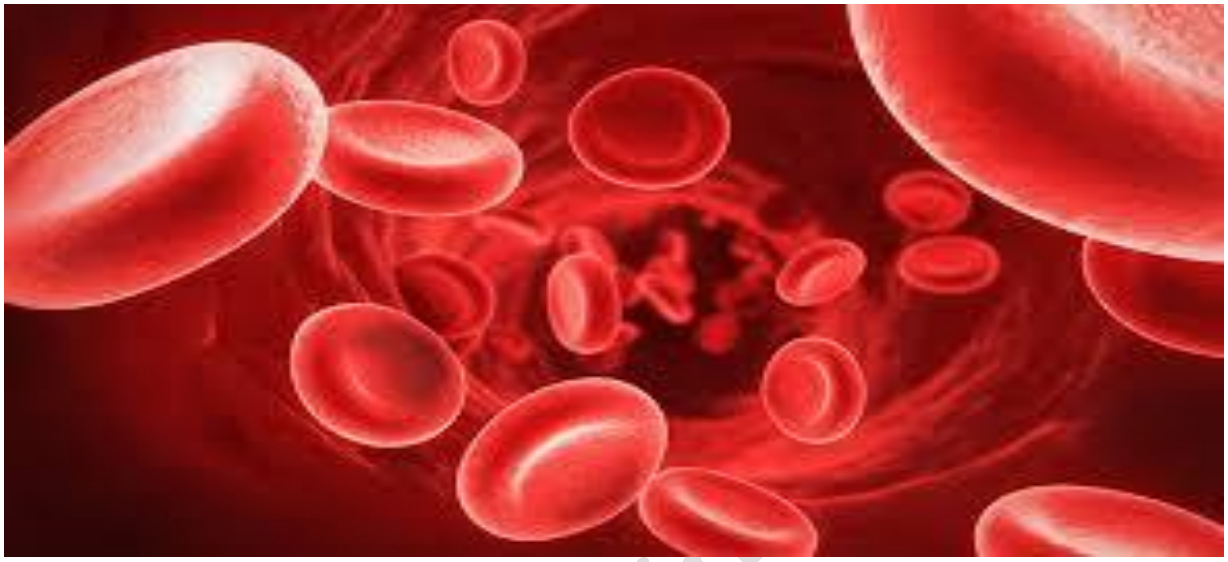
Ministry of Higher Education and
Scientific Research
The southern Technical University
Amara-Technical Institute



Hematology

Practical (1)

For Students of second stage

Medical Laboratory Department

Shaimaa Hussain Altamemem
2024-2025

Syllabus for Hematology practical (1)

Weeks	Topics
1	Identify of hematology laboratory include system
2	Study the erythrocyte Sedimentation rate
3	Study of Packed Cell Volume
4	Study the Hb. Estimation
5	Study the absolute Values include M.C.V, M.C.H, and M.C.H.C
6	Abnormality of R.B.C in Color , Size and Inclusion bodies
7	Abnormality of R.B.C in shape
8	Examination
9	Study the Reticulocyte Count
10	Anemic types
11	Examination
12	Study the abnormal Hb. (Hb.S)
13	Study the hemostasis disorders
14	Study the bleeding time
15	Study the Clotting time

The general aim of study practical hematology (1) :

1-Understanding basic principles of hematology and acquiring essential laboratory skills.

2-Identify and differentiate various normal and abnormal blood cells under the microscope, recognizing key morphological changes indicative of different pathologies.

3-Learning the correct methods for collecting, preserving, and handling blood samples to ensure the accuracy of laboratory results .

4-Learn to identify and address common errors or discrepancies in laboratory procedures and results, ensuring the reliability of diagnostic data.

5-Build a strong foundation of practical skills that are directly applicable to clinical settings, research laboratories, and other hematology-related fields.

6-The ability to classify different types of blood disorders, such as anemia, based on laboratory manifestations.

7-Applying acquired knowledge and skills in a practical setting, qualifying them for work in medical laboratories and hospitals.

8-Training on the use of automated blood analyzers (e.g., complete blood count machines) and understanding their operating principles and result interpretation.

Target group:

Second Year Students / Medical Laboratory Department

Educational Resources and Tools

1-Presentation and Interaction Technologies
- a-Data Show
- b-Interactive Whiteboard
- c-Laptop
- d-Blackboard and Pens

2-Laboratory Equipment
- a-Microscope
- b-Centrifuge
- c-Micro centrifuge
- d-Water Bath

3-Tools and Consumables
- a-Tourniquet
- b-Cotton and Alcohol
- c-Test Tubes
- d-Glass Slides
- e-Coverslips
- f-Pipettes

4-Laboratory Reagents and Solutions
- a-HCl con0.1
- b-PT reagent
- c-Ammonium oxalate 1 %
- d-Methylene Blue stain

Teaching and Learning Methods and Techniques

1-Enhanced Theoretical Lectures.
2-Practical Demonstrations.
3-Hands-on Laboratory Sessions.
4-Case-Based Learning.
5-Interactive and Collaborative Learning.
6-Self-Directed Learning.

Integrated Assessment Methodology

1-Assessment of Foundational Theoretical Knowledge
  a-MCQs
  b-Quiz
2-Assessment of Practical and Laboratory Skills
  a-Objective Structured Practical Examination
  b-Logbook Performance Assessment
3-Assessment of Integrated Professional Competency
  a-Laboratory Reports
  b-Presentations and Seminars

Lecture time : (Two hour)

Week 1

Lecture title :-

Identify of hematology laboratory include system

Learning objective:

By the end of this practical lecture, students will be able to :

1-Define hematology and identify its primary areas of study.

2-Identify the four main areas within a diagnostic hematology laboratory and their respective functions .

3-List the major components of blood and state the primary function of each .

4- Recognize common diseases treated by hematologists .

5-Describe the process of blood sample collection, and the phenomenon of blood clotting.

6-Identify and explain the mechanism of action of common anticoagulants used in hematology .

7-Differentiate between plasma and serum based on their composition and preparation.

8-List and explain common reasons for the refusal of blood samples in a laboratory setting.

Pre-Test

1- Hematology is the study of diseases related to?

a) Bones b) Blood

c) Muscles d) Nerves

2- The primary function of Red Blood Cells (RBCs) is ?

a) Protection against pathogens

b) Maintenance of vascular integrity

c) Oxygen and CO2 transport

d) Blood clotting

3- The process of collecting blood from a patient is called?

a) Urinalysis b) Phlebotomy

c) Biopsy d) Radiography

Identify of hematology laboratory include system

Hematology :- is a branch of medicine, concerning the study of the cause, diagnosis , treatment , and prevention of disease related to blood .

A diagnostic hematology ,usually divided up into four main areas laboratory is :

1-Routine hematology : full blood examinations, morphology and other tests .

2-Coagulation testing : for the proteins and cells involved in clotting .

3-Blood bank : blood and blood product transfusions .

4-Special tests : performed only when required .

Blood component

1- plasma: the liquid component of blood, making up about 55% of its volume. It's a pale yellow liquid that carries blood cells, proteins, nutrients, hormones, and waste products throughout the body.

2- R.B.C (Oxygen & CO2 transport).

3- W.B.C (protection versus , pathogens , Microorganisms) .

4- Platelets (Maintenance of vascular integrity).

Some of diseases treated by hematologists include

1-Iron deficiency a anemia and other types of anemia such as sickle cell anemia .
2-Polycythemia or excess production of red blood cells .
3-Myelofibrosis.
4-Leukemia.
5-Platelet and bleeding disorders such hemophilia, , idiopathic asthrombocytopenic purpura and Von Willebrand disease.
6-The myelodysplastic syndromes.
7-Hemoglobinopathies such as thalassemia and sickle cell disease
8-Multiple myeloma.
9-Malignant lymphoma.
10-Blood transfusion.
11-Bone marrow stem cell transplantation.

Collection of blood samples for testing in the laboratory:-

collected from the venous circulation using a needle and syringe or the vacutainer system. The process of collecting blood from a patient is called venesection. If a sample of blood is removed from the body and placed in a tube the blood will eventually solidify; this process is called clotting. Anticoagulants can be used to stop the clotting process .

The common anticoagulants used are:

1-Ethylenediamine tetra acetic acid (EDTA)

Ethylenediamine tetra acetic acid (pink/purple top tube) is a powder and blood collected into it does not clot. On collection blood must be gently mixed so that the EDTA is dissolved in the blood. EDTA prevents blood from clotting by binding or chelating binding) the calcium ions , Proportion of cells and plasma in a blood sample. from the blood (calcium ions are necessary for blood to clot Blood collected into EDTA is used for **routine hematology tests .**

2- Tri-sodium Citrate (light blue top tube)

Is a liquid anticoagulant that is used to collect blood for coagulation studies Samples must be gently mixed immediately after collection. Tri-sodium citrate chelates calcium ions from the blood (calcium ions are necessary for clotting).used for pt & ptt.

3- Heparin (green top tube)

Heparin is an anticoagulant used for biochemistry tests and some specialized hematology tests. Heparin prevents blood coagulation by inhibiting the action of thrombin. Thrombin is an activated coagulation protein that converts fibrinogen to fibrin. Fibrin formation occurs when blood clots.

EDTA

Tri sodium sitrate

Heparin

Plasma and serum

Plasma:

Above the buffy coat is a pale straw- coloured fluid called plasma. Plasma contains hundreds of different substances proteins, vitamins, hormones and minerals. There are some substances present in plasma, which are of interest to the haematology laboratory. These are the coagulation proteins, which are involved in the clotting of blood.

Serum:
If blood is allowed to clot and then left for a while, the clot will shrink and a straw coloured fluid appears above the clot.This fluid is called serum. For some laboratory tests, , serum is the specimen of choice. Serum is almost the same as plasma except there are no clotting proteins present

Causes of refused blood sample:

1- Clotted blood in an ant coagulated specimen.
2- Collection in the wrong tube.
3- Contaminated specimens and containers .
4- Defective tube.
5- Hemolysis.
6- Improper special handing .
7- Incompletely or inadequately filled tube .
8- Unlabeled or mislabeled specimens.

Post test :

Q1- Define hematology and briefly describe the scope of its study within medicine ?

Q2- Compare and contrast the composition and use of plasma and serum in laboratory testing ?

Q3- List five reasons why a blood sample might be refused by a hematology laboratory ?

Week 2
Lecture title :-

Study the erythrocyte Sedimentation rate

Learning objective:

By the end of this practical lecture, students will be able to :
1-Define Erythrocyte Sedimentation Rate (ESR) and its significance in clinical diagnostics .
2-Explain the three stages of ESR sedimentation and the approximate timeframes for each .
3-Identify and list the various factors that can influence ESR values .
4-Recognize and describe common sources of error during ESR estimation using the Westergren method .
5-List several clinical conditions associated with an increased and decreased ESR .
6-Recall the normal reference ranges for ESR in men, women, and newborns .
7-Accurately read and interpret ESR results .
8- Understand the principle behind using tri-sodium citrate as an anticoagulant and diluent in the Westergren method .

Pre-Test

Q1- What does ESR stand for, and what does it measure?

Q2- Name two factors that can cause an increase in ESR ?

Q3- List one potential source of error when performing an ESR

Study the erythrocyte Sedimentation rate

ESR is a blood test that measures how quickly red blood cells (erythrocytes) settle at the bottom of a test tube in one hour. The estimation of the erythrocyte Sedimentation rate can done by wastergreen method . It is the simplest and widely used method, Tri- Sodium citrate used as a diluents and anticoagulant solution .

ESR takes place in three stages at one hour:

1- stage of Rolex formation (aggregation)10 min .
2- stage of sedimentation (settling) 40 min .
3- stage of packing 10 min .

factors effect on sedimentation rate

1- Age:- In children the E.S.R decrease because the increase in R.B.C .
2- Sex :- In men E.S.R decrease because the increase in R.B.C.
3- During the pregnancy time because the Hemodilution. E.S.R. is high .
4- High places : E.S.R value is different .

Sources of error:

1- Tilting the sedimentation tube.
2- Length of sedimentation tube.
3- increase the temperature above room temperature
4- Unclean Sedimentation tube like dirt or presences of water or alcohol cause hemolysis or error of sedimentation rate
5- Excessive anticoagulant increase sedimentation rate .
6- Clott or old blood decrease sedimentation rate.
7- Failure to mix the blood carefully.
8- Presences of air bubble in blood column.

Condition accompanied by an increase E.S.R a diseases Like:-

1- Acute or chronic infections Like Pneumonia , Leukemia.
2- Anemia .
3- Carcinomatosis .
4- Normal or abnormal pregnancy.

