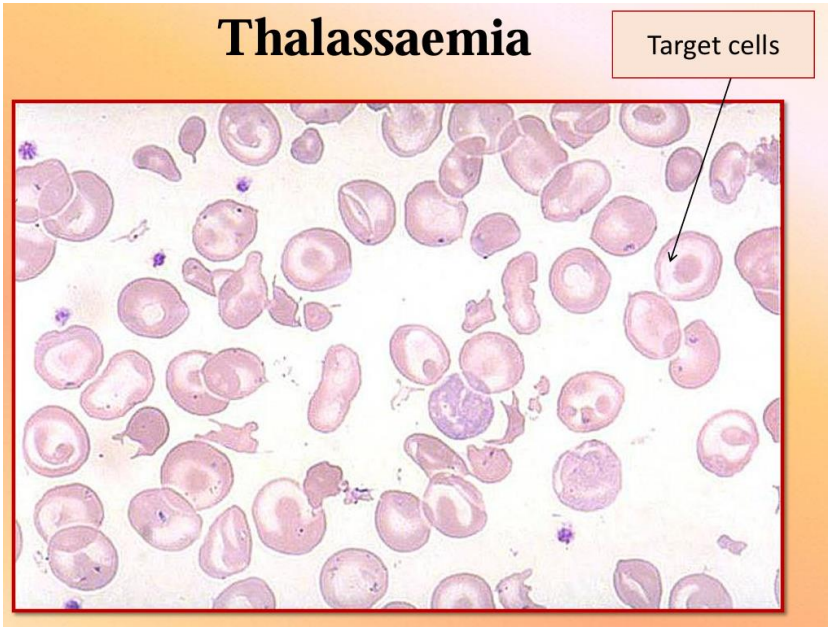


Inherited haemolytic anaemia

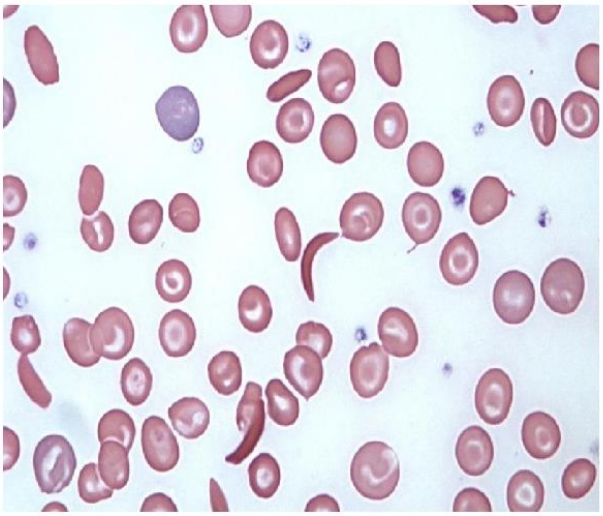
2) Hb abnormalities

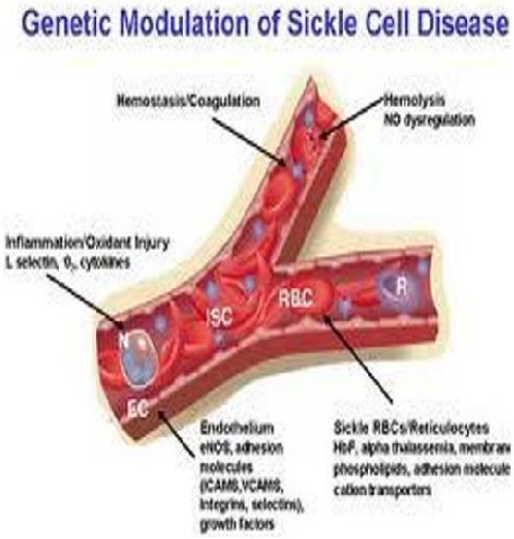
Eg:

- Thalassaemia
- Sickle Cell Anaemia



Sickle Cell Anaemia





Inherited haemolytic anaemia

3)Metabolic disorders of RBCs

Eg:

- Glucose-6-phosphate Dehydrogenase deficiency
- Pyruvate Kinase deficiency

Aquired haemolytic anaemia (Immune)

Eg:

Autoantibodies

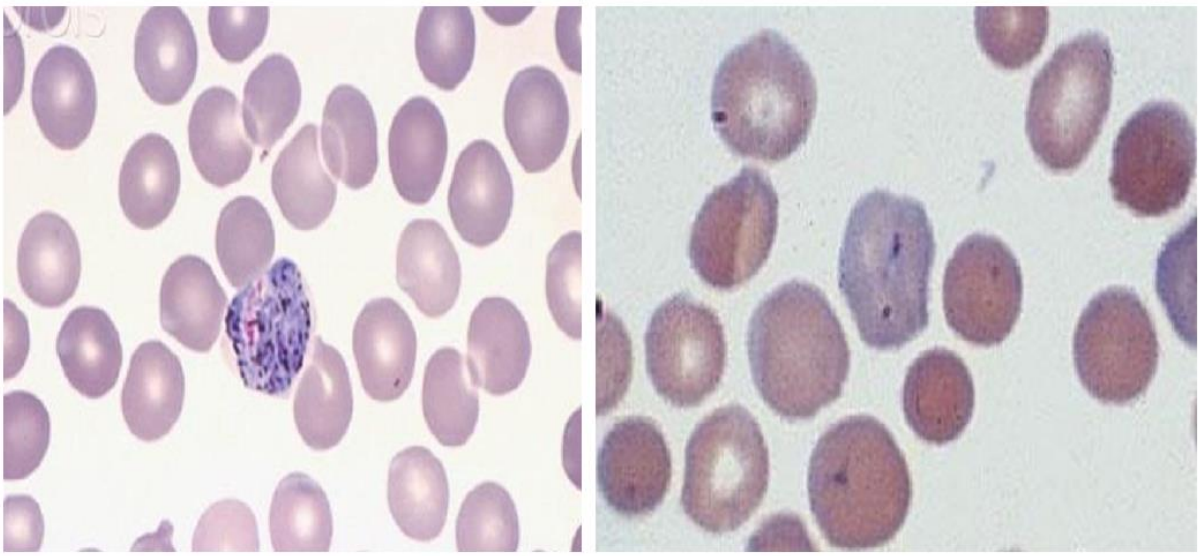
Drug induced Antibodies

AlloAntibodies

Aquired haemolytic anaemia (Non immune)

Eg:

- ✓ MAHA –Micro Angiopathic Haemolytic Anaemia(due to abnormal micro vessels)
- ✓ Parasites – Malaria
- ✓ Burns –Abnormal vessels



Malaria

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

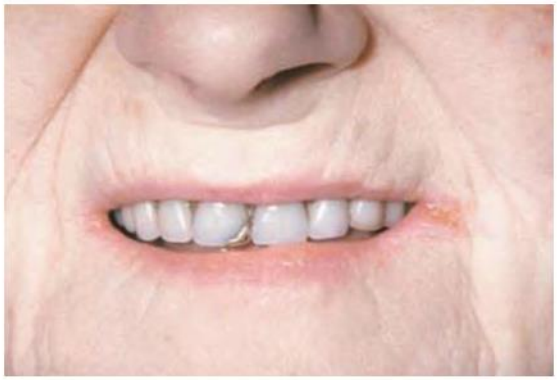
Sixth - Week

Lecture Title: Iron metabolism, iron deficiency anaemia, and Iron overload.

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

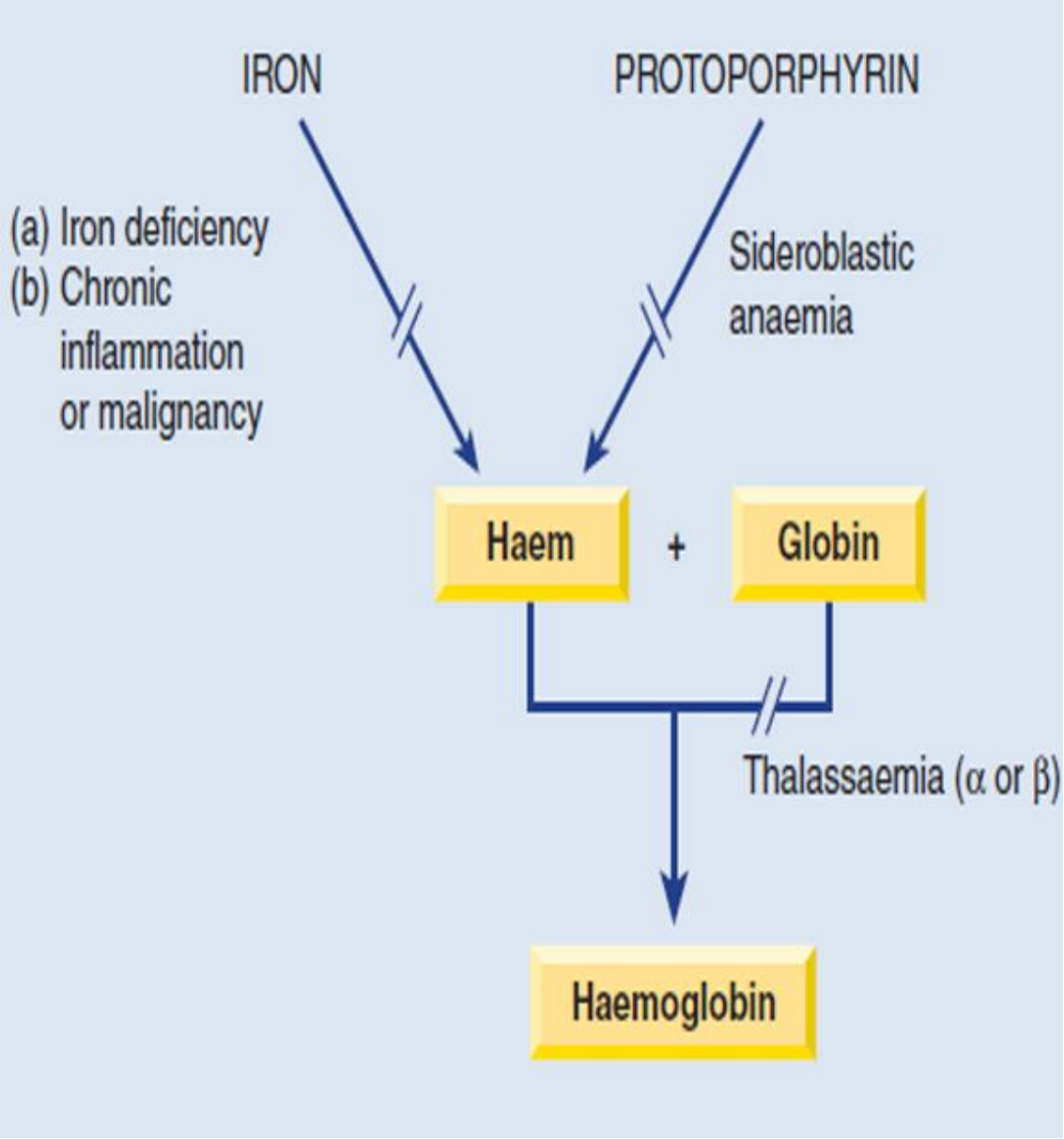
Iron Metabolism

- ✓ Iron is one of the most common elements in the Earth's crust, yet iron deficiency is the most common cause of anemia, affecting about 500 million people worldwide.
- ✓ It is particularly frequent in low-income populations, such as in sub-Saharan Africa or South Asia, where the diet can be of poor quality, and parasites (e.g. hookworm or schistosomiasis), which cause iron loss due to hemorrhage may be present.
- ✓ Moreover, the body has a limited ability to absorb iron. ¬Iron deficiency is the major cause of microcytic, hypochromic anemia, in which the two red cell indices, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), is reduced and the blood film shows small (microcytic) and pale (hypochromic) red cells.
- ✓ This appearance is caused by a defect in hemoglobin synthesis.

The causes of hypochromic microcytic anemia.

These include:

- ➢ Lack of iron (iron deficiency)
- ➢ Iron release from macrophages to serum (anemia of chronic inflammation or malignancy).
- ➢ Failure of protoporphyrin synthesis (sideroblastic anemia).
- ➢ Globin synthesis (α-orβ-thalassemia).
- ➢ Lead also inhibits haem and globin synthesis.


IRON
PROTOPORPHYRIN
(a) Iron deficiency
(b) Chronic inflammation or malignancy
Sideroblastic anaemia
Haem
+
Globin
Thalassaemia (α or β)
Haemoglobin

Nutritional and metabolic aspects of the iron in the Body

Body iron distribution and transport

- The transport and storage of iron are largely mediated by three proteins: transferrin , transferrin receptor 1 (TfR1), and ferritin .
- Transferrin molecules can each contain up to two atoms of iron.
- Transferrin delivers iron to tissues that have transferrin receptors, especially erythroblasts in the bone marrow which incorporate the iron into hemoglobin
- The transferrin is then reutilized.

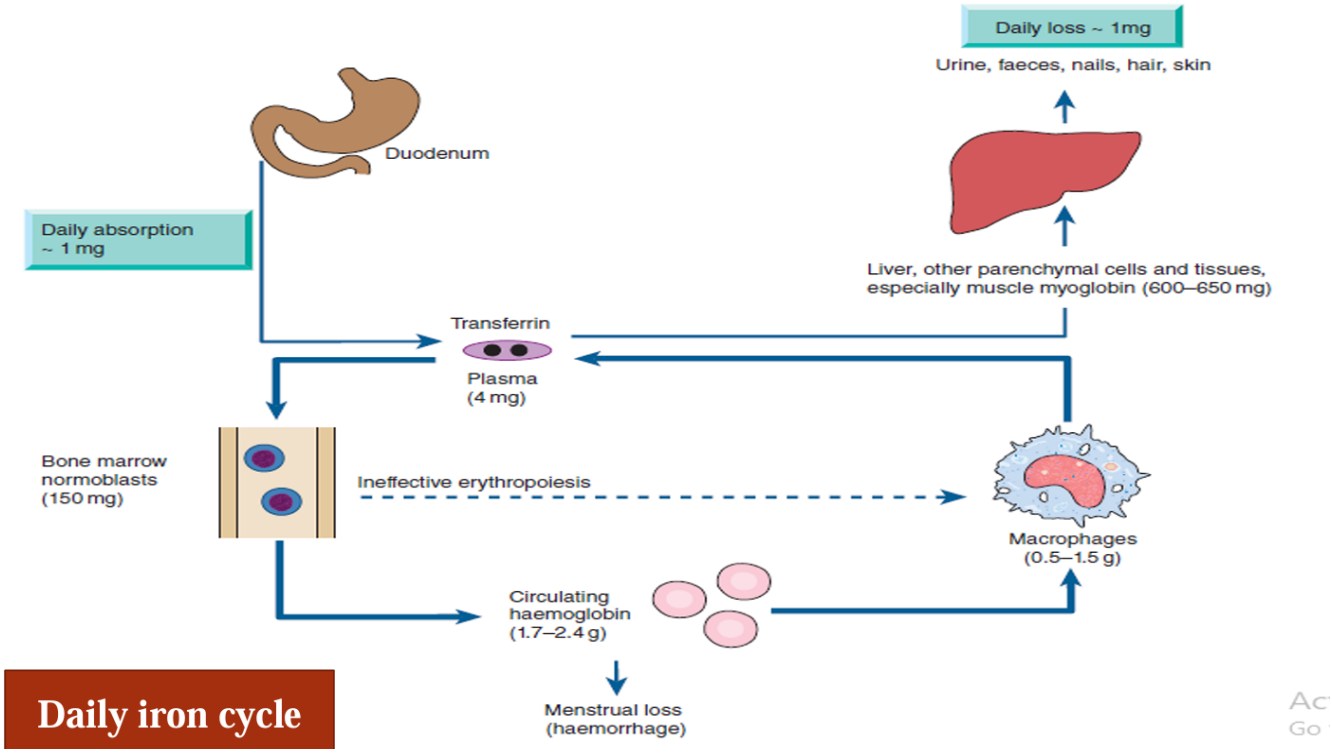
At the end of the red cell's life

- Red cells are broken down in the macrophages of the reticuloendothelial system and the iron is released from hemoglobin, enters the plasma, and provides most of the iron on transferrin.
- Only a small proportion of plasma transferrin iron comes from dietary iron, absorbed through the duodenum and jejunum.
- Som eiron is stored in the macrophages as ferritin and haem osiderin.
- The amount varying widely according to overall body iron status.
- Ferritin is a water-soluble protein–iron complex.
- It is made up of an outer protein shell, apo ferritin, consisting of 22 subunits and an iron–phosphate–hydroxide core

- It contains up to **20%** of its weight as iron and is not visible by light microscopy.
- Haemosiderin is an insoluble protein–an iron complex of a varying composition containing approximately 37% iron by weight.
- It is derived from partial lysosomal digestion of ferritin molecules and is visible in macrophages and other cells by light microscopy.
- Iron in ferritin and haemosiderinis in ferric form.
- It is mobilized after reduction to the ferrous form.
- A copper-containing enzyme, caeruloplasmin, catalyses oxidation of the iron to the ferric form for binding to plasma transferrin.
- Iron is also present in muscle as myoglobin and in most cells of the body in iron-containing enzymes (e.g. cytochrome or catalase).

The distribution of body iron.

Amount of iron in average adult	Male (g)	Female (g)	Percentage of total
Haemoglobin	2.4	1.7	65
Ferritin and haemosiderin	1.0 (0.3–1.5)	0.3 (0–1.0)	30
Myoglobin	0.15	0.12	3.5
Haem enzymes (e.g. cytochromes, catalase, peroxidases, flavoproteins)	0.02	0.015	0.5
Transferrin-bound iron	0.004	0.003	0.1



Daily iron cycle

Regulationofferritinandtransferrinreceptor1 synthesis

- ✓ The levels of ferritin, TfR1, δ-amino laevulinic acid synthase(ALA-S), and divalent metal transporter 1 (DMT-1) are linked to iron status so that iron overload causes a rise in tissue ferritin and a fall in TfR1 and DMT-1, whereas in iron deficiency ferritin and ALA-S are low and TfR1 increased.
- ✓ This linkage arises through the binding of an iron regulatory protein (IRP) to iron response elements (IREs) on the ferritin, TfR1, ALA-S, and DMT-1 mRNA molecules.

- ✓ When plasma iron is raised and transferrin is saturated, the amount of iron transferred to parenchymal cells (e.g. those of the liver, endocrine organs, and heart) is increased and this is the basis of the pathological changes associated with iron loading conditions.
- ✓ There may also be free iron in plasma which is toxic to different organs

.....

Hepcidin

Iron is an essential component for almost all living cells and organisms. However, when present in excess, iron becomes a potential biohazard due to its redox reactivity that promotes oxidative stress.

Thus, balanced iron metabolism is imperative for health and its deregulation leads to disease

- ✓ Hepcidin is a polypeptide (25 amino acids) produced by liver cells.
- ✓ Itis the major hormonal regulator of iron homeostasis in the body.
- ✓ Its unbalanced production contributes to the pathogenesis of several iron-associated disorders.

- ✓ The main actions of hepcidin are to block iron flows into plasma via controlling duodenal iron absorption, release from macrophages, recycling old red blood cells, and organization of stored iron from hepatocytes
- ✓ It inhibits iron release from macrophages and from intestinal epithelial cells by its interaction with the transmembrane iron exporter, **ferroportin**

The production of hepcidin is regulated by mechanisms including its

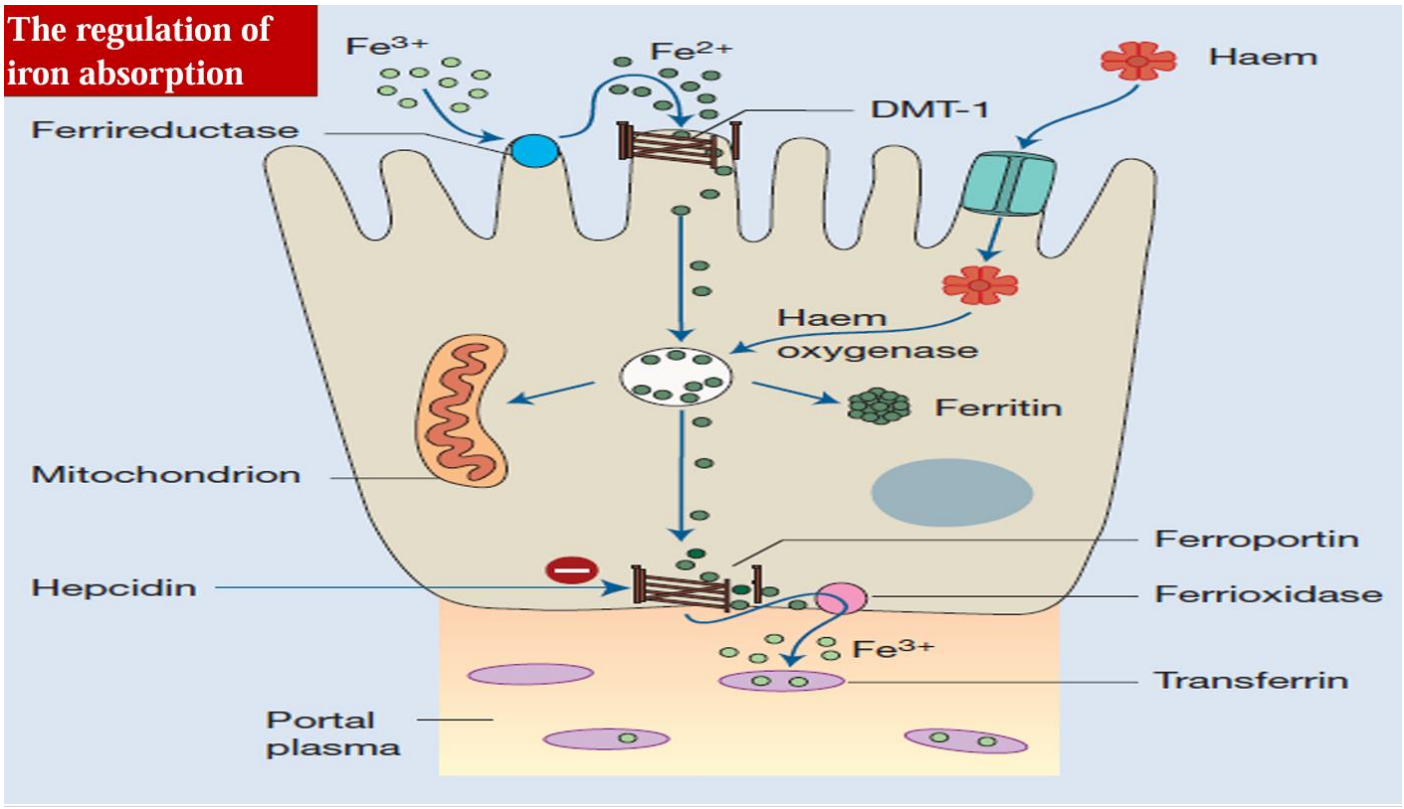
1- Levels are increased by plasma and liver iron as a feedback mechanism to keep body iron levels stable.

2- Levels are decreased by the activity of erythrocytes to maintain iron supply for erythropoiesis.

3- Levels can be increased after inflammation to reduce homeostatic regulation of iron absorption and distribution is controlled by hepcidin

Iron absorption

- Organic dietary iron is partly absorbed as haem and partly broken down in the gut to inorganic iron.
- Absorption occurs through the duodenum. Haem is absorbed through a receptor.
- Haemisthen digested to release iron.
- Inorganic iron absorption by factors such as acid and reducing agents that keep iron in the gut lumen in the Fe^{2+} rather than the Fe^{3+} state.
- The protein DMT-1 is involved in the transfer of iron from the lumen of the gut across the enterocyte microvilli.
- Ferroportin at the basolateral surface controls the exit of iron from the cell into portal plasma.
- The amount of iron absorbed is regulated according to the body's needs by changing the levels of DMT-1 and ferroportin.
- For DMT-1this occurs by an iron regulatory protein (IRP), iron response elements (IREs) binding mechanism, and for ferroportin by hepcidin.
- Ferrireductase present at the apical surface converts iron from the Fe^{3+} to Fe^{2+} state and another enzyme, hephaestin (ferroxidase), converts Fe^{2+} to Fe^{3+} at the basal surface prior to binding to transferrin.



iron Absorbtion

Factors favouring absorption	Factors reducing absorption
Haem iron	Inorganic iron
Ferrous form (Fe^{2+})	Ferric form (Fe^{3+})
Acids (HCl, vitamin C)	Alkalis – antacids, pancreatic secretions
Solubilizing agents (e.g. sugars, amino acids)	Precipitating agents – phytates, phosphates, tea
Reduced serum hepcidin	Increased serum hepcidin
Ineffective erythropoiesis	Decreased erythropoiesis
Pregnancy	Inflammation
Hereditary haemochromatosis	

Iron requirements

- The amount of iron require deach day to compensate for losses from the body and for growth varies with ageand sex;it is highest in pregnancy ,adolescent ,and menstruating females.
- Therefore these groups are particularly likely to develop an iron deficiency if there is additional iron loss or prolonged Reduced intake.

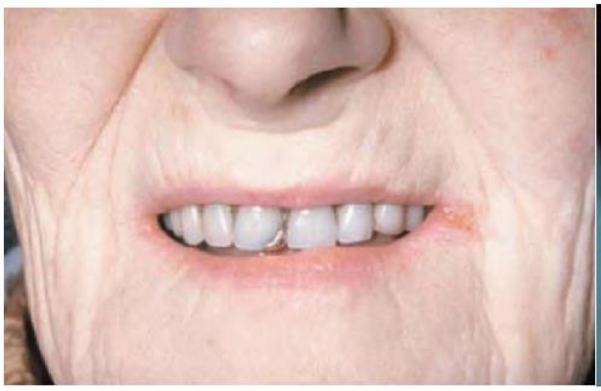
……..

Iron Deficiency Anemia

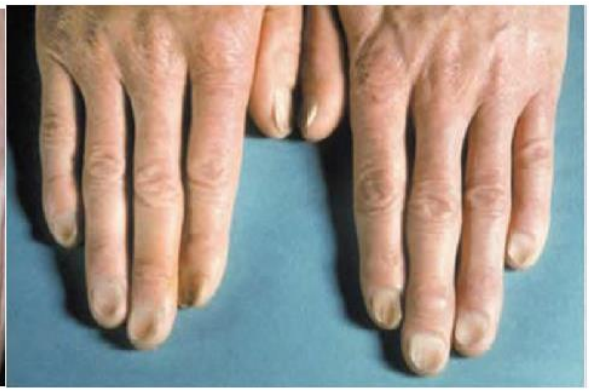
Microcytic Anemia

Clinical features

When iron deficiency is developing, the reticuloendothelial stores (haemosiderin and ferritin) become completely depleted before anemia occurs. As the condition develops, the patient may show the general symptoms and signs of anemia and also painless glossitis, angular stomatitis, brittle, ridged, or spoon nails (koilonychia), and unusual dietary cravings (pica).



Angular stomatitis



brittle, ridged, or spoon nails (koilonychia)

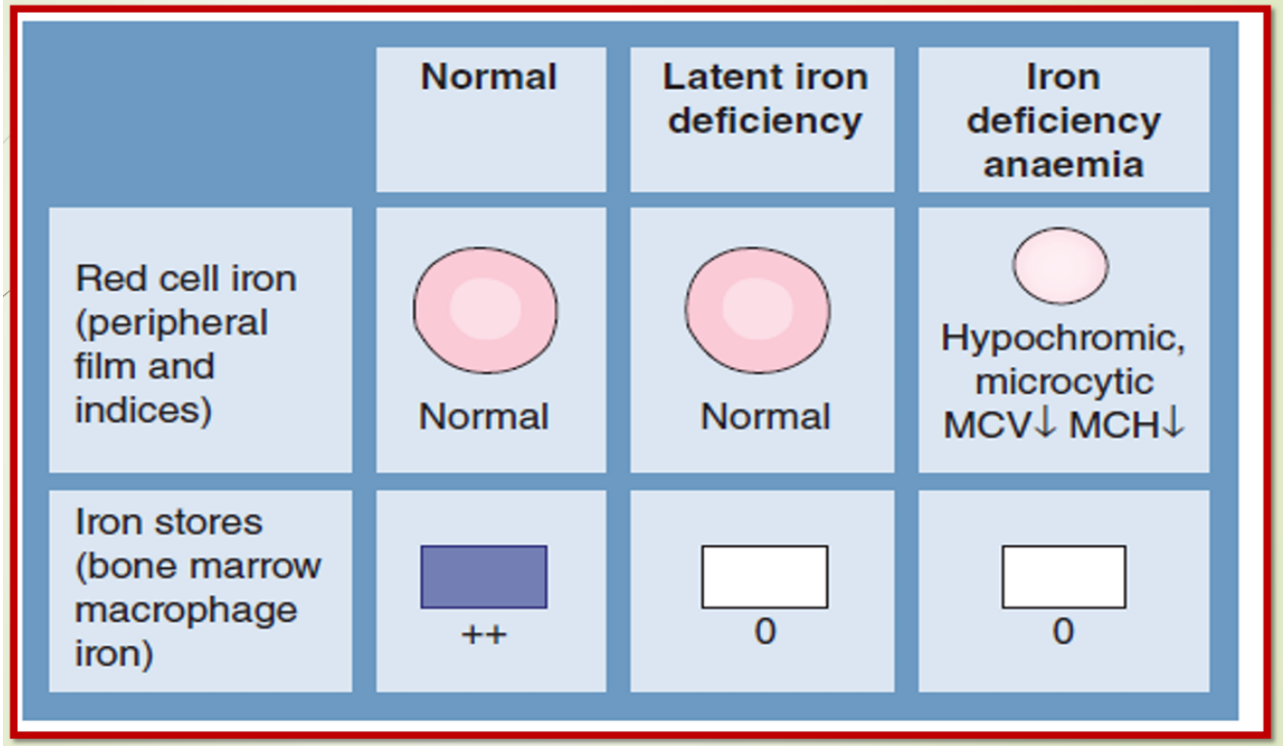
- In children, iron deficiency is particularly significant as it can cause irritability, poor cognitive function, and a decline in psychomotor development.
- There is also evidence that oral or parenteral iron may reduce fatigue in iron-deficient (low serum ferritin) non anemic women.

Causes of iron deficiency

- ✓ In developed countries ,chronic blood loss,especiallyuterine or the gastrointestinal tract, is the dominant cause of iron eficiency and dietary deficiency is rarely a cause on its own.
- ✓ Increased demands during infancy, adolescence, pregnancy, lactation, and in menstruating women account for the high risk of iron deficiency anemia in these particular clinical groups.

- Newborn infants have a store of iron derived from delayed clamping of the cord and the breakdown of excess red cells.
- From **3** to **6** months there is a tendency for a negative iron balance because of growth.
- From 6 months, supplemented formula milk and mixed feeding, particularly with iron-fortified foods, prevent iron deficiency.
- In pregnancy increased iron is needed for an increased maternal red cell mass of approximately **35%**, transfer of 300 mg of iron to the fetus, and because of blood loss at delivery.
- Although iron absorption is also increased, iron therapy is often needed if the hemoglobin (Hb) falls below **90** g/L or the mean cell volume (MCV) is below **80** fL in the third trimester.
- Gluten-induced enteropathy, partial or total gastrectomy, and atrophic gastritis (often autoimmune and with Helicobacter pylori infection) may, however, predispose to iron deficiency.
- In developing countries, iron deficiency may occur as a result of a life-long poor diet, consisting mainly of cereals and vegetables. Hookworm may aggravate iron deficiency, as may repeated pregnancies and menorrhagia in young females.

The development of iron deficiency anemia.



Estimated daily iron requirements. Units are mg/day.

	Urine, sweat, faeces	Menses	Pregnancy	Growth	Total
Adult male	0.5–1				0.5–1
Postmenopausal female	0.5–1				0.5–1
Menstruating female*	0.5–1	0.5–1			1–2
Pregnant female*	0.5–1		1–2		1.5–3
Children (average)	0.5			0.6	1.1
Female (age 12–15)*	0.5–1	0.5–1		0.6	1.6–2.6

*These groups are more likely to develop iron deficiency.

Laboratory diagnosis of a hypochromic anaemia

	Iron deficiency	Chronic inflammation or malignancy	Thalassaemia trait (α or β)	Sideroblastic anaemia
MCV/ MCH	Reduced in relation to severity of anaemia	Normal or mild reduction	Reduced; very low for degree of anaemia	Usually low in congenital type but MCV usually raised in acquired type
Serum iron	Reduced	Reduced	Normal	Raised
TIBC	Raised	Reduced	Normal	Normal
Serum ferritin	Reduced	Normal or raised	Normal	Raised
Bone marrow iron stores	Absent	Present	Present	Present
Erythroblast iron	Absent	Absent	Present	Ring forms
Haemoglobin electrophoresis	Normal	Normal	Hb A_2 raised in β form	Normal

1-Red cell indices and blood film

- Even before anemia occurs, the red cell indices fall and they fall progressively as the anemia becomes more severe.
- The blood film shows hypochromic, microcytic cells with occasional target cells and pencil-shaped poikilocytes .
- The reticulocyte count is low in relation to the degree of anemia.
- When iron deficiency is associated with severe folate or vitamin **B_{12}** deficiency, a ‘dimorphic’ film occurs with a dual population of red cells of which one is macrocytic and the other microcytic and hypochromic; the indices may be normal.

- Adimorphic blood film is also seen in patients with iron deficiency anaemia who have received recent iron therapy and produced a population of new haemoglobinized normal-sized red cells and when the patient has been transfused.
- The platelet count is often moderately raised in iron deficiency, particularly when hemorrhage is continuing.

……..

2-Bonemarrowiron

- Bone marrow examination is not essential to assess iron stores except in complicated cases.
- In iron deficiency anemia there is a complete absence of iron from stores (macrophages) and from developing erythroblasts.
- The erythroblasts are small and have a ragged cytoplasm.

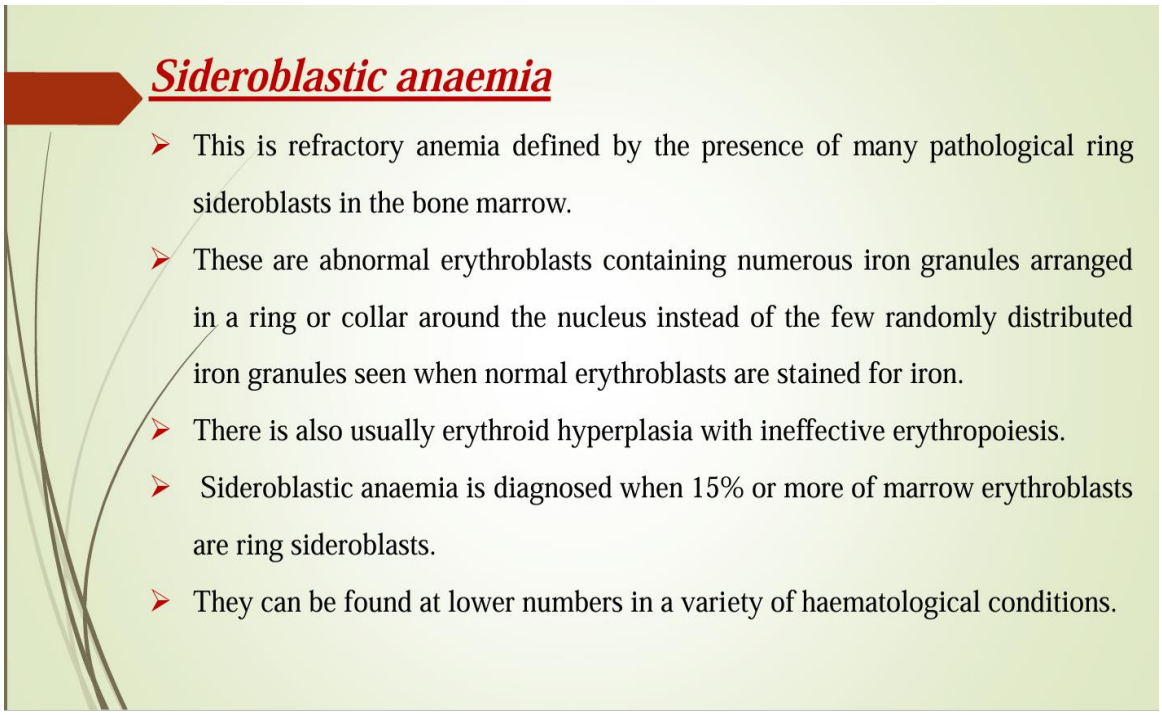
……

3-Serumironandtotaliron-bindingcapacity

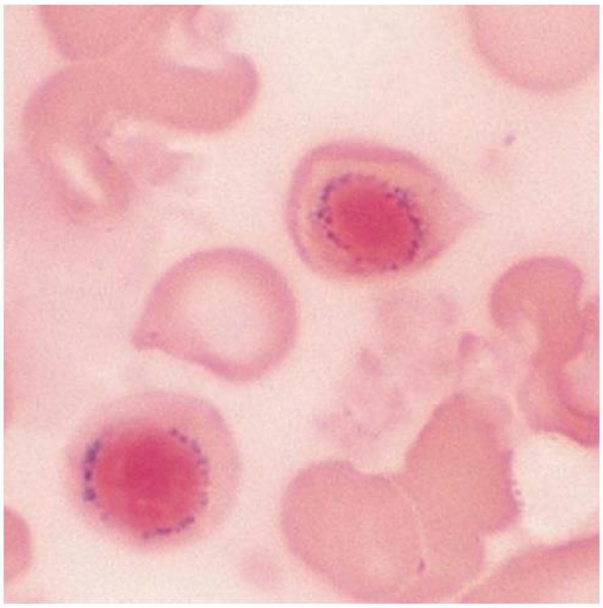
- The serum iron falls and total iron-binding capacity (TIBC) rises so that the TIBC is less than **20%** saturated.
- This contrasts with the anemia of chronic disorders the serum iron and the TIBC are both reduced, and with other hypochromic anemias the serum iron is normal or raised.