Decrease in

1- Polycythemia.
2- Congestive heart failure.
3- Dehydration , burns , allergies.

Normal value :

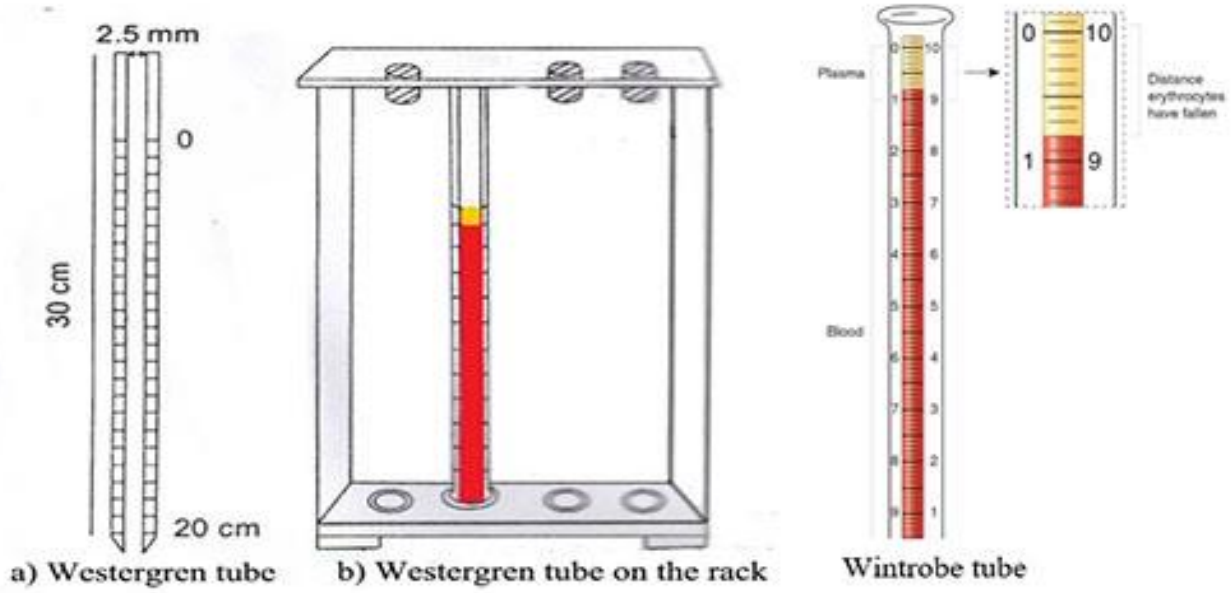
-Man :- 0 - 15 mm/1h
-Women:- 0 - 20 mm/1h
-Newborn :- 0 – 10 mm/ 1h

Material

1- Syringe , Alcohol , Cotton.
2- Westergreen capillary tube (0 – 200) .
3-Westergreen rack .
4- Sodium citrate or oxalate.
5- Test tube.

Procedure:-

1- Collect (2 mL) 0f venous blood in anticoagulant tube.
2- Mix 1 volume of diluents (0.5 mL) with 4 volume of blood (2 mL) by inverting the test tube for (2) minutes.
3- Draw the mixture into westergreen tube to (0) mark.
4- Place the tube vertically in the rack and Leave it for (1 hour).
5- Read the height of the clear plasma above the upper limit of the column of Sedimenting cells.



a) Westergren tube b) Westergren tube on the rack Wintrobe tube

Post test

Q1- Define Erythrocyte Sedimentation Rate (ESR) ?

Q2- Enumerate at least five distinct sources of error that can affect the accuracy of an ESR measurement ?

Q3- List four diseases or conditions that are typically accompanied by an increased ESR?

Q4- State the normal reference values for ESR in
a. Man b. Woman c. Newborn

week 3
Lecture title :

Study of Packed Cell Volume

Learning objective:

By the end of this practical lecture, students will be able to :
1-Define Packed Cell Volume (PCV) / Hematocrit (HCT) and explain its significance as a screening test.
2- Describe the underlying principle of hematocrit measurement.
3- Identify conditions that cause an increase in PCV .
4- physiological or pathological reasons that cause a decrease in PCV .
5-List the essential materials and equipment required for performing the capillary tube method for PCV measurement .
6-Outline the step-by-step procedure for measuring PCV using the capillary tube method .
7- Identify and label the distinct layers observed in a centrifuged .
8- State the normal reference ranges for PCV in adult males, adult females, and newborns .
9-Recognize potential sources of error that can affect the accuracy of PCV results and describe ways to minimize them .

Pre test

Q1-What does PCV stand for?
a) Plasma Cell Volume b) Platelet Count Volume
c) Protein Concentration Value d) Packed Cell Volume

Q2-Which of the following conditions typically causes an increase in PCV?
a) Acute anemia b) Pregnancy
c) Dehydration d) Kidney disease

Study of Packed Cell Volume

Is the processes to measure the percentage of volume red blood cells in a volume of whole blood . and it also known as (**hematocrit**).

It is screening test for :
1- **Anemia** .
2- **Polycythemia** .

Principle :-

Hematocrit :- Ratio of the height of red blood cells column to the whole blood in the tube .

P.C.V increase in polycythemia due to :-
1- Physiological cases (as high altitude).
2- Pathological cases (as Dehydration) .

P.C.V decrease in:-
1- Acute anemia.
2- Pregnancy.
3- Kidney disease.
4- Liver and Splenic disease.

Method to measure P.C.V :

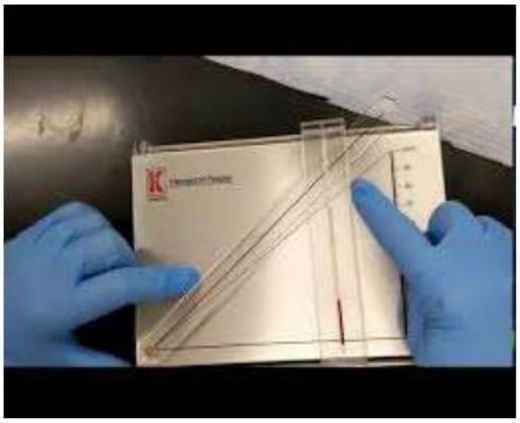
1- Capillary tube method :-
we need a few amount of blood take it from finger.

2- WinTrobe method :-
we need more amount of blood take it from vein.

Materials:

1- Micro centrifuge (heamatocrite centrifuge) .
2- Heamatocrite reader .
3- Capillary tube contain Heparin (anticoagulant).
4- Lancet , Alcohol, Cotton .
5- Wax (Sealing material) .

Capillary tube contain heparin



The hematocrit reader

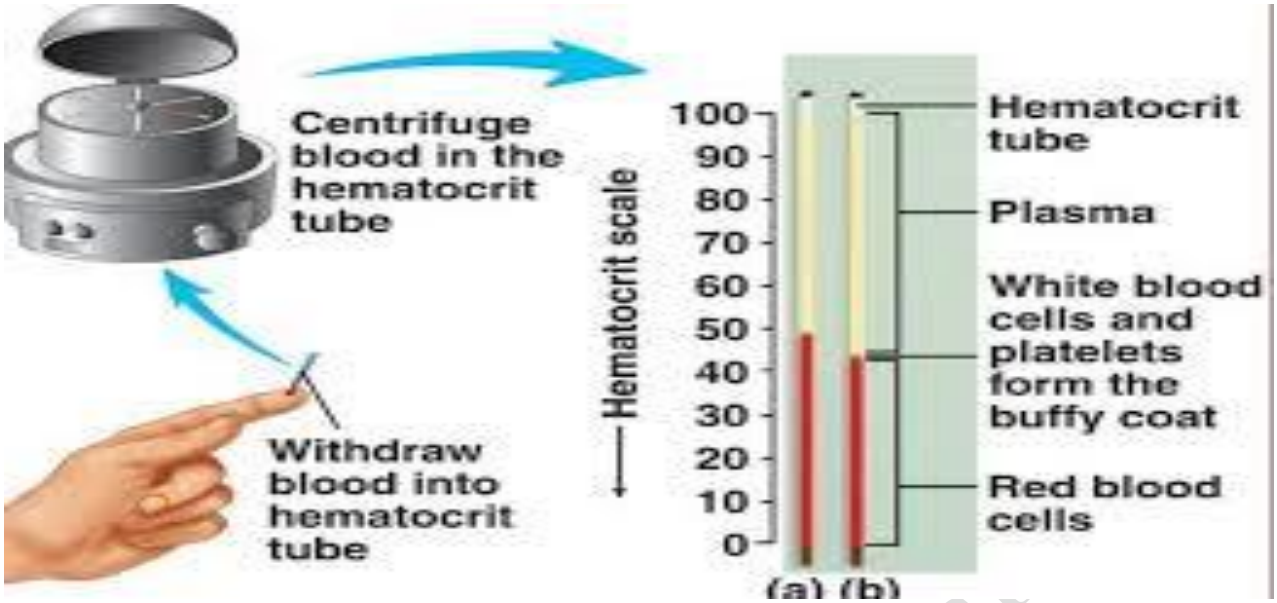
Procedure:

1- Full the capillary tube with blood to 3/4 of its length .

2- Seal the capillary tube by sealing material.

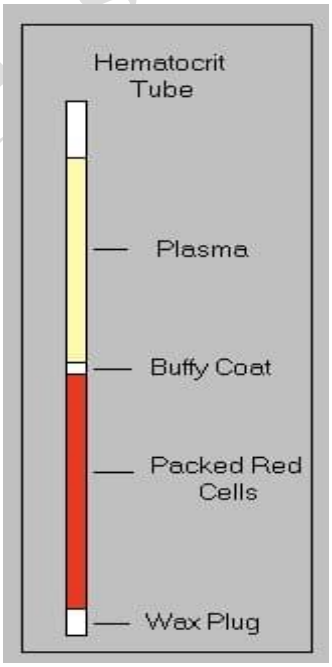
3- Spin the capillary tube by the micro centrifuge (heamatocrite centrifuge) for (5) minute at 5000 R.P.M (revolution per minute)

4- Using the heamatocrite reader to determine the value .



The layers after centration:

Layer R.B.C + Buffy coat (WBCs+ Platelets) layer + Plasma layer



Normal Range : -

- Man : 40 - 54 %
- Women: 37 - 47 %
- New born : 50 - 64 %

Sources of errors :

1 - Bad Sealing .
2 - Vibrating irregular spinning .
3 - Reading of results.
4 - Excessive anticoagulant .
5 - Variation in the pore of capillary tube.
6 - Hemolyzed sample

Post test

Q1- Define Packed Cell Volume (PCV) and explain why it is considered a screening test for both anemia and polycythemia?

Q2- List and explain three different conditions that can lead to a decrease in PCV?

Week 4

Lecture title :

Study the Hb. Estimation

Learning objective:

By the end of this practical lecture, students will be able to :

1-Define hemoglobin (Hb) and describe its molecular structure .

2-Explain the primary functions of hemoglobin in the human body.

3- Identify the key reasons for measuring hemoglobin levels .

4-List conditions that cause an increase or decrease in hemoglobin levels.

5-State the normal range of hemoglobin for males, females, and children.

6-Compare and contrast the principles of Drabkin's method and Sahli's method for hemoglobin estimation .

7-Identify and describe the components of the Sahli Haemoglobin meter apparatus .

8-Perform the Sahli method for hemoglobin estimation accurately, following all procedural steps .

9-Explain the role of hydrochloric acid (HCl) in the Sahli metho.

Pre-Test

Q1-What is the primary function of hemoglobin ?

a) Carries carbon dioxide .
b) Carries oxygen.
c) Fights infection .
d) Clots blood

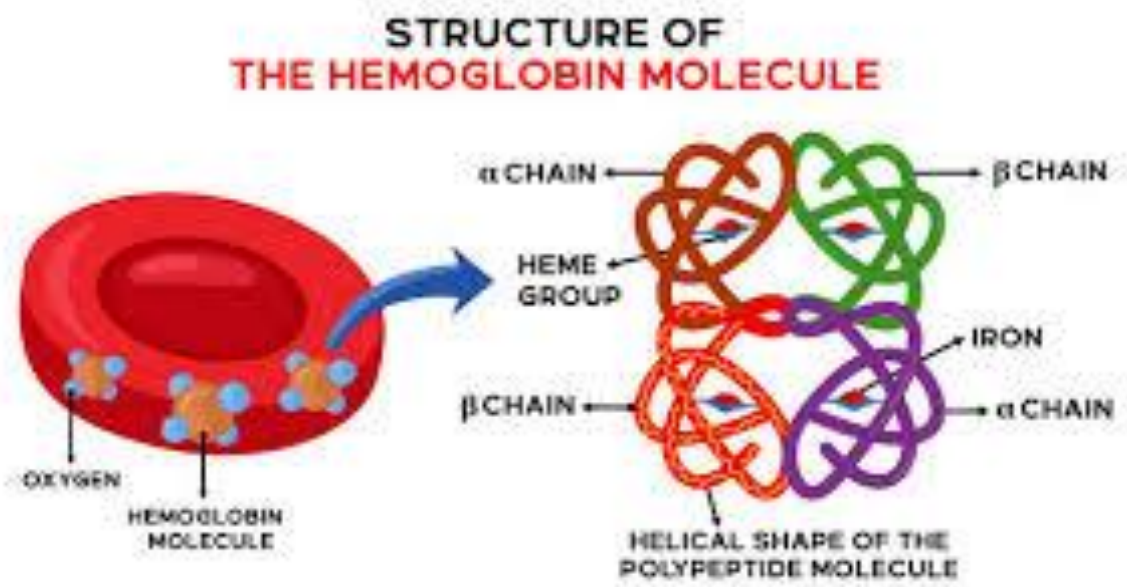
Q2-Hemoglobin is a protein found in which type of blood cell ?

a) White blood cells
b) platelets
c) Red blood cells
d) plasma

Q3-Name one reason why hemoglobin levels might be measured?

Study the Hb. Estimation

Hemoglobin(Hb) :- Is a protein molecule found in red blood cells (erythrocytes) that carries oxygen from the lungs to the rest of the body. Hemoglobin is tetramer with four polypeptide chain (**two alpha α and two beta β**) each of the polypeptide chain has iron atom .The heme group contains a red pigment called (**porphyrin**)



Function of Hb :-

1-Essential for oxygen carrige.
2-Play an essential part in transport of Co2 and regulation of blood reaction .

Why measured of hemoglobin ???

1-Detect anemia and its severity.
2-To monitor anemic patient under the treatment.
3-To check the Hb level of potential donors blood prior of donation.

Hb increase in :-

Polycythemia: Rbc reach more than (7.000.000) cell per cu.mm (cells / mm)

Hb decrease in :-

1- Bleeding .
2- Anemia .
3- Red blood cancer.

Normal Blood Hemoglobin :-

In males :	12 - 16	gm /100 mL of blood
In females :	11.5 -14	gm /100 mL of blood
In children :	14 - 19	gm /100 mL of blood

Methods of Hemoglobin Estimation :-

1- Drabkin's method (Cyanmethemoglobin) Principle :-

Hemoglobin + ferricynide = methemoglobin
Methemoglobin + cyanide = cyanmethemoglobin

2 - Sahli method principle :-

Sahli method depend on colour comparison of the hemoglobin in solution with a standard fixed colour in the form of glass tubes contain acid haematin with standard colour.

The apparatus :-

1-**Sahli Hemoglobin meter** (**Haemometer**) consist from the following:-

A- Two standared glass tubes contain acid haematin with the same colour of standared acid haematin .

B- Small graduated tube closed from lower end open from upper end 20 c , capacity graduated from below upward .

C- Capillary pipette (Micropipette) with 20 mark used for sucking blood from finger puncture.

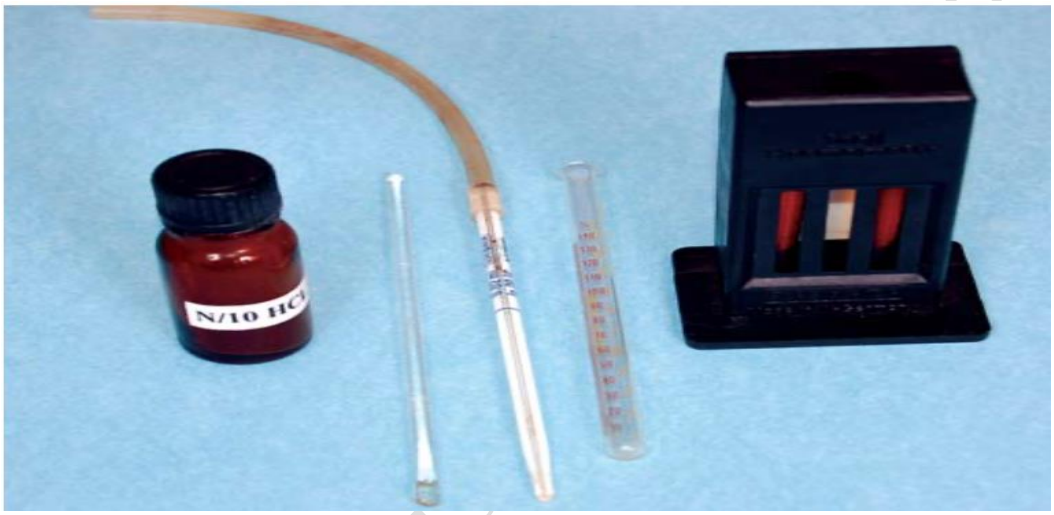
D- Glass rod for mixing .

2- HCL 0.1 N .

3- Distilled water .

4- Cotton and Alcohol .

5- Disposable Lancet .



The procedure :-

1-All the apparatus must be clean and dry.

2-Put in graduated tube HCL (0.1 N) to mark 20.

3-Do make finger puncture .

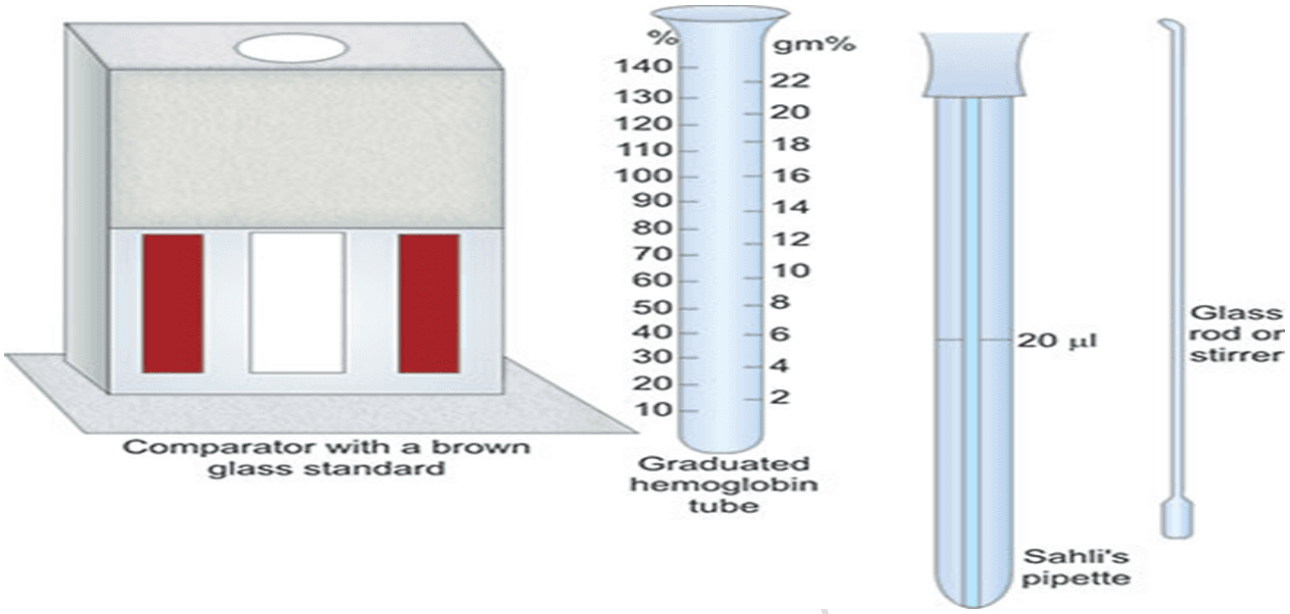
4-With the capillary pipette (Micropipette)suck the blood to mark 20 .

5-Put micropipette immediately and quickly into graduated tube and blow through it so all the blood will be in graduated tube over HCL .

6-Mix the mixture with glass rod and leave for 10 minutes This time is sufficient for haemolysing all the red blood cells leaving the hemoglobin to combine with HCL forming (acid haematin).

7-Add distilled water drop by drop do mixing with glass rod after each adding of the D.W until the colour of the mixture is the same as the colour of standard glass tube.

8-Read the upper limit of the mixture in the graduated tube, and this will represent the percentage of hemoglobin in the blood and the absolute amount of hemoglobin in Gm /100 mL.



Function the HCL (hydrochloric acid) :-

1- Haemolysis the red blood cells.
2- in blood Combine with Hb to form acid haematin.

Post-Test

Q1-Describe the molecular structure of hemoglobin?

Q2-List three specific reasons why a healthcare professional might order a hemoglobin measurement?

Q3-Differentiate between polycythemia and anemia in terms of hemoglobin levels?

Q4-Compare the principles of the Drabkin's method and the Sahli method for hemoglobin estimation?

Week 5
Lecture title:

Study the absolute Values include M.C.V , M.C.H, and M.C.H.C

Learning objective:

By the end of this practical lecture, students will be able to :
1-Define the four main red blood cell indices .
2-State the normal reference ranges for MCV, MCH, and MCHC
3-Explain the clinical significance of each red blood cell index in the morphological classification of anemia.
4-Differentiate between microcytic, normocytic, and macrocytic red blood cells based on MCV values.
5-Identify common conditions and diseases associated with elevated, decreased, or normal values of MCV, MCH, and MCHC.
6-Correlate red blood cell indices with specific types of anemia.
7-Explain the primary uses of a CBC, including diagnosis, monitoring health conditions, and evaluating treatment effectiveness .
8-List and describe common conditions detected by CBC abnormalities.

Pre test

Q1- How is Mean Corpuscular Hemoglobin Concentration (MCHC) different from Mean Corpuscular Hemoglobin (MCH) in terms of what they measure?

Q2-Briefly explain why red blood cell indices are important in diagnosing anemia?

Q3- What does CBC stand for ?

Study the absolute Values include M.C.V, M.C.H and M.C.H.C

Red Blood Cell Indices
The significance of these value is know:
(The Morphological Classification Of Anemia)

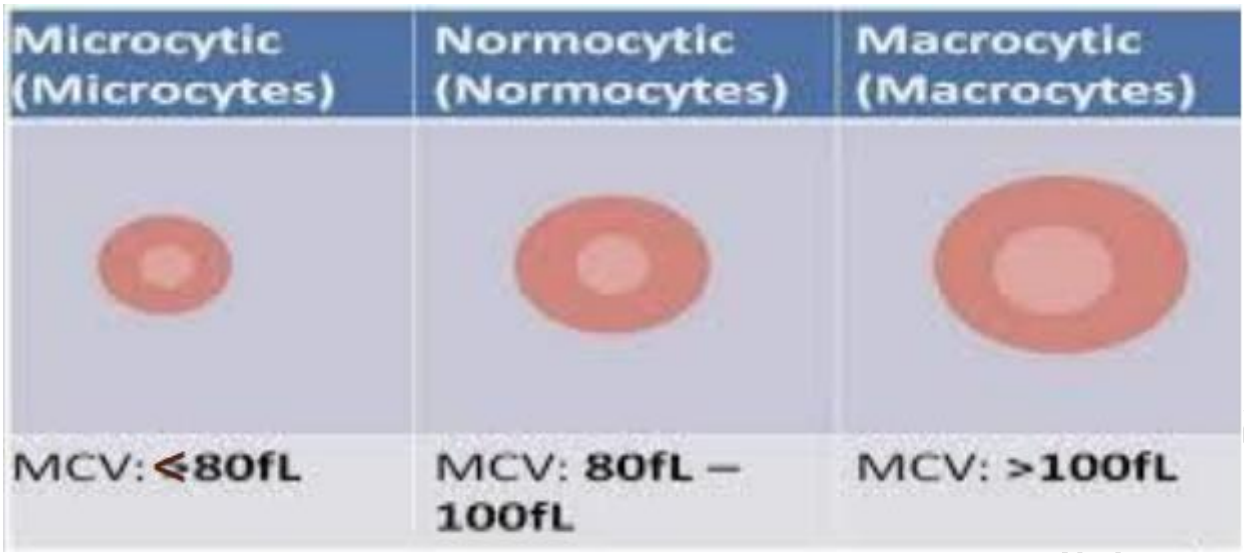
The most commonly used RBC indices are:
1- **Mean Corpuscular Volume (MCV).**
2- **Mean Corpuscular Hemoglobin (MCH).**
3- **Mean Corpuscular Hemoglobin concentration (MCHC).**

It can be directly measured by: **automated hematology analyzer**

Mean corpuscular volume (MCV)
Is the average volume of single Red Blood cell in cuibic micro micro(C.M) or in femto liter (FL).