4- Serum ferritin

- A small fraction of body ferritin circulates in the serum, the concentration being related to tissue, particularly reticuloendothelial, iron stores.
- The normal range in men is higher than in women.
- In iron deficiency anemia the serum ferritin is very low while a raised serum ferritin indicates iron overload or excess release of ferritin from damaged tissues or an acute phase response (e.g. in inflammation).
- The serum ferritin is normal or raised in the anemia of chronic disorders.



……



Ring sideroblasts with a perinuclear ring of iron granules in sideroblastic anaemia.

- ✓ Sideroblastic anemia is classified into different types and the common link is a defect in haem synthesis.
- ➢ **The hereditary form**, anemia is usually characterized by a markedly hypochromic and microcytic blood picture.
- ➢ **Acquired form**,maybedueto alcohol, lead, and drugs.

Lead poisoning

- Lead inhibits both haemand globin synthesis at a number of points. In addition, it interferes with the breakdown of RNA by inhibiting the enzyme pyrimidine 5′ nucleotidase, causing accumulation of denatured RNA in red cells.
- The anemia may be hypochromic or predominantly hemolytic, and the bone marrow may show ring sideroblasts.

……..

Iron overload

- There is no physiological mechanism for eliminating excess iron from the body, so iron absorption is normally regulated to avoid accumulation. Iron overload (hemosiderosis) occurs in disorders associated with excessive absorption or in patients with severe refractory anemias who receive regular blood transfusions.
- Excessive iron deposition in tissues may result in serious damage to organs, particularly the heart, liver, and endocrine organs.

……..

The causes of iron overload

Increased iron absorption

- ❖ Hereditary (primary) haemochromatosis.
- ❖ Ineffective erythropoiesis, e.g. thalassemia intermedia and major .
- ❖ Chronic liver disease.

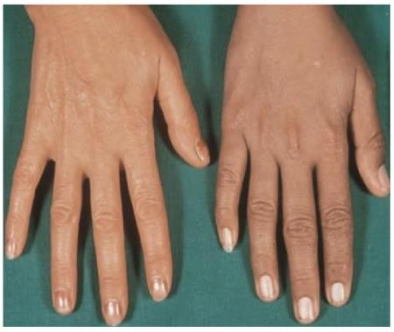
……

- ❖ **Increased iron intake**
  - African siderosis (dietary and genetic)
- ❖ **Repeated red cell**
  - Transfusions
  - Transfusion siderosis

……

Hereditary haemochromatosis

Hereditary haemochromatosis (also called genetic or primary haemochromatosis) is a group of diseases in which there is excessive absorption of iron from the gastrointestinal tract leading to an iron overload of the parenchymal cells of the liver, endocrine organs and, in severe cases, the heart.



Melanin skin pigmentation. The right hand of iron overload is caused by thalassemia major. The left hand is of normal iron status.

Transfusional iron overload

- This develops in patients with chronic anaemia who need to have regular blood transfusions. Each **500** mL of transfused blood contains approximately **250** mg of iron and iron overload is inevitable unless iron chelation therapy is given.
- To make matters worse, iron absorption from food is increased in β-thalassaemia major and many other anaemias secondary to ineffective erythropoiesis because of inappropriately low serum hepcidin levels. This is thought to be due to the release of early erythroblasts that inhibit hepcidin synthesis.
- Non-transferrin-bound iron may appear in the plasma because transferrin is **100%** saturated and causes widespread iron deposition in parenchymal tissues.
- Iron damages the liver and the endocrine organs with failure of growth, delayed or absent puberty, diabetes mellitus, hypothyroidism and hypo parathyroidism. Skin pigmentation as a result of excess melanin and haemosiderin gives a slate grey appearance even at an early stage of iron overload.
- Most importantly, iron can damage the heart.
- In the absence of intensive iron chelation, death occurs in the second or third decade in thalassaemia major, usually from congestive heart failure or cardiac arrhythmias.

Chronic liver diseases

- Moderate iron overload limited to the liver can be found in various chronic viral or metabolic liver diseases in the context of alcohol abuse, necro-inflammatory processes, and the so-called metabolic syndrome.
- These conditions are often characterized by increased serum ferritin, but normal transferrin saturation and slightly increased hepcidin in an inflammatory process.

……

African iron overload

This occurs in sub-Saharan Africa through a combination of increased iron absorption due to a genetic defect, possibly in the ferroportin gene, and consumption of beverages with high iron content due to the use of iron cooking pots.

……

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

Seventh Week

Lecture Title: Megaloblastic anaemia

Third level-First semester

By

Dr. Fidan Fikrat Ahmed

2024-2025

General Objectives of the Module

Providing the necessary skills and developing knowledge among students in identifying the concept of megaloblastic anemia, and classifying the types of anemia that fall within this type of anemia.

Special Objectives of the Module

1. Definition of megaloblastic anemia.

2. Classification of megaloblastic anemia.

3. Definition of vitamin B12 anemia B12 (metabolism, Absorption, Transport, Biochemical function, B12 deficiency anaemia, symptoms and signs, and the necessary laboratory tests to be performed to determine anemia due to vitamin B12 deficiency).

4. Definition of Folate anemia (metabolism, Absorption, Transport, Biochemical function, Folate deficiency anemia, symptoms and signs, and the necessary laboratory tests to be performed to determine anemia due to Folate deficiency).

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology

Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint

of Elsevier Inc. ISBN: 978-1-4557-0830-7.

Health Functions

A sufficient intake of vitamin B12, also known as cobalamin, is important as it helps the body to

- ✓ Convert food into glucose, which is used to produce energy
- ✓ Maintain healthy nerve cells
- ✓ Produce nucleic acids (e.g., DNA), the body's genetic material
- ✓ Regulate, together with vitamin B9 (folate), the formation of red blood cells
- ✓ Control, together with vitamin B6 and vitamin B9, blood levels of the amino acid homocysteine, a potential marker for heart disease risk.

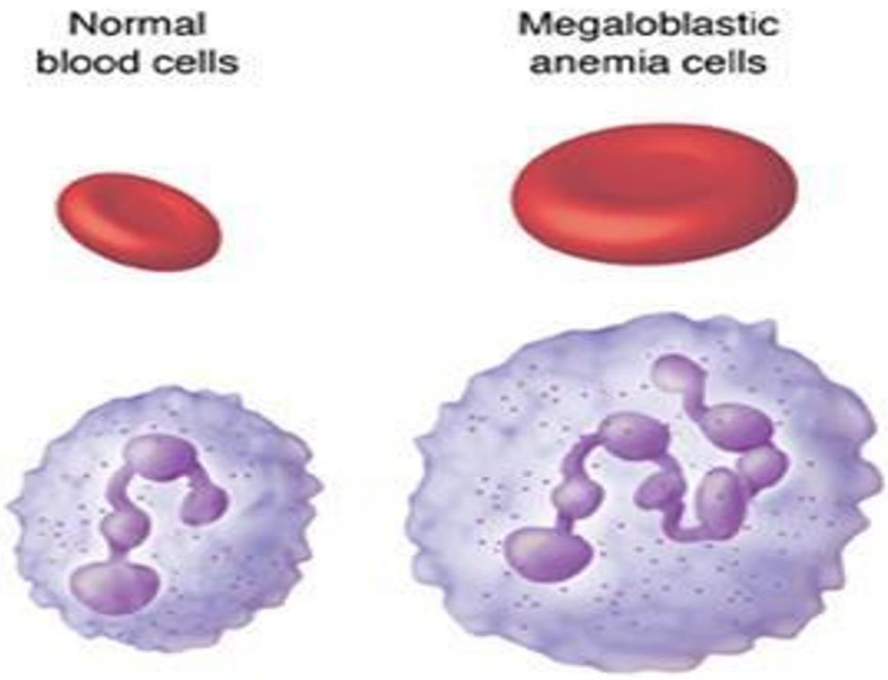
……..

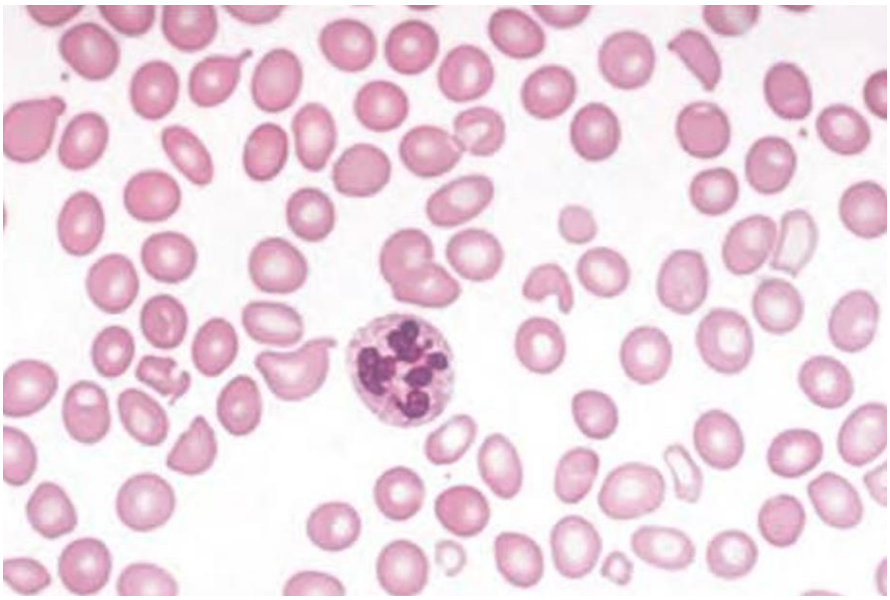
Macrocytic Anemia

➢ In macrocytic anemia, the red cells are abnormally large (mean corpuscular volume, MCV >98 fL). There are several causes but they can be broadly subdivided into

- ➢ Megaloblastic
- ➢ Non-Megaloblastic,
- ➢ Based on the appearance of developing erythroblasts in the bone marrow

……..


Normal
blood cells
Megaloblastic
anemia cells



Megaloblastic anemias

- This is a group of anemias in which the erythroblasts in the bone marrow show a characteristic abnormality– maturation of the nucleus being delayed relative to that of the cytoplasm.
- The underlying defect accounting for the asynchronous maturation of the nucleus is defective DNA synthesis, which is usually caused by a deficiency of vitamin B12 or folate.
- Less commonly, abnormalities of the metabolism of these vitamins or other lesions in DNA.

……..

Synonyms of Vitamin B12

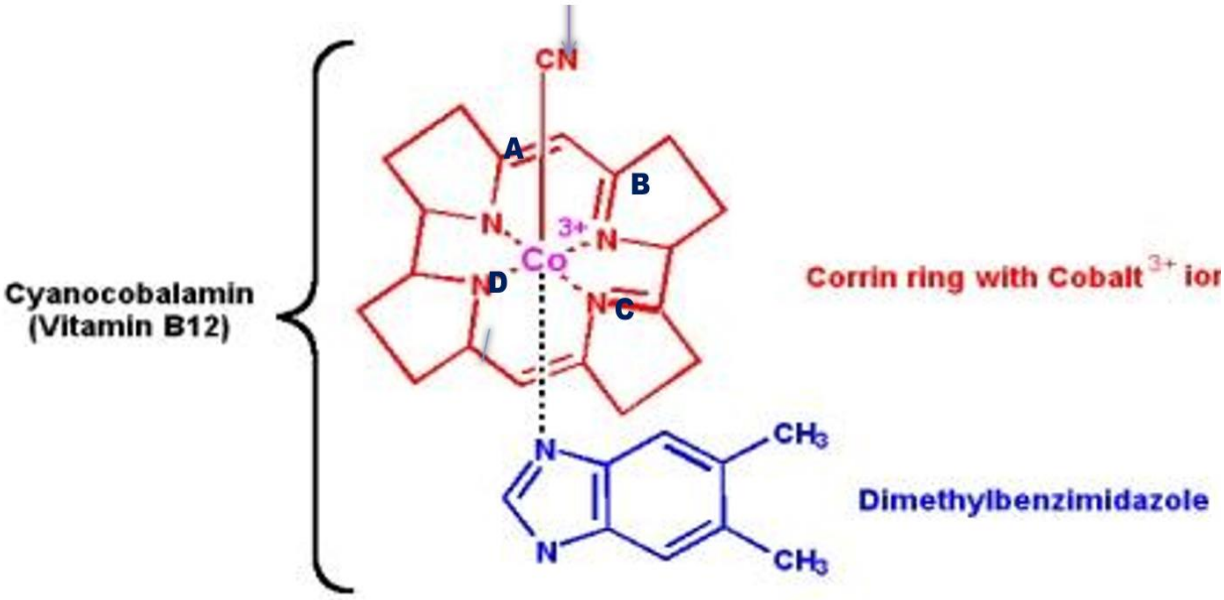
✓ Cobalamin.
✓ Extrinsic factor (EF).
✓ Anti-pernicious anemia factor.

❖ The structure of methylcobalamin (methyl B12), the main form of vitamin B12 in human plasma.
❖ Other forms include deoxyadenosylcobalamin (ado B12), the main form in human tissues.
❖ Hydroxocobalamin (hydroxo B12), the main form used in treatment.

Chemistry

- ❖ Vitamin B12 is water-soluble, heat-stable, and red in color.
- ❖ It contains 4.35% cobalt by weight.
- ❖ Four pyrrole rings co- ordinated with a cobalt atom are called a
- ❖ Corrin ring.
- ❖ The corrin ring has four pyrrole units, like porphyrin

……..



Methyl, Adenosine, acetamide, propionamide

Biochemical function

Vitamin B12 is a coenzyme for two biochemical reactions:

First

- Methyl B12 it is a cofactor for methionine synthase, It is the enzyme responsible for methylation of homocysteine to methionine using methyl tetrahydrofolate (methyl THF) as a methyl donor

Second

- Deoxyadenosine B12 (ado B12) assists in the conversion of methylmalonyl coenzymeA(CoA) to succinyl CoA
- Methylcobalamin: Methyl group replaces the adenosyl group Adenosylcobalamin (Ado-B12).
- When taken up by the cells, these groups are removed and deoxyadenosylcobalamin or Ado-B12 is formed

.....

Metabolism

- In food, vitamin B12 is present as a complex with proteins.
- The free form of vitamin B12 is released by cooking.
- HCL present in gastric juice and proteolysis by pepsin in the stomach.

Mechanism

- The absorbance of vitamin B12 requires an intrinsic
- factor .
- Intrinsic factor:- is a glycoprotein secreted by parietal cells
- of the stomach.
- Vitamin B12 combines with intrinsic factor.
- The vitamin B12– intrinsic factor complex reaches the ileum where it is absorbed
- In the ileum, the complex attaches to a specific receptor and is taken up by the mucosal cell.
- In the mucosal cell, vitamin B12 is released from its complex and reach the portal circulation

……

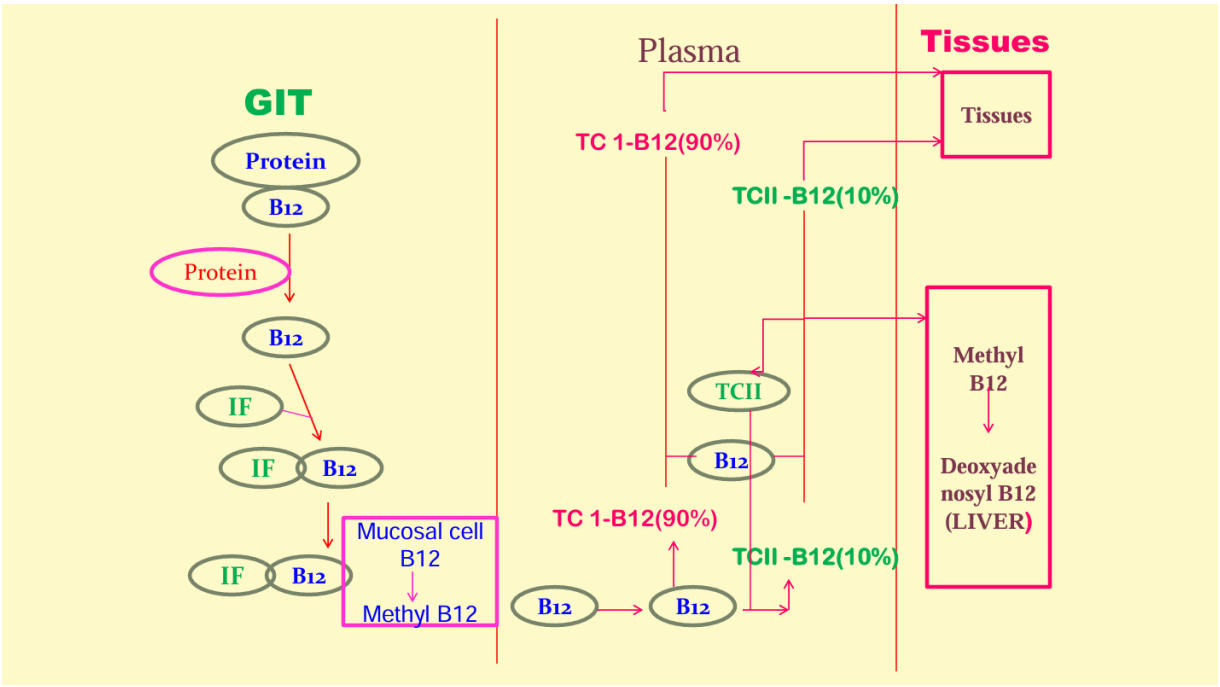
Transport

- In the portal blood, it is transported in combination with transcobalamin II.
- Vitamin B12 is presented to cells where it is taken up by the cells through receptor-mediated endocytosis.

……

Storage

- It is mainly stored in the liver, leukocytes and gastric mucosa



Dietary sources

- ✓ Rich sources of vitamin B12 are meats, egg, milk, sea foods
- ✓ B12 is synthesized by microorganisms
- ✓ Vitamin B12 is not present in Plant sources

B12 Deficiency Anemia

Causes:

Inadequate intake-seen in pure vegetarians and rarely in alcoholism.

Impaired absorption

This is mainly caused by lack of intrinsic factor

Lack of intrinsic factor is called a pernicious

- Anemia is caused by the destruction of gastric mucosa
- Impaired absorption is also seen in small intestinal disorders
- **Impaired storage and transport:**
- Inadequate utilization of vitamins occurs because of liver diseases and abnormalities of transport proteins
- Increased requirements are seen in hyperthyroidism, infancy & thalassemia
- Increased excretion occurs in nephrotic syndrome

……

Autoimmune

Pernicious anemia is an autoimmune condition in which antibodies to intrinsic factors are produced. Anti-intrinsic factor antibodies bind to and inhibit the effects of intrinsic factor, resulting in an inability of B12 to be absorbed by the terminal ileum.

Malabsorption

- ✓ Parietal cells in the stomach produce intrinsic factors; therefore, any patient with a history of gastric bypass surgery may be at risk for developing a B12 deficiency because their new alimentary pathway bypasses the site of intrinsic factor production.
- ✓ In patients with normal intrinsic factor production, any damage to the terminal ileum, such as surgical resection due to Crohn's isease, will impair the absorption of B12 and lead to a deficiency.
- ✓ Other damage to the small intestine, such as inflammation from celiac disease or infection with the tapeworm Diphyllobothrium latum, may also result in a B12 deficiency.

.....

Clinical Features

- Vitamin B12 deficiency is characterized by

Megaloblastic anemia:

Feature of megaloblastic anemia include

- pallor,
- Fatigue.
- glossitis (beefy red tongue).
- slight yellow discoloration of the conjunctiva due to increased unconjugated bilirubin
- Progression of anemia may result in angina & congestive cardiac failure

Gastrointestinal dysfunction:

- GIT epithelial cells are undergoing rapid turnover& dependent on vitamin B12
- B12 deficiency results in
- weight loss.
- diarrhea.

Demyelination of nervous tissue

- Damage to the nervous system is seen in B12 deficiency.
- There is demyelination affecting the cerebral cortex as well as the dorsal column.
- Symmetrical paresthesia of extremities (an abnormal sensation, typically tingling or pricking (“pins and needles”), caused chiefly by pressure on or damage to peripheral nerves), alterations of tendon & deep senses & reflexes.

Achlorhydria

The absence of acid in gastric juice (absence of hydrochloric acid in the gastric secretions) is associated with B12 deficiency

Romberg's sign & positive Babinski's sign

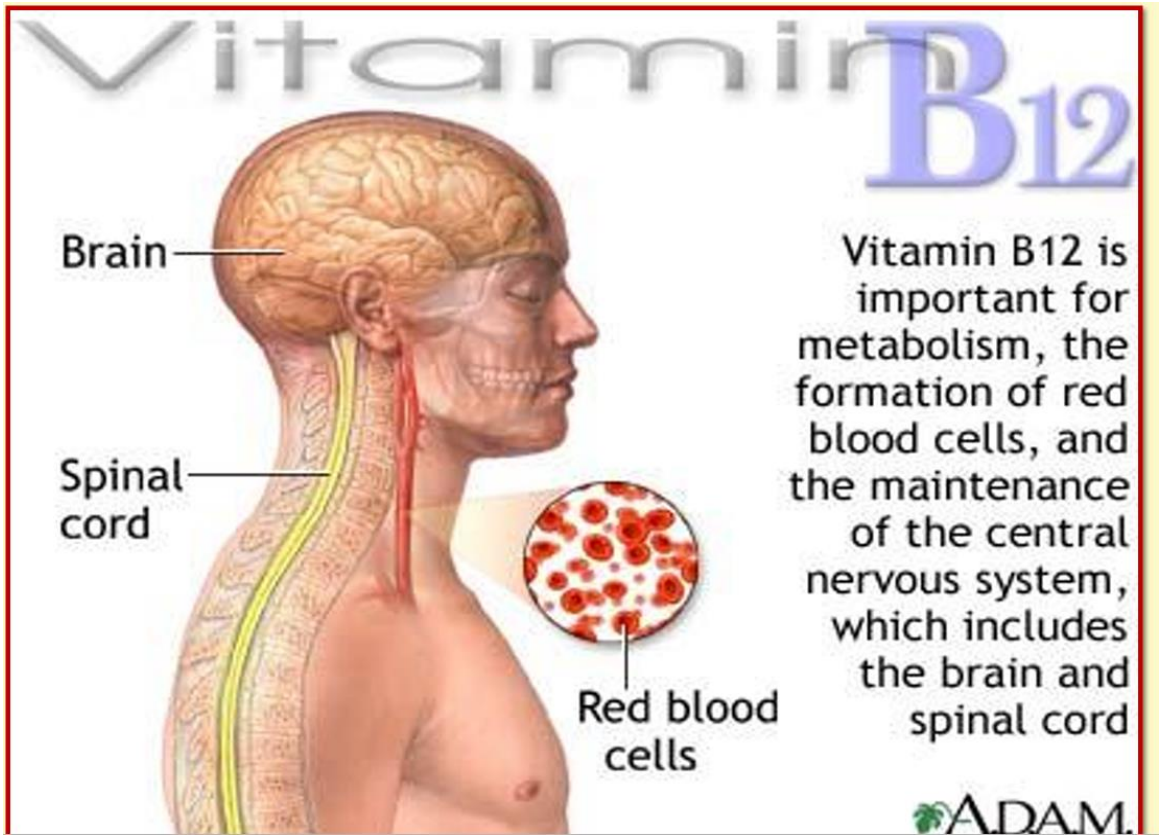
Assessment of B12 deficiency

- Serum B12: It is quantitated by radio-immunoassay or by ELISA.
- Peripheral smear: Peripheral blood & bone marrow morphology shows megaloblastic anemia.
- Homocysteinuria: Excretion of homocysteine in urine.
- Methylmalonic acid is excreted in the urine.

Laboratory tests for vitamin B12 and folate deficiency

			Result in	
Test	Normal values*		Vitamin B_{12} deficiency	Folate deficiency
Serum vitamin B_{12}	160–925 ng/L	120–680 pmol/L	Low	Normal or borderline
Serum folate	3.0–15.0 µg/L	4–30 nmol/L	Normal or raised	Low
Red cell folate	160–640 µg/L	360–1460 nmol/L	Normal or low	Low

* Normal values differ with different commercial kits.



FOLIC ACID Deficiency Anemia

Folic Acid

- Folate is an essential water-soluble vitamin, naturally present in food, especially in fruits, green leafy vegetables, and liver
- Folic acid is the synthesized form of folate present in fortified foods and supplements and has a higher bioavailability than naturally occurring folate.
- Folate has been added to grains to prevent congenital disabilities, especially neural tube defects, as it is necessary for the formation of several coenzymes in many metabolic systems, particularly for purine and pyrimidine synthesis, nucleoprotein synthesis and maintenance in erythropoiesis.

……..

Synonyms of Folic acid

It is also named to as

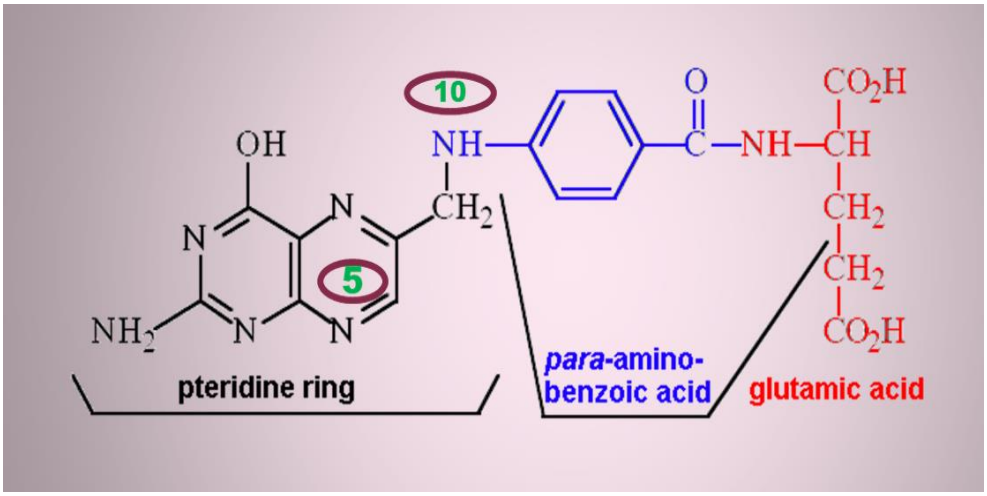
- ✓ Folate
- ✓ Vitamin B9
- ✓ Folacin

Active form

Tetrahydrofolate (THF or FH4) is the active form of folic acid

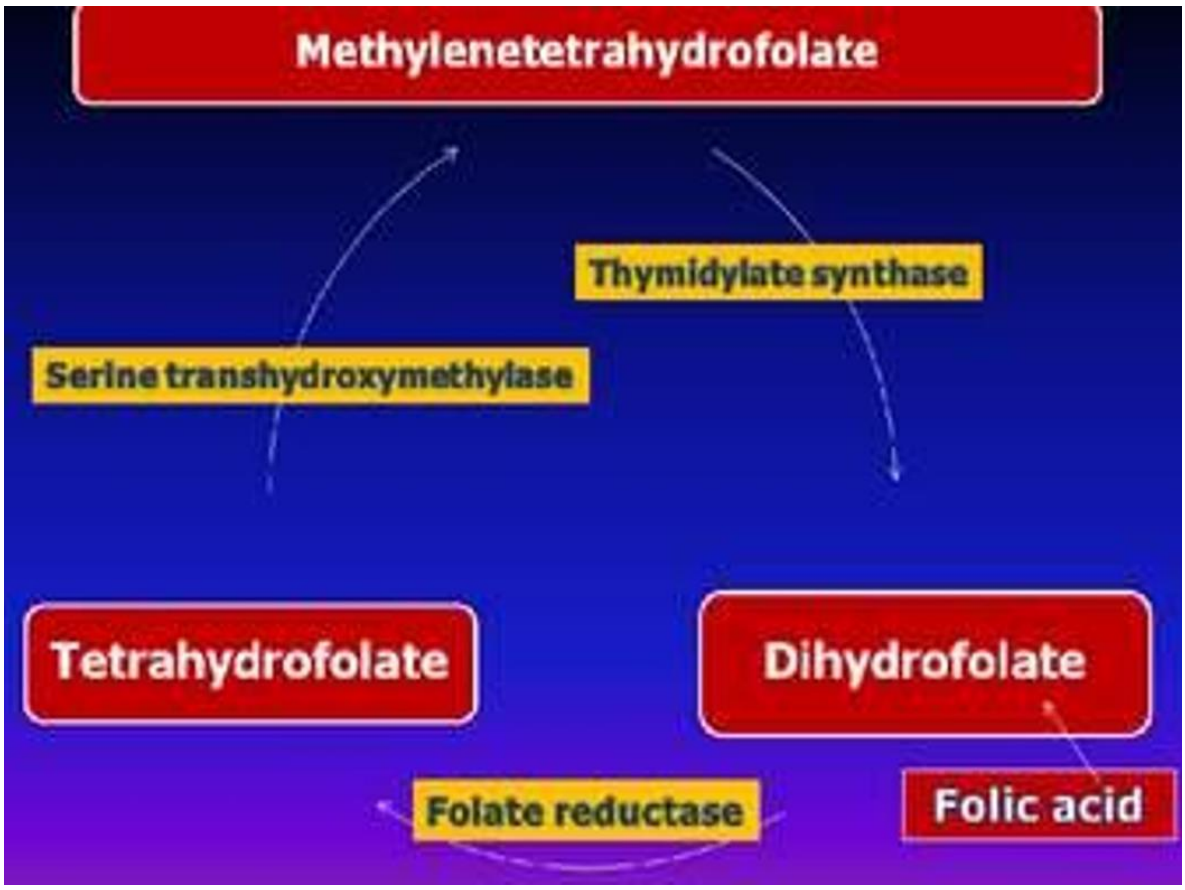
Chemistry

- Folic acid mainly consists of three components
- Pteridine ring
- PABA (para-amino benzoic acid)
- Glutamic acid residue
- Hence it is known as Pteroylglutamic acid



Storage

- Inside the cells, tetrahydrofolates are found as
- polyglutamate (with 5-6 amino acid residues),
- which are biologically most potent
- polyglutamate is the storage form of folic acid
- It is mainly stored in the liver (10-20 mg)
- The coenzymes of folic acid are actively involved in
- the one carbon metabolism
- THF acts as an acceptor or donor of one carbon units
- (formyl, methyl etc.) in reactions involving amino acid
- & nucleotide metabolism.



Dietary Sources

- ❖ Rich sources are green leafy vegetables such as
- ❖ spinach, cauliflower, Carrots Corn Beets.
- ❖ Poor sources are liver, kidney, milk, fruit

Folic Acid deficiency

• Dietary deficiency is the most common cause of folic acid

Dietary deficiencies are caused by

❖ Overcooking of food resuls in loss of folic acid activity
❖ Impaired absorption due to small intestinal diseases.

Increased demand of folic acid seen in

• Pregnancy

• Hemolytic anemia

Hence folic acid preparations are prescribed in pregnancy &hemolytic anemia.

• Loss of folic acid seen in patients undergoing dialysis

• Folate is absorbed in the jejunum by active and passive transport mechanisms across the intestinal wall.

• Hence, diseases such as celiac disease, short bowel syndrome, amyloidosis, gastric bypass can inhibit folate absorption resulting in a deficiency.

• Elevated pH, as occurs in achlorhydria (absence of hydrochloric acid in the gastric secretions), can also lead to poor folate absorption.

• Drugs such as methotrexate, phenytoin, sulfasalazine, and trimethoprim can antagonize folate utilization, inhibit its absorption or conversation to its active form resulting in folate deficiency.

• Congenital deficiencies of enzymes required in folate metabolism can lead to folate deficiency.

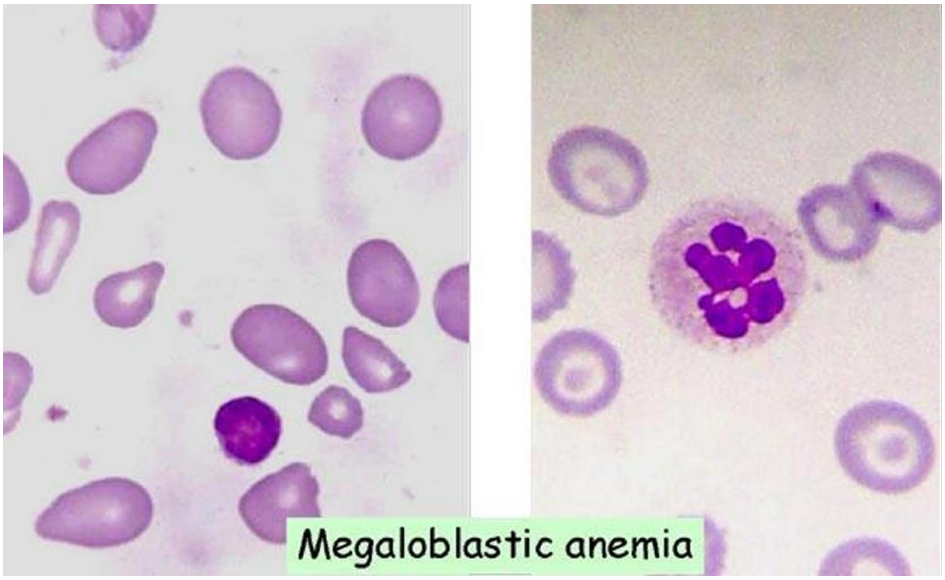
• Folic acid deficiency can occur subsequent to vitamin B-12 deficiency due to an impairment of methionine synthase resulting in the trapping of folate as methyltetrahydrofolate whereby methylene THFA accumulates in serum leading to folate trap phenomenon and increased urinary excretion of folate.

• Alcoholism is a significant cause of folate deficiency.

Clinical Features

•Megaloblastic anemia characterized by hyperchromic macrocytic anemia

• Megaloblastic changes are seen in bone marrow & mucosa



- ✓Patients look pale (mildly jaundiced), because of the excess breakdown of hemoglobin resulting from increased ineffective erythropoiesis in the bone marrow.
- ✓Glossitis (a beefy- red sore tongue)

Laboratory Findings

- Peripheral smear shows macrocytic hyperchromic anemia
- Hypersegmentation of neutrophils is common
- Complete blood count (CBC) test to determine if you have folic acid deficiency anemia.
- Bone marrow shows megaloblastic changes characterized by abnormally large size of erythroid cells with cytoplasmic maturation but impaired nuclear maturation due to defective DNA synthesis
- Defective red cell production

.....

Biochemical Findings

•Low plasma folic acid levels (<3ng/ml)

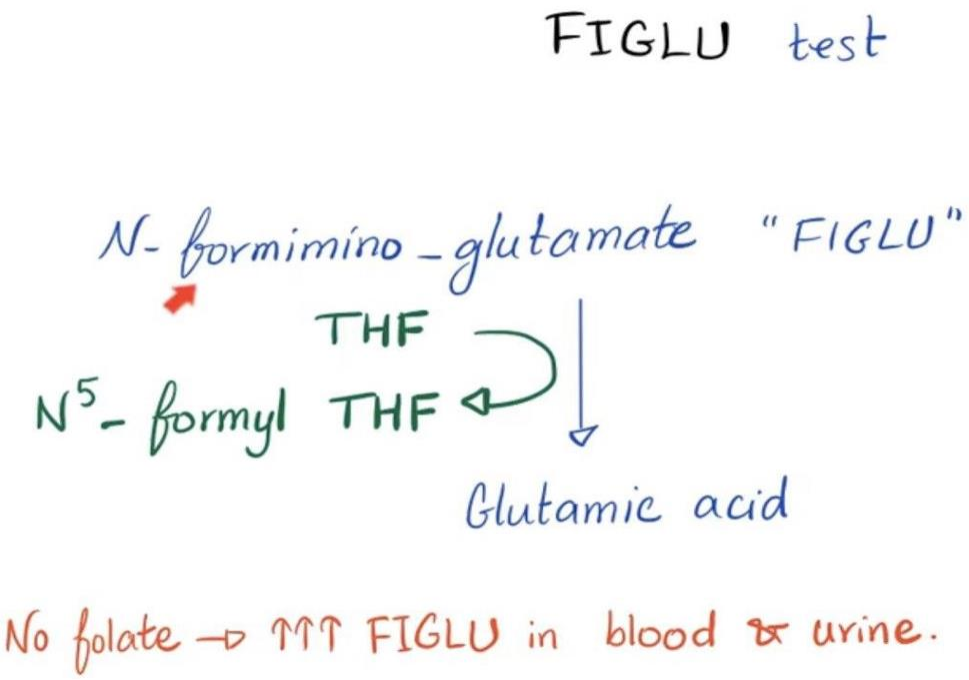
• Low red cell folic acid levels (<150 ng/ml)

• Normal plasma Vitamin B12 levels

FIGLU Excretion Test:

✓ Folic acid deficiency is associated with increased excretion of formiminoglutamate (FIGLU) in urine.

✓ Due to impaired conversion of FIGLU to glutamate in a reaction requiring FH4



Folic Acid Deficiency & Neural Tube Defects

- Folic acid supplementation during pregnancy helps to prevent neural tube defects
- Mainly involved in the brain & spinal cord
- Folic acid is involved in nucleic acid & amino acid metabolism
- Deficiency results in impaired & aberrant neural development

……..

Neural tube defects

• Neural tube defects are serious birth defects of the brain and spinal cord.

• The two most common neural tube defects are s.p(a spinal cord defect) and anencephaly (a brain defect).

• Neural tube defects develop very early during pregnancy when the neural tube—which forms the early brain and the spinal cord—does not close properly.

……..

- ✓ Spina Bifida-60%
- ✓ Anencephaly-30%
- ✓ Encephalocele-10%

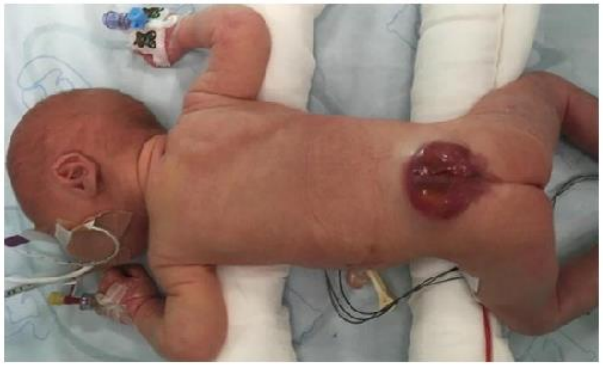
Spina Bifida

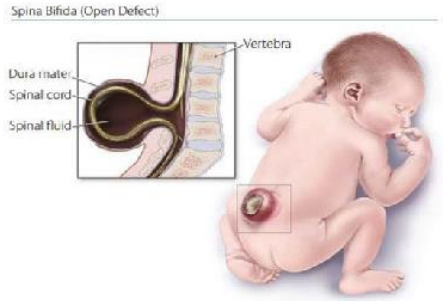
• Is a condition that affects the spine and is usually apparent at birth.

• It is a type of neural tube defect (NTD). Spina bifida can happen anywhere along the spine if the neural tube does not close all the way.

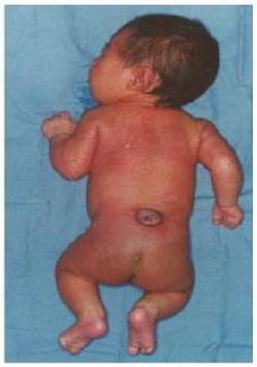
• When the neural tube doesn't close all the way, the backbone that protects the spinal cord doesn't form and close as it should.

• This often results in damage to the spinal cord and nerves.

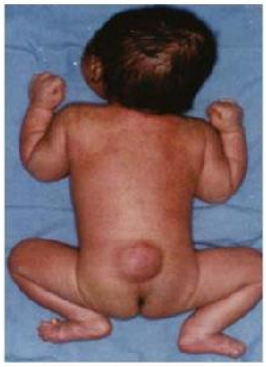




relomeningocele (open spina bifida)

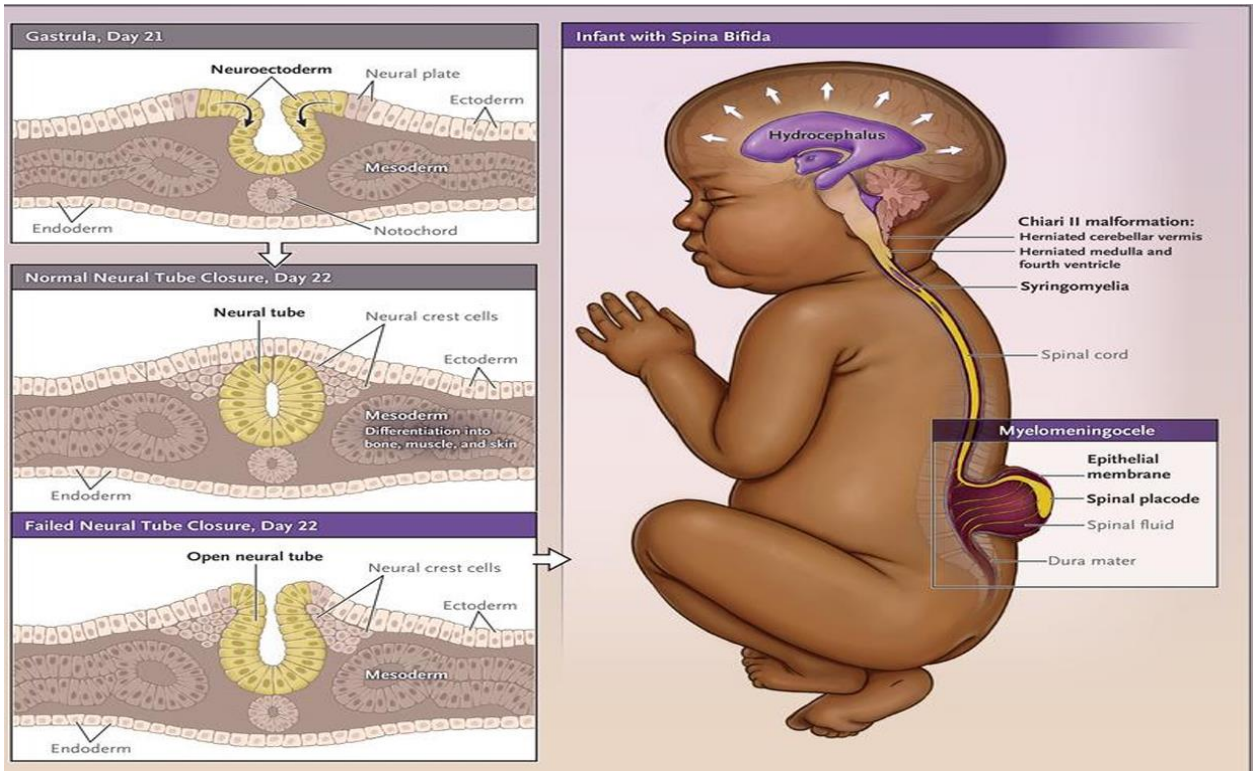


b. Meningocele (closed spina bifida)



a. Myelomeningocele (open spina bifida)

b. Meningocele (closed spina bifida)



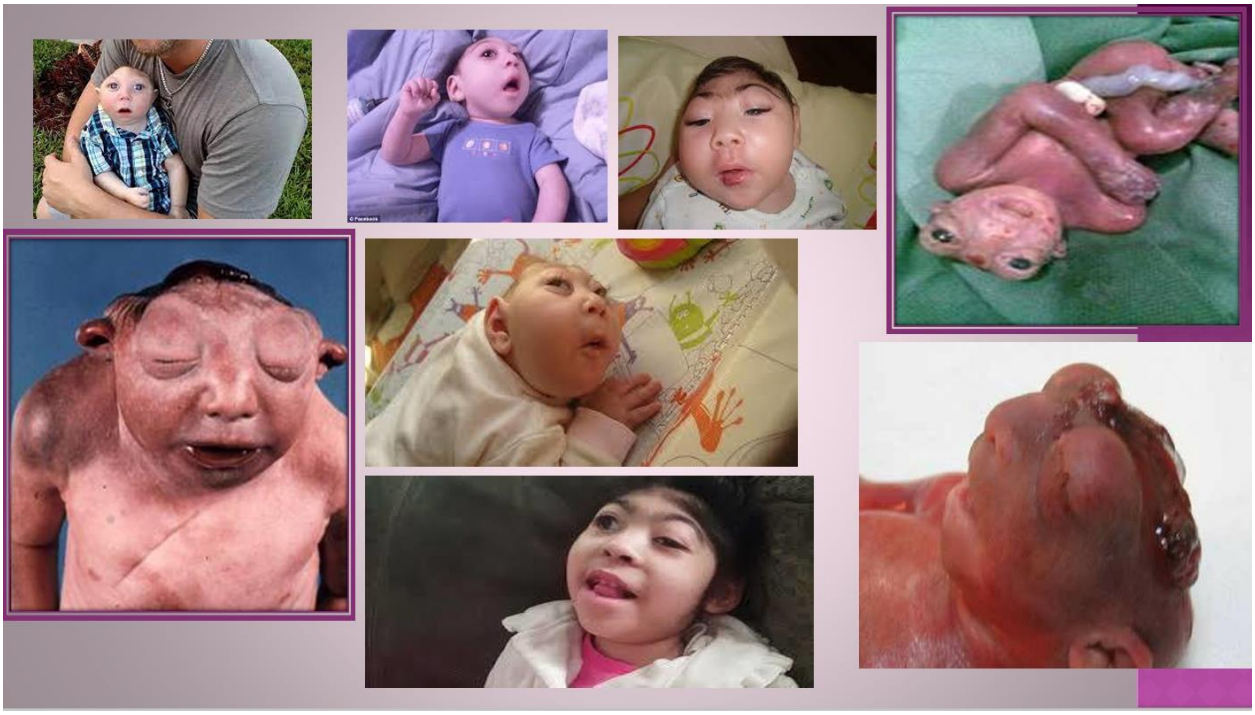
Anencephaly brain

• Happens if the upper part of the neural tube does not close all the way.

• This often results in a baby being born without the front part of the brain (forebrain),and the thinking and coordinating part of the brain (cerebrum).

• The remaining parts of the brain are often not covered by bone or skin.

• About 1 in every 4,600 babies is born with anencephaly.



Encephalocele

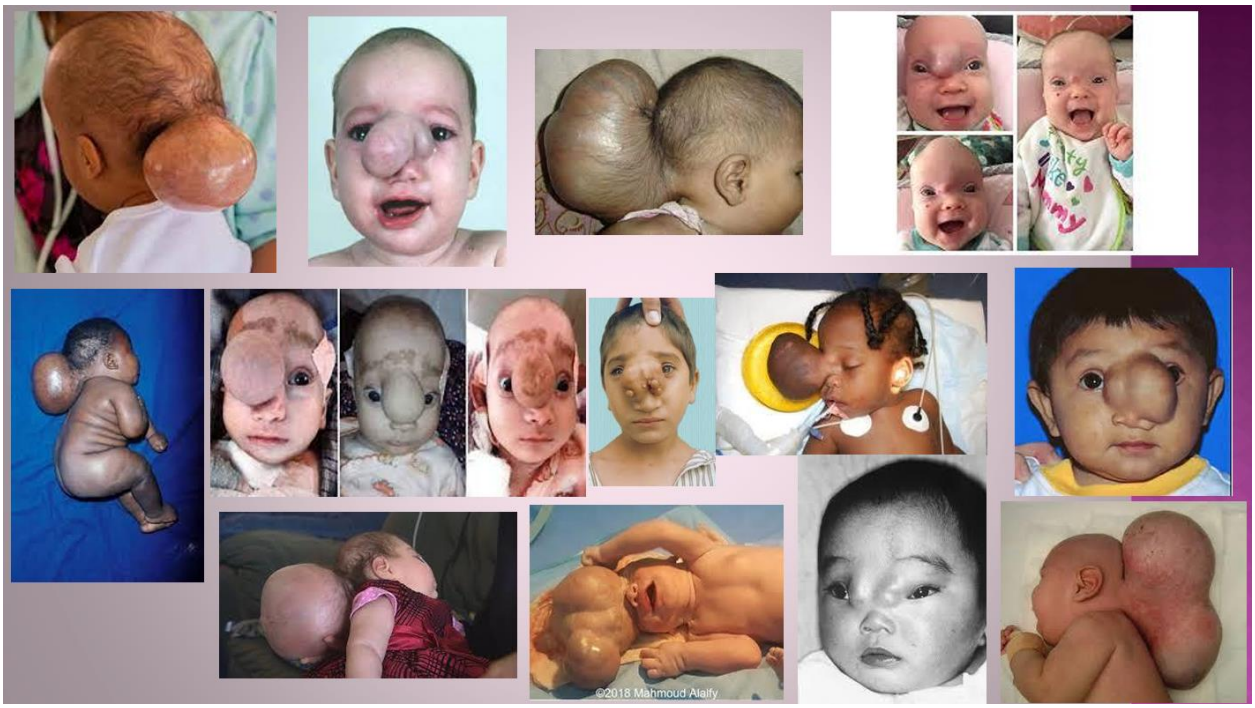
• Is a neural tube defect characterized by sac-like protrusions of the brain and the membranes that cover it through openings in the skull.

• These defects are caused by the failure of the neural tube to close completely during fetal development.

• Encephaloceles cause a groove down the middle of the skull,

between the forehead and nose, or on the back side of the skull.

• The severity of encephalocele varies, depending on its location.



Folic Acid Deficiency & Homocysteinemia

• Homocysteine is a risk factor.

• Folic acid is required for the conversion of homocysteine to methionine.

• Deficiency is associated with increased plasma levels of homocysteine.

•Folic acid supplementation decreases plasma homocysteine levels.

•Homocysteine levels are also increased in Vitamin B12 & B6 deficiency.

Signs and Symptoms

Symptoms of folic acid deficiency include:

- Fatigue
- Mouth sores
- Swollen tongue
- Poor growth

……..

Once anemia occurs, you might experience the following:

- Fatigue
- Dizziness
- Feeling cold
- Irritability
- Headache
- Difficulty breathing
- Pale skin
- Diarrhea
- Weight loss
- Loss of appetite
- Difficulty concentrating

Who Is at Risk for Folic Acid Deficiency Anemia?

Factors that increase your likelihood of developing this condition include:

- Eating overcooked foods
- Consuming a vitamin-poor diet
- Heavy alcohol drinking (alcohol interferes with folate absorption)
- Medical conditions (like sickle cell disease)
- Medications
- Pregnancy

Factors associated with increased risk of NTDs

❖ **Family history of neural tube defects**

People who've had one baby with an NTD have a 2% to 3% increased

risk of having a second baby with an NTD.

❖ **Certain medications**

❖ **Folic acid deficiency**

Folate deficiency increases the risk of neural tube defects, and inadequate concentrations of folate in a pregnant woman can therefore lead to the development of a fetus with congenital defects and malformations.

Diabetes

People with poorly managed diabetes who are pregnant have a higher risk of having a baby with an NTD.

Obesity

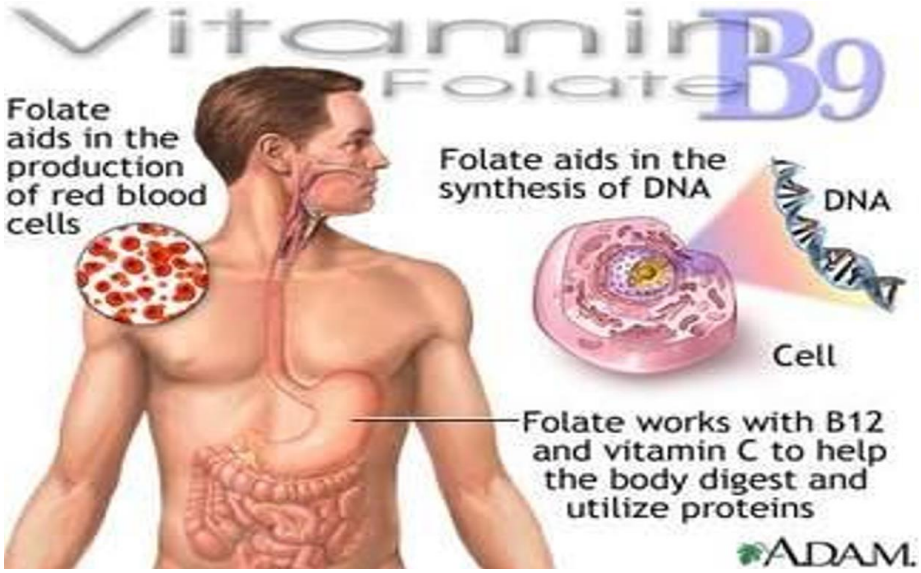
People who have obesity before pregnancy have an

Increased risk of having a baby with an NTD

Increased body temperature in early pregnancy: Increases in core body temperature (hyperthermia) in the early weeks of pregnancy due to a prolonged fever or hot tub have been associated with a slightly increased risk of NTDs.

Opioid

Use in early pregnancy: Opioids are a class of very powerful and highly addictive drugs that reduce pain. Pregnant people who've taken opioids in the first two months of pregnancy have an increased chance of having a baby with an NTD, in addition to other complications.



Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

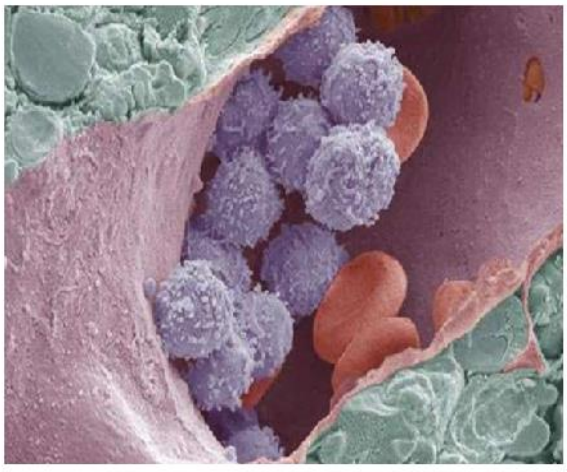
8th - Week

Lecture Title: Haemolytic anaemia.

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for the students in identifying the concept of Haemolytic anaemia (Normal red cell destruction(Classification, Hereditary hemolytic anemia, Acquired hemolytic anemia.

Special Objectives of the Module

1. Definition of Haemolytic anaemia.

2. Explain the process of destruction of red blood cells.

3. Classification of haemolytic anaemia.

4. Definition of Hereditary hemolytic anemia, Acquired hemolytic anemia (Causes, Clinical features, Symptoms, Signs, laboratory findings, diagnosis).

References:

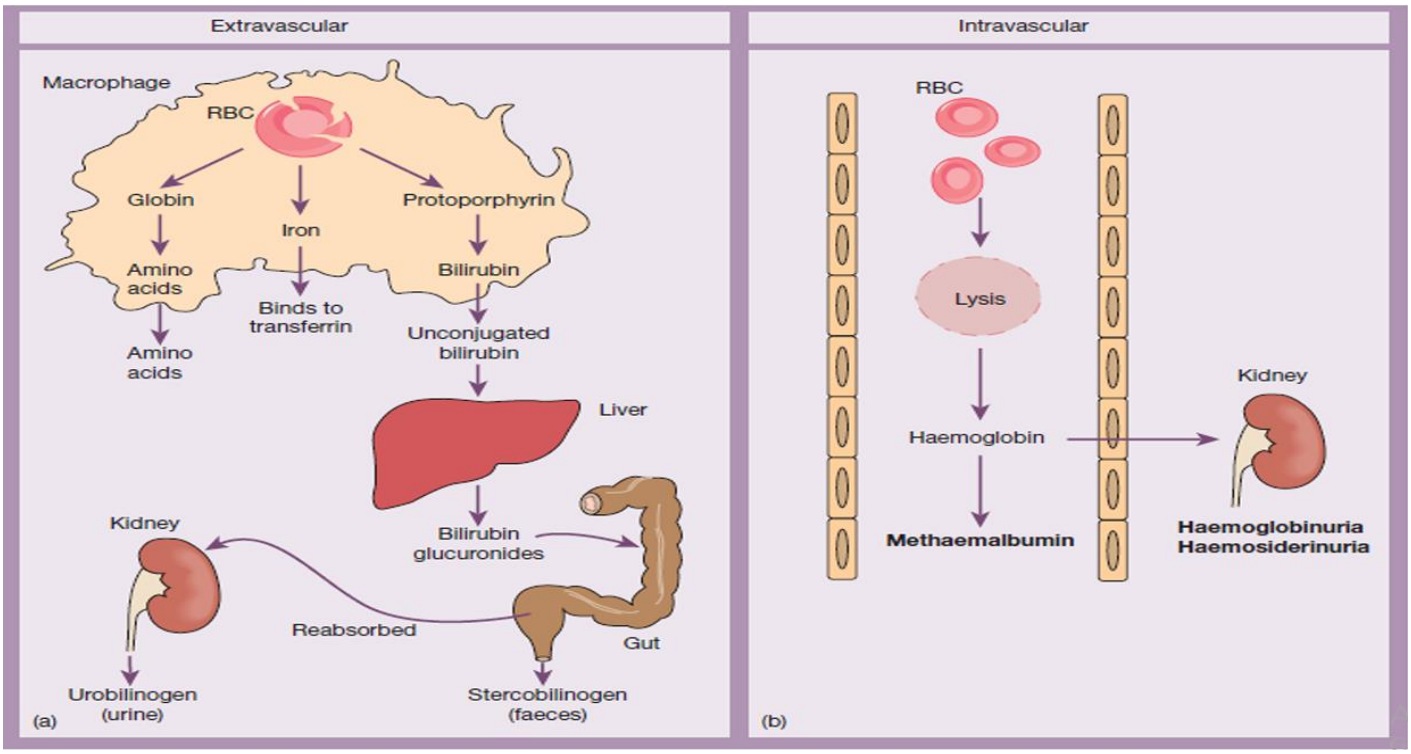
1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

- ✓ Isaform of anemia due to hemolysis.
- ✓ The abnormal breakdown of red blood cells (RBCs), either in the blood vessels (intravascular hemolysis) or elsewhere in the human body (extravascular) .
- ✓ Increased red cell destruction (and increased erythropoiesis) cells are being produced at the same rate as they are hemolyzed; this can develop into anemia if :
- ✓ Erythrocyte destruction accelerates beyond the compensatory capacity of the marrow
- ✓ Red cell destruction usually occurs after a mean lifespan of 120 days when the cells are removed extravascularly by the macrophages of the reticuloendothelial (RE) system, especially in the marrow and also in the liver and spleen.
- ✓ As the cells have no nucleus, red cell metabolism gradually deteriorates as enzymes are degraded and the cells become non-viable. The breakdown of haem from hemoglobin liberate iron for recirculation via plasma transferrin mainly to marrow erythroblasts, and protoporphyrin, which is broken down to bilirubin.
- ✓ Bilirubin circulates to the liver where it is conjugated to glucuronides, which are excreted into the gut via bile and converted to stercobilinogen and stercobilin (excreted in feces)
- ✓ Stercobilinogen and stercobilin are partly reabsorbed and excreted in urine as urobilinogen and urobilin.
- ✓ Globin chains are broken down into amino acids which are reutilized for general protein synthesis in the body.
- ✓ Haptoglobins are proteins in normal plasma which bind hemoglobin.
- ✓ The hemoglobin–haptoglobin complex is removed by the RE system.



Haemolytic anaemias:

Anemias that result from an increase in the rate of red cell destruction. Because of erythropoietic hyperplasia and extension of bone marrow, red cell destruction may be increased several-fold before the patient becomes anaemic.

Haemolytic anaemia may not be seen until

- ✓ Theredcell lifespan is less than 30 days.
- ✓ Itleads to a marked reticulocytosis.

Classification

Table 1.1 is a classification of the haemolytic anaemias.

1.Hereditary haemolytic anaemias are the result of intrinsic red cell defects.

2.Acquired haemolytic anaemias are the result of an xtracorpuscular or environmental change. Paroxysmal nocturnal haemoglobinuria (PNH) is the exception because although it is an acquired disorder, the PNH red cells have an intrinsic defect.

Table 1.1 Classification of haemolytic anaemias.

Hereditary	Acquired
Membrane Hereditary spherocytosis, hereditary elliptocytosis	**Immune** *Autoimmune* Warm antibody type Cold antibody type
Metabolism G6PD deficiency, pyruvate kinase deficiency	*Alloimmune* Haemolytic transfusion reactions Haemolytic disease of the newborn Allografts, especially stem cell transplantation
Haemoglobin Genetic abnormalities (Hb S, Hb C, unstable); see	*Drug associated* **Red cell fragmentation syndromes** **March haemoglobinuria** **Infections** Malaria, clostridia **Chemical and physical agents** Especially drugs, industrial/domestic substances, burns **Secondary** Liver and renal disease **Paroxysmal nocturnal haemoglobinuria**

Intravascular

and extravascular haemolysis

1. There are two mechanisms whereby red cells are destroyed in haemolytic anaemia. There may be excessive removal of red cells by macrophages of the RE system (extravascular haemolysis) or they may be broken down directly in the circulation (intravascular haemolysis).

……

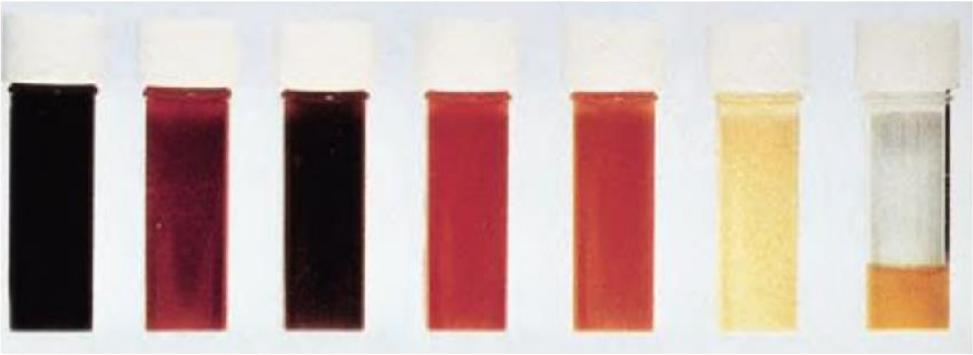
- ✓ In intravascular haemolysis, free haemoglobin is released which rapidly saturates plasma haptoglobins and the excess free haemoglobin is filtered by the glomerulus.
- ✓ Themainlaboratory features of intravascular haemolysis are:
  1. Haemoglobinaemia (is an excess of hemoglobin in the blood plasma.) and haemoglobinuria (The presence of hemoglobin in the urine).
  2. Haemosiderinuria (existence of hemosiderin in the urine).
  3. Methaemalbuminaemia(isan albumin complex consisting of albumin and heme and detected by Schumm's test).

Causes of intravascular

- haemolysis
- Mismatched blood transfusion (ABO)
- G6PD deficiency with oxidant stress
- Red cell fragmentation syndromes
- Some severe autoimmune haemolytic anaemias
- Some drug- and infection-induced haemolytic anaemias
- Paroxysmal nocturnal haemoglobinuria
- March haemoglobinuria
- Unstable haemoglobin

Clinical features:

The patient may show pallor of the mucous membranes and splenomegaly. There is no bilirubin in urine but this may turn dark on standing because of excess urobilinogen and some patients (particularly with sickle cell disease) develop ulcers around the ankle.



Progressive urine samples in an acute episode of intravascular haemolysis showing haemoglobinuria of decreasing severity.

Laboratory findings:

The laboratory findings are divided into three groups:

1. Features of increased red cell breakdown:

- ❖ Serumbilirubin raised, unconjugated and bound to albumin.
- ❖ Urine urobilinogen increased.
- ❖ Serum haptoglobins absent because the haptoglobins become saturated with haemoglobin and the complex is removed by RE cells.

2. Features of increased red cell production:

(a) reticulocytosis;

(b) bone marrow erythroid hyperplasia; the normal marrow myeloid : erythoid ratio of 2 : 1 to 12 : 1 is reduced to 1 : 1.

3. Damagedred cells:

(b) osmotic fragility;

(a) morphology (e.g. microspherocytes, elliptocytes, fragments);

(c) specific enzyme, protein or DNA tests.

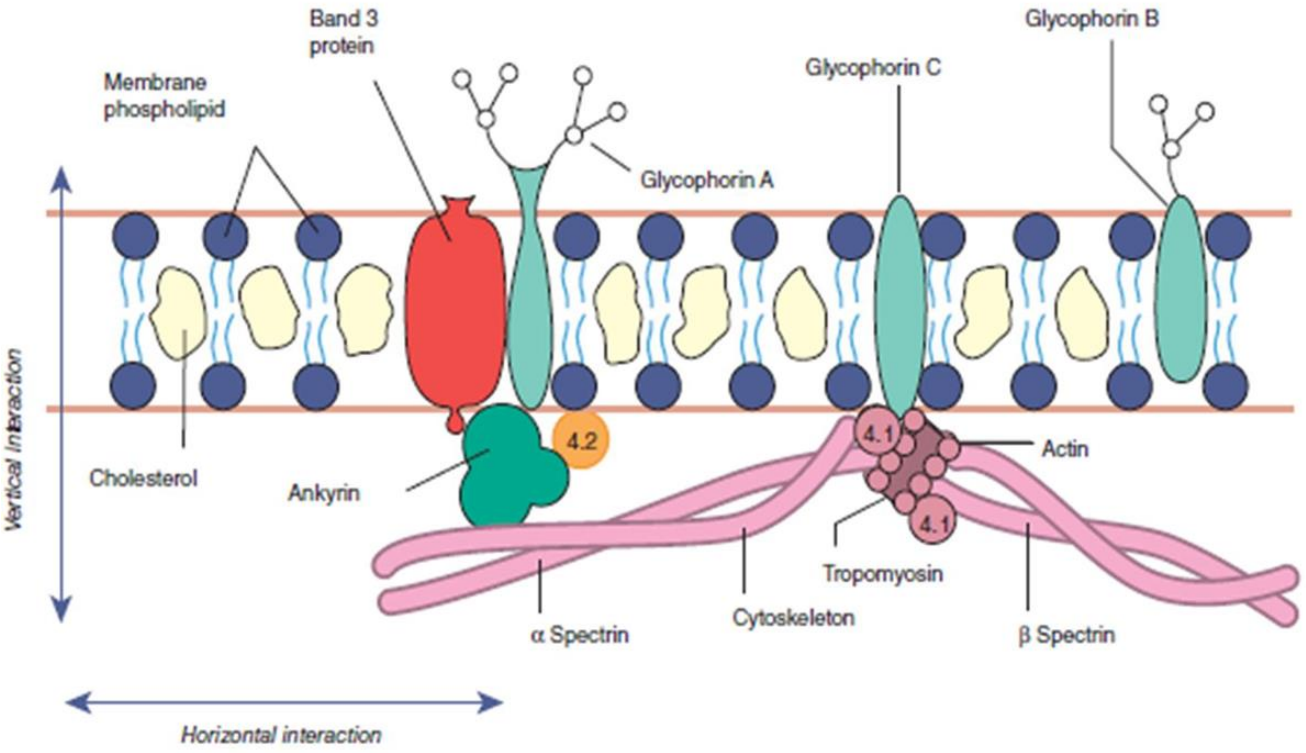
Hereditary Haemolytic Anaemias

- ***Membrane Defects***

1. Hereditary Spherocytosis

- Hereditary spherocytosis (HS) is the most common hereditary haemolytic anaemia in northern Europeans.
- HS is usually caused by defects in the proteins involved in the vertical interactions between the membrane skeleton and the lipid bilayer of the red cell.

- ✓ Hereditary spherocytosis is caused by defects in the genes that code for the red blood cell proteins spectrin (alpha and beta), ankyrin, band 3 protein, protein 4.2 of red blood cell membrane. These proteins are necessary to maintain the normal shape of a red blood cell, which is a biconcave disk.
- ✓ In HS, the marrow produces red cells of normal biconcave shape but these lose membrane and become spherical as they circulate through the spleen and the rest of the RE system.
- ✓ The spherocytes are unable to pass through the splenic microcirculation where they die prematurely.
- ✓ Theinheritance of HS is autosomal recessive or autosomal dominant and 75%of patients are autosomal dominant trait.

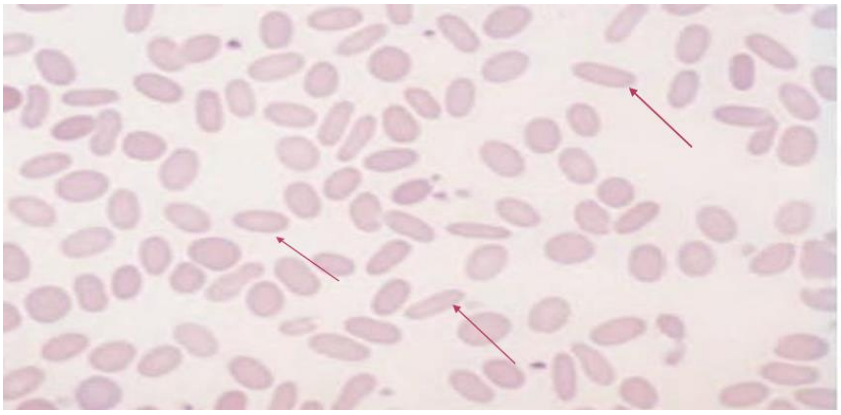


Lab . Finding includes:

- ✓ Reticulocytes are usually 5– 20% and the blood film shows microspherocytes that are densely staining with smaller diameters than normal red cells.
- ✓ The direct antiglobulin (Coombs) test is normal.

2. Hereditary Elliptocytosis

- ✓ It is also known as ovalocytosis has similar clinical and laboratory features to HS except for the appearance of the blood film but it is usually a clinically milder disorder.
- ✓ It is usually discovered by chanceon a blood filmand there may be no evidence of haemolysis.
- ✓ The basic defect is due to α or β- spectrin mutants leading to defective spectrin dimer formation that leading to defective in spectrin– ankyrin associations.



Lab . Finding:

Blood film showed at least 25% of erythrocytes in the specimen have elliptical shape. Also osmotic fragility test, an autohaemolysis test, and direct protein assaying by gel electrophoresis can be done for confirming the disease .

DefectiveRed Cell Metabolism

1. Glucose-6-phosphate Dehydrogenase Deficiency

- Glucose-6-phosphate dehydrogenase (G_6PD)is anenzyme in the pentose phosphate.
- Pathway that function to reduce nicotinamide adenine dinucleotide phosphate (NADP).
- Glucose-6-phosphate dehydrogenase deficiency results from mutations in the G_6PD gene that code for glucose-6-phosphate dehydrogenase.
- G_6PD enzyme protects red blood cells from harmful molecules called Reactive Oxygen Species (ROS), which are results from normal cellular functions.
- Deficiency of this enzyme results in reactive oxygen species accumulate and damage red blood cells and factors such as infections, certain drugs, or ingesting fava beans can increase the levels of reactive oxygen species, causing red blood cells to be destroyed faster than the normal.
- The inheritance of G6PD is sex-linked, affecting males. The female heterozygotes have an advantage of resistance to Falciparum malaria.
- The main races affected are in West Africa, the Mediterranean, the Middle East and South- East Asia.

There are some agents that cause haemolytic anaemia in G6PD deficiency:

1. Fava beans.

2. Infections and other acute illnesses (e.g. diabetic ketoacidosis).

3. Drugs:

Antimalarials,

Sulphonamides, Nitrofurans, Chloramphenicol, Aspirin, Antihelminths, Vitamin K.

.....

Agents that may cause haemolytic anaemia in glucose-6-phosphate dehydrogenase (G6PD) deficiency.

- ❖ Infections and other acute illnesses (e.g. diabetic ketoacidosis)
- ❖ Drugs
- ❖ Antimalarials (e.g. primaquine, pamaquine, chloroquine,Fansidar, Maloprim)
- ❖ Other antibacterial agents (e.g. nitrofurans, chloramphenicol)
- ❖ Analgesics (e.g. aspirin), moderate doses are safe
- ❖ Antihelminths (e.g. β-naphthol, stibophen)
- ❖ vitamin K analogues, naphthalene.
- ❖ Fava beans.

Symptoms

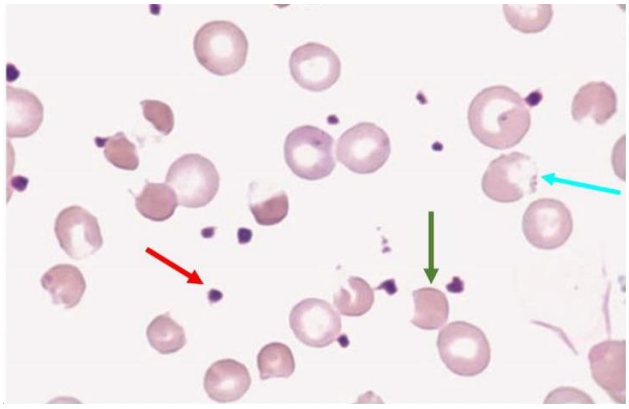
- primary symptoms
- neonatal hyperbilirubinemia on day 2-4
- acute hemolytic anemia following exposure to precipitants, typically within 24-72 hours after ingestion
- fatigue
- jaundice
- dark urine
- back pain

.....

Lab Finding

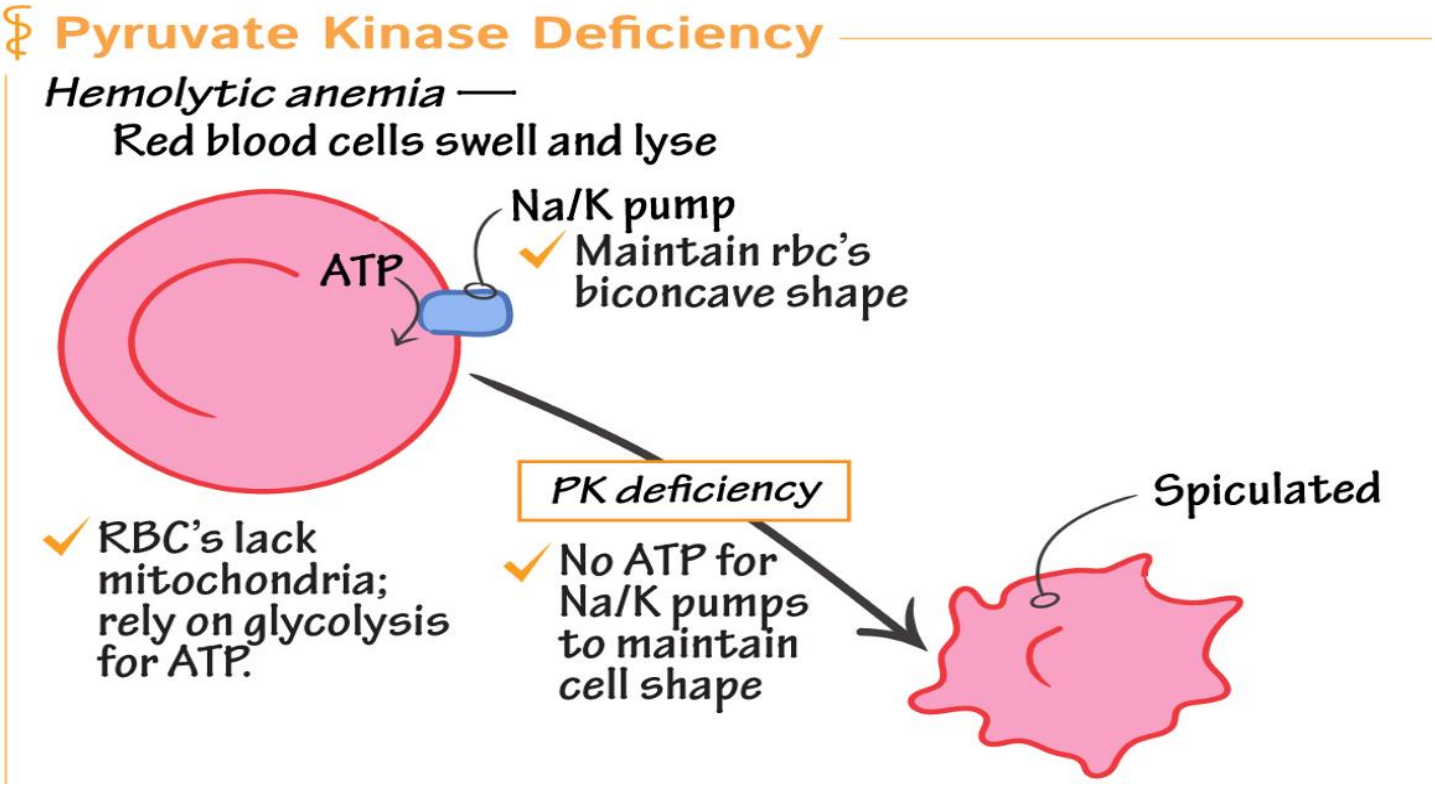
1. During a crisis the blood film may show contracted and fragmented cells (bite cells and blister cells) which have had Heinz bodies removed by the spleen.