Normal range : (80 - 96) FL or C.M

MCV elevated or decreased accordance with average R.B.C size.
with average red Blood cells size
- **High** (MCV) indicates (**macrocytic**)
  large average R.B.Cs size
- **Low** (MCV) indicates (**microcytic**)
  small average R.B.Cs size
- **Normal** (MCV) indicates (**normocytic**)
  normal average R.B.Cs size



Calculation

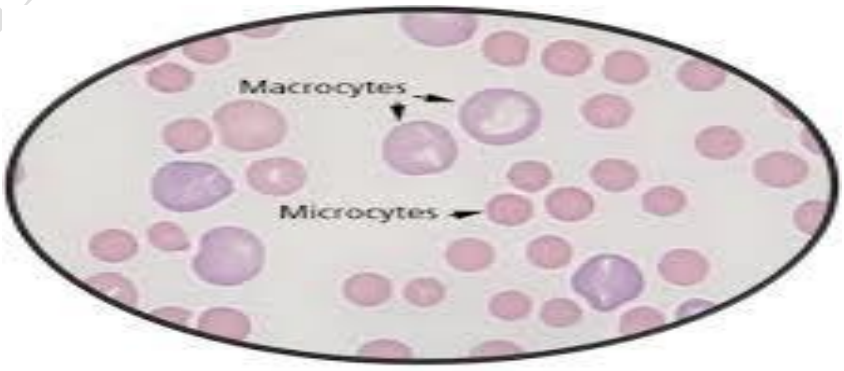
$$MCV = PCV / RBC \times 10^{12}$$

It decrease in -

1- Iron deficiency anemia.
2- Thalassemia.
3- Anemia of chronic blood loss.
4- Chronic disease.

Increase in -

1- Pernicious anemia and deficiency of vitamin B12.
2- Alcoholism.
3- Liver diseases.



-Mean corpuscular hemoglobin MCH

It mean average of hemoglobin in a single red blood cell.

Units of Measurement:
Pico-gram (pg) or micro grams (µg)

Calculation

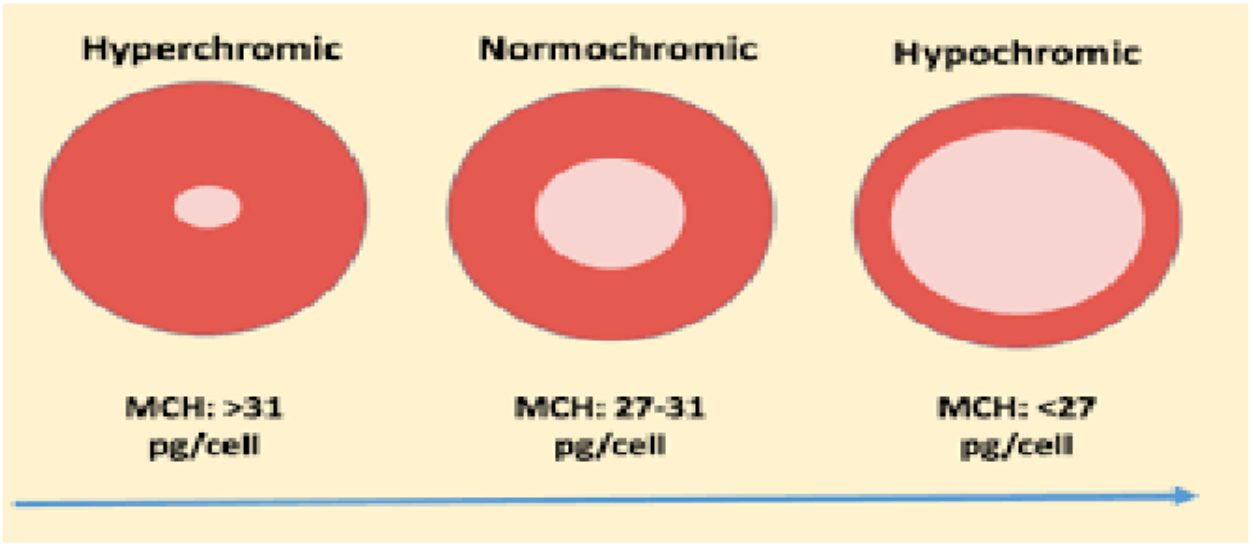
$$MCH = HB / RBC\ (x10^{12})$$

Normal range: (27- 32) pg

The value is frequently higher in newborns and infants than adult
- When MCH values are **high**, the red blood cells called (**Hyperchromic**) the Rbc will be deeper in color

- When MCH values are **low**, the red blood cells called (**Hypochromic**) the Rbc will be pallor in color)

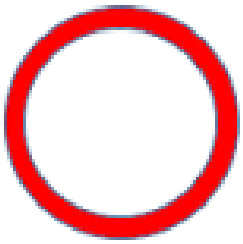
- When MCH values are **normal**, the red blood cells called (**Normochromic**) the Rbc will be normal central pallor in color.



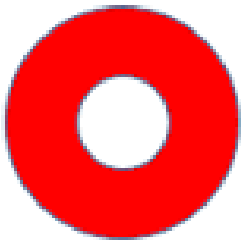
-Mean corpuscular hemoglobin concentration (MCHC)

Is the average Concentration of hemoglobin in the red blood cells, it percentage of hemoglobin in 100 ml of packed red blood cell but not in 100 ml of whole blood.

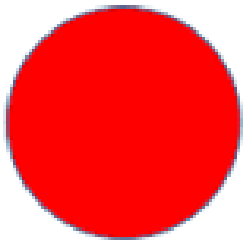
When MCHC is **high**, the red blood cells referred to as being (**Hyperchromic**) possible causes of high MCHC (Autoimmune Hemolytic Anemia)
When MCHC is **low**, the red blood cells referred to as being (**Hypochromic**) possible causes include (Iron Deficincy Anemia)



Hypochromic RBC
Low MCHC



Normochromic RBC
Normal MCHC



Hyperchromic RBC
High MCHC

Calculation

$$\textbf{MCHC} = \textbf{HB(g/dl)} \times 100 / \textbf{PCV}$$

Normal range: (30-35)g/dl

Increase in
1- Sideroblastic anemia .
2- Severe dehydration .

It decrease
1.Iron deficiency anemia .
2.Thalassemia .

In more rare cases low MCHC and hypochromic microcytic anemia can caused by:

1- Cancer including cancers that cause internal blood loss .
2- Parasitic infection like hook worm infection .
3- Lead poisoning .

These indicators are available with **automated hematology analyzer** (CBC)

CBC Is a group of blood tests that measure the number and size of the different cells in the blood.
It is one of the most commonly ordered blood tests, C.B.C is the calculation of the cellular (formed elements) of blood .
A complete blood count common blood test that gives doctors information about five major parts of the blood:
three types of cells (**red blood cells, white blood cells, and platelets**) and two value (**hemoglobin and hematocrit values).**
Normal ranges may be slightly different for men and women.

C.B.C used for?
A complete blood count is often part of a routine checkup. It is also used to monitor a condition or treatment that may affect in the blood cell counts such as **infections**, **anemia, immune system disorders**, and **blood cancers.**
1-Diagnose a health problem:- Doctor may order a CBC if have unexplained symptoms like weakness, fever, redness, swelling, ,bleeding.
2-Monitor a health problem:- Doctor may regularly order CBCs to monitor condition if have been diagnosed with a disorder that affects blood cell counts.
3-Monitor the treatment :- Certain medical treatments can affect the blood cell counts and may require regular CBCs. Doctor can evaluate how well treatment is working based on the CBC .

CBC blood test check for?

1- **Anemia** (low levels of red blood cells or hemoglobin).
2- Erythrocytosis (high concentrations of red blood cells).
3- Leukocytosis (high white blood count).
4- Leukopenia (low white blood count).
5- Thrombocytosis (high platelet count).
6- Thrombocytopenia (low platelet count).

The main parts of the CBC are :-

1- White blood cell (WBC) count.
2- Red blood cell (RBC) count .
3- Hemoglobin (HGB) value.
4- Hematocrit (HCT) value.
5- Platelet count.

Procedure :-

the complete blood count (C.B.C) test is performed by obtaining a few milliliters of blood sample directly from the patient , the skin is wiped clean with an alcohol pad ,and a needle is inserted through the area of cleaned skin into to patient`s vein.
the blood is pulled from the needle by a syringe or by a collection to a special vacuumed vial where it is collected, this sample is taken to the for analysis by use (**device automated hematology analyzers)** .

Condition by measuring the following:

1- RBC (red blood cell count):- This is the number of red blood cells. These are important because they carry oxygen through the body. They also help filter carbon dioxide. If RBC count is too low, may have **anemia** or another condition. The normal range for men is **5 million to 6 million cells/mcL**; for women it's **4 million to 5 million cells /mcl.**

2- White blood cells (WBCs):- These help to fight infections. If WBC levels is high, it refer to inflammation or infection somewhere in the body. If it's low, could refer to risk of infection. The normal range is (**4,500 to 11,000)** cells per microliter (cells/mcL).

3- Hb (hemoglobin):- This is the protein in the blood that holds the oxygen. The normal range for **men is (12 to 16)** grams per deciliter gm/dL, for women it's(**11 to 14)**gm/dL.

4- Hct (hematocrit):- the percentage of volume red blood cells in a volume of whole blood,The normal range for men is between **40% and 54%.** For women the range is between **37% and 47%.**

5- Platelets:- play a role in clotting. This test measures the number of platelets in the blood. The normal range is (**150,000 to 450,000) cells/mcL**.

6- MCV (mean corpuscular volume):- This is the average size of the red blood cells .

7- MCH (mean corpuscular hemoglobin):- How much hemoglobin (a protein) is in the typical red blood cell.

8- MCHC (mean corpuscular hemoglobin concentration):- This measures how concentrated the hemoglobin is in the typical red blood cell.

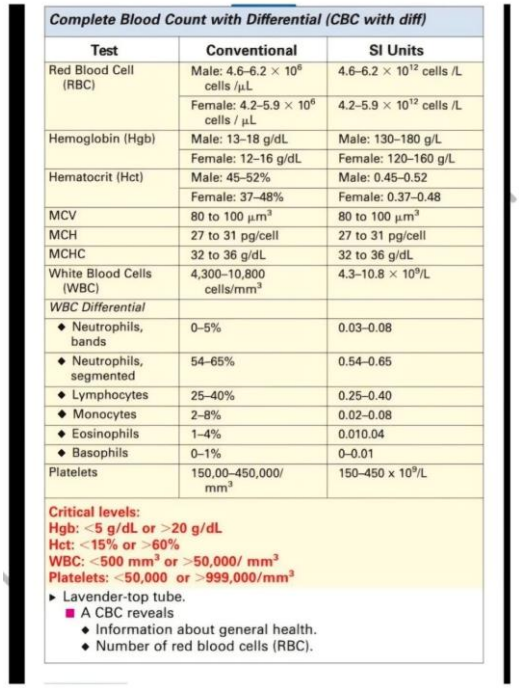
9- RDW (red cell distribution width):- How the much the red blood cells vary in size.

10-Reticulocyte Count. the results help in figure out what could be causing anemia .normal vaiue (0.2%- 2%).

11-MPV (mean platelet volume):- The size of the platelets in the blood.

12-PDW (platelet distribution width):-How much the platelets vary in size.