2. There are also features of intravascular haemolysis.



2 . Pyruvate Kinase Deficiency

- This is an inherited autosomal recessive and cause due to a mutation in the PKLR gene is located on chromosome 1q22 that coded for Pyruvate kinase isozymes R (RBC isoform) /L (Liver isoform) enzyme that catalyzes the production of pyruvate and ATP from phosphoenol pyruvate.
- The red cells become rigid as a result of reduced adenosine triphosphate (ATP) formation.
- The blood film shows poikilocytosis and distorted prickle cells, particularly post splenectomy.
- Direct enzyme assay is needed to make the diagnosis and direct DNA sequencing.
- Their red blood cells cannot produce the necessary amount of ATP to sustain Na+/K+ pump function.
- Red blood cells rely on glycolysis for ATP production because they lack
- mitochondria and cannot perform oxidative phosphorylation. As a result,
- pyruvate kinase deficiency will deprive red blood cells of their ATP supply. Red blood cells need ATP to power Na+/K+ pumps, which are responsible for maintaining their biconcave shape.



Signs & symptoms

Signs & symptoms

chronic hemolytic anemia

splenomegaly

jaundice

neonatal

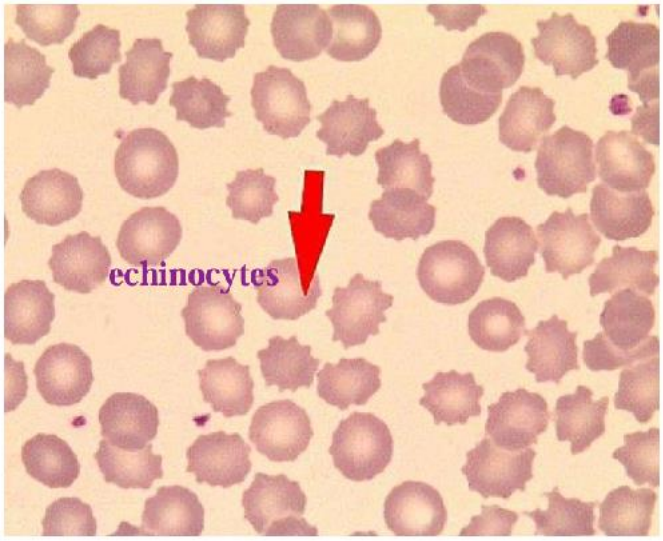
hepatomegaly

hyperbilirubinemia

gallstones

skin ulcer

secondary hemochromatosiss



Echinocytes (RBC with thorny projections) different from acanthyocytes

When peripheral blood is stained by new methylene blue or brilliant cresyl blue, reticulocytes seen. Reticulocytes are immature red blood cells that have lost their nuclei just prior to entering the circulation. In normal individuals reticulocytes comprise 0.5% to 1.7% of the total erythrocytes. An increase in the percentage of reticulocytes (reticulocytosis) is an important sign in any patient suffering anemia. In PKLR deficiency the level of reticulocytosis results in a reticulocyte level of 4% to 15%. In association with the reticulocytosis there is a reduction in hematocrit (packed cell volume) to around 17% to 37% where it ranges from 41% to 50% in normal individuals. Hemoglobin measurement also shows a decrease to 6–12g/dL where normal ranges from 12 16.5g/dL. Examination of bone marrow from PKLR deficient patients will show normoblastic erythroid hyperplasia.

Haemolytic anaemias:

Anemias that result from an increase in the rate of red cell destruction. Because of erythropoietic hyperplasia and extension of bone marrow, red cell destruction may be increased several- fold before the patient becomes anaemic.

Haemolytic anaemia may not be seen until

- ❖ Theredcell lifespan is less than 30 days.
- ❖ Itleads to a marked reticulocytosis.

……

Classification

Table 1.1 is a classification of the haemolytic anaemias.

1.Hereditary haemolytic anaemias are the result of intrinsic red cell defects.

2.Acquired haemolytic anaemias are the result of an extracorpuscular or environmental change. Paroxysmal nocturnal haemoglobinuria (PNH) is the exception because although it is an acquired disorder, the PNH red cells have an intrinsic defect.

Table 1.1 Classification of haemolytic anaemias.

Hereditary	Acquired
Membrane Hereditary spherocytosis, hereditary elliptocytosis	**Immune** *Autoimmune* Warm antibody type Cold antibody type
Metabolism G6PD deficiency, pyruvate kinase deficiency	*Alloimmune* Haemolytic transfusion reactions Haemolytic disease of the newborn Allografts, especially stem cell transplantation
Haemoglobin Genetic abnormalities (Hb S, Hb C, unstable); see	*Drug associated* **Red cell fragmentation syndromes** **March haemoglobinuria** **Infections** Malaria, clostridia **Chemical and physical agents** Especially drugs, industrial/domestic substances, burns **Secondary** Liver and renal disease **Paroxysmal nocturnal haemoglobinuria**

Acquired hemolytic anemias

Immune Haemolytic Anaemias

1 . Autoimmune HaemolyticAnaemias

- Autoimmune haemolytic anaemias (AIHAs) are caused by antibody production by the body against its own red cells.
- They are characterized by a positive direct antiglobulin test (DAT) also known as the Coombs ' test.
- They are divided into' warm ' and ' cold' types according to whether the antibody reacts more strongly with red cells at 37 ° C or 4 ° C.

Table 1.3 Immune haemolytic anaemias: classification.

Warm type	Cold type
Autoimmune	
Idiopathic	*Idiopathic*
Secondary	*Secondary*
SLE, other 'autoimmune' diseases	Infections – *Mycoplasma* pneumonia, infectious mononucleosis
CLL, lymphomas	Lymphoma
Drugs (e.g. methyldopa)	Paroxysmal cold haemoglobinuria (rare, sometimes associated with infections, e.g. syphilis)
Alloimmune	
Induced by red cell antigens	
Haemolytic transfusion reactions	
Haemolytic disease of the newborn	
Post stem cell grafts	
Drug induced	
Drug–red cell membrane complex	
Immune complex	

CLL, chronic lymphocytic leukaemia; SLE, systemic lupus erythematosus.

Synonyms of Warm Autoimmune Hemolytic Anemia

- ✓ WAHA
- ✓ Warm AIHA
- ✓ WAIHA
- ✓ Warm antibody autoimmune hemolytic anemia

A. Warm Autoimmune HaemolyticAnaemias

- ❖ Antibodies are proteins which are made by the immune system.
- ❖ These proteins normally attach to the surface of bacteria. They act like labels to tell the immune system to destroy the bacteria.
- ❖ In AIHA, the immune system (which normally defends the body against
- ❖ infection) makes an antibody against its own red blood cells.

For many people, the cause of AIHA is not obvious. This is called idiopathic or primary AIHA.

Some people develop AIHA due to other factors; these people have secondary AIHA. Secondary AIHA may be caused by:

- infection e.g. Hepatitis C, HIV, cytomegalovirus (CMV), Epstein-Barr virus (EBV), tuberculosis
- cancer e.g. lymphoma, chronic lymphocytic leukaemia (CLL)
- other autoimmune conditions e.g. SLE (systemic lupuserythromatosus), scleroderma
- certain drugs.

……..

A. Warm Autoimmune HaemolyticAnaemias

- ✓ The red cells are coated with immunoglobulin (Ig), usually immunoglobulin G (IgG) alone or with complement, and are therefore taken up by RE macrophages which have receptors for the Ig Fc fragment (fragment crystallizable region).
- ✓ Whenthe cells are coated with IgG and complement (C3d, the degraded fragment of C3) or complement alone, red cell destruction occurs more generally in the RE system.
- ✓ Warm autoimmune hemolytic anemia (WAHA) is an autoimmune disorder characterized by the premature destruction of healthy red blood cells (hemolysis).
- ✓ Autoimmune diseases occur when one's own immune system attacks healthy tissue.
- ✓ In the case of WAHA and other types of autoimmune hemolytic anemia, red blood cells are "tagged" by antibodies and are then destroyed by other types of immune cells.

- ✓ WAHA is the most common type of autoimmune hemolytic anemia; it affects approximately 1 to 3 per 100,000 people every year and can occur at any age.
- ✓ The disease is termed "warm" because the antibodies are active and cause hemolysis at body temperature.

Clinical Features

- ❖ The disease may occur at any age, in either sex, and presents as a haemolytic anaemia of varying severity.
- ❖ The spleen is often enlarged.
- ❖ It may occur alone or in association with other diseases .
- ❖ When associated with idiopathic thrombocytopenic purpura (ITP), a similar condition affecting platelets , it is called Evans' syndrome.
- ❖ When secondary to systemic lupus erythematosus, the cells typically are coated with immunoglobulin and complement.

Signs & Symptoms

- ❖ WAHA can develop at any age, but the median age of onset is 52 years. This means that one-half of affected individuals will be younger than 52 years of age when the disease begins and that the other half will be above this age.
- ❖ The symptoms of WAHA usually develop slowly over a period of several weeks to months, but in some people can develop suddenly over a few days.
- ❖ Specific symptoms that occur may vary from one person to another and depend on the rate of onset, the degree of hemolysis, and the presence of an underlying disorder.
- ❖ Some individuals, especially those with a gradual onset of anemia, may not have any obvious symptoms (asymptomatic).

- Symptoms of anemia include paleness of the skin (pallor), fatigue, shortness of breath (dyspnea), dizziness, and palpitations.
- In cases of brisk and severe hemolysis, chest pain, decreased alertness (lethargy), confusion, transient loss of consciousness (syncope), and deregulation of heart rate and blood pressure (hemodynamic instability) might occur.
- Hemolysis also leads to increased release of hemoglobin in the blood and urine, which can result in darkly pigmented urine.
- Hemoglobin is degraded into a yellow compound called bilirubin, which can accumulate and lead to yellowing of the skin and whites of the eyes (jaundice).
- An enlarged spleen (splenomegaly).
- Splenomegaly may cause an affected individual to have a bloated or full feeling in the abdomen.
- WAHA is also associated with an increased risk of blood clots in the veins (venous thromboembolism).
- These clots can notably develop in the legs (deep vein thrombosis) and have the potential to detach, circulate in the blood, and occlude the veins of the lungs (pulmonary embolism).
- Thromboembolisms typically occur in the weeks after diagnosis and are more common in patients with more severe hemolysis and in those that are treated with surgical removal of the spleen (splenectomy).
- Rarely, clots can form in the arteries feeding the heart (coronary arteries) and lead to a heart attack (myocardial infarction) or in the arteries of the brain (cerebral arteries) and lead to a stroke.
- Patients that require a splenectomy are also at a higher rate of developing infections.
- After being treated, 30% of patients will be cured, and the rest are at risk of developing recurrent episodes of hemolysis.

- The majority of people with WAHA survive, although a mortality rate of about 5% is seen.
- Mortality is mainly attributed to thromboembolisms and infections.
- The list of causes of secondary WAHA is extensive but notably includes medications, autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis, deficiency of the. immune system (immunodeficiency), leukemias and lymphomas, infections, and pregnancy.
- Identifying the cause of secondary WAHA is important, as it might influence the treatment and management of the underlying condition.

Laboratory Findings

- The haematological and biochemical findings are typical of an extravascular haemolytic anaemia with spherocytosis prominent in the peripheral blood .
- TheDATis positive as a result of IgG,
- The antibodies both on the cell surface and free in serum are best detected at 37°C.

Diagnosis

- Blood tests show an elevated value of immature red blood cells (reticulocytes), which occurs when the body is forced to roduce extra red blood cells to make up for those that are destroyed prematurely.
- Some individuals with hemolytic anemia have elevated values of bilirubin in the blood (hyperbilirubinemia).

- Hemolytic anemia also leads to increased values of lactate dehydrogenase (LDH) in the blood, as it is released from destroyed red blood cells.

.....

Haptoglobin is a hemoglobin scavenger that gets consumed when increased values of hemoglobin are released in the blood due to hemolysis. Haptoglobin values are therefore low in hemolytic anemia.

When hemolytic anemia is suspected to be autoimmune in origin, specialized tests such as a Coombs test may be performed. This test is used to detect antibodies that act against red blood cells. A sample of blood is taken and then exposed to the Coombs reagent. A positive test is indicated when the red blood cells clump in the presence of the reagent. The autoantibodies seen in WAHA are notable for being of the IgG subtype in most cases and being active at body temperature .

Depending on the case, further testing might be performed to attempt to identify a cause of secondary WAHA. The following sequence allows the diagnosis of WAHA:

1) detection of anemia with increased reticulocyte counts
2) determination that the anemia is caused by hemolysis, based on elevated bilirubin and LDH and low haptoglobin.
3) determination that WAHA is the cause of hemolytic anemia with a Coombs test.
4) possible investigation for a secondary cause of WAHA.

Affected Populations

- WAHA affects 1 to 3 people per 100,000 each year in the general population. A total of about 1 in 8,000 individuals live with this condition.
- People of any age, including children, may develop WAHA, but it is more common among adults, with a peak incidence between 50-70 years.
- The median age at onset is 52 years.
- It is possibly slightly more common in women compared to men.
- Secondary WAHA is more common in people with predisposing conditions, such as those with lymphomas and leukemias or those with a disease affecting the immune system.

B. Cold autoimmune haemolytic anaemias

In these syndromes the autoantibody, whether monoclonal (as in the idiopathic cold haemagglutinin syndrome or associated with lymphoproliferative disorders) or polyclonal (as following infection, e.g. infectious mononucleosis or Mycoplasma pneumonia) attaches to red cells mainly in the peripheral circulation where the blood temperature is cooled.

- Theantibody is usually IgM and binds to red cells best at 4° C . IgM antibodies are highly efficient at fixing complement and both intravascular and extravascular haemolysis can occur.
- Mild jaundice and splenomegaly may be present. The patient may develop acrocyanosis at the tip of the nose, ears, fingers and toes caused by the agglutination of red cells in small vessels.

……..

Laboratory findings

Are similar to those of warm AIHA except that spherocytosis is less marked, red cells agglutinate in the cold and serum shows a high titre of " cold " autoantibodies to red cells.

Synonyms of Cold Agglutinin Disease

- CAD
- Cold agglutinin hemolytic anemia
- Cold antibody hemolytic anemia
- Cold antibody disease

Affected Populations

- ✓ CAD most commonly affects people between the ages of 40 and 80.
- ✓ The median age at symptom onset is around 65 years, meaning that half of the affected individuals develop symptoms before this age, and the other half after this age.
- ✓ The disease is present in about 16 people per million (prevalence), and develops in one person per million every year (incidence).
- ✓ The disease is almost twice as common in women compared to men.

2. Alloimmune HaemolyticAnaemias

In these anaemias, antibody produced by one individual reacts with red cells of another.

Two important situations are:

1- Transfusion of ABO- incompatible blood.

2- Rh disease of the new born.

The increased use of allogeneic transplantation for renal, hepatic, cardiac and bone marrow diseases has led to the recognition of alloimmune haemolytic anaemia resulting from the production of red cell antibodies in the recipient by donor lymphocytes transferred in the allograft.

……..

3. Drug- Induced Immune Haemolytic Anaemias

- ✓ Drugs can cause immune haemolytic anaemias via three mechanisms :

1. Antibody directed against a drug– red cell membrane complex (e.g. penicillin, ampicillin). This only occurs with massive doses of the antibiotic.

2. Deposition of complement via a drug– protein– antibody complex onto the red cell surface (e.g. quinidine).

3. A true autoimmune haemolytic anaemia in which the role of the drug is unclear (e.g. methyldopa).

In each case, the haemolytic anaemia gradually disappears when the drug is discontinued.

Red Cell Fragmentation Syndromes

- This is caused by red cells passing through abnormal small vessels and may be caused by deposition of fibrin strands often associated with disseminated intravascular coagulation (DIC) or platelet adherence as in thrombotic thrombocytopenic purpura (TTP).
- Theperipheral blood contains many deeply staining red cell fragments.
- These arise through physical damage to red cells either on abnormal surfaces (artificial heart valves or arterial grafts), arteriovenous malformations, or microangiopathic hemolytic anemia.

Chemical and physical agents

- ✓ Certain drugs in high doses cause oxidative intravascular haemolysis with Heinz body formation in normal subjects.
- ✓ Chemical poisoning (e.g. with lead or arsine) can cause severe haemolysis.
- ✓ Severe burns damage red cells causing acanthocytosis or spherocytosis.

Secondary HaemolyticAnaemias

- ✓ In many systemic disorders red cell survivalis shortened. This is may contribute to anaemia.

Paroxysmal Nocturnal Haemoglobinuria

- ✓ PNHoccurs when mutations lead to loss of PIGAgene.
- ✓ The mutations happen . in "hematopoietic stem cells." that led to production of abnormal blood cells.
- ✓ The loss of the PIGA gene means that the red cell lack a protective protein layer on the outside of its membrane.
- ✓ It's not a disease that can inherit from parents. The disease is acquired through genetic mutations that occur throughout the patientlife.

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

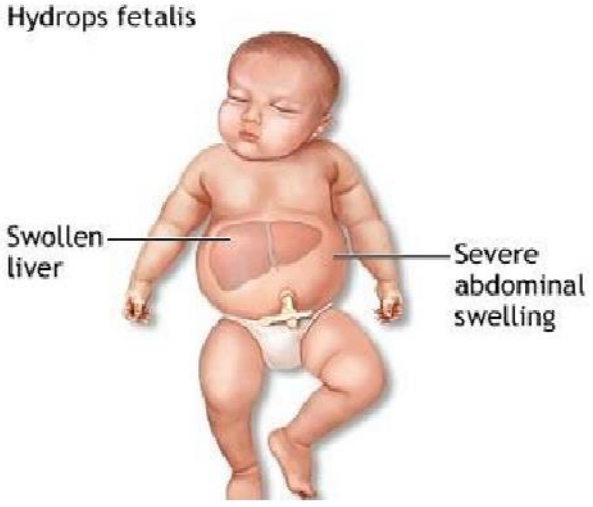
9th - Week

Lecture Title: Thalassemia

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for the students in identifying the concept of thalassemia (Classification, Causes, Clinical features, Symptoms, Signs, laboratory findings, diagnosis).

Special Objectives of the Module

1. Definition of thalassemia.
2. Classification of thalassemia.
3. Differentiate between the types of thalassemia.
4. Explain each type with (Causes, Clinical features, Symptoms, Signs, laboratory findings, diagnosis).

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.
2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.
3. Learning Guide Series Hematology.
4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas.
Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

Thalassemia was first recognized in **1925** by a Detroit physician, Cooley and Lee, who described a series of infants who became profoundly anemic and developed splenomegaly and bone change over the first year of life.

George and William **(1932)**, described the pathological changes of the condition for the first time, recognized that many of their patients came from the Mediterranean region, and hence invented the word thalassemia from the Greek words ("thalassa": meaning sea and ("aima": meaning blood).It was only after **1940** that the true genetic character of this disorder was fully appreciated.

Thalassemia is considered the most common genetic disorder orldwide. Thalassemia is found in some **60** countries with the highest prevalence in the Mediterranean region, parts of North and West Africa, the Middle East, the Indian subcontinent, southern Far East and southeastern Asia, especially Thailand and southern China

Definition

Thalassemia are a heterogeneous group of genetic disorder of hemoglobin synthesis characterized by a reduction in the synthesis of one or more of the globin's chains leads to imbalanced globin-chain synthesis, defective hemoglobin production causing anemia.

Classification

The two main types are called

Alpha thalassemia.

Beta thalassemia.

Depending on which part of globin chain is produced in reduced amounts .

.....

Alpha thalassemia

Normally, alpha globin chain is made by four genes (two from each parent), two on each strand of chromosome 16. The alpha halassemia are caused by a decrease in production of alpha globin's chains due to deletion or mutation of one or more of the four alpha globin's genes located on chromosome 16 Two α thalassemia phenotypes are recognized ,The former phenotype has been referred to as

- **α -thalassemia 1**
- **α -thalassemia 2.**

It is now recognized that the α- thalassemia 1 determinants are associated with complete absence of α-globin synthesis and the α thalassemia 2 phenotypes with only a reduction in α-globin synthesis. Accordingly, these two major α thalassemia variants are now designated thalassemia α° and α+

Alpha (0) thalassemia– More than 20 different

genetic mutations that result in the functional deletion of both pair of α-globin genes have been identified. Individuals with this isorder are not able to produce any functional α-globin and thus are unable to make any functional hemoglobin A, F, or A2.

Alpha (+) thalassemia– There are more than 15 different genetic mutations that result in decreased production of α-globin usually due to the functional deletion of 1 of the 4 alpha globin genes. ased on the number of inherited alpha genes, alpha (+) thalassemia is ub classified into 4 general forms:

A-Thalassemia (-α/α α) is characterized by inheritance of 3 normal α-genes. These patients are referred to clinically as silent carrier of alpha thalassemia. Other names for this condition are alpha thalassemia minima, alpha thalassemia-2 trait, and heterozygosity for alpha (+) thalassemia minor. The affected individuals exhibit no abnormality clinically and may be hematologically normal or have mild reductions .

B-Inheritance of 2 normal alpha genes due to either

Heterozygosity for alpha (+) thalassemia (-α /- α) (one from each of two chromosomes) called a "trans deletion“-- Homozygosity for alpha (+) thalassemia (α α/--) (two on the same chromosome) called a "cis deletion" results in the development of alpha thalassemia minor or alpha thalassemia-1 trait. When parents are carriers of the cis deletion, there is a one in four, or 25 percent, chance with each pregnancy, to have a baby with alpha thalassemia major.

C- Inheritance of one normal alpha gene (-α/--) results in abundant formation of hemoglobin H composed of tetramers of excess beta chains. This condition is known as Hb Hdisease.

D- The loss of all four alpha genes produces a condition that is incompatible with life. The gamma chains produced during fetal life associate in groups of four to form an abnormal hemoglobin called "hemoglobin Bart's"

There are four subtypes of alpha thalassemia that range from mild to sever in their effect on the body :

(1) Silent carrier state

This is the one-gene deletion alpha thalassemia condition. This condition generally causes no symptoms or signs of anemia and will not need treatment because the lack of alpha protein is so small that the hemoglobin functions normally. It is called "silent carrier" because it is difficult to identify α thalassemia silent carrier state by standard hematological studies. They are detected only by DNA Studies

(2) Alpha Thalassemia Trait

Also known as mild alpha-thalassemia. These patients have lost two alpha globin genes. Patients with this condition have small red cells and a mild anemia but they do not have clear symptoms. They look and feel normal but may be discovered upon routine testing.

(3) Alpha Thalassemia

Intermedia Also known as hemoglobin H disease . These patients have lost three alpha globin genes. Patients with this condition have a severe anemia, and often require blood transfusions to survive. Infants born with alpha thalassemia intermedia appear normal at birth but often develop anemia and splenomegaly by the end of their first year. Hepatomegaly is not a common finding and there may be some association with mental retardation. Due to the hemolytic nature of this anemia, there may be an increase in respiratory infections, leg ulcers and gallstones. Skeletal changes are not commonly seen in hemoglobin H disease.

The severe imbalance between the alpha chain production (now powered by one gene, instead of four) and beta chain production (which is normal) causes an accumulation of beta chains inside the red blood cells. Normally, beta chains pair only with alpha chains. With three-gene deletion alpha thalassemia, however, beta chains begin to associate in groups of four, producing abnormal hemoglobin, called "hemoglobin H". The condition is called "hemoglobin H disease".

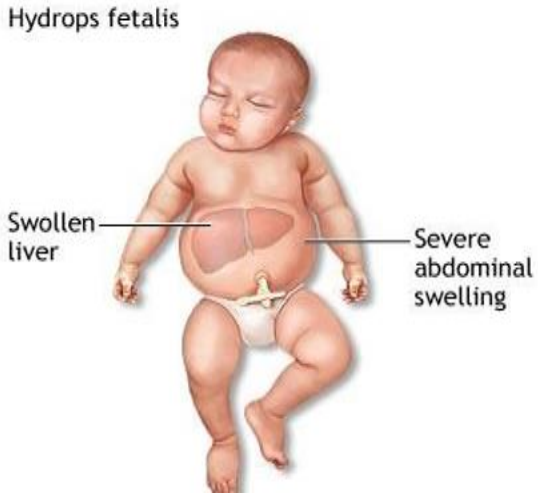
Hemoglobin H has two problems

. • First it does not carry oxygen properly, making it functionally useless to the cell.

• Second, hemoglobin H protein damages the membrane that surrounds the red cell, accelerating cell destruction. The combination of the very low production of alpha chains and destruction of red cells in hemoglobin H disease produces a severe, life-threatening anemia. Untreated, most patients die in childhood or early adolescence

(4) Alpha Thalassemia Major

Also known as hydrops fetalis. In this condition, there are no alpha genes in the individual's DNA, which causes four gamma globins produced by the fetus to form abnormal hemoglobin called hemoglobin Bart's. Most individuals with this condition die before or shortly after birth.



Beta thalassemia

There are more than 200 mutations within the beta-globin gene found worldwide to produce beta-thalassemia.Unlike the deletion that constitutes most of the alpha thalassemia syndromes,beta-thalassemia is caused by a mutation on **chromosome 11** that affect all aspect of beta-globin production: transcription, translation, and the stability of the beta-globin production.

The molecular defects in β thalassemia result in absent or reduced β chain production .Alpha chain synthesis is unaffected and hence there is imbalanced globin chain production leading to an excess of α chains. In the absence of their partners, they are unstable and precipitate in the red cell precursors, giving rise to large intracellular inclusions, which interfere with red cell maturation. Hence, there is a variable degree of intramedullary destruction of red cell precursors (i.e. ineffective erythropoiesis). Those red cells that mature and enter the circulation contain α chain inclusion, which interfere with their passage through the microcirculation, particularly in the spleen.

These cells, which show a variety of abnormalities of membrane structure and permeability, are prematurely destroyed and thus the anemia of β thalassemia results from both ineffective erythropoiesis and a shortened cell survival. The anemia acts as a stimulus to erythropoietin production and this causes expansion of the bone marrow, which may lead to serious deformities of the skull and long bones. Because the spleen is being constantly bombarded with abnormal red cells, it hypertrophies .

There are three general categories of beta thalassemia that also range from mild to severe in their effect on the body.

(1)Beta thalassemia Minor

Also known as thalassemia Trait. In this condition, one of the two beta globin genes is abnormal but the lack of beta protein is not great enough to cause problems in the normal functioning of the hemoglobin. Alpha chain production continues at a near normal rate. The alpha chains combine with the available beta chains resulting in decreased levels of hemoglobin A here still remains excess alpha chains and this stimulates the increased production of delta chains. The alpha and delta chains combine to form increased amounts of hemoglobin A2.

This if there is still an excess of alpha chains the normal mechanism which switches off gamma chain production does not function correctly and the rate of gamma chain Production is greater than in a normal adult. results in the formation of increased amounts of hemoglobin F A person with this condition simply carries the genetic trait for thalassemia and have a 50/50 chance to pass the gene to their offspring, who would also have thalassemia minor and will usually experience no health problems other than possible mild anemia .

(2) Beta thalassemia

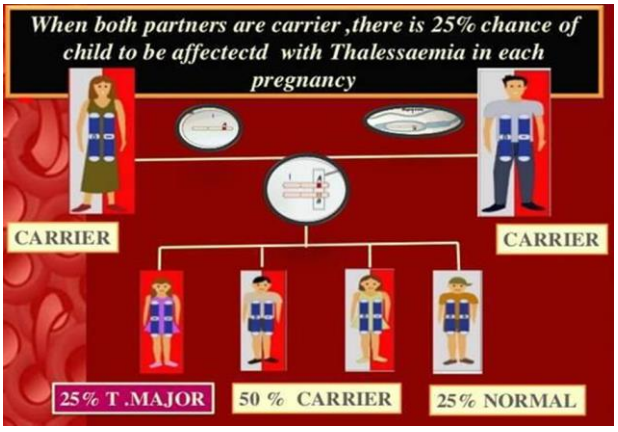
Intermedia In this condition, an affected person has two abnormal genes but is still producing some beta globin. In this condition the lack of beta protein in the hemoglobin is great enough to cause a moderately severe anemia and significant health problems, including fatigue or shortness of breath, bone deformities, mild jaundice and enlargement of the spleen.

(3) Beta thalassemia Major

It is also called Cooley's anemia , named after the doctor who first described it in 1925. Beta thalassemia Major is the most severe form of beta thalassemia in which the complete lack of beta globin production, preventing the production of significant amounts of Hb A. The severe imbalance of globin chain synthesis (alpha >> beta) results in ineffective erythropoiesis and severe microcytic hypochromic anemia. The excess unpaired alpha-globin chains aggregate to form precipitates that damage red cell membranes, resulting in intravascular hemolysis. Premature destruction of erythroid precursors results in intramedullary death and ineffective erythropoiesis.

The profound anemia typically is associated with erythroid hyperplasia and extramedullary hematopoiesis At birth the baby with thalassemia major seems entirely normal. This is because the predominant hemoglobin at birth is still fetal hemoglobin (Hb F). Hb F has two alpha chains (like Hb A) and two gamma chains (unlike Hb A). It has no beta chains so the baby is protected at birth from the effects of thalassemia major. Anemia begins to develop within the first year after birth. It becomes progressively more and more severe. The infant fails to thrive (to grow normally) and often has problems feeding (due to easy fatigue from lack of oxygen, with the profound anemia), bouts of fever (due to infections) to which the severe anemia the child and diarrhea and other intestinal problems.

Without treatment, the spleen, liver, and heart become enlarged, and bones can become thin and brittle ,the result is death before age twenty. This anemia requires lifelong regular blood transfusions and considerable ongoing medical care. Over time, these frequent transfusions lead to excessive amounts of iron in the body. Left untreated, this excess iron can deposit into the liver, heart and other organs and can lead to a premature death from organ failure



β thalassemia major

• The pathophysiologic mechanisms that result from a lack of β chain production can be classified into four categories

• reduced Hb A

• compensatory production of other hemoglobin

• ineffective erythropoiesis with hemolysis

• erythroid hyperplasia

• In β thalassemia major reduced synthesis of β chains results in an

excess of free α chains and a β to α chain ratio of <0.25 .

• The excess free α chains cannot form hemoglobin tetramers, so they

• precipitate within the cell, damaging the cell membrane, and leading to chronic hemolysis

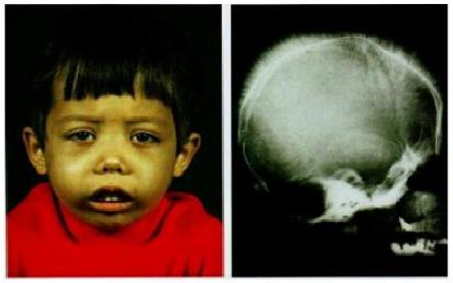
.....

β thalassemia major

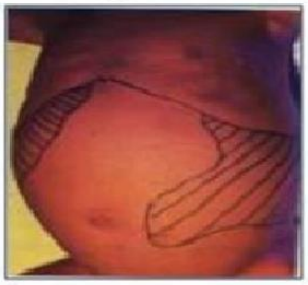
Clinical feature of β thalassemia major :

1. Sever anemia
2. Enlargement spleen and liver
3. Expansion of bone and Osteoporosis
4. Iron overload (increase destruction of RBC)
5. Infection (Hepatitis B&C)
6. Liver disease and Hepatocellular carcinoma
7. Jaundice which is a yellowing of the skin or the whites of the eyes

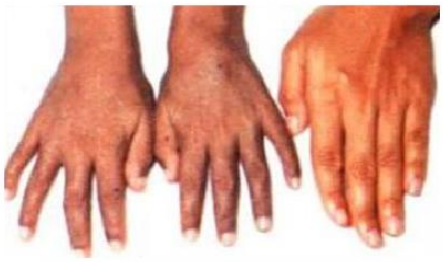
β thalassemia major



β Thalassemia Major



Hepatosplenomegaly



Dark skin due to iron overload

β thalassemia major

Laboratory Results :

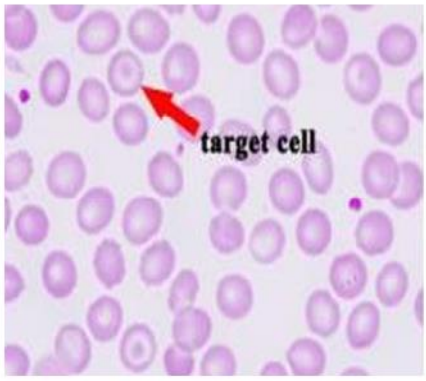
• The hemoglobin level can be as low as 2 or 3 g/dL (20–30 g/L) in the

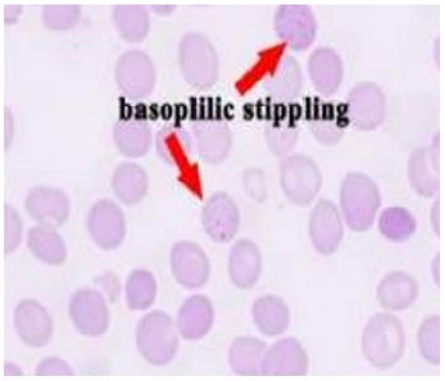
• more severe forms of the disease.

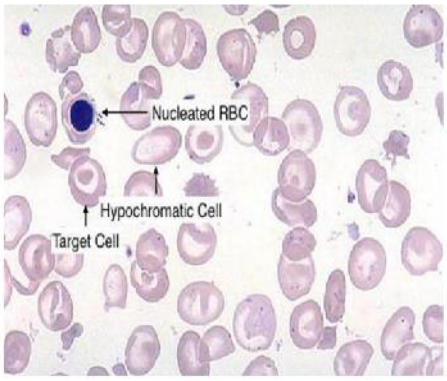
• The anemia is markedly microcytic and hypochromic with an MCV of <67 fL and a markedly reduced MCH and MCHC.

• The peripheral blood smear shows marked anisocytosis and poikilocytosis

β thalassemia major







β thalassemia minor

•The RBC count is within the reference interval or slightly elevated.

•the Hb A level is 92% to 95%

•the Hb A2 level is characteristically elevated and can vary from 3.5% to 7.0%.

……

3-Non transfusion dependent (intermediate) :

• is a term used to describe anemia that is more severe than β thalassemia minor but does not require regular transfusions to maintain hemoglobin level and quality of life (transfusion independent)

• patients with β thalassemia intermedia typically maintain a hemoglobin level greater than 7 g/dL, it is the clinical features rather than the emoglobin level that determine the diagnosis

• Moderate severe of thalassemia

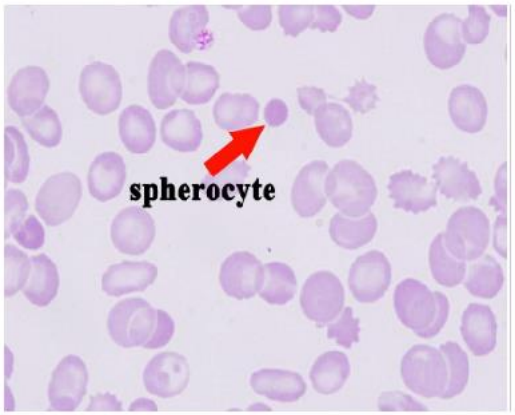
• Not need for regular blood transfusion

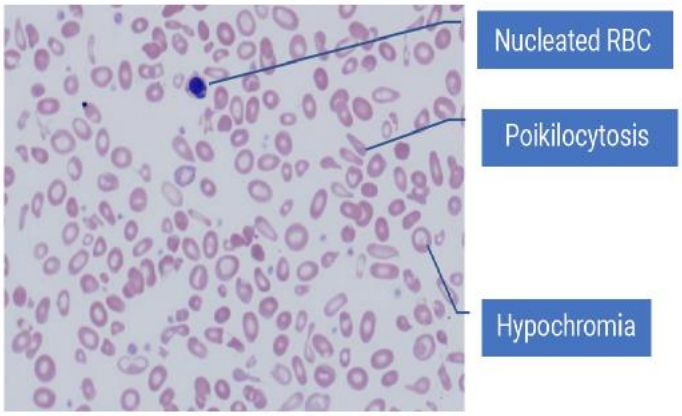
• Caused by either mild defect in β chain synthesize or by β thalassemia trait in association with mild globin abnormalities such as Hb Lepore.