13-White Blood Cell Differential:- There are five types of white blood cells. This test shows how many of each type : neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

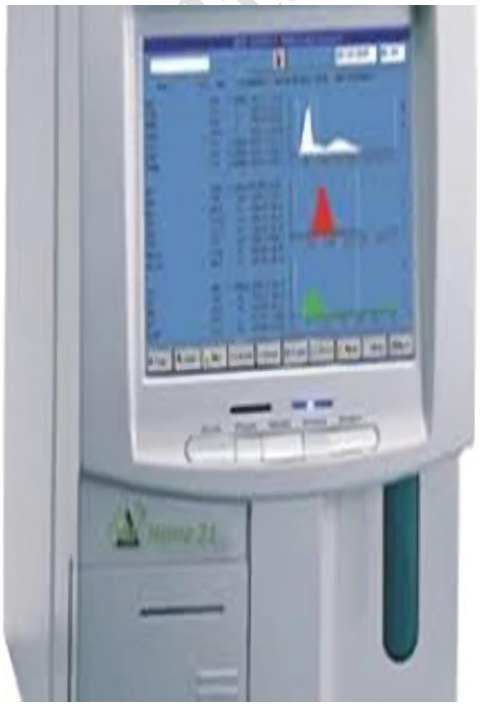


Complete Blood Count with Differential (CBC with diff)

Test	Conventional	SI Units
Red Blood Cell (RBC)	Male: 4.6–6.2 × 10^6 cells /μL	4.6–6.2 × 10^{12} cells /L
	Female: 4.2–5.9 × 10^6 cells / μL	4.2–5.9 × 10^{12} cells /L
Hemoglobin (Hgb)	Male: 13–18 g/dL	Male: 130–180 g/L
	Female: 12–16 g/dL	Female: 120–160 g/L
Hematocrit (Hct)	Male: 45–52%	Male: 0.45–0.52
	Female: 37–48%	Female: 0.37–0.48
MCV	80 to 100 μm^3	80 to 100 μm^3
MCH	27 to 31 pg/cell	27 to 31 pg/cell
MCHC	32 to 36 g/dL	32 to 36 g/dL
White Blood Cells (WBC)	4,300–10,800 cells/mm^3	4.3–10.8 × 10^9/L
WBC Differential		
◆ Neutrophils, bands	0–5%	0.03–0.08
◆ Neutrophils, segmented	54–65%	0.54–0.65
◆ Lymphocytes	25–40%	0.25–0.40
◆ Monocytes	2–8%	0.02–0.08
◆ Eosinophils	1–4%	0.010.04
◆ Basophils	0–1%	0–0.01
Platelets	150,00–450,000/ mm^3	150–450 x 10^9/L

Critical levels:
Hgb: <5 g/dL or >20 g/dL
Hct: <15% or >60%
WBC: <500 mm^3 or >50,000/ mm^3
Platelets: <50,000 or >999,000/mm^3

▸ Lavender-top tube.
■ A CBC reveals
◆ Information about general health.
◆ Number of red blood cells (RBC).



CBC Report **CBC Device**

Post test

Q1-Explain the difference between hypochromic and hyper chromic red blood cells ?

Q2-Discuss the significance of red blood cell indices in the morphological classification of anemia?

Q3-List three conditions that can lead to an elevated MCHC ?

Q4-Which of the following is a primary reason a doctor might order a CBC as part of a routine checkup?

a) To monitor blood glucose levels .
b) To assess kidney function .
c) To diagnose a health problem such as unexplained fever or weakness.
d) To check cholesterol levels .

Week 6
Week 7
Lecture title:

Abnormality of R.B.C in Color , Size and Inclusion Bodies

Learning objective:

By the end of this practical lecture, students will be able to :
1-Define and describe normal erythrocyte morphology, including size, shape, and color.
2-identify and differentiate various abnormalities in erythrocyte size.
3-Identify and differentiate various abnormalities in erythrocyte color.
4-Recognize and classify different abnormal erythrocyte shapes and link them to specific hematological disorders.
5-List and describe common erythrocyte inclusion bodies and other abnormal features observed in peripheral blood smears.
6-Explain the importance of a well-spread, well-stained blood film in the diagnosis of hematological disorder.

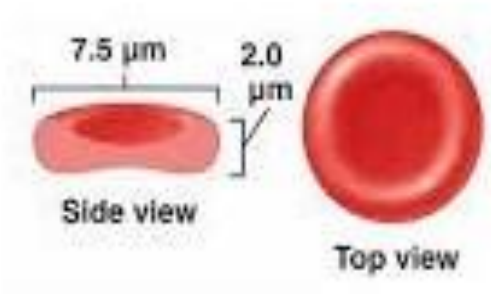
Pre test

Q1-What is the approximate normal diameter of an erythrocyte ?
a) 5.0 µm b) 6.2 µm
c) 7.2 µm d) 8.5 µm

Q2- A red blood cell with an area of central pallor larger than one-third of its diameter is described as
a) Normochromic b) Hyper chromic
c) Hypochromic d) Polychromic

Abnormality of R.B.C in Color , Size and Inclusion Bodies

Examination of well spread, well stained blood film is one essential part of hematology. The erythrocyte biconcave disks, round, smooth and diameter (7.5 to 8.7 µm).Variable proportion of the cells in well spread film may be contracted and have an irregular contour or loss of their substances as a result of fragmentation.



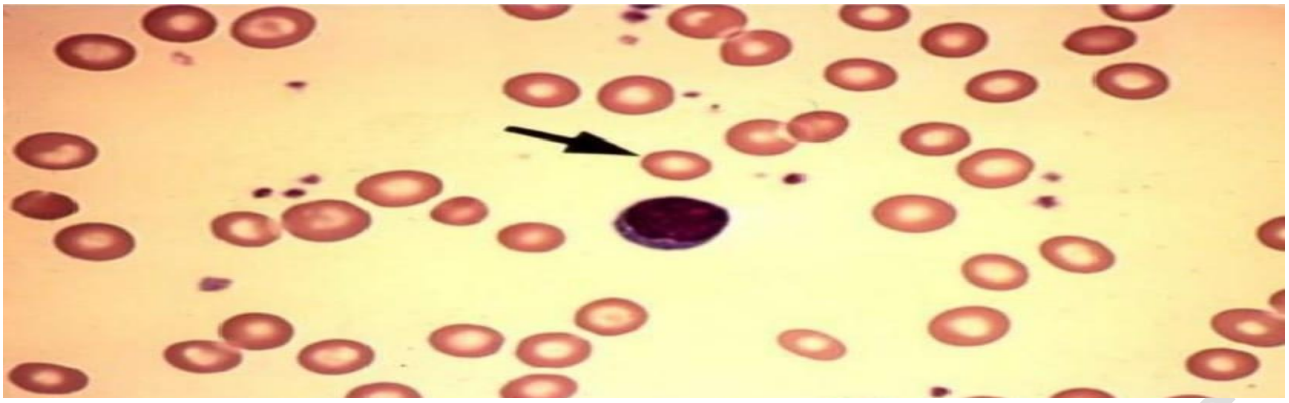
Abnormality of erythrocyte in size include

Anisocytosis : is a medical term meaning that a patient's red blood cells are of unequal size. This is commonly found in anemia and other blood conditions.

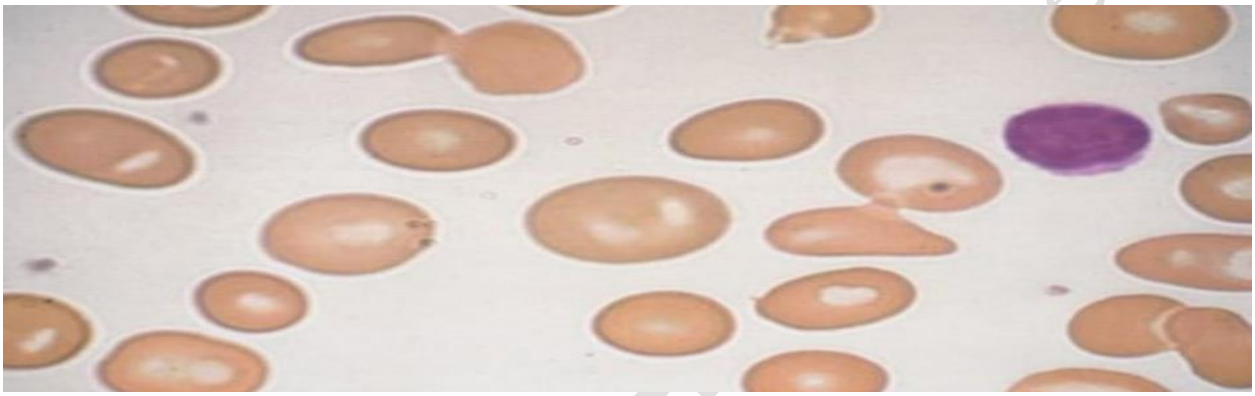
1- **Microcytosis** :- R.B.Cs smaller than normal size are considered as microcytes.

Microcytosis is seen in Iron deficiency anemia. Thalassemia ,lead poisoning ,and anemia of chronic disorders

2- **Macrocytosis** :- R.B.Cs larger than the normal size(7.2M) are considered as macrocytes. Macrocytosis is seen in liver disease , hypothyroidism, megaloblastic anemia.



Microcytosis



Macrocytosis

2-Abnormality of erythrocyte in color include

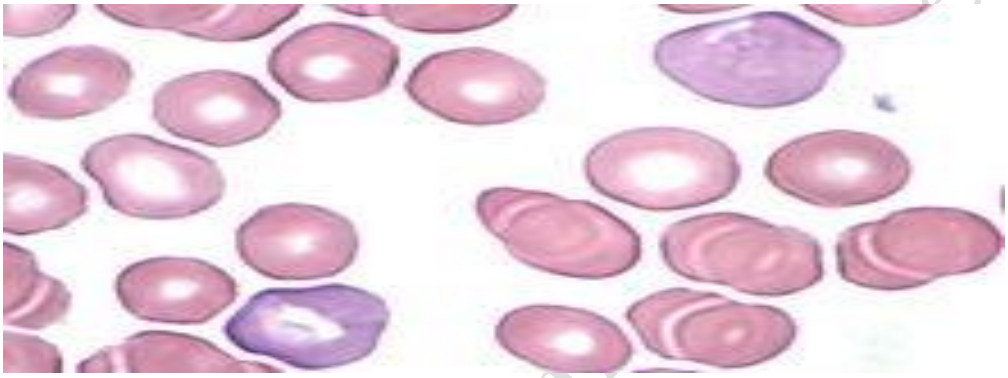
Normochromic R.B.Cs that appear disc shaped and having an area of central pallor. One – third of the cell diameter.
(containing normal amount of hemoglobin).

Variation in color:-

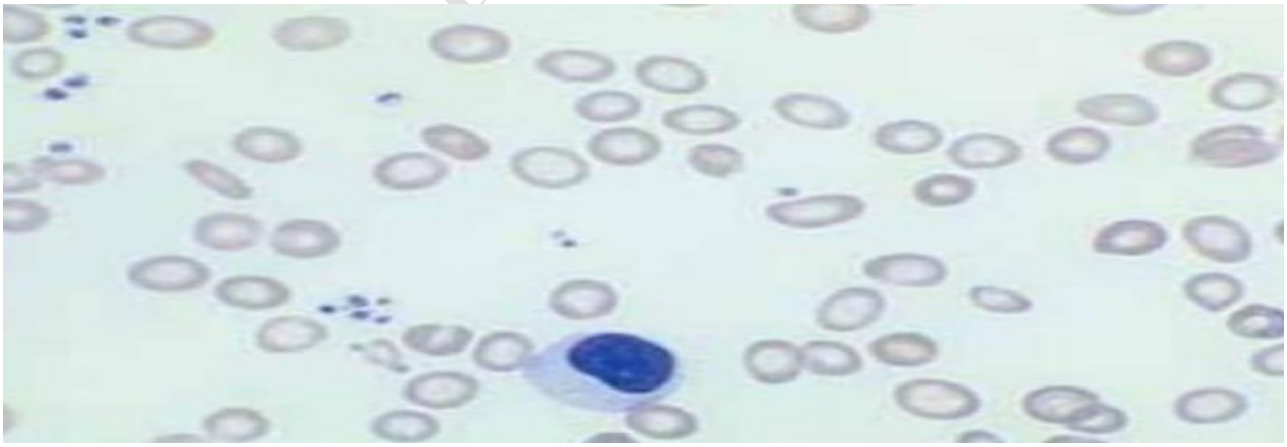
1- **Hypochromic** :- R.B.Cs that have an area of pallor that is smaller than the normal are called hypochromic this variation is seen in :-
Iron deficiency anemia , anemia of chronic disease and thalassemia.

2-**Hyperchromic**: larger amount than normal range seen in Autoimmune Hemolytic Anemia.

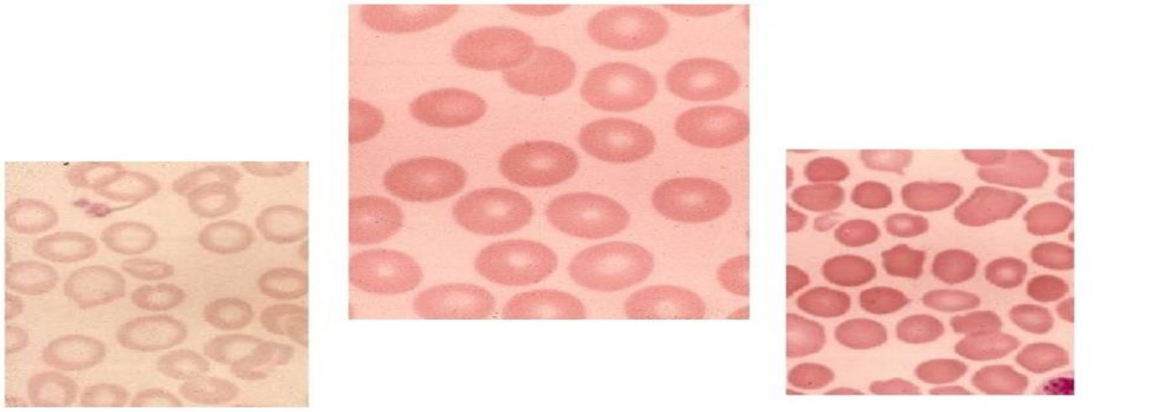
3-**Polychromasia** :- is a disorder where there is an abnormally high number of immature Red blood cells found in the bloodstream as a result of being prematurely released from the bone marrow during blood formation . Red blood cells typically appear pink or salmon-colored on a PBS, With olychromasia, there may be several blue, bluish-gray or purple cells .