……

Laboratory diagnosis for general types of thalassemia

1- There is a severe hypochromic, microcytic anaemia with target cells and basophilic stippling in the blood film

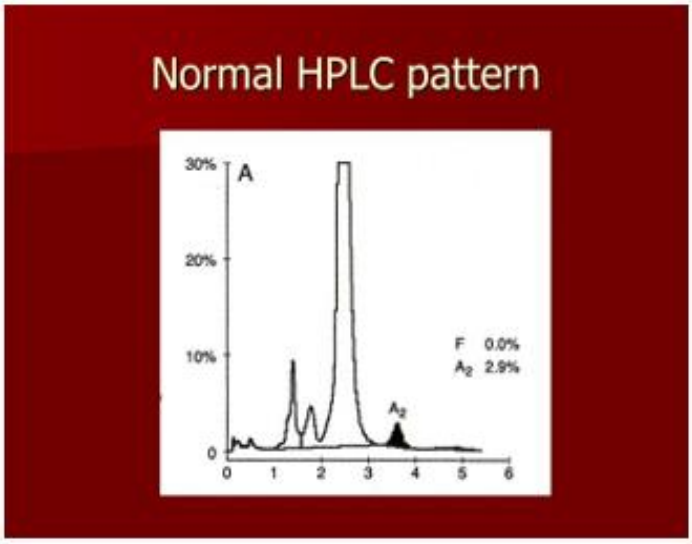




Laboratory diagnosis for general types of thalassemia

2- High performance liquid chromatography (HPLC) is now usually used as the first-line method to diagnose haemoglobin disorders.

3- DNA analysis is used to identify the defection each allele important in antenatal



Laboratory diagnosis for general types of thalassemia

4- haemoglobin electrophoresis show :

a- reveals absence or almost complete absence of Hb A

b- with almost all the circulating haemoglobin being Hb F.

C- The Hb A2 percentage is normal, low or slightly raised

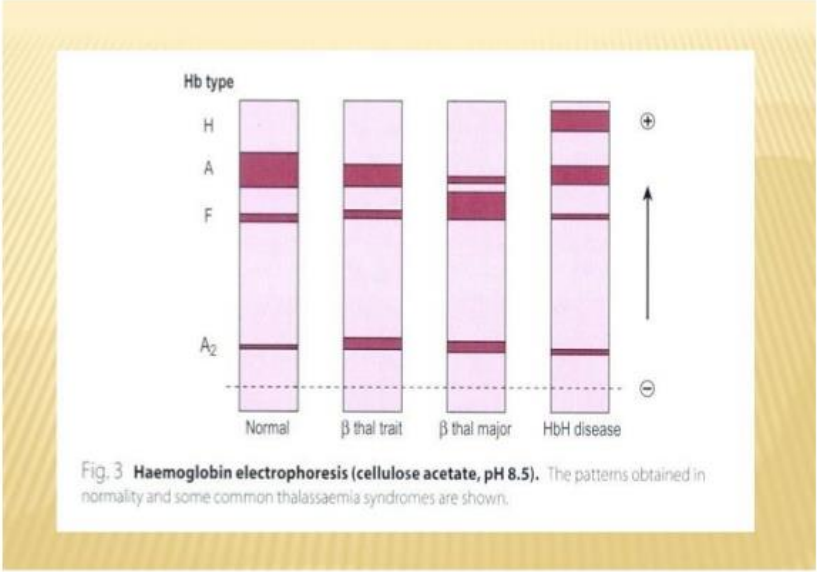


Fig. 3 **Haemoglobin electrophoresis (cellulose acetate, pH 8.5).** The patterns obtained in normality and some common thalassaemia syndromes are shown.

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

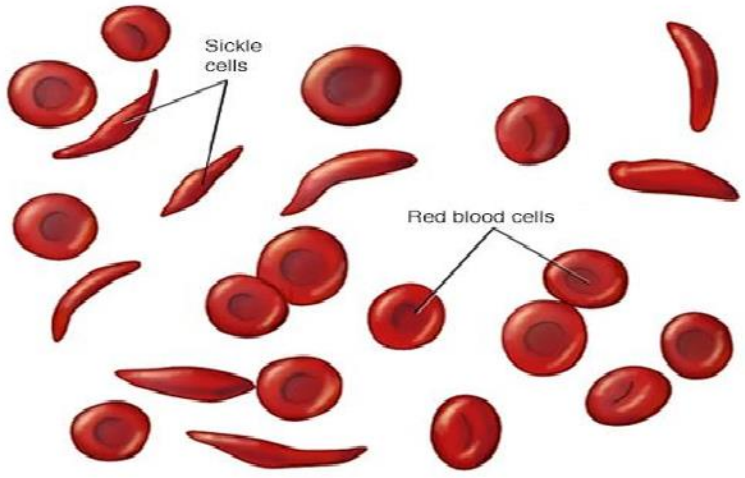
10th - Week

Lecture Title: Sickle cell anaemia

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for the students in identifying the concept of Sickle cell anaemia (Causes, Clinical features, Symptoms, Signs, laboratory findings, diagnosis).Special Objectives of the Module

1. Definition of Sickle cell anaemia.

2. Explain the causes, Clinical features, Symptoms, Signs, laboratory

findings, and diagnosis of Sickle cell anaemia).

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

Sickle Cell Animea

- ✓ Sickle cell anemia, or sickle cell disease (SCD), is a genetic disease of the red blood cells (RBCs). Normally, RBCs are shaped like discs, allowing them to travel through even the smallest blood vessels. However, with this disease, the RBCs have an abnormal crescent shape resembling a sickle. This makes them sticky, rigid, and prone to getting trapped in small vessels, which blocks blood from reaching different body parts. This can cause pain and tissue damage.
- ✓ SCD is an autosomal recessive condition. You need two copies of the gene to have the disease. If you have only one copy of the gene, you are said to have a sickle cell trait.
- ✓ Asickle cell disease is a group of hemoglobin disorders resulting from the inheritance of the sickle β-globin gene.
- ✓ The sickle β-globin abnormality is caused by the substitution of valine for glutamic acid in position 6 in the β chain.
- ✓ Thesickle-shaped cells are not flexible and cannot change shape easily. Many of them burst apart as they move through your blood vessels.
- ✓ The sickle cells usually only last 10 to 20 days, instead of the normal 90 to 120 days. Your body may have trouble making enough new cells to replace the ones that you lost. Because of this, you may not have enough red blood cells. This is a condition called anemia, and it can make you feel tired.
- ✓ The sickle-shaped cells can also stick to vessel walls, causing a blockage that slows or stops the flow of blood. When this happens, oxygen can't reach nearby tissues. The lack of oxygen can cause attacks of sudden, severe pain, called pain crises. These attacks can occur without warning. If you get one, you might need to go to the hospital for treatment.

What causes sickle cell disease (SCD)?

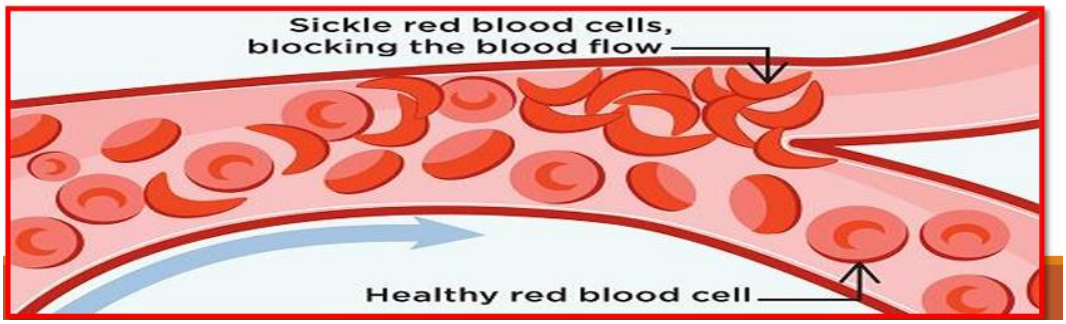
The cause of SCD is a defective gene, called a sickle cell gene. People with the disease are born with two sickle cell genes, one from each parent. If you are born with one sickle cell gene, it's called the sickle cell traits. People with sickle cell trait are generally healthy, but they can pass the defective gene on to their children.

Who is at risk for sickle cell disease (SCD)?

In the United States, most of the people with SCD are African Americans:

•About 1 in 13 African American babies is born with the sickle cell trait

•About 1 in every 365 black children is born with sickle cell disease SCD also affects some people who come from Hispanic, southern European, Middle Eastern, or Asian Indian backgrounds.



What are the types of sickle cell disease?

Hemoglobin is the protein in red blood cells that carries oxygen. It normally has two alpha chains and two beta chains. The four main types of sickle cell anemia are caused by different mutations in these genes. People who have sickle cell disease have abnormal hemoglobin, called hemoglobin S or sickle hemoglobin, in their red blood cell. People who have sickle cell disease inherit two abnormal hemoglobin genes, one from each parent.

.....

The types of sickle cell disease include the following:

- ✓ Hemoglobin Sβ0 thalassemia
- ✓ Hemoglobin Sβ+ thalassemia
- ✓ Hemoglobin SC Hemoglobin SD
- ✓ Hemoglobin SE Hemoglobin SS

Hemoglobin SS disease

Hemoglobin SS disease is the most common type of sickle cell disease. It occurs when you inherit copies of the hemoglobin S gene from both parents. This forms hemoglobin known as Hb SS. As the most severe form of SCD, individuals with this form also experience the worst symptoms at a higher rate.

Hemoglobin SC disease

Hemoglobin SC disease is the second most common type of sickle cell disease. It occurs when you inherit the Hb C gene from one parent and the Hb S gene from the other. Individuals with Hb SC have similar symptoms to individuals with Hb SS. However, the anemia is less severe.

Hemoglobin SB+ (beta) thalassemia

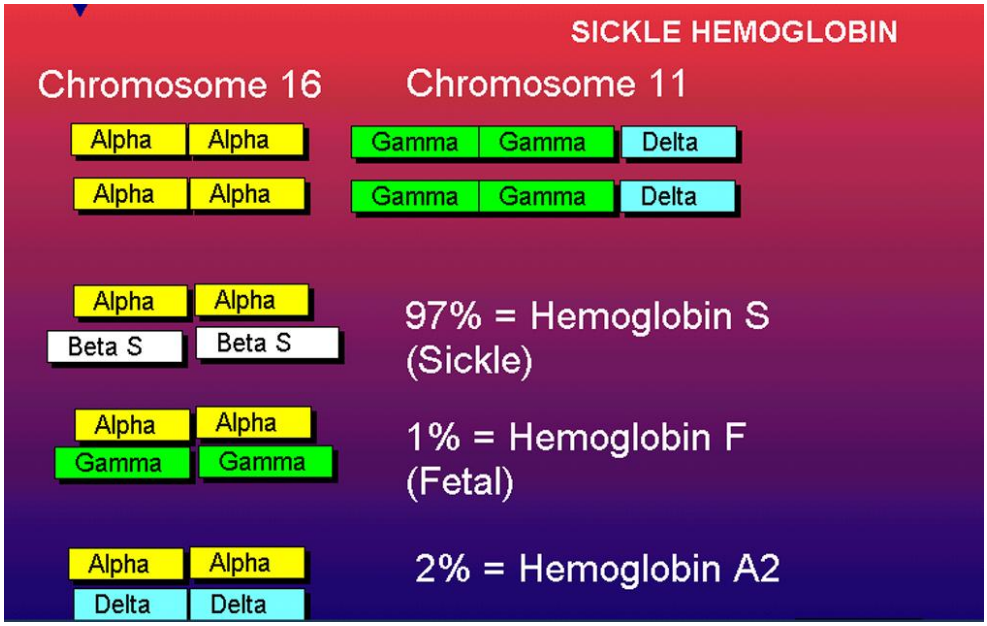
Hemoglobin SB+ (beta) thalassemia affects beta globin gene production. The size of the red blood cell is reduced because less beta protein is made. If inherited with the Hb S gene, you will have hemoglobin S beta thalassemia. Symptoms are not as severe.

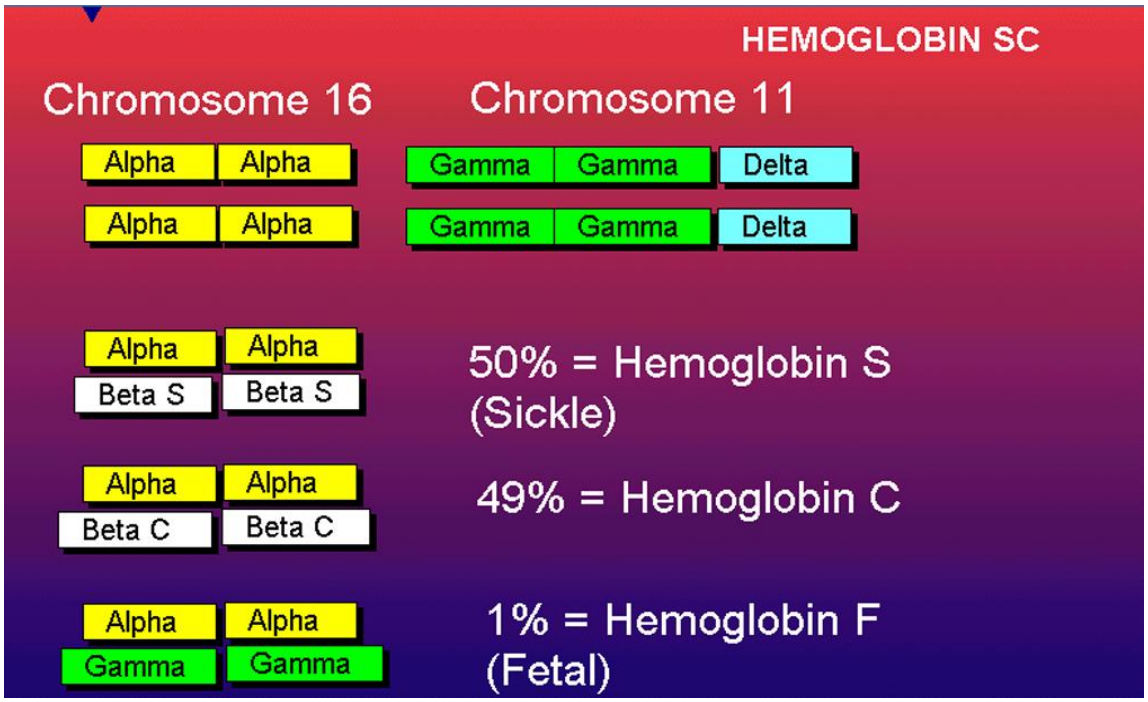
Hemoglobin SB 0 (Beta-zero) thalassemia

Sickle beta-zero thalassemia is the fourth type of sickle cell disease. It also involves the beta globin gene. It has similar symptoms to Hb SS anemia. However, sometimes the symptoms of beta zero thalassemia are more severe.

Hemoglobin SD, hemoglobin SE, and hemoglobin SO

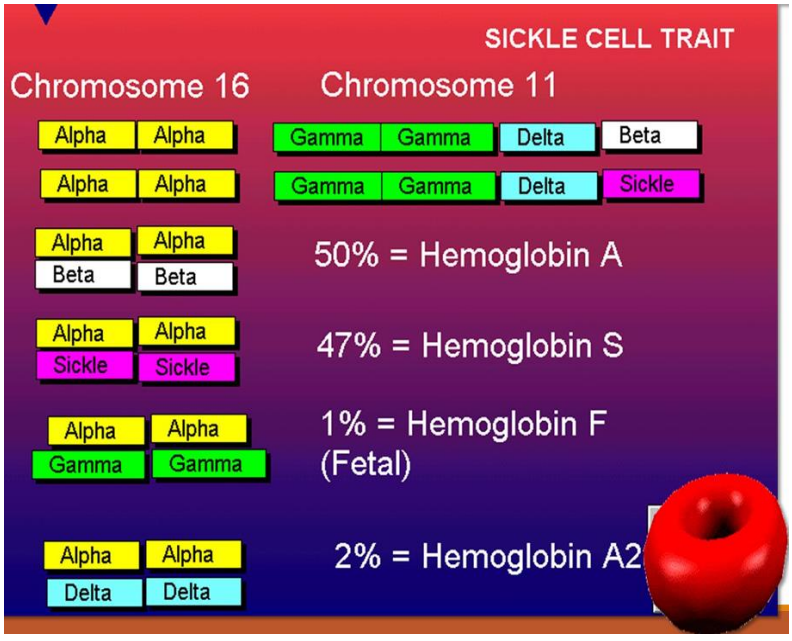
These types of sickle cell disease are rare and usually don't have severe symptoms.

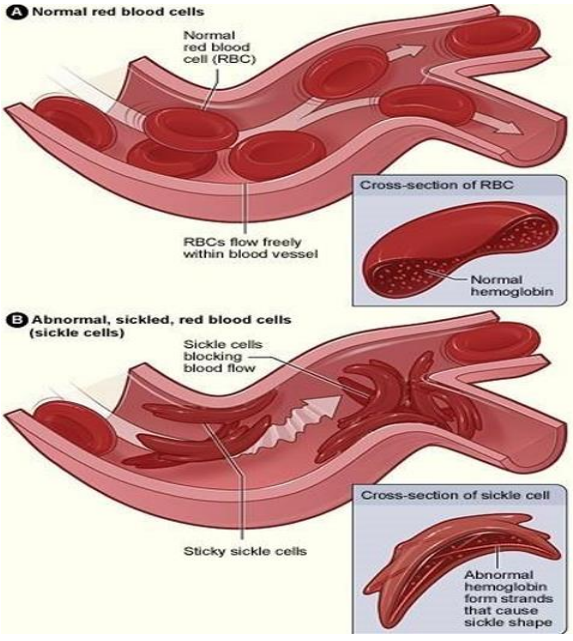




Sickle cell trait

People who only inherit a mutated gene (hemoglobin S) from one parent are said to have sickle cell trait. They may have no symptoms or reduced symptoms.

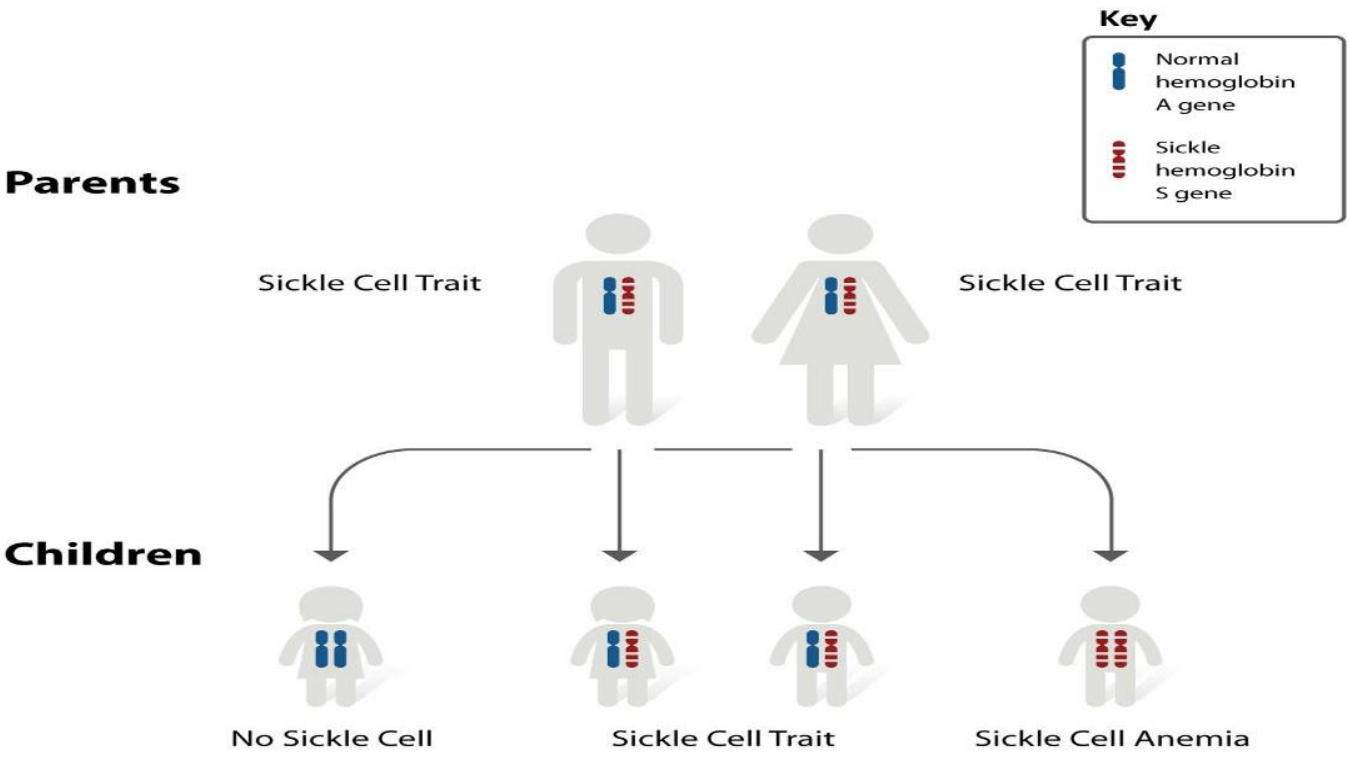




Normal vs. Sickle red cells

How is the hemoglobin S gene inherited?

- ✓ When the hemoglobin S gene is inherited from only one parent, and a normal hemoglobin gene—hemoglobin A—is inherited from the other, that person will have sickle cell trait. People who have sickle cell trait are generally healthy.
- ✓ Only rarely do people who have sickle cell trait have complications similar to those seen ipeople who have sickle cell disease. But people who have sickle cell trait are carriers of a defective hemoglobin S gene, so they can pass it on when they have a child.
- ✓ If the child's other parent also has sickle cell trait or another abnormal hemoglobin gene, such as beta-thalassemia, hemoglobin C, hemoglobin D, or hemoglobin E, that child has a chance of having sickle cell disease.



In the image above, each parent has one normal hemoglobin A gene and one hemoglobin S gene, which means each of their children has:

- ✓ A 25% chance of inheriting two normal hemoglobin A genes. In this case, the child does not have sickle cell trait or disease.
- ✓ A 50% chance of inheriting one normal hemoglobin A gene and one hemoglobin S gene. This child has sickle cell trait.
- ✓ A 25% chance of inheriting two hemoglobin S genes. This child has sickle cell disease.
- ✓ It is important to keep in mind that each time this couple has a child, the chances of that child having sickle cell disease remain the same. In other words, if the firstborn child has sickle cell disease, there is still a 25% chance that the second child will also have the disease. Both boys and girls can inherit sickle cell trait, sickle cell disease, or normal hemoglobin.

What are the symptoms of sickle cell anemia?

Symptoms of sickle cell anemia usually show up at a young age. They may appear in babies as early as 4 months old, but generally occur around the 6 month mark.

While there are multiple types of SCD, they all have similar symptoms, which vary in severity. These include:

- Excessive fatigue or irritability, from anemia
- Fussiness, in babies
- Bedwetting, from associated kidney problems
- Jaundice, which is the yellowing of the eyes and skin

.....

- Swelling and pain in hands and feet
- Frequent infections
- Pain in the chest, back, arms, or legs
- Periods of pain that can last a few hours to a few days.
- Blood clots.
- Joint pain that resembles arthritis.
- Chronic neuropathic pain (nerve pain).

.....

Early signs and symptoms

Early symptoms of sickle cell disease may include:

A yellowish color of the skin, known as jaundice, or whites of the eyes, known as icterus, occurs when a large number of red cells

undergo hemolysis

Fatigue or fussiness from anemia

Painful swelling of the hands and feet, known as Ductility's

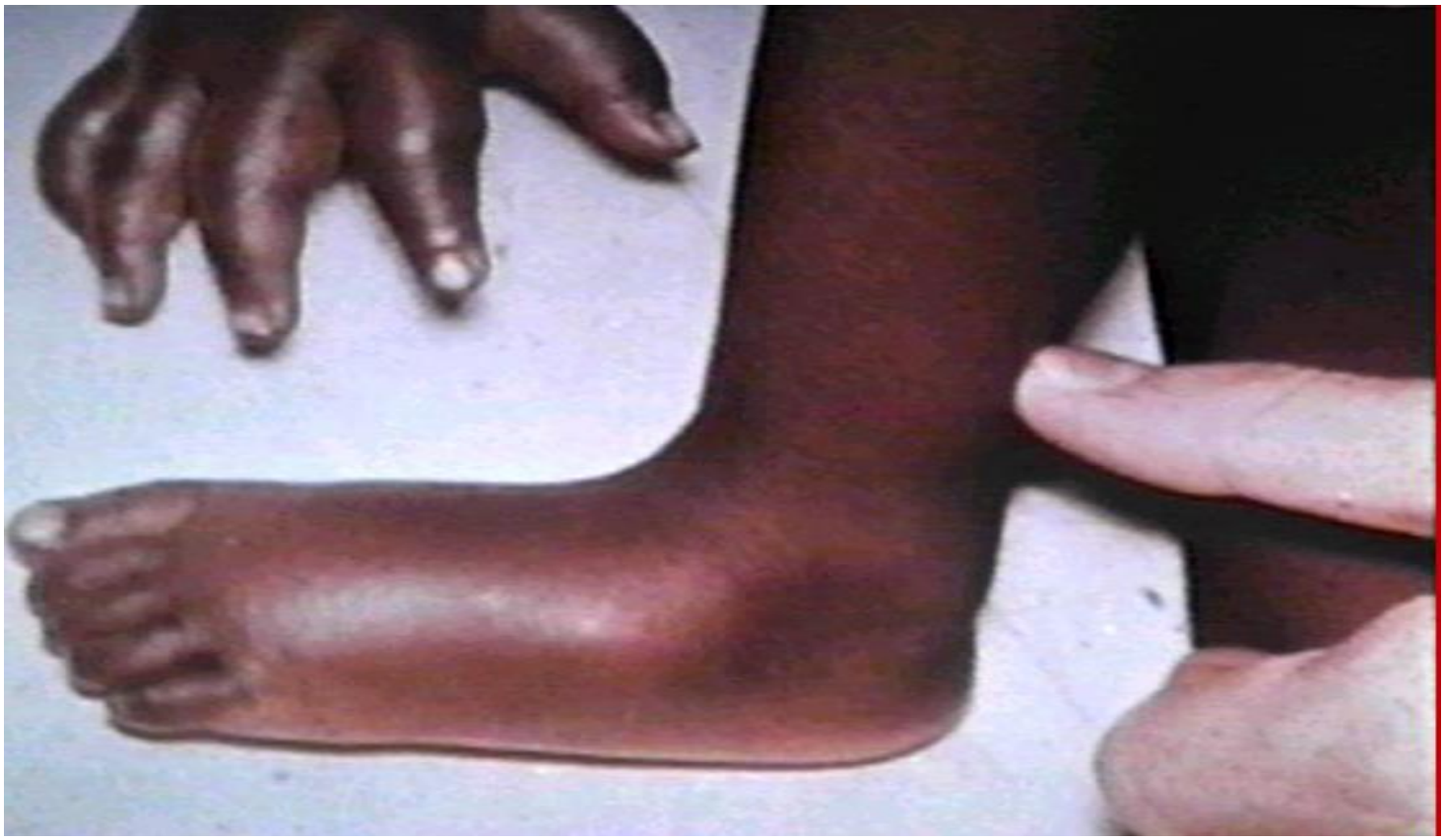
……

Complications

Complications of sickle cell disease include:

•**Acute chest syndrome**. Sickling in the blood vessels of the lungs can deprive lungs of oxygen. This can damage lung tissue and cause chest pain, fever, and difficulty breathing. Acute chest syndrome is a medical emergency.

Acute pain crisis. Also known as sickle cell or vaso-occlusive crisis, this can happen without warning when sickle cells block blood flow. People describe this pain as sharp, intense, stabbing, or throbbing. Pain can strike almost anywhere in the body and in more than one spot at a time. Common areas affected by pain include the abdomen, chest, lower back, or arms and legs. A crisis can be brought on by high altitudes, dehydration, illness, stress, or temperature changes.



•**Delayed growth and puberty**. Children who have sickle cell disease may grow and develop more slowly than their peers because of anemia. They will reach full sexual maturity, but this may be delayed.

•**Eye problems**. Sickle cell disease can injure blood vessels in the eye, most often in the retina. Blood vessels in the retina can overgrow, get blocked, or bleed. This can cause the retina to detach . These problems can lead to vision loss.

•**Gallstones.** When red blood cells break down, in a process called hemolysis, they release hemoglobin. Hemoglobin then gets broken down into a substance called bilirubin. Bilirubin can form stones, called gallstones, that get stuck in the gallbladder.

•**Heart problems**. including coronary heart disease and pulmonary hypertension. Frequent blood transfusions may also cause heart damage from iron overload.

•**Infections**. The spleen is important for protection against certain kinds of infections. If you have sickle cell disease, a damaged spleen raises the risk for certain infections, including chlamydia, salmonella, and staphylococcus.

.....

•**Joint problems.** Sickling in the hip bones and, less commonly, the shoulder joints, knees, and ankles, can decrease oxygen flow and result in a condition called avascular or aseptic necrosis, which severely damages the joints. Symptoms include pain and problems with walking and joint movement. Over time, you may need pain medicines, surgery, or joint replacement.

•**Kidney problems**. Sickle cell disease may cause the kidneys to have trouble making the urine as concentrated as it should be. This may lead to a need to urinate often and to bedwetting or uncontrolled urination during the night. This often starts in childhood.

•**Leg ulcers**. Sickle cell ulcers are sores that usually start small and then get larger and larger. Some ulcers will heal quickly, but others may not heal and may last for long periods of time. Some ulcers come back after healing. People who have sickle cell disease usually do not get ulcers until after age 10.

•Liver problems. Sickle cell intrahepatic cholestasis is an uncommon but severe type of liver damage that happens when sickled red cells block blood vessels in the liver. This blockage prevents enough oxygen from reaching liver tissue. These episodes are usually sudden and may happen more than once. Children often recover, but some adults may have chronic problems that lead to liver failure. Frequent blood transfusions can lead to liver damage from iron overload.

.....

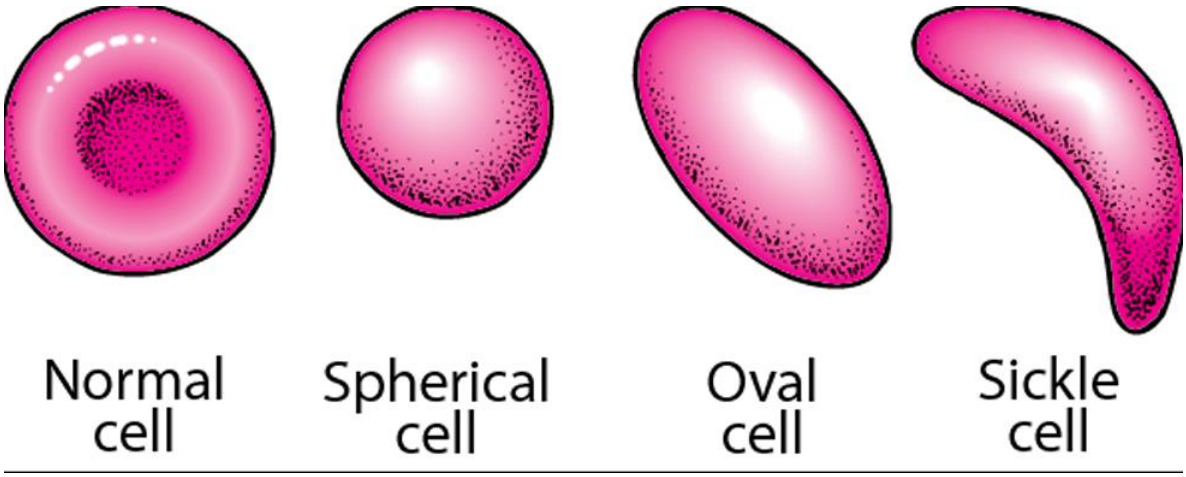
Laboratory findings

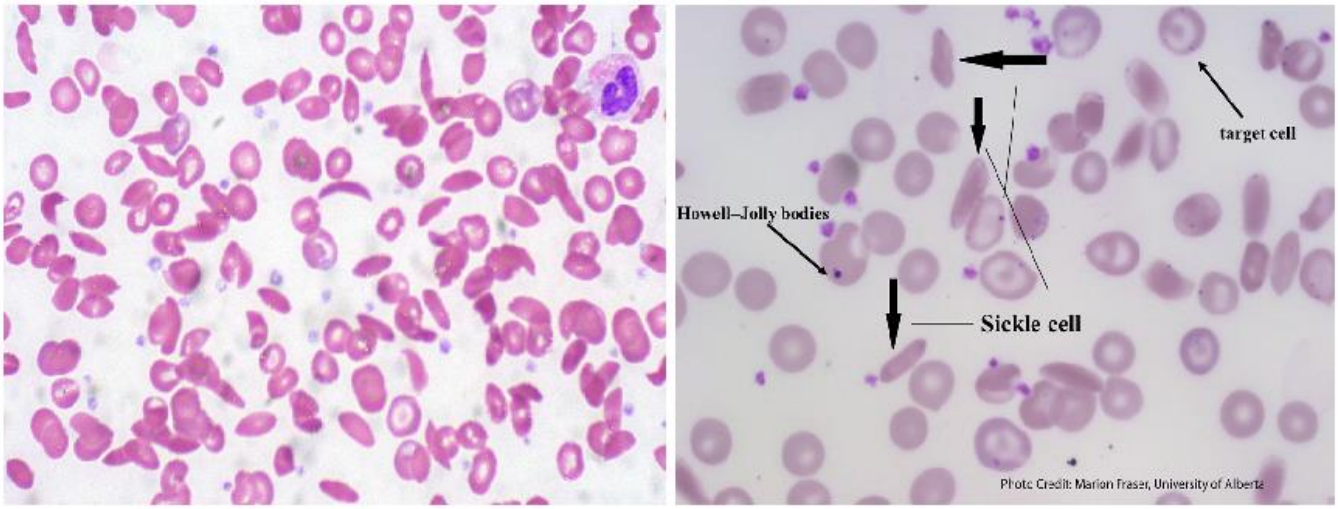
1- The haemoglobin is usually 60–90 g/L – low in comparison to mild or no symptoms of anaemia.

2- Sickle cells and target cells occur in the blood . Features of splenic atrophy (e.g. Howell–Jolly bodies) may also be present.

3- Screening tests for sickling are positive when the blood is deoxygenated.

4- Haemoglobin electrophoresis : in Hb SS, no Hb A is detected. The amount of Hb F is variable and is usually 5–15.





Managing Sickle Cell

- Fluids
- Antibiotics
- Rest
- Pain Medication
- Prevention of infections
- Healthy lifestyle

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

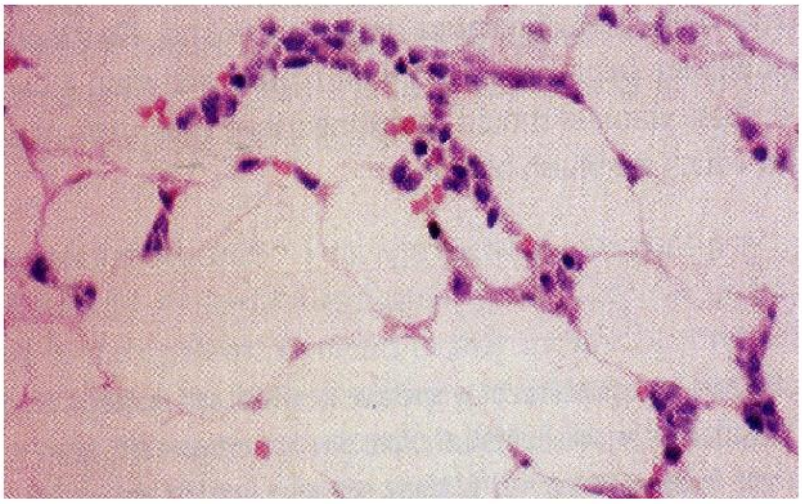
11th - Week

Lecture Title: Aplastic Anaemia.

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for the students in identifying the concept of aplastic anemia, and classification of anemia.

Special Objectives of the Module

1. Definition of aplastic anemia.

2. Explain the causes, Clinical features, Symptoms, Signs, laboratory findings, and diagnosis of aplastic anemia.

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

Aplastic Anemia

• An acquired stem cell disorder.

• Idiopathic (unknown etiology) in 70% of the cases.

• Diagnosis of exclusion.

• Important to differentiate from other causes of Bone Marrow Failure.

• Aplastic anemia is a severe, life-threatening syndrome in which the production of erythrocytes, WBCs, and platelets has failed.

• Aplastic anemia may occur in all age groups and both genders.

• The disease is characterized by peripheral pancytopenia and accompanied by a hypocellular bone marrow.

Pathophysiology

• The primary defect is a reduction in or depletion of ematopoietic precursor stem cells with decreased production of all cell lines. his is what leads to the peripheral pancytopenia.

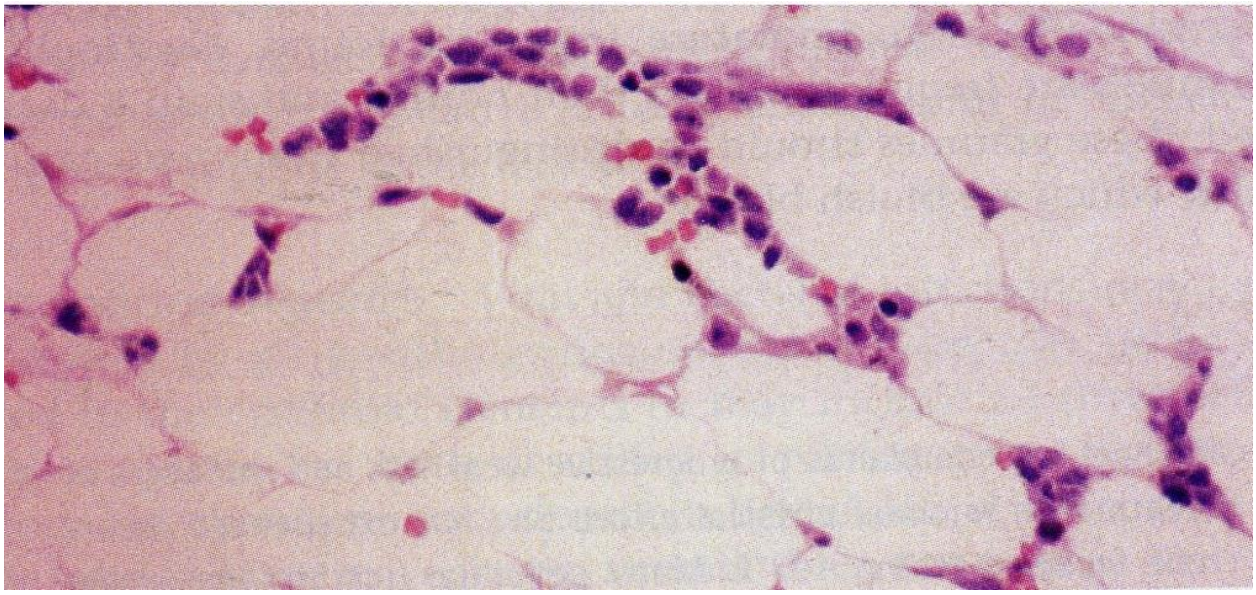
• This may be due to quantitative or qualitative damage to the luripotential stem cell.