Polychromasia



Hypochromic RBC



HYPOCHROMIC HYPERCHROMIC

3- Abnormality of erythrocyte in inclusion bodies:-

1- **Erythroblast**.

2- **Haemoconia**: small particles of lipids formed by fragmentation of the stroma of erythrocytes.

3- **Basophilic**:- is the presence of numerous basophilic granules that are dispersed through the cytoplasm of erythrocytes in a peripheral blood smear. Normoblast.

4- **polychromatic normoblast**.

5- **Orthrochromatic normoblast.**

6- **Reticulocyte**.

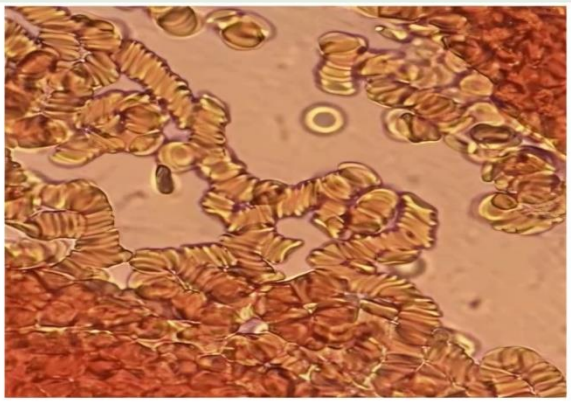
7- **Megaloblastic**.

8- **Jowal jolly body** :- is a basophilic nuclea emnants (clusters of DNA) in circulating erythrocytes.

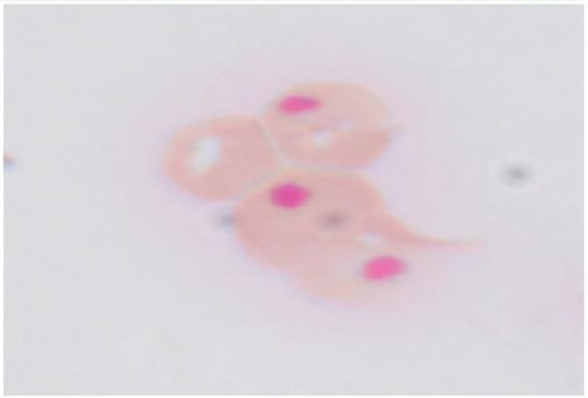
9- **Heinze bodies** :- appear as small round inclusions within the red cell body, formed by damage to the hemoglobin component molecules.

10- **Rolex** :- stacks or aggregations of red blood cells (RBCs) that form because of the unique discoid shape of the cells.

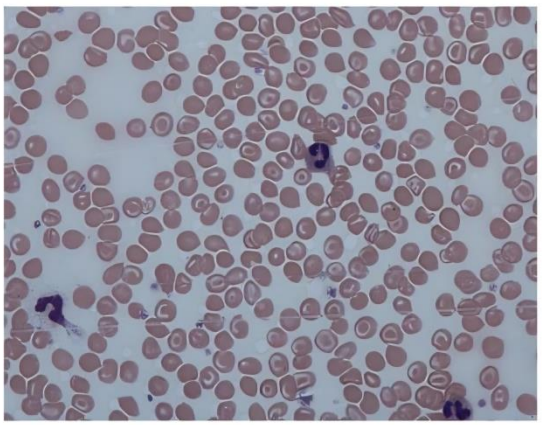
11-**Malaria parasite**.



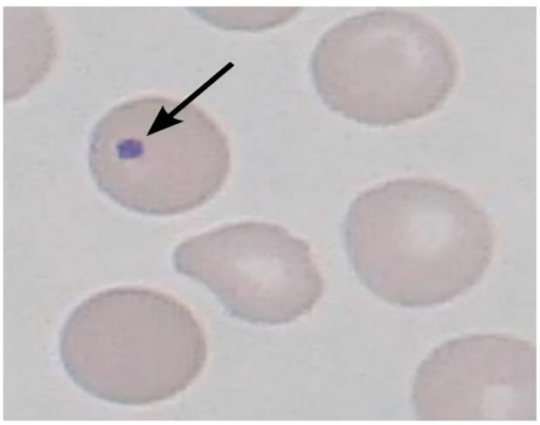
Rbc Rouleaux



Heinz body



Codocytes



Jowall jolly body

Abnormality of erythrocyte in shape include :-

Poikilocytosis :- (variation in the shape of R.B.Cs).
The term Poikilocytosis refers to a condition where 10% or more of the red blood cells are abnormally shaped due to other medical conditions, include:

1- **Elliptocyte** :- abnormally shaped Rbcs that appear oval or elongatedas , as in hereditary elliptocytosis.

2-**Echinocyte**:- Rbcs that has an abnormal cell membrane characterized by many small, evenly spaced thorny projections.
3- **Spherocyte** :- as in hereditary spherocytosis.

4- **Dacrocyte** :- shaped like a teardrop (teardrop cell) found primarily in diseases with bone marrow fibrosis, such as: primary myelofibrosis.

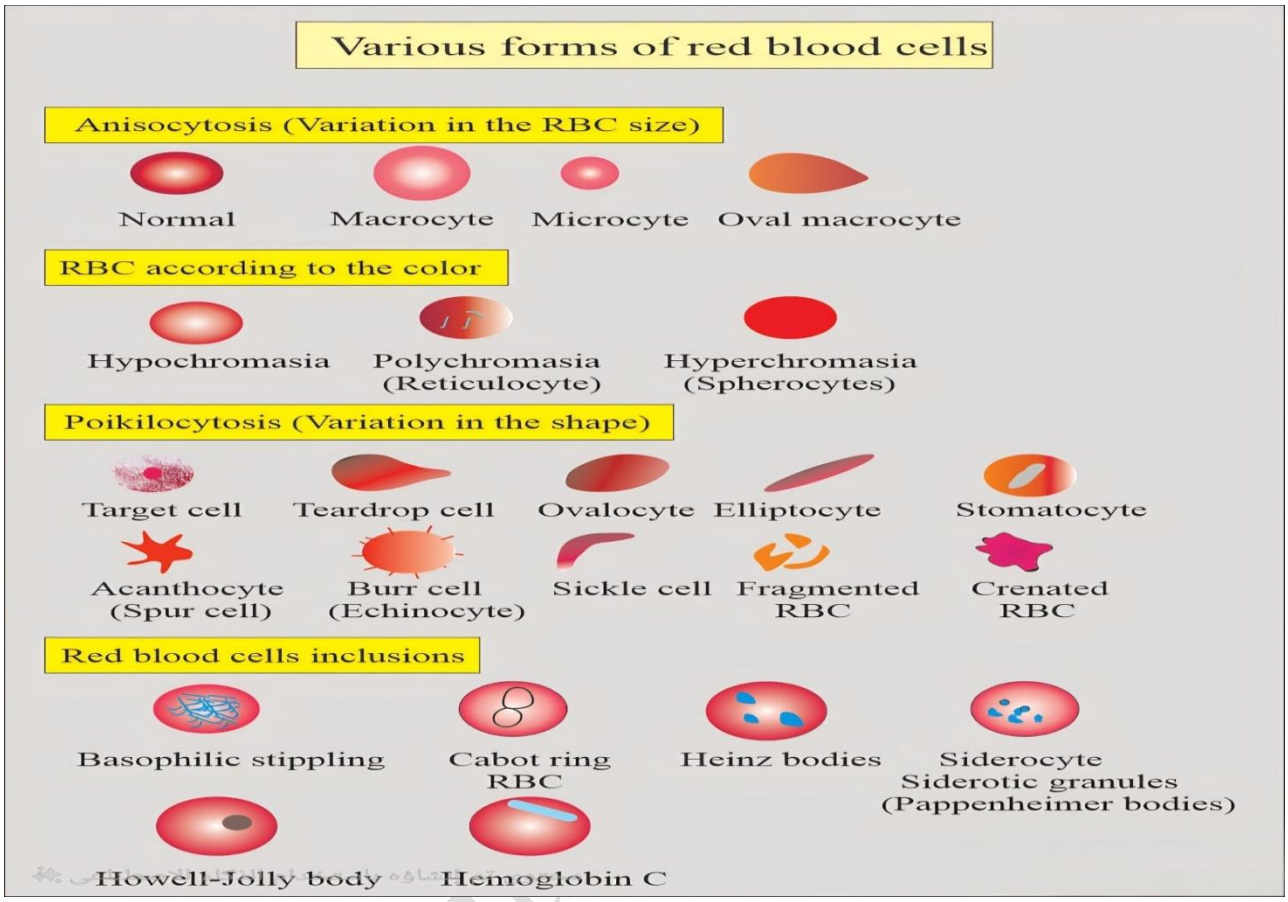
5-**Target cell** :- also known as (Codocytes) Rbcs that have the appearance of a shooting target. In optical microscopy these cells appear to have a dark center (a central, hemoglobinized area) surrounded by a white ring (an area of relative pallor),as in thalassemia.

6- **Stomatocyte** :- Rbc create the appearance of a slit-like area of central pallor (in alcoholism).

7-**Acanthocyte**:- refers to an abnormal form of Rbc that has a spiked cell membrane , in chronic liver disease .

8- **Sickel cell** :- It results in an abnormality in the oxygen-carrying protein haemoglobin found in Rbcs in sickle cell anemia .

9-**Schistocyte** :- (Helmet cell or Fragment cell) typically irregularly shaped, jagged, and have two pointed ends.in hemolytic anemia .



Post test

Q1-Define the following terms in the context of red blood cell morphology:

a) Anisocytosis b) Poikilocytosis

Q2-Compare and contrast microcytosis and macrocytosis?

Q3-Describe the appearance of the abnormal red blood cell shapes on a peripheral blood smear and name one condition in which each can be found:

a) Target cell b) Sickle cell

c) Schistocyte d) Acanthocyte

week 8 **: Examination**
Week 9
Lecture title:

Study the Reticulocyte Count

Learning objective:

By the end of this practical lecture, students will be able to :
1-Define a reticulocyte and describe its key characteristics, including its cellular components and typical percentage in human blood.
2-Explain the role of reticulocytes in erythropoiesis and their maturation process.
3-Identify the primary reasons for performing a reticulocyte count, linking it to the assessment of red blood cell production and bone marrow function
4-List the essential materials required for performing a reticulocyte count.
5-Describe the step-by-step procedure for preparing and staining a blood smear for reticulocyte counting.
6-State the normal reference ranges for reticulocyte counts in adults and newborns.
7-Interpret the clinical significance of high and low reticulocyte counts.

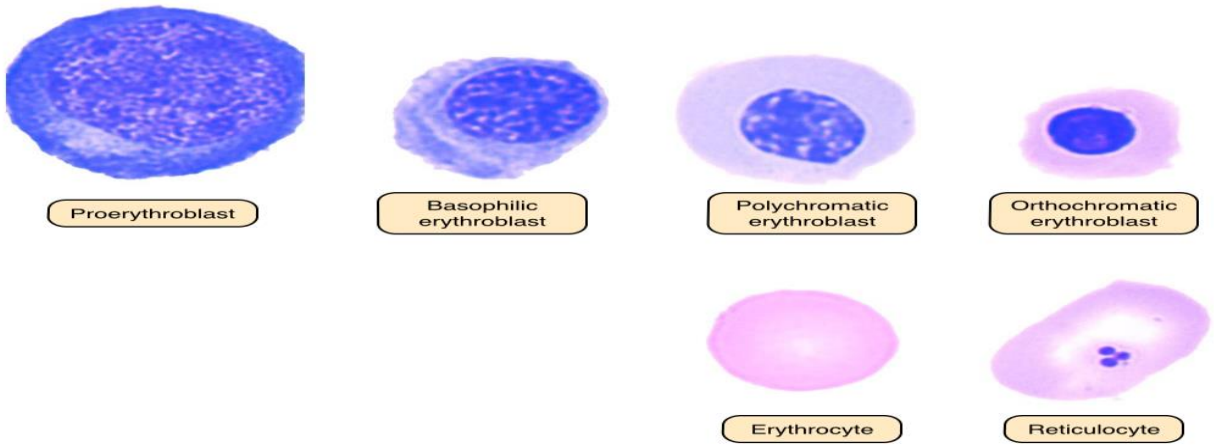
Pre test :

Q1- Which of the following best describes a reticulocyte?
a) A mature red blood cell
b) A white blood cell involved in immunity
c) An immature red blood cell containing remnant RNA
d) A platelet involved in clotting

Q2- In which part of the body do reticulocytes primarily mature?
a) Spleen b) Liver
c) Bone marrow d) Kidneys

Study the Reticulocyte Count

Reticulocytes are immature red blood cells (RBCs) produced in the bone marrow and released into the peripheral blood, where they mature into RBCs within 1 to 2 days. An increase or decrease in reticulocyte count can be an indicator of erythropoiesis activity or failure, especially relative to anemias and bone marrow dysfunction.