• In rare instances, it is the result of abnormal hormonal stimulation of stem cell proliferation or the result of a defective bone marrow microenvironment or cellular or humoral immunosuppression of hematopoiesis.

- Direct damage to DNA/chromosomes by a chemical or radiation.
- Inappropriate immune system activation by a trigger (infection, drugs, autoimmune disorder) which causes immune attack on bone marrow causing failure.

.....

Hypocellular bone marrow in aplastic anemia



.....

Aplastic Anemia: clinical presentation

- Low blood counts
- Anemia fatigue, shortness of breath, pallor
- Thrombocytopenia bleeding, bruising
- Neutropenia infections , fever

.....

Aplastic Anemia: Laboratory Studies

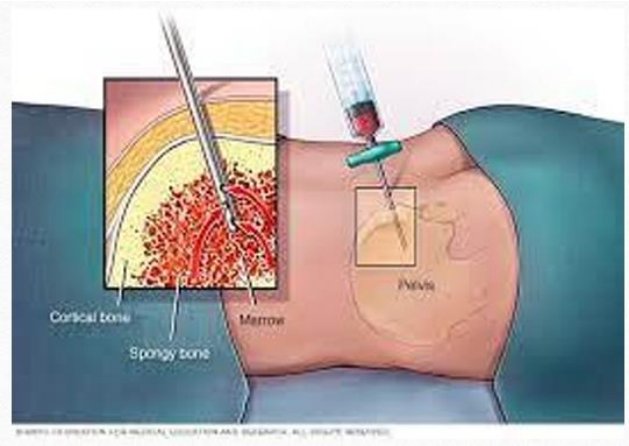
- Pancytopenia (all cell lines are low)
- Low reticulocyte count

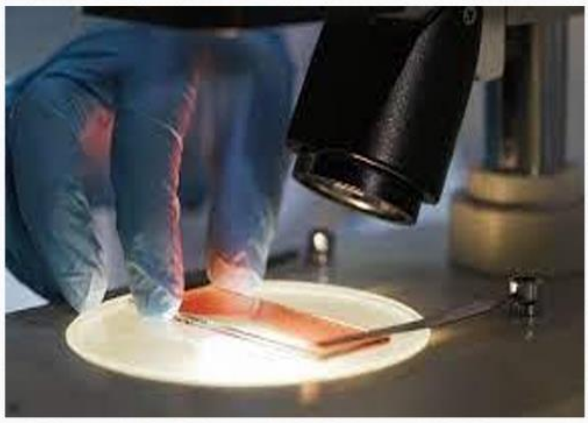
Epidemiology

- 2 per million in Western countries and 4-6 per million in Asia
- 2 incidence peaks: young adults and elderly
- > 25% of pediatric patients and 5-15% of adults < 40 years old have an inherited cause
- First case described in 1888, term “aplastic anemia” first used in 1904.

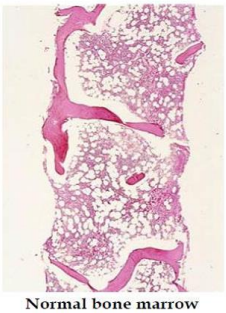
.....

Bone Marrow Biopsy

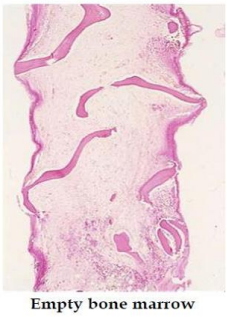




The bone marrow in Aplastic Anemia



Normal bone marrow



Empty bone marrow

Causes of Aplastic Anemia

- Idiopathic (unknown etiology) 75%
- Chemicals (benzene, insecticides, pesticides)
- Radiation
- Pregnancy
- Medications (chloramphenicol, antibiotics, NSAIDs, quinines)
- Infections – HIV, hepatitis, COVID , rarely other viruses- CMV, EBV
- Eating disorders

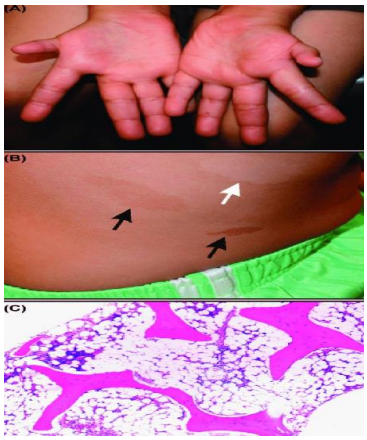
Genetic Bone Marrow Failure syndromes

- Rare, early in life
- Growth failure
- Often associated with physical abnormalities
- Associated with increased risk of leukemia and other cancers

.....

Fanconi Anemia

- Short stature
- Skin hypo/hyperpigmented areas
- Skeletal abnormalities particularly affecting the thumb



Fanconi Anemia

Fanconi's anemia – the disorder usually becomes symptomatic ~ 5 years of age and is associated with progressive bone marrow hypoplasia. Congenital defects individuals. such as skin hyperpigmentation and small stature are also seen in affected Familial aplastic anemia – a subset of Fanconi's anemia in which the congenital defects are absent.

Fanconi Anemia

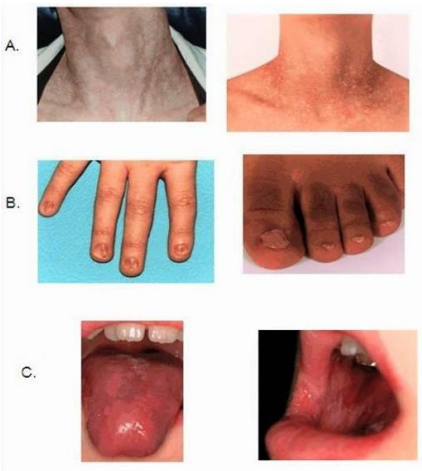
Clinical manifestations

- Fatigue
- Heart palpitations
- Pallor
- Infections
- Petechiae
- Mucosal bleeding

……..

Dyskeratosis Congenita

- Nail dystrophy
- Lacy skin pigmentation
- Oral leukoplakia



Emberger Syndrome

- Peripheral lymphedema
- GATA2 mutation

Aplastic anemia vs Hypoplastic

- Dysplasia is typically in precursors only
- Dysplasia in > 10% of cells in multiple cells lines (red cells, platelets, white cells)
- Cytogenetics abnormalities chromosome 5, 7

Prognosis of Aplastic Anemia

- Untreated, SAA has a one-year mortality of over 70 %

Related disorders

Disorders in which there is peripheral pancytopenia, but the bone cellular elements marrow is normocellular, hypercellular, or infiltrated with bnormal Myelopthesic anemia – replacement of bone marrow by fibrotic, granulomatous, or neoplastic cells.

Pure Red Cell Aplasia

Pure red cell aplasia is characterized by a selective decrease in erythroid precursor cells in the bone marrow. WBCs and platelets are unaffected

Pure Red Cell Aplasia

Acquired

- Transitory with viral or bacterial infections
- Patients with hemolytic anemias may suddenly halt erythropoiesis
- Patients with thymoma– T-cell mediated responses against bone arrow erythroblasts or erythropoietin are sometimes produced.

……

Pure Red Cell Aplasia

Congenital

Diamond-Blackfan syndrome – occurs in young children and is progressive. It is probably due to an intrinsic or regulatory defect in the committed erythroid stem cell.

……

Other hypoproliferative anemias

Renal disease – due to decreased erythropoietin function Endocrine deficiencies – may lead to decreased erythropoietin production. decreased androgens in males; decreased pituitary.

Management

1. Identification and elimination of the underlying cause
2. Supportive therapy
3. Hematopoietic stem cell transplantation
4. Immunosuppressive treatment
5. Androgens
6. Growth factors (GM, CSF, G-CSF, EPO)

.....

Supportive Therapy

- Supplementation of blood products
- Prevention and treatment of hemorrhage
- Prevention and treatment of infection

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

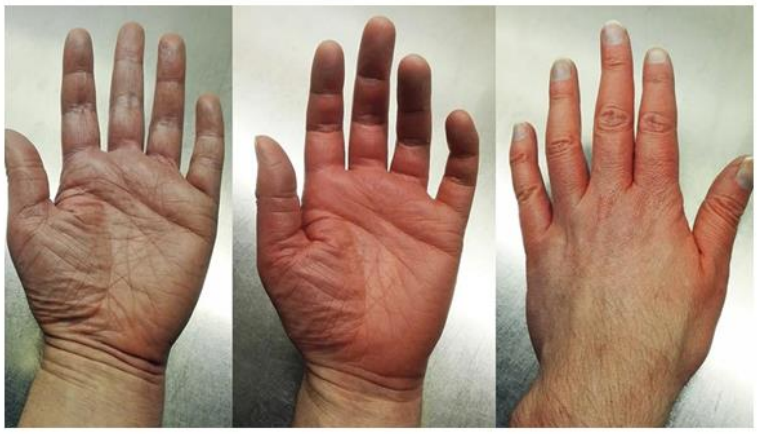
12th - Week

Lecture Title: Polycythaemia.

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for the students in identifying the concept of Polycythaemia.

Special Objectives of the Module

1. Definition of polycythemia.

2. Classification of polycythemia.

3. Explain the causes, Clinical features, Symptoms, Signs, laboratory findings, and diagnosis of Sickle cell polycythaemia).

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

Polycythaemia

Polycythemia is a blood disorder in which the body produces hormone. too many blood cells as a result of a problem with the bone marrow or an increased production of the erythropoietin(EPO) hormone.

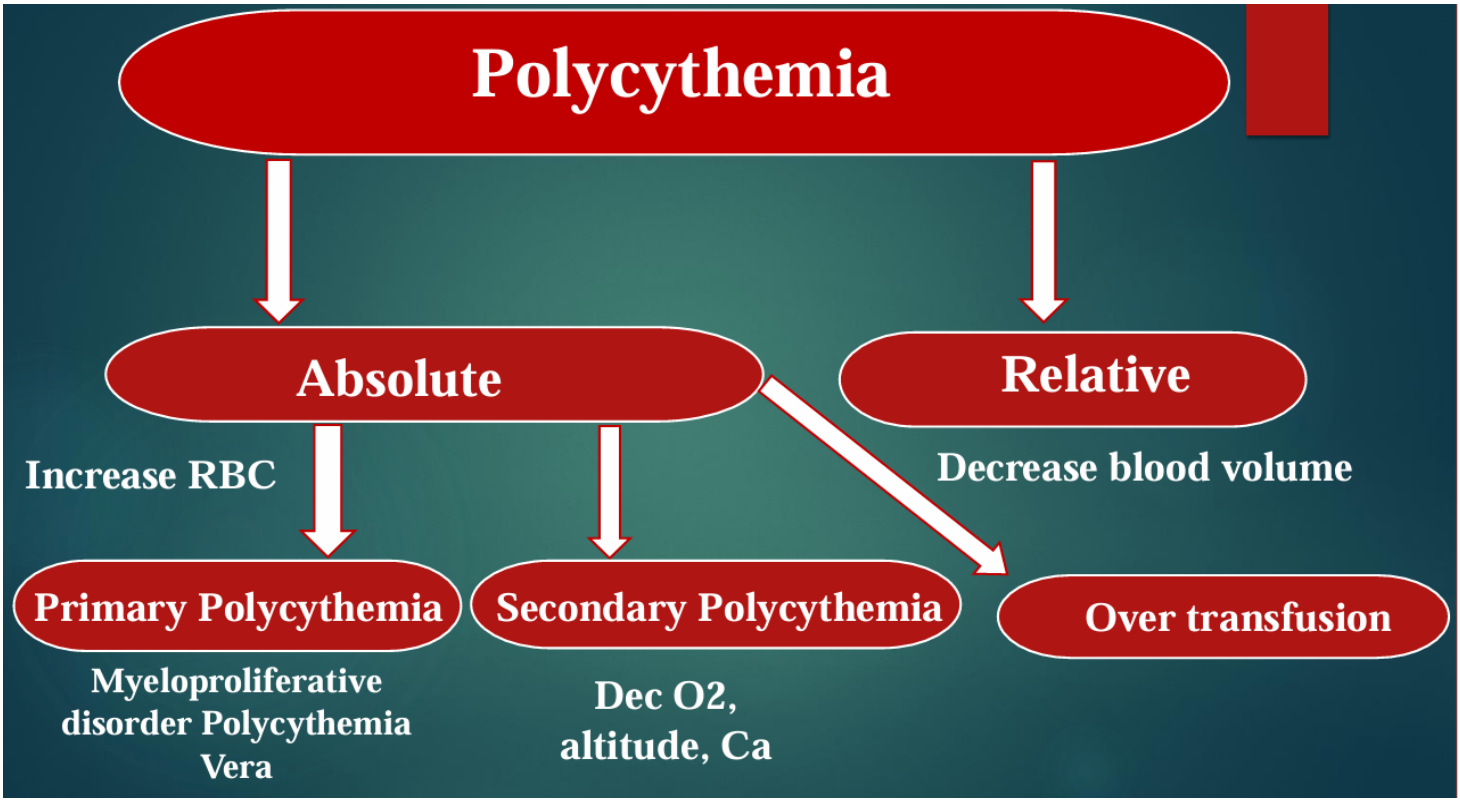
• Polycythaemia or erythrocytosis refers to an elevation of the haemoglobin or haematocrit (haemoglobin >16.0 g/dL in women or >16.5 g/L in men; haematocrit >48% in women or 49% in men) or increased red cell mass (>25% above the mean normal predicted value).

• An initial concerning result on routine testing should always be confirmed by a second measurement in a non-fasting state.

Increasing in RBCs and hemoglobin count per liter (High PCV) leading to increase in blood viscosity. Polycythemia is an increased number of RBCs in the blood.

In polycythemia, the levels of Hgb ,HCT or RBCs count may be elevated when measured in CBC as compared to normal.

Hgblevels greater than 16.5 g/dL (grams per deciliter) in women and greater than 18.5 g/dL in men suggest polycythemia. In terms of HCT, a value greater than 48 in women and 52 in men is indicative of polycythemia.



Relative Polycythaemia

Relative polycythaemia is secondary to a decreased plasma volume (without an increased red cell mass). This is generally the consequence of intravascular fluid depletion, which may be due to dehydration (including that induced by diuretics and caffeine), movement of fluid into the third-space, tobacco smoking and ovarian hyperstimulation syndrome (IVF).

Major Criteria

1. Haemoglobin >16.5 g/dL in men or >16.0 g/dL in women, or

Hematocrit>49% in men or 48% in women, or Increased red cell mass (>25% above mean normal predicted value)

2. Bone marrow biopsy showing hypercellularity for age with trilineage growth Minor Criteria (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)

3. Presence of JAK2 V617F or JAK2 exon 12 mutation

……

Minor Criteria

Subnormal serum EPO level

Relative Polycythaemia

Low Volume States

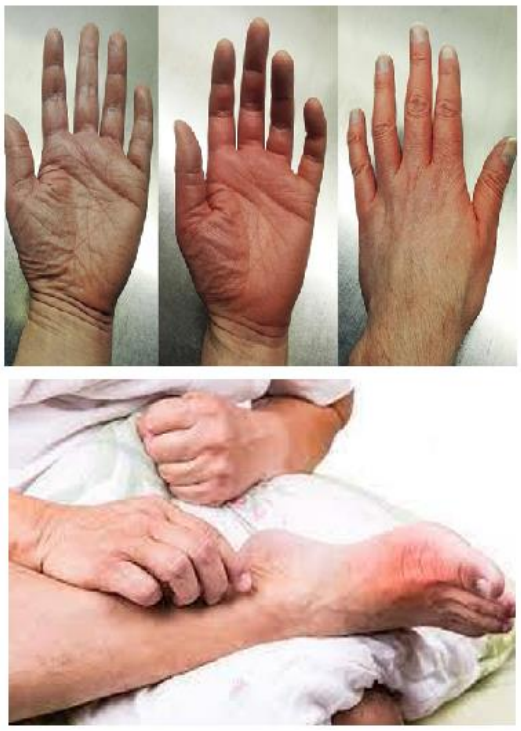
1. Dehydration
2. Burns
3. Prolonged vomitings
4. Diarrhea
5. Excessive diuretics
6. Stress or Gaisbock syndrome

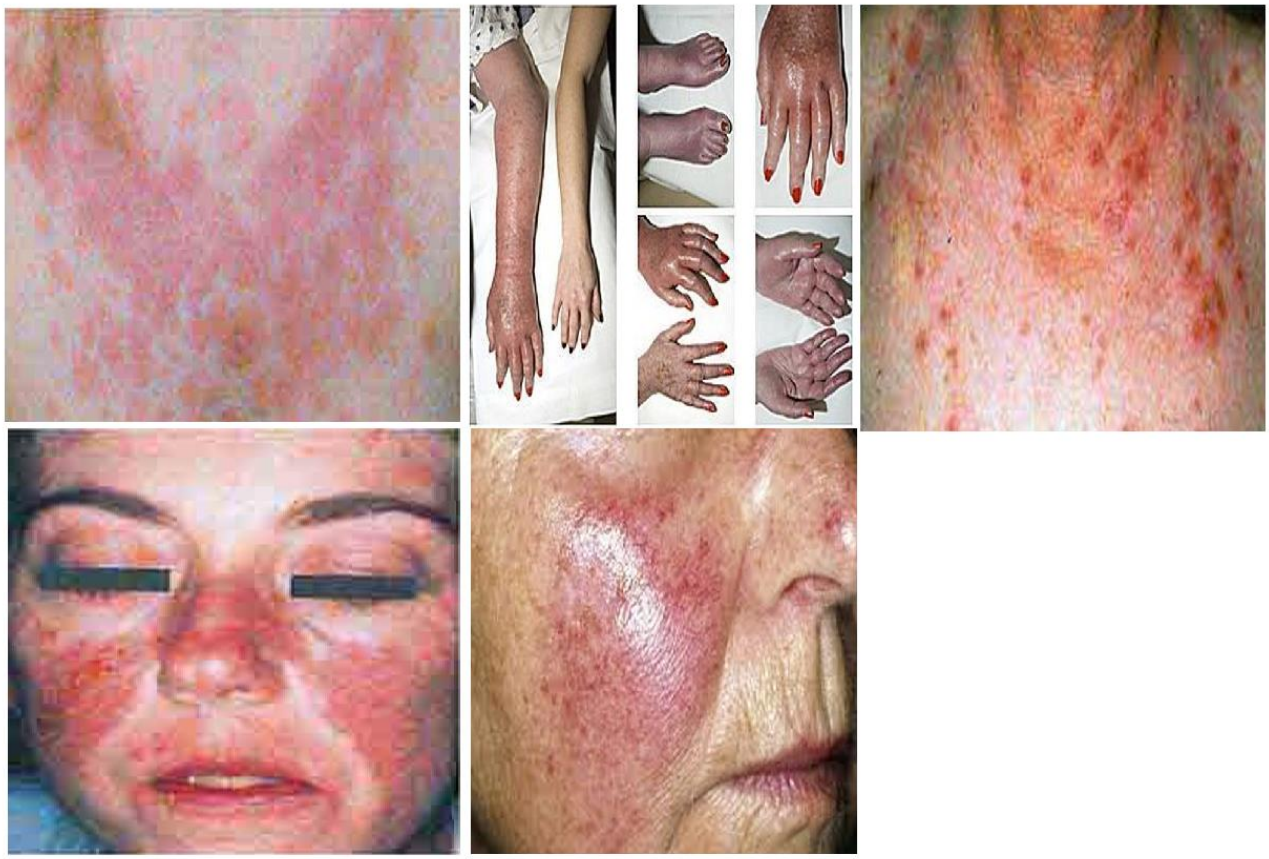
Absolute Polycythaemia

- ✓ Primary Polycythemia
- ✓ Secondery

Absolute polycythaemia is a true increase in red cell mass resulting in an elevated haemoglobin, haematocrit or red blood cell count. It is clinically significant and can arise as a primary or secondary phenomenon.

Primary polycythaemia manifests when a congenital or acquired disorder leads to abnormal erythropoiesis. The most commonly encountered condition is polycythaemia vera (PV), a clonal myeloproliferative neoplasm (MPN) almost invariably accompanied by the JAK2 V617F mutation and frequently by a low serum erythropoietin (EPO).





……

Secondary polycythaemia is caused by exogenous stimulation of erythropoiesis. It is generally associated with an elevated EPO level, which may be physiologically appropriate (stimulated by hypoxia) or inappropriate (in the absence of hypoxia).

Primary Polycythemia

- Polycythemia vera is a type of blood cancer. It causes your bone marrow to make too many red blood cells. These extra cells thicken the blood and slow its flow, which can cause serious problems, such as blood clots. True spherocytosis is a rare disease.
- Polycythemia vera is caused by mutations in the Janus kinase 2 (JAK2) gene, which produces a protein (enzyme) that stimulates the excessive production of blood cells.
- Hypererythrocytosis can occur in any age group, but it is most common in adults between the ages of 50 and 75. Men are more likely to develop erythrocytosis, but women who develop it are more often of a younger age group.

❖ This is a disease of older subjects with an equal sex incidence. Clinical features are the result of hyperviscosity, hypervolaemia or hypermetabolism.

1. Headaches, dyspnoea, blurred vision and night sweats. itching, characteristically after a hot bath, can be a severe problem.
2. Retinal venous engorgement.
3. Splenomegaly in 75% of patients
4. Haemorrhage or thrombosis either arterial or venous
5. Hypertension in one-third of patients.
6. Gout (as a result of raised uric acid production
7. Peptic ulceration occurs in 5-10% of patients.

Laboratory Findings

1. The haemoglobin, haematocrit and red cell count are increased. The total red cell volume (TRCV) is increased.
2. A neutrophil leucocytosis is seen in over half of patients, and some have increased circulating basophils.
3. A raised platelet count is present in about half of patients
4. The JAK2 mutation is present in the bone marrow and peripheral blood granulocytes in nearly 100% of patients.
5. The neutrophil alkaline phosphatase (NAP) score is usually increased.
6. Increased serum vitamin B12 and vitamin B12-binding capacity (due to increased in haptocorrin*)
7. The bone marrow is hypercellular withprominent megakaryocytes, bestassessedby a trephine.
8. Serum erythropoietin usually low.
9. Haptocorrin function is to protect B12 from acid while it passes through the stomach.

Diagnosis

Diagnosis involves the use of clinical and laboratory findings to establish whether there is a true polycythaemic state (absolute versus relative) and if present, whether it is primary or secondary in nature.

History and examination should include a search for cardiorespiratory, renal, and liver disease as well as complications of hyperviscosity. Familial conditions, occupational risks, smoking, snoring, and medication exposures (such as diuretics, androgens, anabolic steroids, and erythropoietin therapy) should all be considered. Common symptoms of hyperviscosity of any cause include cardiocerebral compromise (chest pain, transient ischaemic attack, headache, fatigue, blurred vision).

Symptoms more specific

to PV include itch, erythromelalgia, gout, or splenic discomfort. Erythromelalgia is a rare condition that primarily affects the feet and, less commonly, the hands (extremities). It is characterized by intense, burning pain of affected extremities, severe redness (erythema), and increased skin temperature that may be episodic or almost continuous in nature.

……..

Complications

Haemorrhage and thrombosis can complicate both primary and secondary cases of polycythemia, however, the risk of therapy can be instituted. progression to marrow fibrosis or leukemia is exclusive to PV. It is vital to ensure correct diagnosis so that appropriate therapy can be instituted.

……..

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

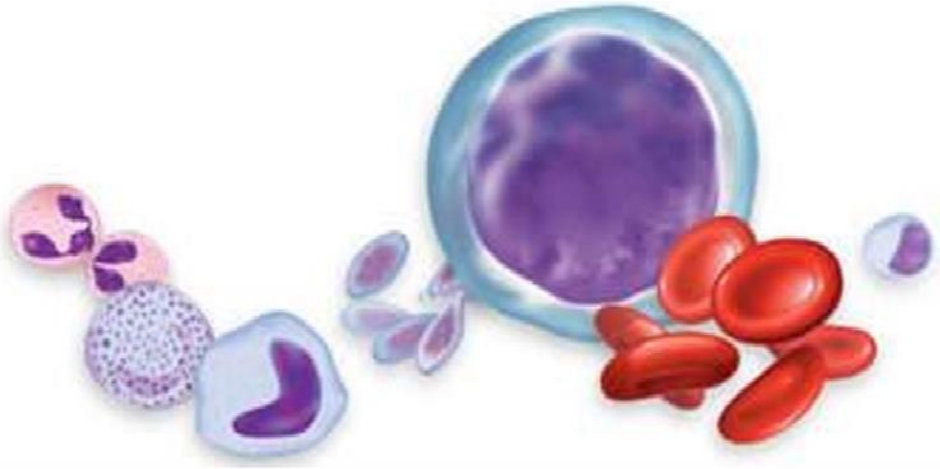
13th - Week

Lecture Title: Leukaemia.

Third level-First semester

By

Dr. Fidan Fikrat Ahmed



2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for the students in identifying the concept of leukaemia, and classification of leukaemia.

Special Objectives of the Module

1. Definition of leukaemia.

2. Classification of leukaemia.

3. Differentiates between the types of leukaemia.

4. Explain the causes, Clinical features, Symptoms, Signs, laboratory findings, and diagnosis of leukaemia).

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

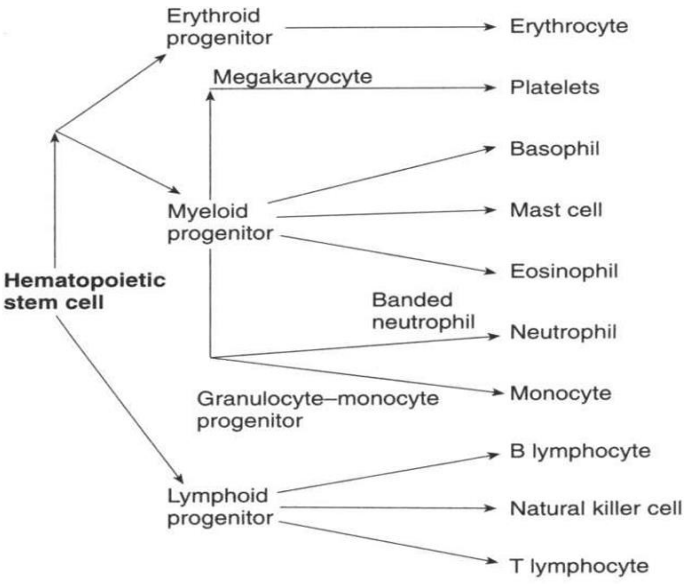
2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

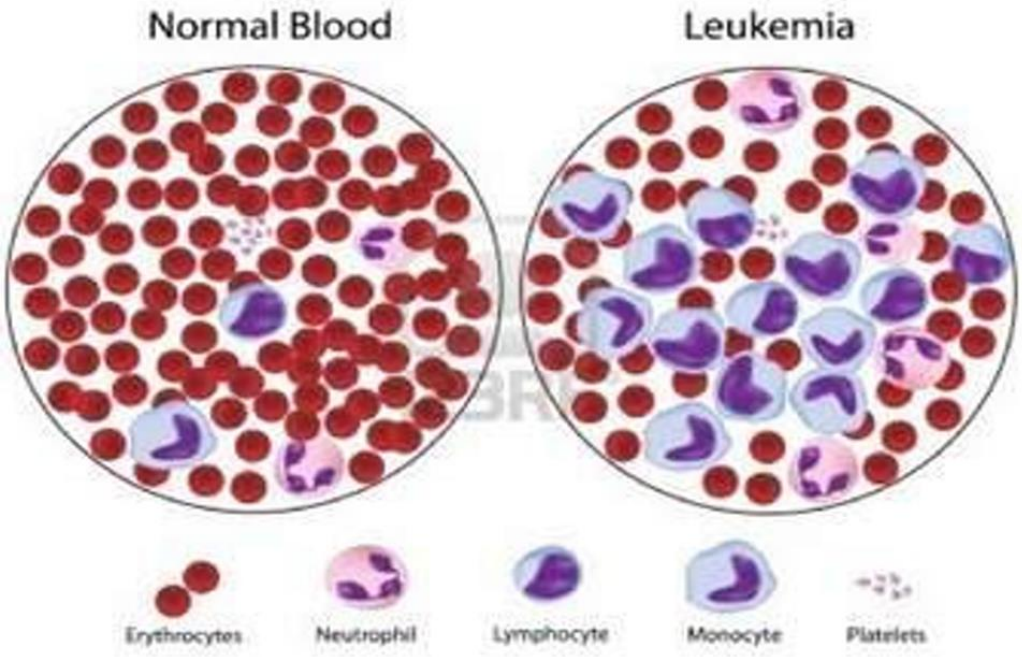
3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas. Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

Leukemia

- The word Leukemia comes from the Greek leukos which means "white" and aima which means "blood".
- Leukemia is the general term for some different types of blood cancer.
- It is a group of malignant disorders, affecting the blood and blood–forming tissue of the bone marrow, lymph system, and spleen.
- The stem cells are committed to producing specific types of blood cells.
- Lymphoid stem cells produce either T or B lymphocytes.
- Myeloid stem cells differentiate into three broad cell types: RBCs, WBCs, and platelets.
- **In Leukemia:** stem cell disorder is characterized by a malignant neoplastic proliferation and accumulation of immature hematopoietic cells in the bone marrow
- Incidence is higher in men than in women.





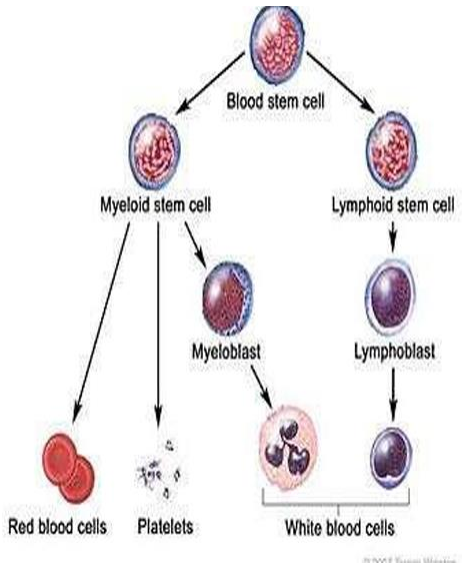
Etiology

Combination of predisposing factors including

- ❖ Genetic and environmental influences.
- ❖ Environmental factors
- ➢ Ionizing radiation
- ➢ Chemical carcinogens or Chronic exposure to a chemical such as benzene
- ➢ Radiation exposure and Certain drugs.
- ➢ Cytotoxic therapy of breast, lung, and testicular cancer.
- ➢ Infections: Human T cell leukemia-lymphoma virus I (HTLV-I) .
- ➢ Association with diseases of immunity: Immunodeficiency diseases like AIDS and iatrogenic immunosuppression induced by chemotherapy or radiation.

Pathophysiology

- ✓ The lack of control causes normal cell in bone marrow to be replaced by immature and undifferentiated leukocytes or blast cells.
- ✓ Abnormal immature leukocytes then circulate in the blood and infiltrate the blood-forming organs (liver, spleen, lymph nodes) and other sites throughout the body.



Different types of leukemia

- ➢ Acute leukemia
- ➢ Chronic leukemia.

Acute leukemia gets worse very fast and may make feel sick right away.

Chronic leukemia gets worse slowly and may not cause symptoms for years.

Leukemias are also subdivided into:

✓ **Lymphocytic Leukemia .**

✓ **Myelogenous Leukemia** .

According to the type of affected blood cell.

- If the cancerous transformation occurs in the type of marrow that makes lymphocytes, the disease is called lymphocytic leukemia.
- If the cancerous change occurs in the type of marrow cells that
- produce red blood cells, other types of white cells, and platelets, the disease is called myelogenous leukemia

……

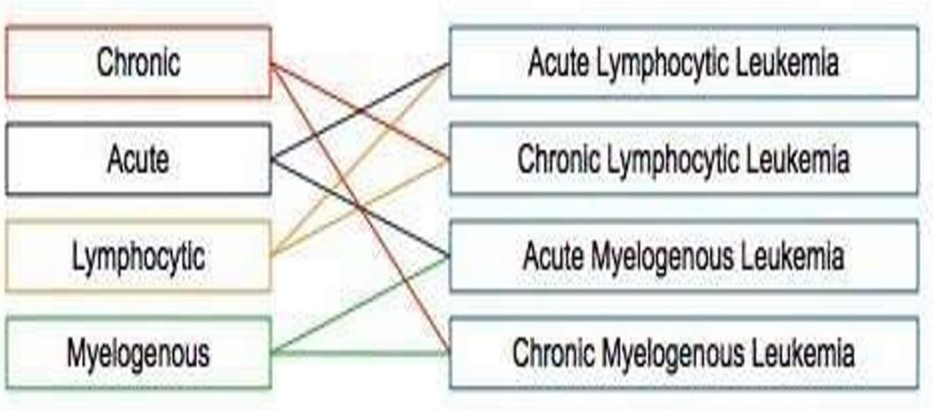
There are four main types of leukemia:

✓ Acute lymphoblastic (lymphocytic) leukemia (ALL)

✓ Acute myeloid (myelogenous) leukemia (AML)

✓ Chronic lymphocytic leukemia (CLL)

✓ Chronic myeloid (myelogenous) leukemia (CML)



Acute:

1. Acute myeloblastic leukemia(AML): at all ages
2. Acute lymphoblastic leukemia(ALL): primarily a disease of

Children and young adults

Chronic:

1. Chronic myeloid leukemia(CML): middle age
2. Chronic lymphocytic leukemias(CLL): elderly

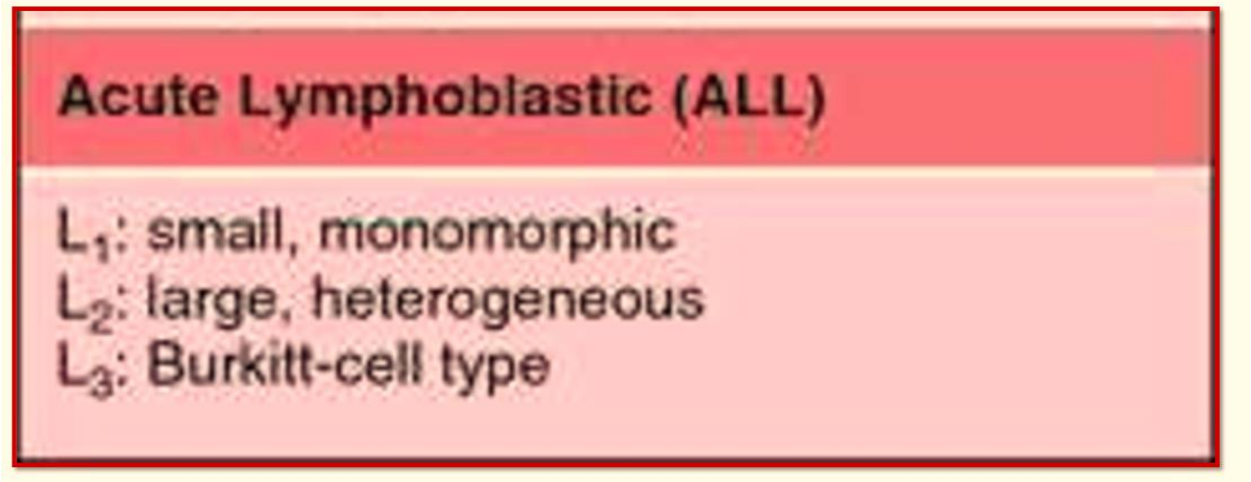
- It is important to know that patients are affected and treated differently for each type of leukemia.
- These four types of leukemia do have one thing in common – they begin in a cell in the bone marrow.
- The cell undergoes a change and becomes a type of leukemia cell.
- ALL and AML (acute leukemias) are each composed of young cells, known as lymphoblasts or myeloblasts. These cells are sometimes called blasts.
- CLL and CML have few blast cells.

……

Classification of leukemia FAB & WHO Classification of acute leukemia

1- French-American –British (FAB) Classification Of Acute Leukemia

Classification Of Acute Leukemia (FAB) Acute Myeloid Leukemia	
Mo	Acute Myeloblastic Leukemia, with minimal differentiation
M1	Acute Myeloblastic Leukemia, without maturation
M2	Acute Myeloblastic Leukemia, with maturation
M3	Promyelocytic Leukemia , hypergranular
M3v	Promyelocytic Leukemia , hypogranular
M4	Acute Myelomonocytic Leukemia
M4eo	Acute Myelomonocytic Leukemia, with eosinophilia
M5a	Acute Monoblastic Leukemia, without differentiation
M5b	Acute Monoblastic Leukemia, with differentiation
M6	Acute Erythroleukemia
M7	Acute Megakaryocytic Leukemia



2. World Health Organization

- ✓ Cellular morphology and cytochemical stain, cytogenetics, molecular abnormalities & clinical syndrome
- ✓ Acute leukemia as > 20% bone marrow blasts.

Cytochemical stains are the special stains that can be performed on peripheral blood smears and bone marrow smears. They play a very important role in the diagnosis, differentiation, and classification of Leukemia.

Relate to problems caused by Bone marrow failure.

- Overcrowding by abnormal cells.
- Inadequate production of normal marrow elements.
- Anemia, thrombocytopenia, ↓ number and function of WBCs.