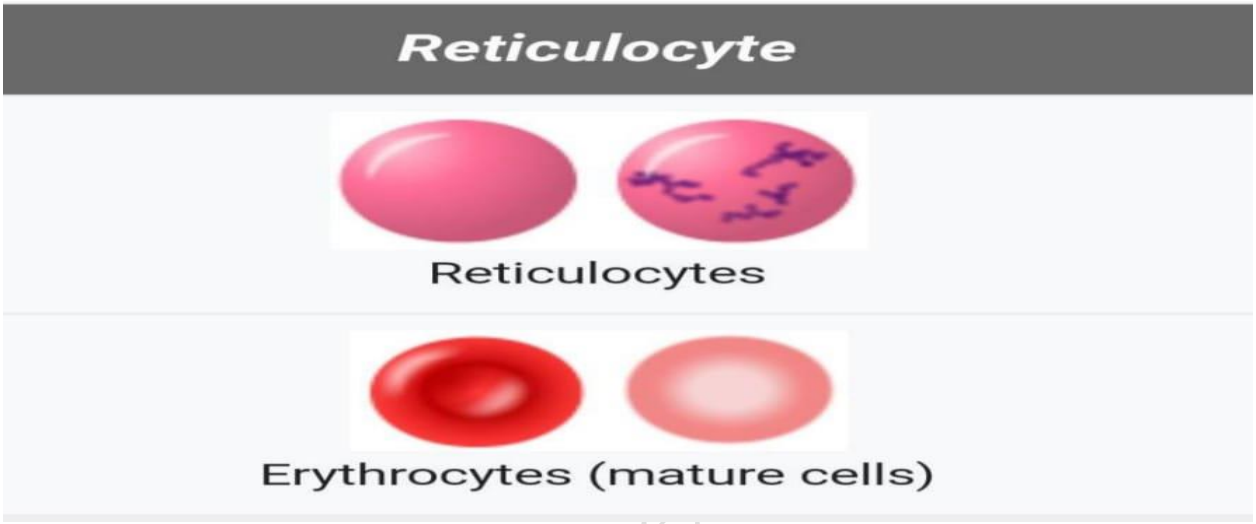


Reticulocyte count

Its young , immature RBC, which still contain RNA (the nuclear material) in cytoplasm,is the remnant of ribonuclear protein which was present in cytoplasm,typically composing about 1 % of the red blood cells in the human body .

In the process of erythropoiesis ,reticulocyte develop and mature in the bone marrow and then circulate for about a day in the blood stream before developing into mature red blood cells.

Like mature red blood cells , in mammals reticulocyte do not have a cell nucleus . they are called reticulocyte because of a reticular network of ribosomal RNA that becomes visible under a microscope with certain stains such as (Methylene Blue stain).
A reticulocyte count is blood test that measures how fast red blood cells called reticulocyte are made by the bone marrow and released into the blood.



Reticulocyte count used for:-
1- Diagnose specific types of anemia .
2- See if treatment for anemia is working .
3- See if bone marrow is producing the right amount of red blood cells .
4-Check bone marrow function after chemotherapy or a bone marrow transplant .

Materials:
1- EDTA whole blood is the preferred anticoagulant .
2- (Brilliant cresyle blue) (Methylene Blue stain) solution (is used to precipitate the RNA into dark – blue filaments or granules to identify retics).
3- Cotton , alcohol ,syringe.
4- Slide .
5- Pipette , tubes.

Procedure

1- Put 2 drops of new methylene blue in the small test tube .
2- Add drops of blood to the tube.
3- Mix blood/stain mixture, The mixture color should be smoky-gray. Adjust if needed.
4- add more blood if mixture is too blue.
5- Shake& put in water bath at 37c for 15 minutes .
6- Make blood film on clean dry slide , important Prepare (2- 4) good smears, LABEL and let dry.
7- Counting: Using oil/100x power, it is count in 10 fields.

-NORMAL VALUE :- (0.2 % - 2 %)

-NEW BORN :- (3 % – 6 %)

Result:

Reticulocytes count are a useful clinical indicator of anemias and bone marrow response to anemia.
When a patient is anemic and the bone marrow is unable to respond, the reticulocyte count will be low. When the bone marrow can respond appropriately, the reticulocyte count will increase.

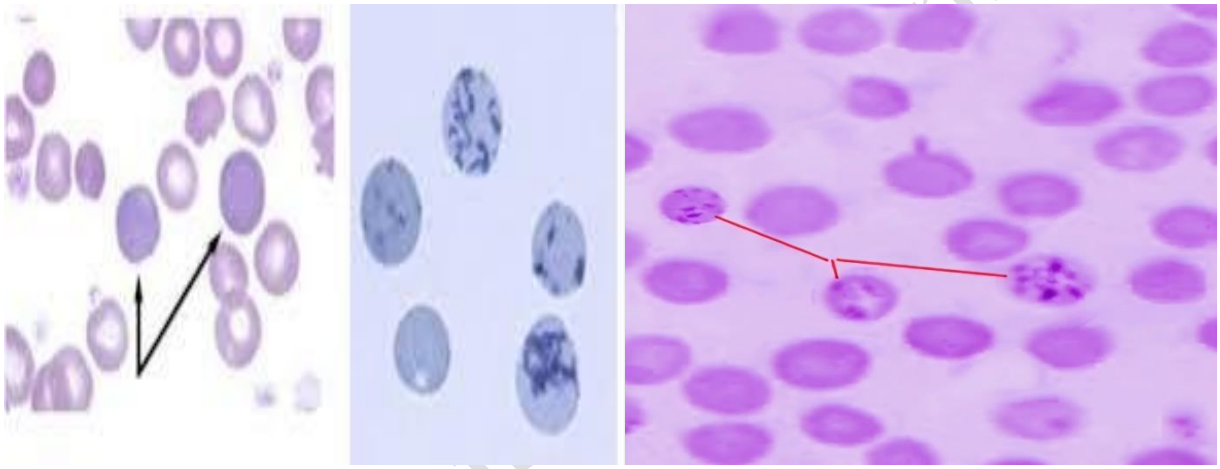
High values:

A high reticulocyte count mean more red blood cell are being made by the bone marrow (Reticulocytosis) this can occur:-
1-After a lot of bleeding .
2-In high altitude .
3-Certain types of anemia these condition cause red blood cell to break down (hemolysis).
4-After the treatment for pernicious anemia , iron deficiency anemia.
5-Starts working.

Low values:

A low reticulocyte count mean fewer red blood cells are being made by the bone marrow (Reticulosytopenia)This can be caused by:-

1- Aplastic anemia or other types anemia , such as Iron deficiency anemia .
2- Exposure to radiation .
3- Chronic infection.
4- Certain medicines that damage the bone marrow.



Post-Test

Q1- List at least three essential materials needed to perform a reticulocyte count?

Q2- Which stain is typically used to visualize the reticular network in reticulocytes?

a) Wright's stain .
b) Gram stain .
c) Brilliant Cresyl Blue .
d) Giemsa stain .

week 10

Lecture title:

Anemic types

Learning objective:

By the end of this practical lecture, students will be able to :

1-Define anemia based on hemoglobin levels as per the World Health Organization (WHO) guidelines .

2-Identify the common signs and symptoms associated with anemia .

3-List and describe the various potential causes of anemia.

4-Explain the diagnostic methods used to identify anemia and its underlying causes.

5-Differentiate between various types of anemia .

6-Understand the basic principles of treatment for different types of anemia.

Pre test

Q1-What is the primary function of red blood cells in the body?

a) Fighting infections .

b) Carrying oxygen .

c) Clotting blood .

d) Producing hormones .

Q2-Which of the following is a common symptom of anemia ?

a) Increased energy .

b) Reddened skin .

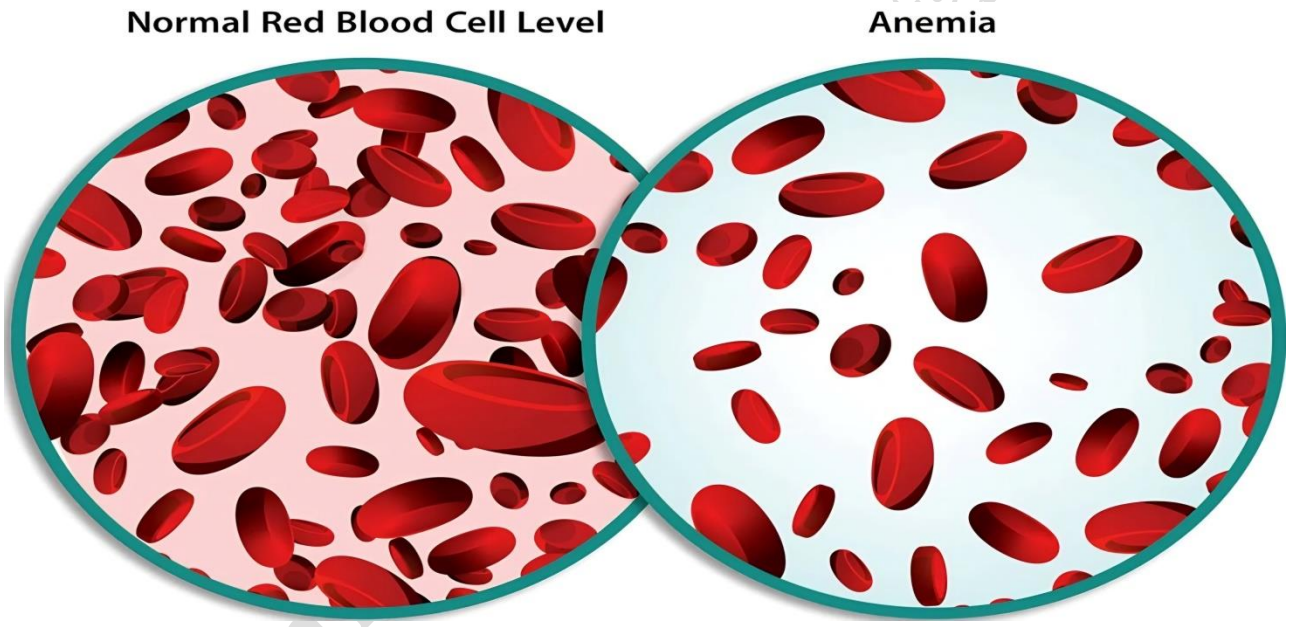
c) Fatigue .

d) Weight gain .

Anemic types

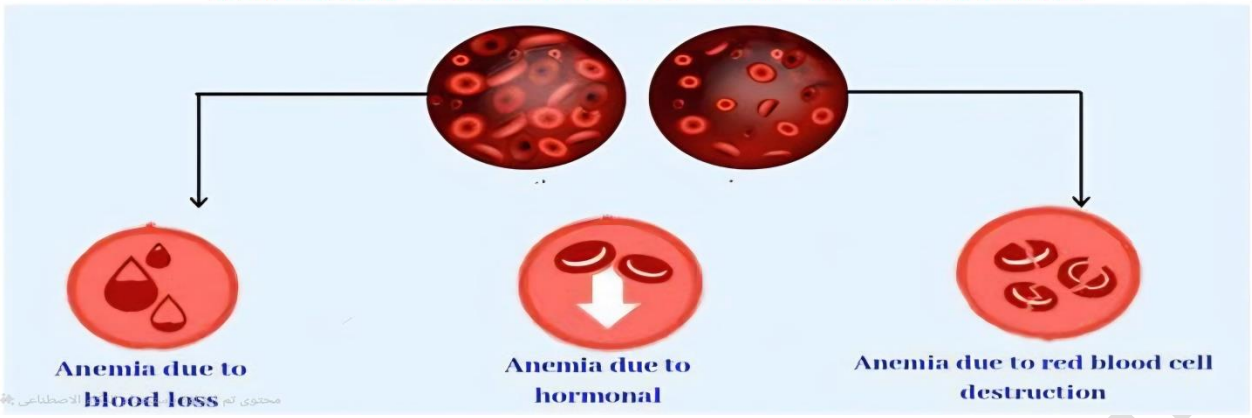
Anemia : Is a condition in which the body does not have enough healthy red blood cells. Red blood cells provide oxygen to body tissues.