Relate to problems caused by Leukemic cells infiltrating patient's organs

- Splenomegaly
- Hepatomegaly
- Lymphadenopathy
- Bone pain, meningeal
- irritation, oral lesions (chloromas)

Classification of leukemia

1- A - Acute lymphatic leukemia (ALL)

Usually occurs before 14 years of age peak incidence is between 2-9 years of age, and older adult

Pathophysiology

It arises from a single lymphoid stem cell, with impaired maturation and accumulation of the malignant cells in the bone marrow.

Signs and symptomS

Anaemia, bleeding, lymphadenopathy, infection ,Fever.

Clinical manifestation

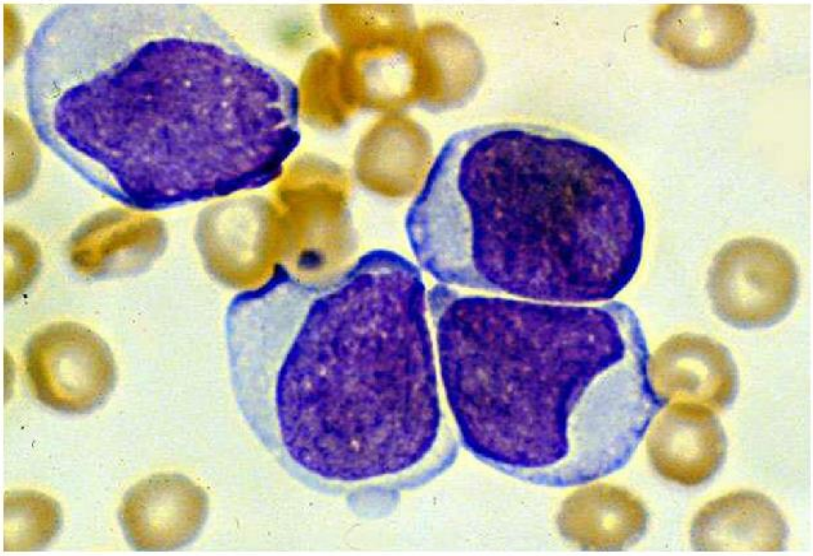
- ✓ Fever
- ✓ Pallor
- ✓ Bleeding
- ✓ Anorexia
- ✓ Fatigue
- ✓ Weakness
- ✓ Bone, joint, and abdominal pain
- ✓ Increase intracranial press
- ✓ Generalized lymphadenopathy
- ✓ Infection of the respiratory tract
- ✓ Anemia and bleeding of the mucus membrane
- ✓ Weight loss
- ✓ Mouth sore

Diagnosis

- Low RBCs count.
- Low Hb.
- Low Hct.
- Low platelet count.
- Low normal or high WBC count.
- A blood smear shows immature lymphoblasts.

.....

ALL Histology



Treatment and Complications

Treatment of anemia and hemorrhage.

1. Anemia and hemorrhage are managed by fresh blood transfusions and platelet concentrates.

2. Patients with severe thrombocytopenia (platelet count below 20,000/µl) require regular platelet transfusions.

- ❖ hemorrhages an important cause of death in these cases
- ❖ Treatment and prophylaxis of infection
- ❖ Cytotoxic drug therapy
- ❖ Bonemarrowtransplantation

……

1-B -Acute Myelogenous (myeloid) Leukaemia (AML)

- ❖ In these diseases, the original acute leukemia cell goes on to form about a trillion more leukemia cells.
- ❖ These cells are described as nonfunctional because they do not work like normal cells.
- ➢ They also crowd out the normal cells in the marrow. This causes a decrease in the number of new normal cells made in the marrow.
- ➢ This further results in low red cell counts (anemia), low platelet counts (bleeding risk), and low neutrophil counts (infection risk).

1-B -Acute Myelogenous (myeloid) Leukaemia (AML)

It occurs at any age but occurs most often at adolescence and after the age of 55

Pathophysiology

Characterized by the development of immature myeloblasts in the bone marrow.

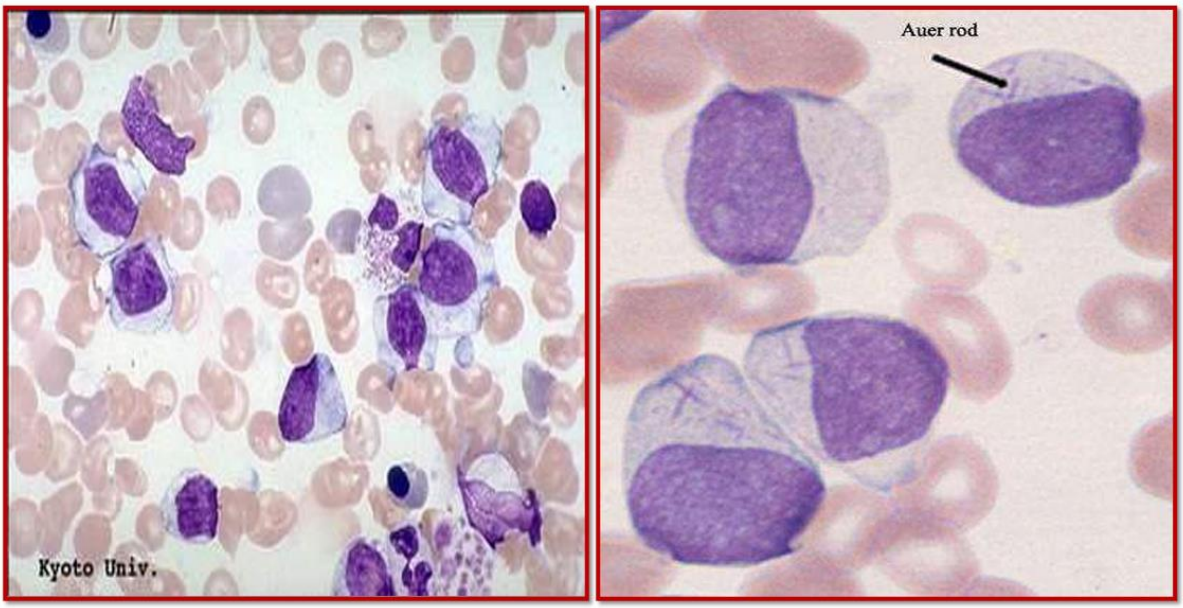
Clinical manifestation

Similar to ALL plus sternal tenderness.

Diagnosis

Low RBC, Hb, Hct, low platelet count, low to high WBC count with myeloblasts.

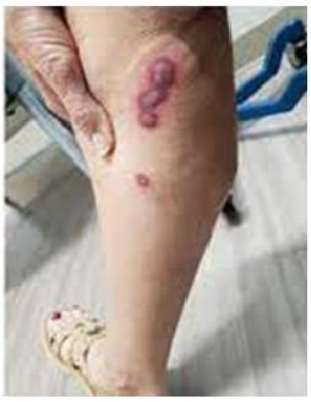
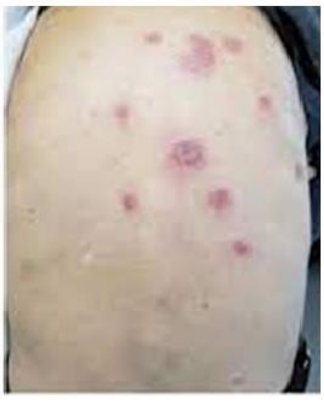
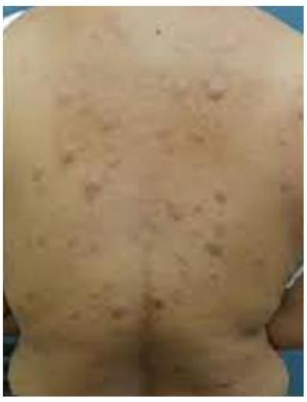
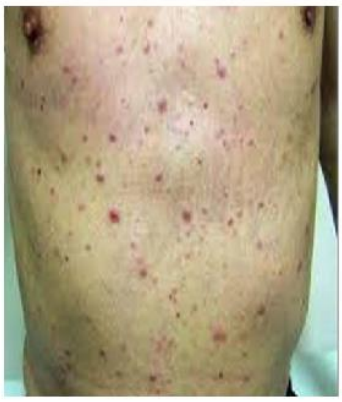
AML Histology



Acute leukemia's: acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Common side effects may include

- Changes to blood counts
- Mouth sores
- Nausea
- Vomiting
- Diarrhea
- Hair loss
- Rash
- Fever.

.....

2- A - Chronic lymphocytic Leukaemia(CLL)

The incidence of CLL increases with age and is rare under the age of 35. It is common in men.

Pathophysiology

- It is characterized by the proliferation of small, abnormal, mature B lymphocytes, often leading to decreased synthesis of immunoglobulin and depressed antibody response.
- The number of mature lymphocytes in peripheral blood smear and bone marrow is greatly increased

Clinical Manifestation

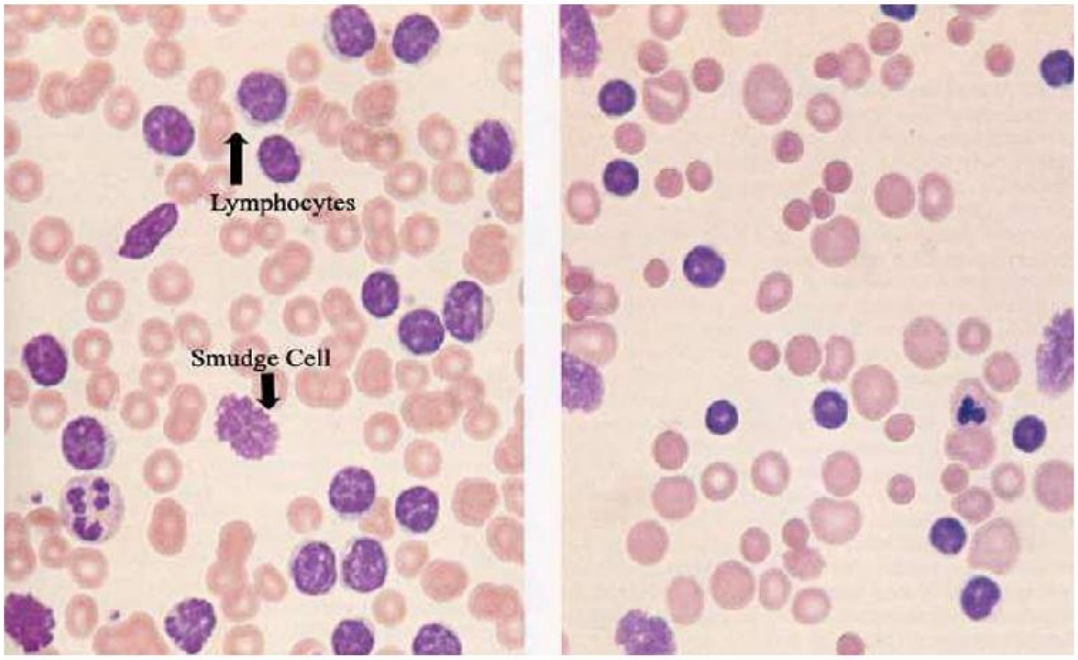
Usually, there are no symptoms.

Chronic fatigue, weakness, anorexia, splenomegaly, lymphadenopathy, hepatomegaly.

Signs and Symptoms

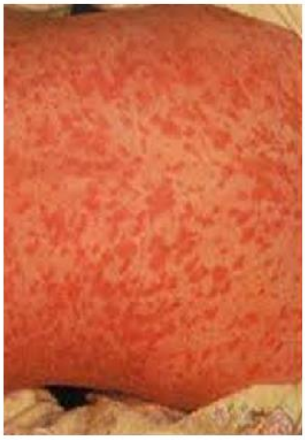
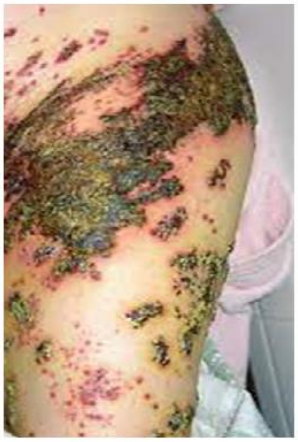
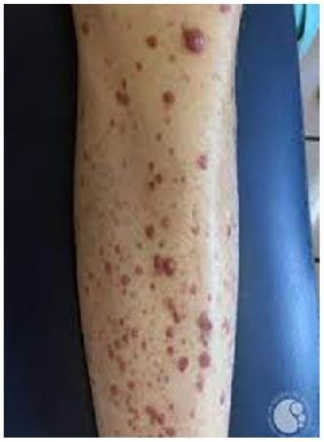
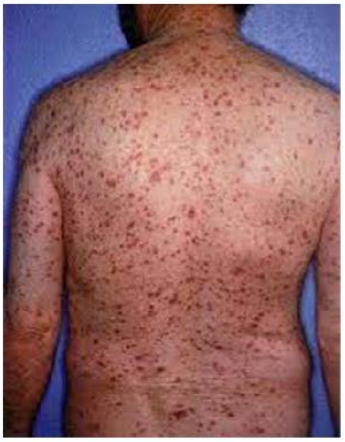
- Pruritic vesicular skin lesions .
- Anemia
- Thrombocytopenia.
- The WBC count is elevated to a level between 20,000 to 100,000.
- Increase blood viscosity and clotting episode.

CLL Histology



Chronic lymphocytic leukemia (CLL). Common side effects may include:

- Extreme tiredness
- Hair loss
- Changes to blood counts
- Upset stomach
- Mouth sores
- Diarrhea.

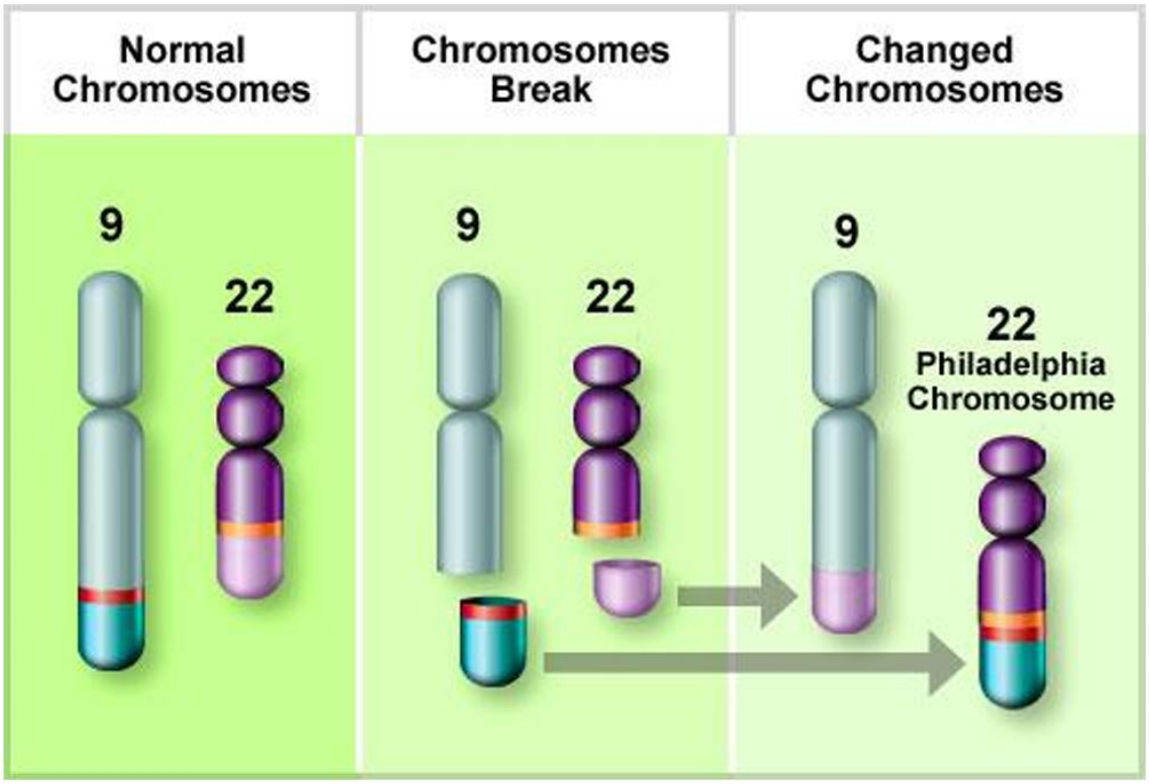
2- B - Chronic Myelogenous Leukaemia(CML)

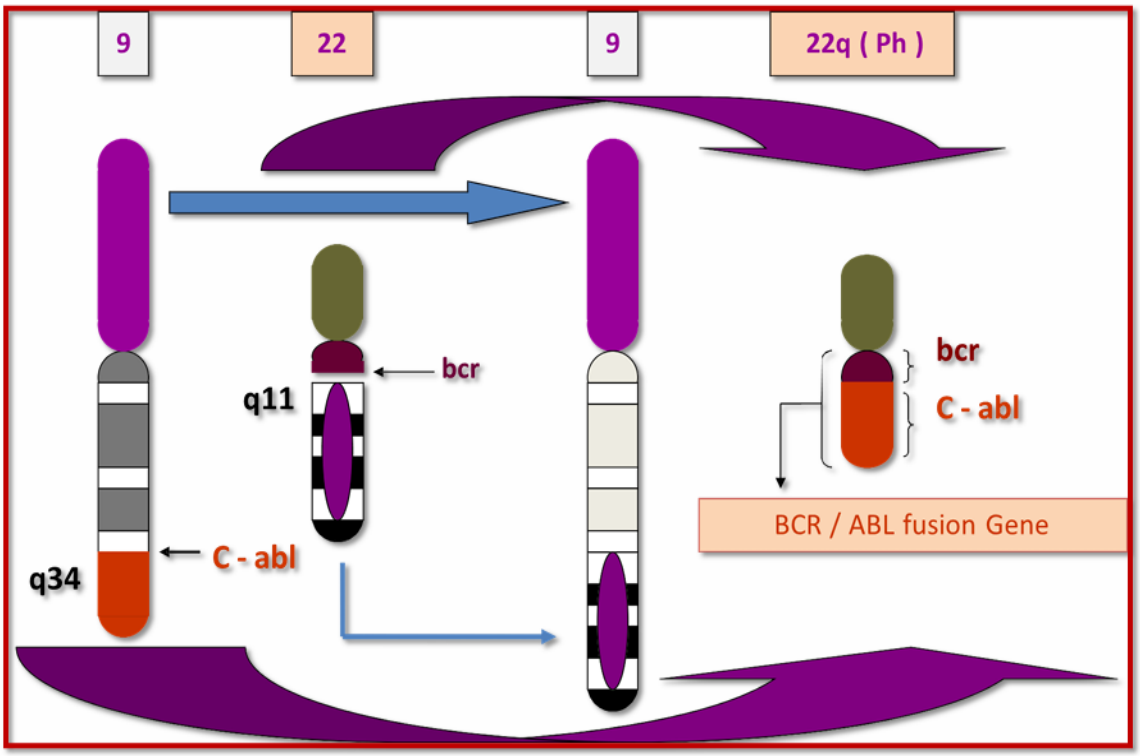
- Philadelphia chromosome
- The chromosome abnormality that causes chronic myeloid leukemia.
- Occurs between 25-60 years of age. Peak 45 year
- It is caused by benzene exposure and high doses of radiation

……

Philadelphia chromosome

- The chromosome abnormality that causes chronic myeloid leukemia(CML) (9 &22)
- Genetic marker
- Chronic, stable phase followed by an acute, aggressive (blastic) phase


Normal Chromosomes
Chromosomes Break
Changed Chromosomes
9
22
9
22
9
22
Philadelphia Chromosome


9
22
9
22q (Ph)
bcr
q11
C - abl
q34
bcr
C - abl
BCR / ABL fusion Gene

Disease Progression

- ❖ Chronic Phase
- ❖ Accelerated Phase
- ❖ Blast Crisis

.....

Clinical Manifestation

There are no symptoms in the disease.

The classic symptoms include:

- ❖ Fatigue, weakness, fever.
- ❖ Weight loss, joint & bone pain.
- ❖ Massive splenomegaly
- ❖ The accelerated phase of the disease(blastic phase) is characterized by an increasing number of granulocytes in the peripheral blood.
- ❖ There is corresponding anemia and thrombocytopenia.

Diagnosis

• Lower RBC count, Hb, Hct, high platelet count early, lower count later.

• A normal number of lymphocytes and normal or low number of monocytes in WBC.

Laboratory Findings

The diagnosis of CML is generally possible on blood pictures alone. However, bone marrow, cytochemical stains, and other investigations are also useful

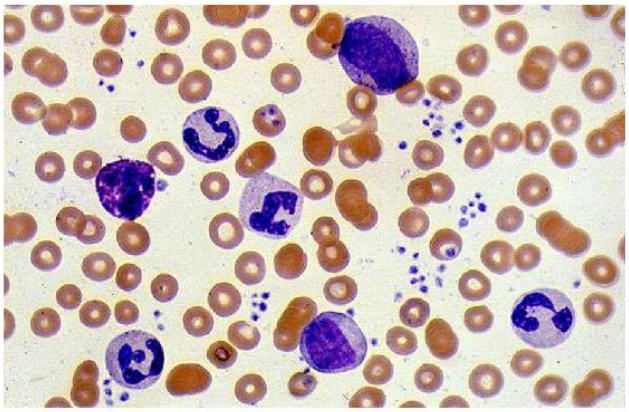
Blood Picture

Anaemia. Anemia is usually of moderate degree and is normocytic normochromic in type.

White blood cells. Characteristically, there is marked leukocytosis (approximately 200,000/μl or more at the time of presentation).

Platelets. Platelet count may be normal but is raised in about half the cases.

CML HISTOLOGY



Accelerated phase

Criteria of accelerated phase

- Blasts in blood or bone marrow 10-19%
- Basophilic ≥ 20%
- Thrombocytopenia <100,000/cm
- Thrombocytosis >100,000/cm
- splenomegaly or leukocytosis

When to suspect progression

- Decreasing platelet counts
- Increasing basophil count
- Increasing total counts
- Lymphadenopathy

……

Blast phase (blast crisis) of CML

- ✓ Criteria of blast phase
- ✓ Blasts ≥20%
- ✓ Extramedullary blast proliferation
- ✓ Large foci of blasts in the bone marrow

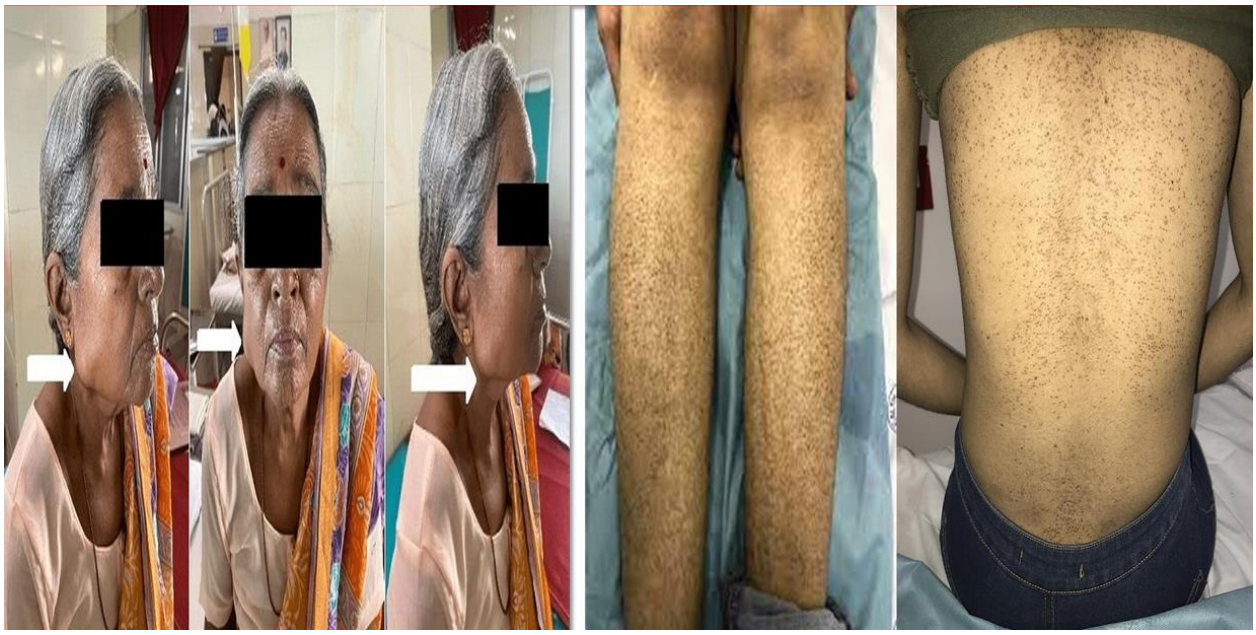
Phenotype of blasts

- ✓ Myeloblasts– 60-70%
- ✓ Lymphoblasts-10-30%
- ✓ Acute myelofibrosis

……

Chronic myeloid leukemia (CML). Common side effects from tyrosine kinase inhibitor therapy such as Gleevec may include

- Changes to blood counts
- Diarrhea
- Muscle cramps and joint pain
- Nausea
- Swelling or fluid retention.



……..

MANAGEMENT

- ❖ Watchful waiting,
- ❖ Chemotherapy,
- ❖ Radiation therapy
- ❖ Stem cell transplant.

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

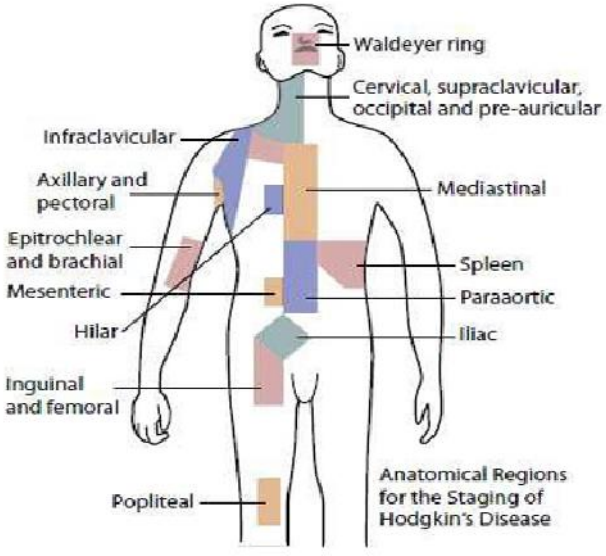
14th - Week

Lecture Title: Lymphoma Hodgkin lymphoma & Non- Hodgkin lymphoma.

Third level-First semester

By

Dr. Fidan Fikrat Ahmed

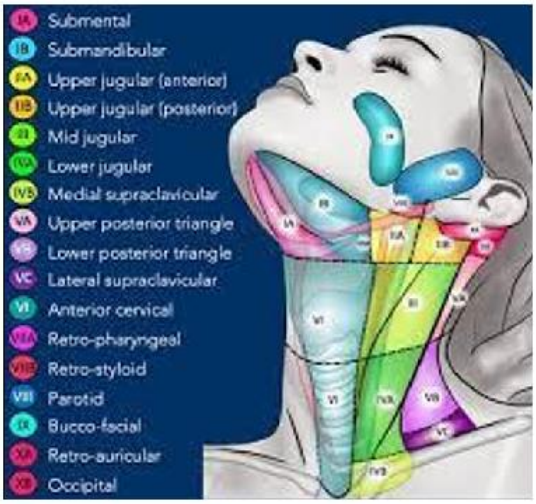


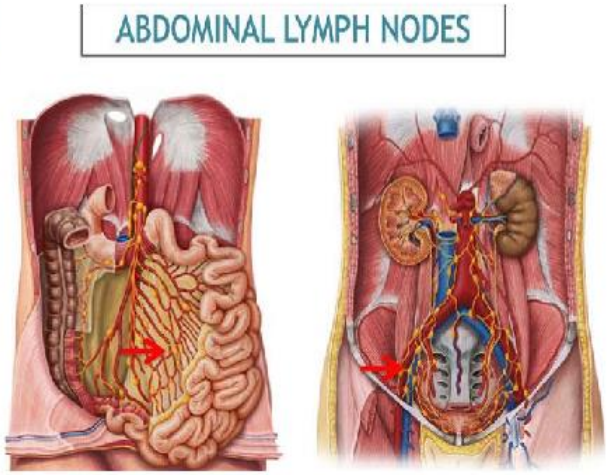
2024-2025

Lymphoma

Lymphatic System

- The lymphatic system is part of the immune system.
- The normal immune system helps to protect the body from infection.
- The marrow, lymph nodes, and spleen are some of the parts of the immune system.
- There are about 600 lymph nodes throughout the body. Lymph nodes and other lymphoid tissues that are commonly involved in lymphoma are those around the ears and jaw, in the tonsils and adenoids, in the front and back of the neck, above and below the collar bone, in the armpit, near the elbow, in the chest, in the abdomen, in the pelvis, and in the groin.
- The spleen contains many clusters of lymphocytes that can become malignant and grow, leading to the enlargement of the spleen.
- The gut-associated (intestinal) lymph tissue may also be the site of





lymphoma development.

Lymphoma is a general name for a group of cancers that affect the ymphatic system. The two major types of lymphoma are

- ✓ **Hodgkinlymphoma(HL)**
- ✓ **Non-Hodgkinlymphoma(NHL).**

Most forms of HLare highly curable. Lymphoma originates in the ymphocyte which is part of the broad group of diseases called Hematological Neoplasms.

.....

Thomas Hodgkin (17August 1798– 5April 1866) British physician and pathologist

• 1832, in a paper titled ”On Some MorbidAppearances of theAbsorbent Glands and Spleen”

• In 1856, Samuel Ilks, published a paper entitled "Cases of Enlargement of the lymphatic glands and spleen“

• Theodor Langhans and WS Greenfield first described the microscopic characteristics of Hodgkin's lymphoma in 1872 and 1878

• In 1898 and 1902, respectively, Carl Sternberg and Dorothy Reed independently described the cytogenetic features of the malignant cells of Hodgkin's lymphoma.

Epidemiology Of Lymphomas

• 5th most frequently diagnosed cancer overall for both males and females

• Males > females

• Hodgkin lymphoma stable

• NHL increasing over time (Stage III or IV at Diagnosis)

.....

Incidence

Approximately 4 new cases/100 000 population / year

Age

HLis most likely to be diagnosed in people in their 20s or early 30s. It is less common in middle age but becomes more common again after age 65-70

Sex Ratio

Slight male excess (1.5:1)

.....

Causes and Risk Factors

Most cases of HL occur in people who do not have identifiable risk factors;

most people with identifiable risk factors do not develop HL. The following are examples of risk factors.

- ❖ Patients who have a history of a blood test confirming mononucleosis have a 3-fold increased risk of HL compared to the general population.
- ❖ People infected with human T-cell lymphocytotropic virus (HTLV) or human immunodeficiency virus (HIV) or Immunosuppression also have an increased probability of developing HL.
- ❖ Family history of lymphoma. There is an increase in the incidence of HL in siblings of patients with the disease.
- ❖ Connective tissue disease.
- ❖ Infectious agents.
- ❖ Ionizing radiation.

Clinical presentation of Hodgkin lymphoma

1-Enlarged, painless(painful after drinking alcohol), rubbery, non-erythematous, non-tender lymph nodes are the hallmark of the disease.

2-25% of Patients may develop ‘’B’’ symptoms which are:

a- Drenching night sweats. b-10% weight loss c-Fever

3-Although pruritus is common in the disease it is not one of the ‘’B’’

symptoms.

4-Cervical, supraclavicular and axillary lymphadenopathy are initial signs of the disease.

5-Extralymphatic sites involved more common with nonhodgkin lymphoma

such as (Spleen, Liver, Bone marrow, Lung, CNS)

Signs and Symptoms

The most common early sign of HL is a painless swelling (enlargement) of one or more lymph nodes. The vast majority of patients with HL have affected lymph nodes in the upper part of the body—usually in the neck or upper chest. Sometimes the affected lymph node is in the armpit, abdomen, or groin.

Other HLsymptoms include

- ❖ Fever
- ❖ Persistent fatigue
- ❖ Persistent cough and shortness of breath (if HL is located in the chest)
- ❖ Sweating, especially at night (drenching sweats of the whole body, not just the neck area or chest area)
- ❖ Weight loss
- ❖ Enlarged spleen
- ❖ Itching

Diagnosis

Imaging. A doctor may first order imaging tests and the patient's medical history and physical examination diagnosis of HL. The imaging test may show enlarged lymph nodes in the chest or abdomen or both. Tumor masses can also occur outside the lymph nodes in the lung, bone, or other body tissue.

Lymph node biopsy.

Immunophenotyping. A technique called "immunophenotyping" is ometimes used to distinguish HL from other types of lymphoma or other noncancerous conditions. The hematopathologist looks for the presence of Reed-Sternberg and Hodgkin cells to confirm a diagnosis of HL.

Gross Features

• Lymph nodes enlarged

• Appearance is somewhat dependent on the microscopic subtypes

• Consistency varies from soft to hard depending on the amount of fibrosis

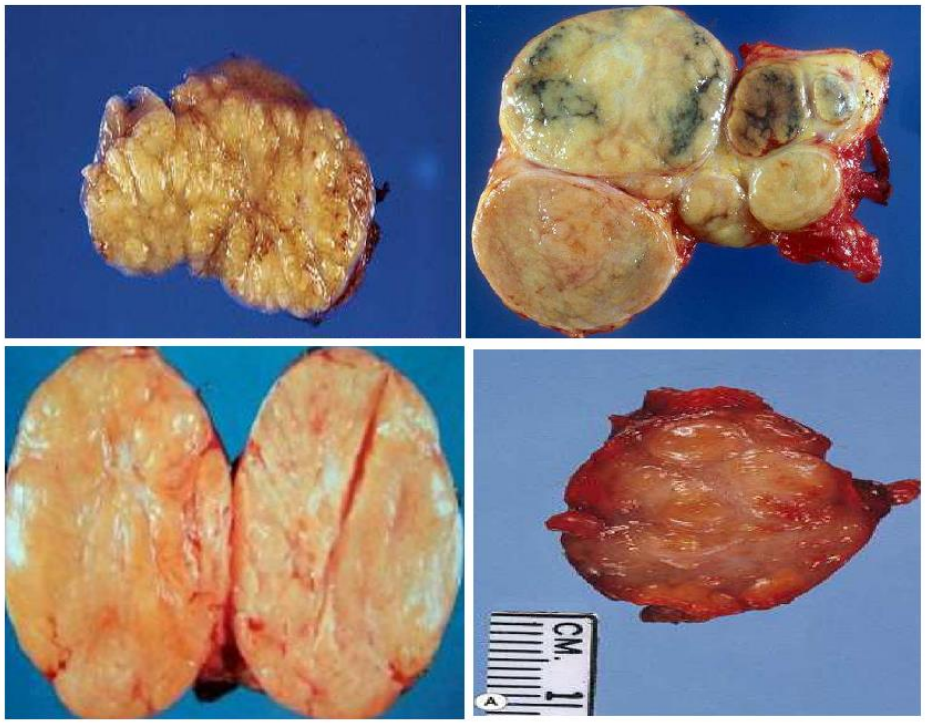
• Some degree of nodularity is often appreciated

• Foci of necrosis +/

• Cut surface of the node has a more heterogeneous appearance except for in Lymphocyte predominant type

• In advanced cases, several nodes from the same group become matted together

……..



Subtypes of Hodgkin

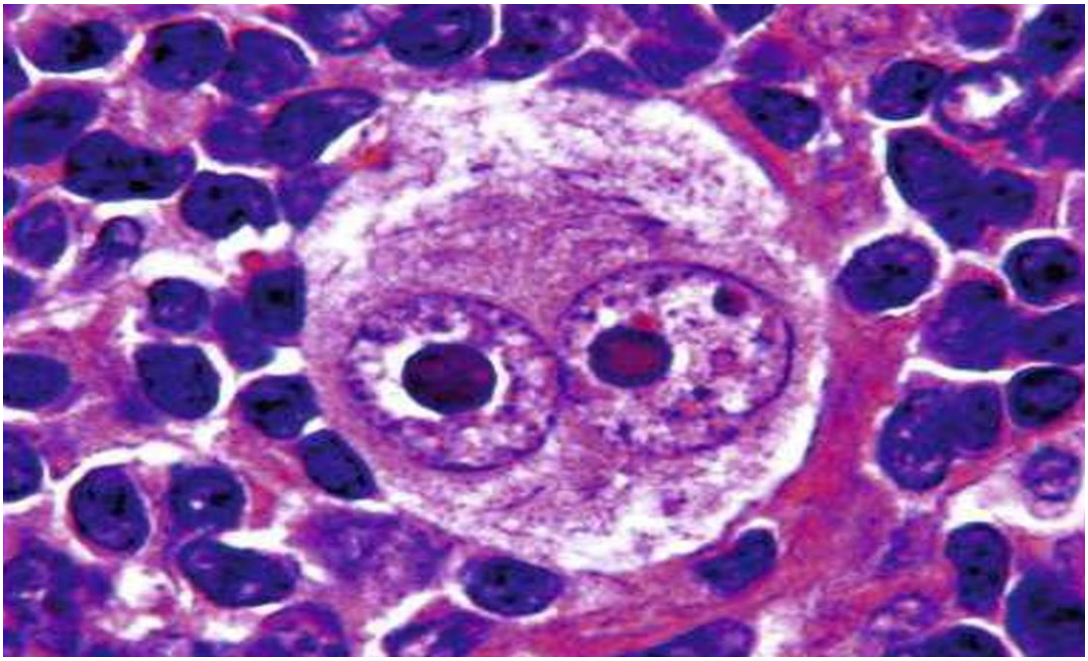
Lymphoma

There are two main HL subtypes:

1-Classical Hodgkin lymphoma.

2-Nodular lymphocyte-predominant Hodgkin lymphoma.

About 95 percent of HL patients have the classical subtype. Knowing the patient's subtype is important for making treatment decisions. HL is distinguished from other types of lymphoma by the presence of " Reed-Sternberg cells" (named for the scientists who first identified them). Reed-Sternberg cells are usually B cells and have differences and variations to them. The frequency with which these cells are seen and the variations observed help determine a patient's subtype. Other cells associated with the disease are called "Hodgkin cells."



Variants Of Rs Cells

1. Classical – seen in mixed cellularity type, lymphocyte rich type HL

2. Mononuclear– lacking nuclear lobation with a large inclusion-like nucleolus.– seen in mixed cellularity type, lymphocyte rich type HL.

3. Lacunar– delicate, folded, or multilobate nuclei and abundant pale cytoplasm that is often disrupted during the cutting of sections, leaving the nucleus sitting in an empty hole (a lacuna) :

Artifact induced by formalin fixation (not seen in tissues fixed in Zenker's or B5 fixative)– Seen in Nodular Sclerosis type HL

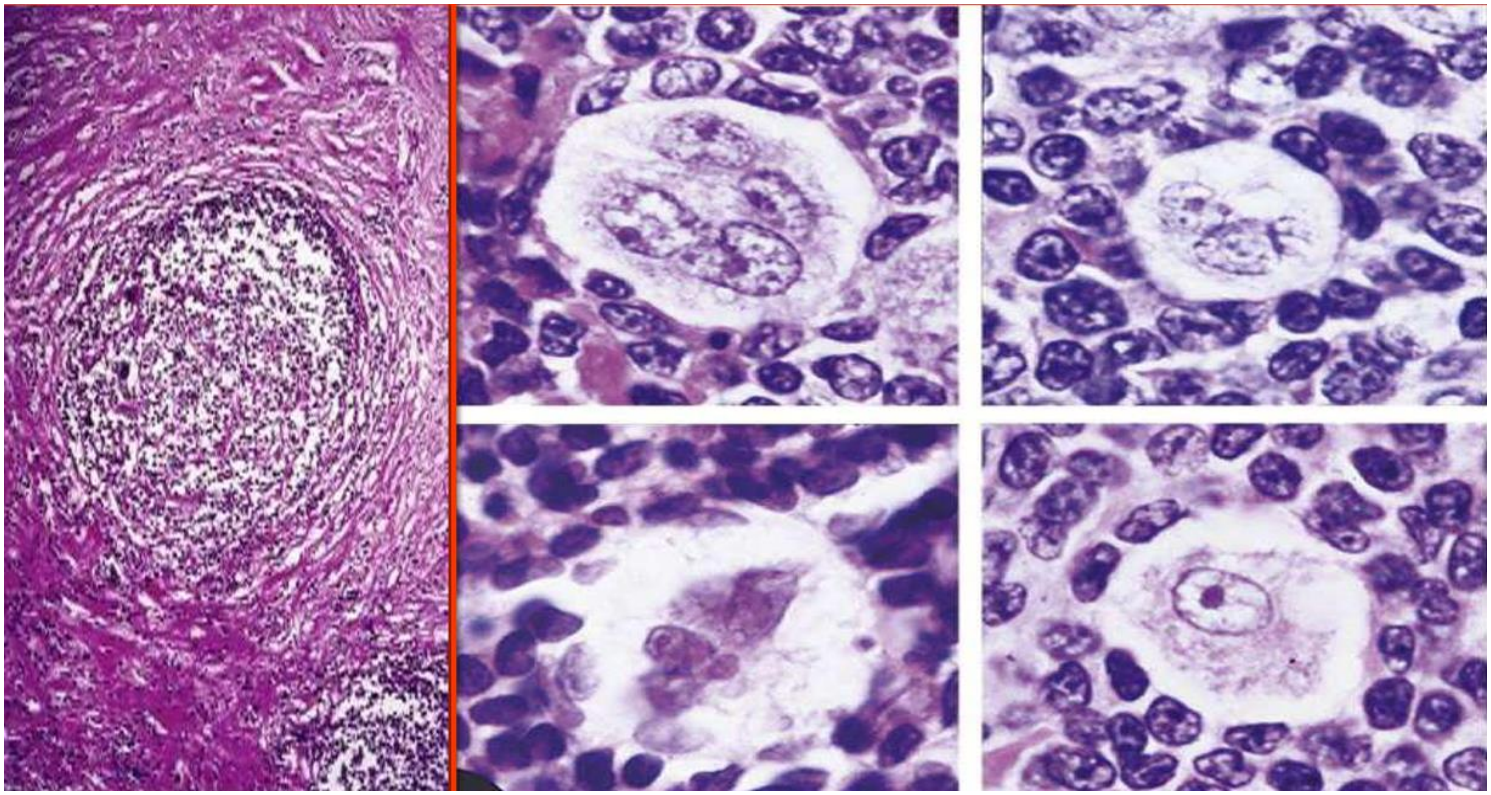
4. Lymphohistiocytic (L&H)– polypoid multilobed nucleus resembling a popcorn kernel,inconspicuous nucleoli, and moderately abundant cytoplasm :"popcorn cell"– Seen in NLPHL

1-Classical Hodgkin Lymphoma.

Classical HL can be further subdivided. Four major subtypes have been identified.

1-Nodular Sclerosis Hodgkin lymphoma .

Nodular sclerosis is the most common subtype, representing about 60 to 70 percent of HL cases. ounger patients are more likely to have this type. It is the most common type in young adults age 15-34 years. The nodes first affected are often those located in the center of the chest (the mediastinum). This subtype is characterized by fibrous tissue, visible under the microscope, among the Hodgkin cells. This tissue forms scars, and sometimes after reatment there can be persistent abnormalities, such as small lumps. These may be benign, consisting of scar tissue (also called "residual fibrosis") that remains after the disease cells have been eliminated. This form of classical HL is highly curable.

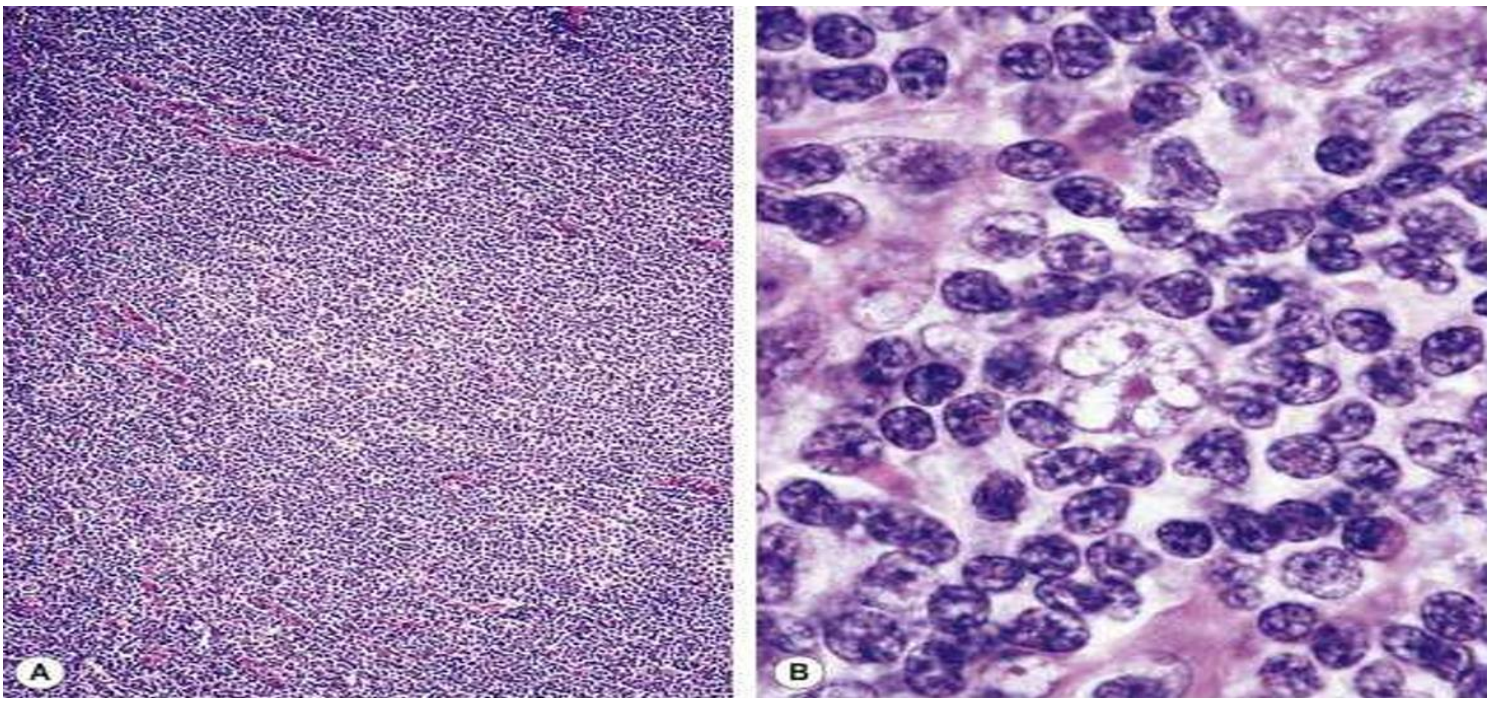


2-Mixed Cellularity Hodgkin lymphoma. Mixed cellularity is the

second most common subtype. It occurs in about 25 percent of patients and mostly in older patients (55-74 years), children (0-14 years), and those with immune disorders such as AIDS. It is a somewhat more aggressive subtype, although just as curable, as nodular sclerosis HL.

3-Lymphocyte-Depleted Hodgkin lymphoma.

This subtype occurs in about 4 percent of patients, nearly always in older patients as well as those with HIV. It usually indicates extensive disease with a relatively poor outlook and may be misdiagnosed as non-Hodgkin lymphoma.



4-Lymphocyte-Rich Classical Hodgkin lymphoma .

This subtype is similar to the nodular lymphocyte predominant subtype nder the microscope but has more clinical characteristics in common with classical HL.

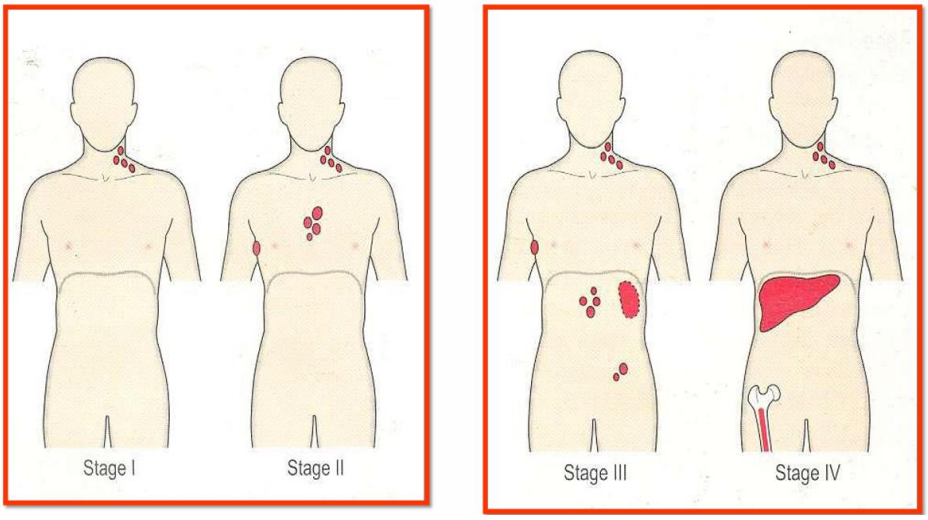
……

2-Nodular Lymphocyte-Predominant Hodgkin Lymphoma.

- ✓ The nodular lymphocyte-predominant (NLPHL) subtype occurs in about 5 percent of patients.
- ✓ The cells in NLPHL, known as “lymphocytic” and “histolytic” cells, are different from classic Reed-Sternberg B cells.
- ✓ Patients with this subtype may have no symptoms and are usually diagnosed with very limited disease.
- ✓ Itis most common in young men.
- ✓ The NLPHL subtype is indolent (slow-growing) and is associated with
- ✓ long-term survival. However, there is a 3 percent risk that this subtype will transform to non-Hodgkin lymphoma.
- ✓ Thetreatment is somewhat different from the treatment for other subtypes.

Staging

Doctors use physical examinations and imaging tests (also called “diagnostic radiology”) to determine the extent of the disease. This is called “staging.” Staging provides important information for treatment planning. The staging system commonly used for HL is the Modified Ann Arbor Staging System.



Stages and Categories of Hodgkin Lymphoma

Lymphoma Stage I: Apparent involvement of a single lymph node region or a single organ, such as bone.

Stage II:

Involvement of two or more lymph node regions that are close to each other; for example, all in the neck and chest, or all in the abdomen and on the same side of the diaphragm (a thin muscle below the lungs).

Stage III:

Involvement of several lymph node regions in the neck, chest, and abdomen (on both sides of the diaphragm).

Stage IV:

Widespread involvement of lymph nodes on both sides of the diaphragm and in other organs, such as the lungs, liver, and bones. Categories A, B, X, and E. The four stages of HL can be divided into categories.

……

The A category indicates the absence of fever, exaggerated

sweating, and weight loss.

The B category indicates that patients have a fever, excessive

sweating, and weight loss.

The X category indicates bulky disease (large masses of

lymphocytes).

The E category indicates organs involved outside of the lymph system.

Physical Examination and Imaging Tests.

The physical examination and imaging tests help the doctor evaluate

- ❖ The location and distribution of lymph node enlargement
- ❖ Whether organs other than lymph nodes are involved
- ❖ Whether there are very large masses of tumors in one site or another. Imaging tests include
- ❖ Chest x-ray
- ❖ Computed tomography (CT) scan of the chest, abdomen and pelvis
- ❖ Magnetic resonance imaging (MRI) in select cases
- ❖ [18F] Fluorodeoxy glucose positron emission tomography (FDG-PET)
- ❖ (evaluates the whole body).

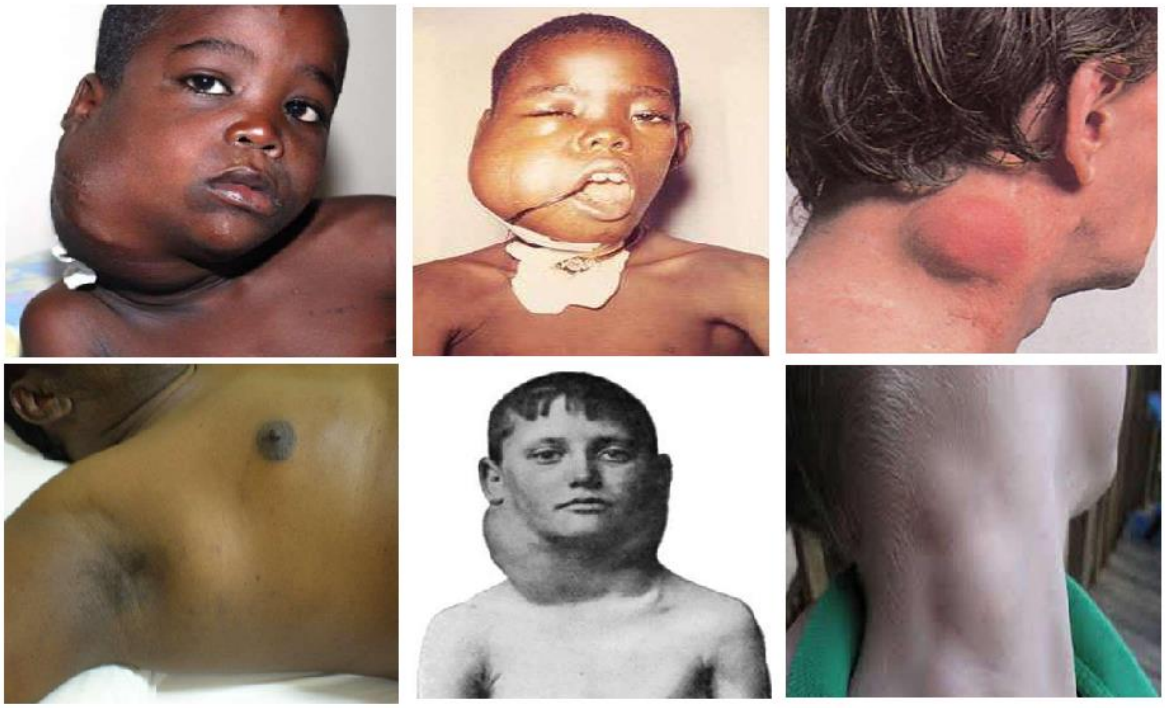
In many centers, patients have CT scans of the neck, chest, abdomen, and pelvis—all the areas where lymph nodes are present—to see whether there are other areas of disease. The CT scan can also show whether there is the involvement of the lungs, liver, and other organs, which is information that is helpful in staging .

The use of PET or PET-CT scans in managing HL is becoming more ommon. Currently, PET is widely used for staging and response assessment after the completion of therapy. It is used to a lesser extent for assessment of response during therapy. PET cannot replace CT scan or bone marrow biopsy in taging HL. However, it can provide complementary information.

Blood and Bone Marrow Tests.

Patients have blood cell counts and other blood tests done to check ndicators of disease severity such as blood protein levels, uric acid levels, erythrocyte sedimentation rate (ESR) and liver functions.

Some patients who have been diagnosed with HL may have a bone marrow biopsy. Your doctor will decide if it is necessary to perform depending on certain features such as where the disease is in your body. A bone marrow biopsy may not be required for patients with early-stage disease and low-risk clinical features, such as no symptoms of fever, night sweats, weight loss or bulky disease (large masses of lymphocytes).



Non-Hodgkin lymphoma (NHL)

1-The neoplastic transformation of either B or T cell lineages of lymphatic cells.

2-NHL causes the accumulation of neoplastic cells in both the lymph nodes as well as more often diffusely in extra lymphatic organs and the loodstream.

3-Absent reed-Sternberg cells.

……

Risk factors of Non-Hodgkin lymphoma (NHL)

A- Diseases - Infections:

1-Human immunodeficiency virus (HIV)

2-Epstein-Barr virus (EBV): linked to Burkitt lymphoma.

3-Helicobacter pylori: Extranodal tissues generating lymphoma include

mucosa- associated lymphoid tissue (MALT)

4-Human T-cell leukemia/lymphoma virus(HTLV-1)

5-Hepatitis C virus

B-Age:

Most people with non-Hodgkin lymphoma are older than 60.

Clinical Presentation of Hodgkin lymphoma

1-Clinical presentation is the same as for Hodgkin lymphoma.

2-The difference is that Hodgkin is localized to cervical and supraclavicular nodes 80%-90% of the time.Whereas NHL is localized 10-20% of the time.

3-CNS involvement is more common with NHL.

4-HIV positive patients often have CNS involvement.

Staging and diagnosis

1-Staging and Diagnosis are the same as for Hodgkin lymphoma.

2-Differences:Bone marrow biopsy is more central in the initial staging of NHL Because the presence of bone marrow involvement means the patient has stage IV disease and therefore needs combination chemotherapy.

Symptoms

1-Mass Effect – Lymphadenopathy (occipital, Posterior auricular, preauricular, mandibular, submental, cervical, supra & infraclavicular, Axillary, inguinal, Popliteal, Hepatosplenimegaly, mediastinal, Abdominal,Pelvic, testicular, CNS masses.

2- Hematologic – Anemia,Thrombocytopenia,lymphocytosis

3- Constitutional B-Symptoms , Fatigue, Anorexia, Pruritus.

4- Paraneoplastic Syndromes

Physical Examination

1. Lymph nodes examination for:

1- Size

2- Multiplicity

3- Consistency

4- Tenderness

.....

Treatment

1- Surgery

2- Radiotherapy

3- Chemotherapy

4-Combined Chemotherapy.

5- Autologous stem cell transport

Grades:-NHL divided into Low or high grade

High Grade	Low Grade
1- Lymphoma has cells that look quite different from normal cells.	1- Lymphomas have cells that look much like normal cells.
2-Celltend to grow fast (aggressive).usually look follicular. Incurable. Wider dissemination at presentation.	2-Cell multiply slowly(indolent).usually look diffuse. Long term treatment maybe achievable
3-Treatment of aggressive, forms of lymphoma can result in a cure in the majority of cases. the prognosis for patients with a poor response to therapy is worse.	3-They don't cure the lymphoma, they can alleviate the symptoms. Patients with these types of lymphoma can live near-normal lifespans, but the disease is Incurable

.....

Northern Technical University

College of Health and Medical

Technical /Kirkuk

Medical Laboratory Department

Module of General Hematology

15th - Week

Lecture Title: Bleeding disorders.

Third level-First semester

By

Dr. Fidan Fikrat Ahmed

2024-2025

General Objectives of the Module

Acquiring the necessary skills and developing knowledge for the students in identifying the concept of bleeding disorders.

Special Objectives of the Module

1. Definition of bleeding disorders.

2. Definition of haemostasis.

3. Explain the mechanism of clotting.

4. Enumerate the causes, Clinical features, Symptoms, Signs,

laboratory findings, and diagnosis of bleeding disorders.

5. Explain the causes of deficiency of clotting factors.

6. Checks for factors that control the clotting.

References:

1. A. Victor Hoffbrand and Paul A. H. Moss. Hoffbrand's Essential Haematology. Seventh Edition. This edition was first published 2016 © 2016 by John Wiley & Sons Ltd.

2. Gamal Abdul Hamid. CLINICAL HEMATOLOGY. 2013.

3. Learning Guide Series Hematology.

4. Bernadette F. Rodak and Jacqueline H. Carr. Clinical Hematology Atlas.

Fourth Edition. Copyright © 2013 by Saunders, an imprint of Elsevier Inc. ISBN: 978-1-4557-0830-7.

Bleeding Disorders

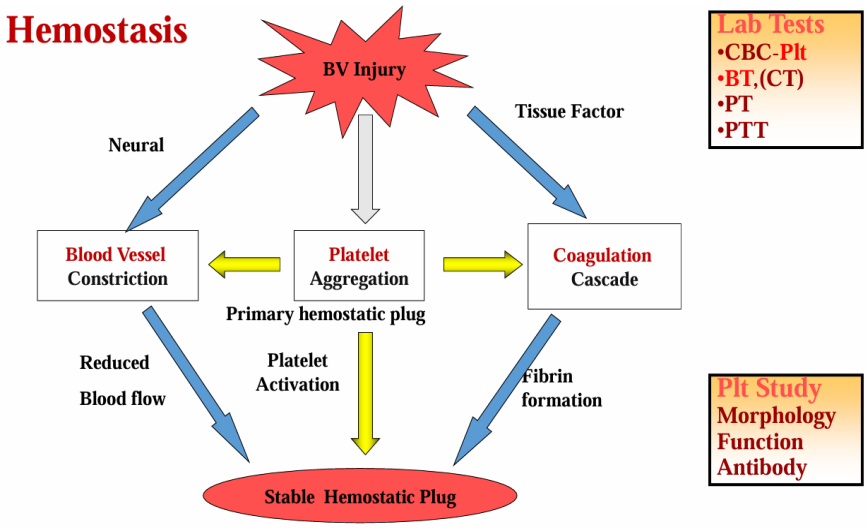
Hemostasis

1. Vascular phase

2. Platelet phase

3. Coagulation phase

4. Fibrinolytic phase



Normal Clotting

Response to vessel injury

1. Vasoconstriction to reduce blood flow

2. Platelet plug formation (von willebrand factor binds damaged vessle and platelets)

3. Activation of clotting cascade with generation of fibrin clot formation

4. Fibrinlysis (clot breakdown).

Clotting Cascade

Normally the ingredients, called factors, act like a row of dominoes toppling against each other to create a chain reaction. If one of the factors is missing this chain reaction cannot proceed.

……

Vascular Phase

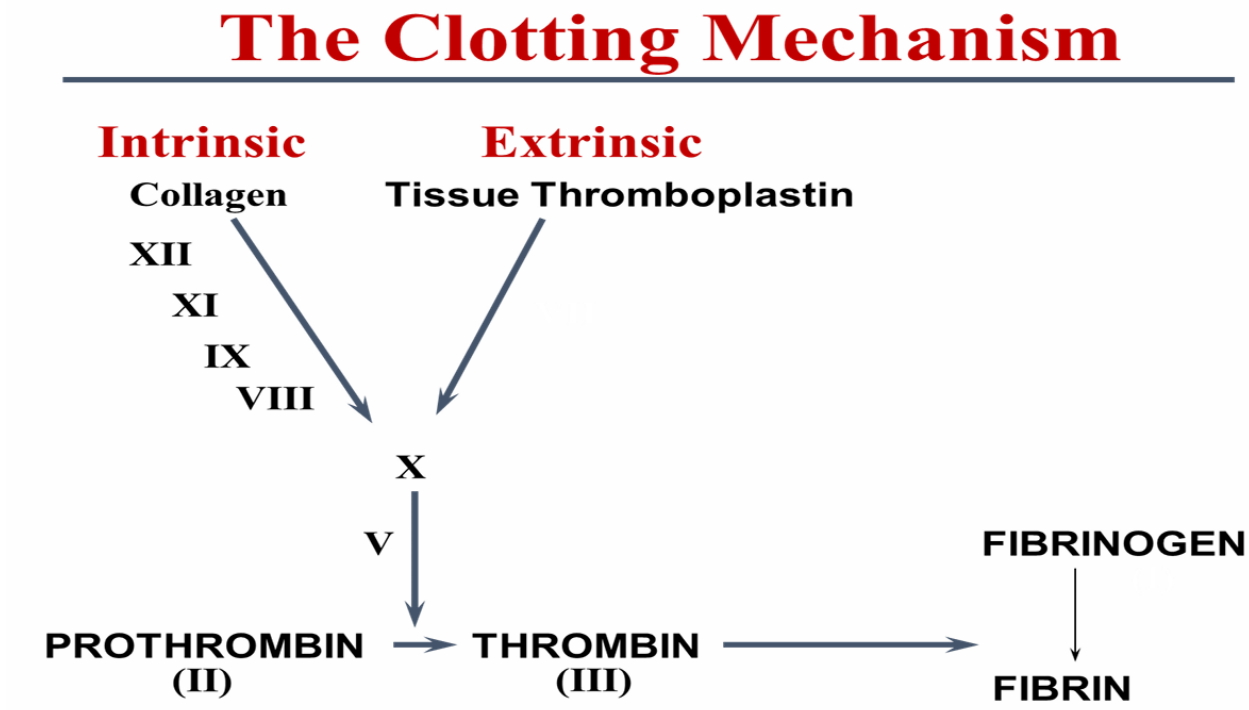
When a blood vessel is damaged, vasoconstriction results.

Platelet Phase

Platelets adhere to the damaged surface and form a temporary plug.

Coagulation Phase

Through two separate pathways the conversion of fibrinogen to fibrin is complete.



Fibrinolytic Phase

Anticlotting mechanisms are activated to allow clot disintegration and repair of the damaged vessel.

……

Hemostasis

Dependent Upon:

•Vessel wall integrity

•Adequate numbers of platelets

• Proper functioning platelets

•Adequate levels of clotting factors

• Proper function of fibrinolytic pathway

……

Laboratory Evaluation

•Platelet count.

• Bleeding time (BT).

• Prothrombin time (PT).

• Partial thromboplastin time (PTT).

• Thrombin time (TT).

Platelet Count

❖ NORMAL	100,000 - 400,000 CELLS/MM^3
< 100,000	**Thrombocytopenia**
50,000 - 100,000	**Mild Thrombocytopenia**
< 50,000	**Sever Thrombocytopenia**

Bleeding Time

Normal Value

2-8 minutes

Provides assessment of platelet count and function

Prothrombin Time

• Measures Effectiveness of the Extrinsic Pathway

• Mnemonic – PET

Normal Value

10-15 SECS

Partial Thromboplastin Time

• Measures Effectiveness of the Intrinsic Pathway

•Mnemonic – PITT

Normal Value

25-40 SECS

Thrombin Time

• Time for Thrombin To Convert

Fibrinogen ⇨ Fibrin

• A Measure of Fibrinolytic Pathway

Normal Value

9-13 SECS

So What Causes Bleeding Disorders?

- ❖ Vessel Defects
- ❖ Platelet Disorders
- ❖ Factor Deficiencies
- ❖ Other Disorders

Vessel Defects

- ❖ Vitamin C Deficiency
- ❖ Bacterial & Viral Infections
- ❖ Acquired & Hereditary Conditions

Vascular defect - cont.

Infectious and hypersensitivity vasculitides

- Rickettsial and meningococcal infections
- Henoch-Schonlein purpura (immune

Platelet Disorders

- •Thrombocytopenia
- • Thrombocytopathy

Thrombocytopenia

Inadequate Number of Platelets

Thrombocytopathy

Adequate number but abnormal function

Thrombocytopenia

- ❖ Drug Induced
- ❖ Bone Marrow Failure
- ❖ Hypersplenism
- ❖ Other Causes

.....

Other Causes

- ❖ Lymphoma
- ❖ HIV Virus
- ❖ Idiopathic Thrombocytopenia Purpura (ITP)

Thrombocytopathy

- Uremia
- Inherited Disorders
- Myeloproliferative Disorders
- Drug Induced

Factor Deficiencies

(Congenital)

- ✓ Hemophilia A
- ✓ Hemophilia B
- ✓ Von Willebrand's Disease

Factor Deficiencies

❖ Hemophilia A (Classic Hemophilia)

• 80-85% of all Hemophiliacs

• Deficiency of Factor VIII

• Lab Results - Prolonged PTT

❖ Hemophilia B (Christmas Disease)

• 10-15% of all Hemophiliacs

• Deficiency of Factor IX

• Lab Test - Prolonged PTT

Factor Deficiencies

✓ Von Willebrand's Disease
✓ Deficiency of VWF & amount of Factor VIII
✓ Lab Results - Prolonged BT, PTT

Other Disorders

❖ Oral anticoagulants
- Coumarin
- Heparin

❖ Liver disease
❖ Malabsorption
❖ (Acquired)
❖ Broad-spectrum antibiotics

Inhibitors

• 30%ofpeople with haemophilia develop an antibody to the clotting factor they are receiving for treatment. These antibodies are known as inhibitors.

• These patients are treated with high does of FVIIa for bleeds or surgery. This overrides defect in FVIII or FIX deficiency.

• Longterm management involves attempting to eradicate inhibitors by administering high dose FVIII (or FIX) in a process called immune tolerance.

……..

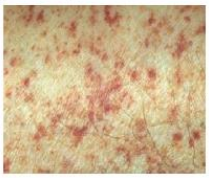
Bleeding Disorders

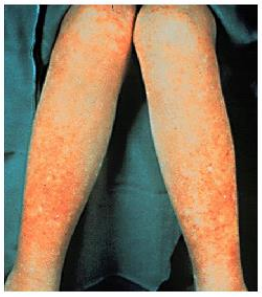
Clinical Features of Bleeding Disorders

	Platelet disorders	Coagulation factor disorders
Site of bleeding	Skin Mucous membranes (epistaxis, gum, vaginal, GI tract)	Deep in soft tissues (joints, muscles)
Petechiae	Yes	No
Ecchymoses (“bruises”)	Small, superficial	Large, deep
Hemarthrosis / muscle bleeding	Extremely rare	Common
Bleeding after cuts & scratches	Yes	No
Bleeding after surgery or trauma	Immediate, usually mild	Delayed (1-2 days), often severe

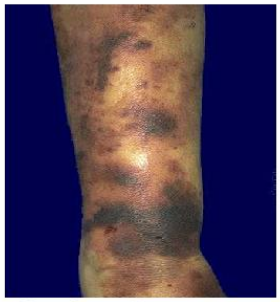
Petechiae

(typical of platelet disorders)



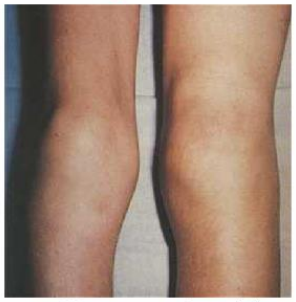


Platelet



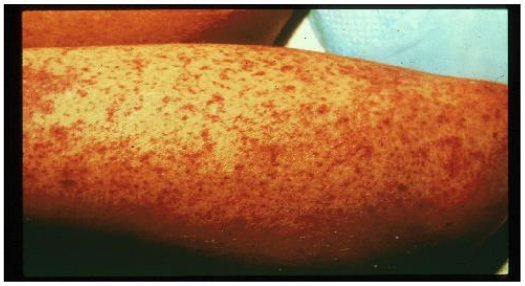
Petechiae, Purpura

Coagulation

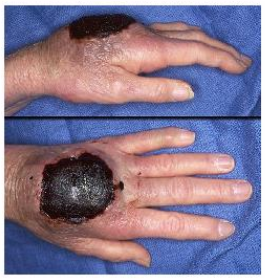


Hematoma, Joint bl.

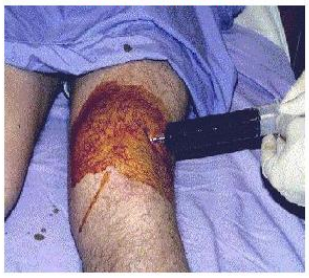
Petechiae



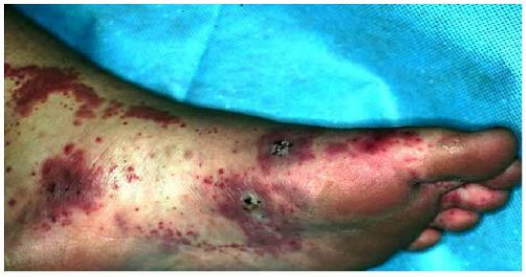
Hematoma



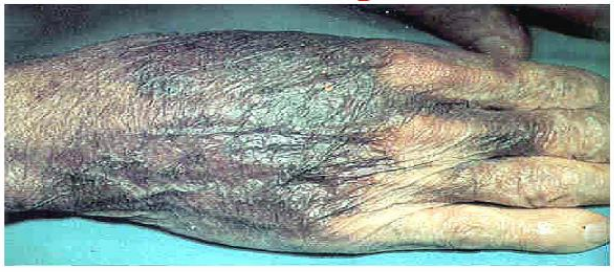
Hemarthrosis

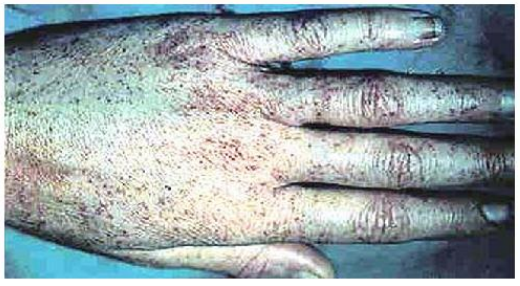


Purpura

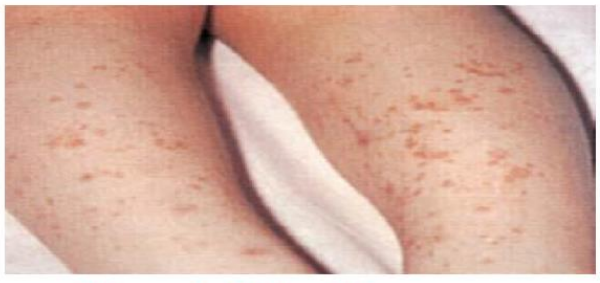


Senile Purpura





Petechiae



Henoch-Schonlein purpura

Coagulation Factor Disorders

Inherited bleeding disorders

- Hemophilia A and B
- VonWillebrands disease
- Other factor deficiencies

Acquired bleeding disorders

- Liver disease
- Vitamin K deficiency/warfarin overdose
- DIC

Hemophilia A and B

	Hemophilia A	Hemophilia B
Coagulation factor deficiency	Factor VIII	Factor IX
Inheritance	X-linked recessive	X-linked recessive
Incidence	1/10,000 males	1/50,000 males
Severity	Related to factor level <1% - Severe - spontaneous bleeding 1-5% - Moderate - bleeding with mild injury 5-25% - Mild - bleeding with surgery or trauma	
Complications	Soft tissue bleeding	

Hemophilia

Clinical manifestations (Hemophilia A & B are indistinguishable)

Hemarthrosis (most common)

Fixed joints

Soft tissue hematomas (e.g., muscle)

Muscle atrophy

Shortened tendons

Other sites of bleeding

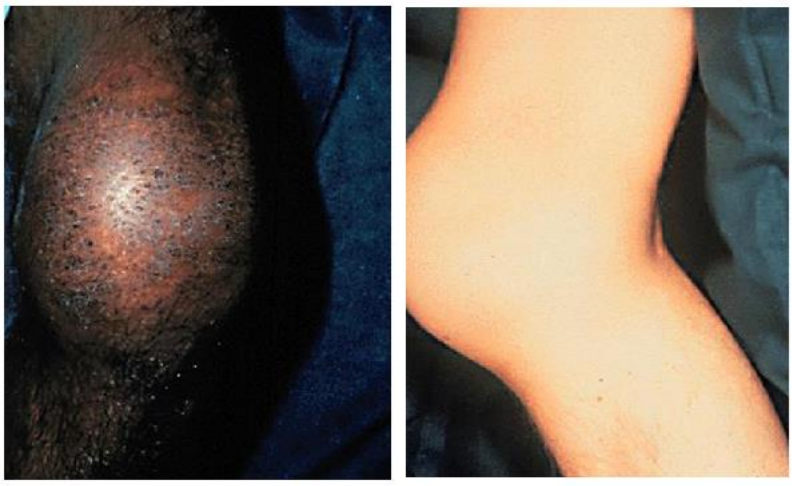
Urinary tract

CNS, neck (may be life-threatening)

Prolonged bleeding after surgery or dental extractions

.....

Hemarthrosis(acute)



Classification of platelet disorders

- **Quantitative disorders**
  - Abnormal distribution
  - Dilution effect
  - Decreased production
  - Increased destruction
- **Qualitative disorders**
  - Inherited disorders (rare)
  - Acquired disorders
    - Medications
    - Chronic renal failure
    - Cardiopulmonary bypass

.....

Thrombocytopenia

Immune-mediated
- Idioapthic
- Drug-induced
- Collagen vascular disease
- Lymphoproliferative disease
- Sarcoidosis

Non-immune mediated
- DIC
- Microangiopathic hemolytic anemia