According to the World Health Organization (WHO), anemia is defined as hemoglobin (Hb) levels <12.0 g/dL in women and <13.0 g/dL in men.



The symptoms of anemia:-

1- Fatigue**:** feeling too tired is the most noticeable anemia symptom.
2- Chest pain.
3- Dizziness.
4- Frequent infections.
5- Heart palpitations.
6- Headache.
7- Pallor (skin color that's paler than usual).
8- Shortness of breath (dyspnea).



Possible causes of anemia include:-

1- Iron deficiency.
2- Vitamin B12 deficiency.
3- Foliate deficiency.
4- Certain medicines.
5- Destruction of red blood cells earlier than normal (which may be caused by immune system problems).
6- Long-term (chronic) diseases such as chronic kidney disease, cancer, ulcerative colitis, or rheumatoid arthritis.
7- Inherited such as thalassemia or sickle cell anemia.
8- Pregnancy.
9- Problems with bone marrow such as multiple myeloma.
10- Slow blood loss such as, heavy menstrual periods or stomach ulcers.
11- Sudden heavy blood loss.

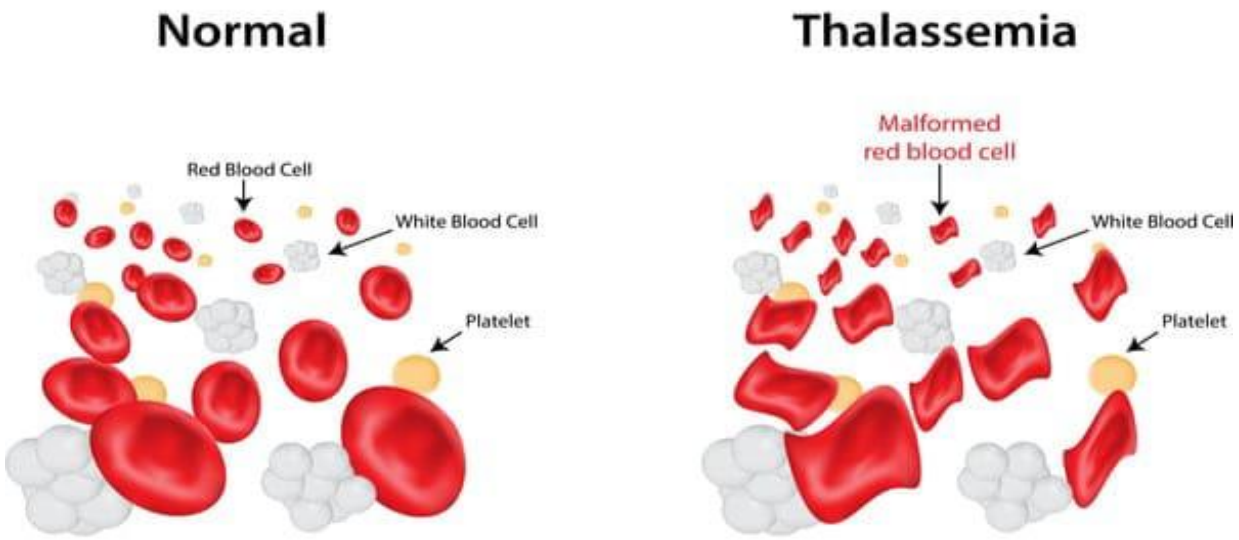
How is anemia diagnosed?

1- Complete blood count (CBC).
2- Hemoglobin Test (Hb).
3- Hematocrit. Test (HCT).
4- Peripheral blood smear.
5- Reticulocyte count.

Types of Anemia

1- Thalassemia

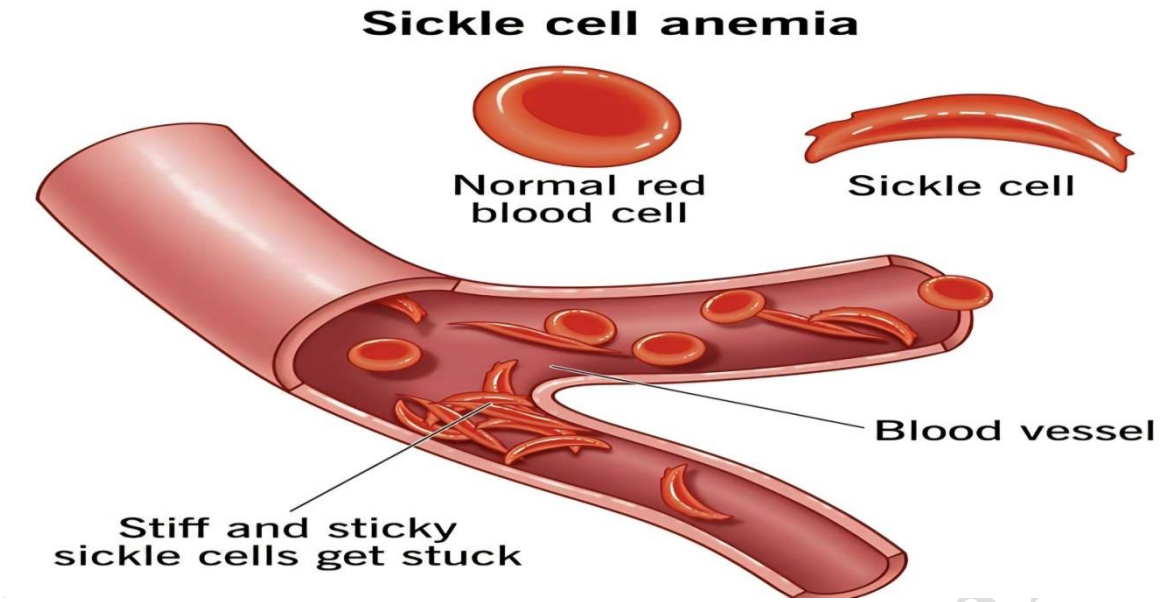
is an inherited blood disorder that affects the body ability to produce hemoglobin and healthy red blood cells. Types include(**alpha and beta**).Thalassemia may cause symptoms that range from **mild to severe**. Treatment can consist of blood transfusions and iron chelation therapy.



2- Sickle cell anemia

Is one of a group of inherited disorders ,It affects the shape of red blood cells, which carry oxygen to all parts of the body.

Red blood cells are usually round and flexible, so they move easily through blood vessels. In sickle cell anemia, some red blood cells are shaped like sickles or crescent moons. These sickle cells also become rigid and sticky, which can slow or block blood flow.The treatment is to relieve pain and help prevent complications of the disease.



3- Pernicious anemia

One of the causes of vitamin B12 deficiency, is an autoimmune condition that prevents the body from absorbing vitamin B12. Without adequate vitamin B12,the body have fewer red blood cells carrying oxygen throughout the body.

The patient can have pernicious anemia for several years before noticing changes in his body. Left untreated, pernicious anemia can cause serious medical complications, including irreversible damage to the nervous system. Healthcare providers treat pernicious anemia by prescribing vitamin B12 supplements.

RBC are macrocyte & hypersegmented neutrophil as in megaloblastic anemia (MCV increase, MCHC normal) .

4- Fanconi anemia (FA)

is a rare inherited blood disorder that leads to bone marrow failure FA is a type of aplastic anemia . That prevents the bone marrow from making enough new blood cells for the body to work normally, this can lead to serious health problems , such as Leukemia .

FA is very rare. It affects 1 in 160,000 people worldwide. Most of the time, FA is diagnosed during childhood or young adulthood.

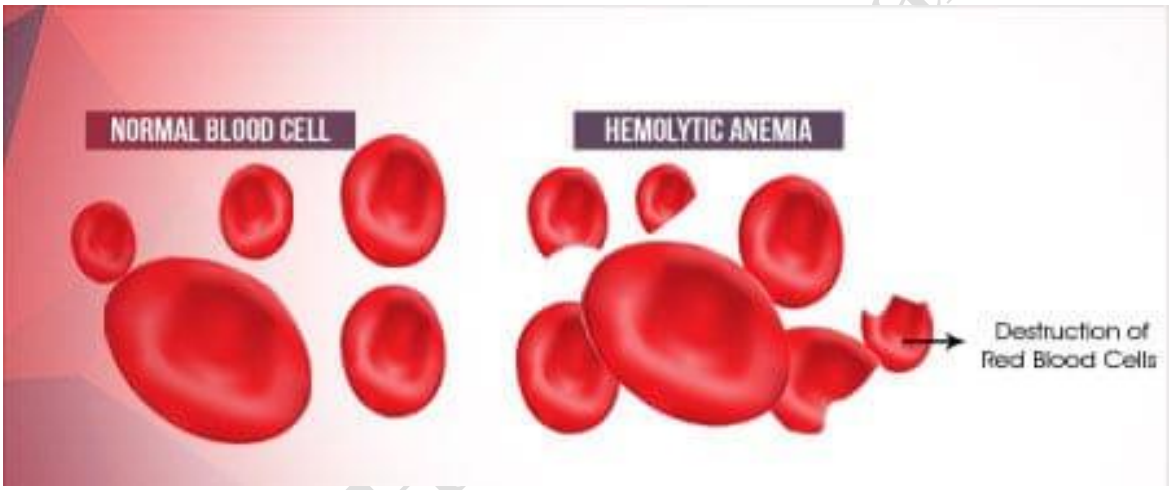
5- Hemolytic anemia

Is a disorder in which red blood cells are destroyed faster than they can be made. The destruction of red blood cells is called hemolysis.

Hemolytic anemia can be inherited or acquired:

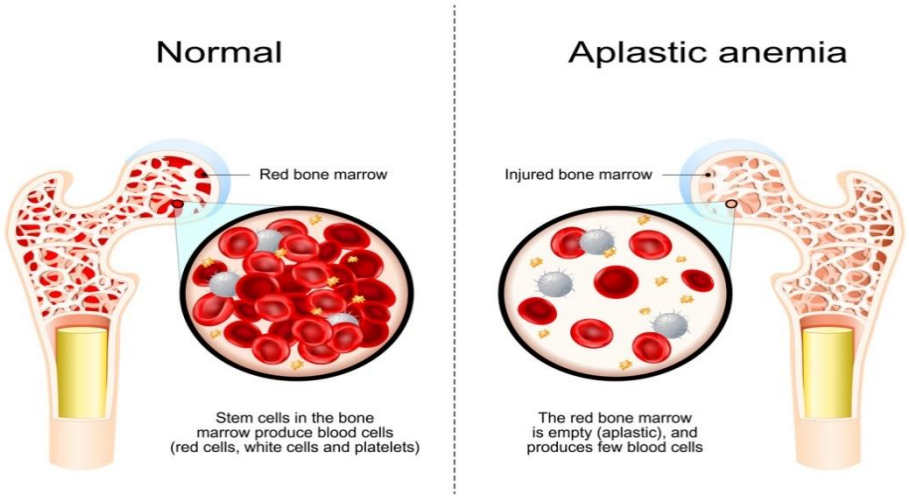
-Inherited hemolytic anemia:-happens when parents pass the gene for the condition on to theirchildren.

-Acquired hemolytic anemia:-is not something you are born with. You develop the condition later. Sometime the cause of hemolytic anemia isn`t known.



6- Aplastic anemia

Is a rare but serious blood disorder. It happens when the bone marrow can’t make enough blood cells and platelets. People with aplastic anemia have an increased risk of serious infections, bleeding issues, heart issues and other complications. There are treatments to manage aplastic anemia symptoms, but a stem cell transplantation is the only cure. aplastic anemia can develop at any age .



7-Iron-deficiency anemia

Is a blood disorder that affects the red blood cells. It's the most common form of anemia. It happens when the body doesn't have enough iron to make hemoglobin, a substance in the red blood cell that allows them to carry oxygen throughout the body.

As a result, iron deficiency may cause short of breath or tired. These symptoms develop over time. in iron deficiency **the blood test show .MCV ,MCH ,MCHC all decreased.**

When iron deficiency is diagnosed, iron supplements its useful .

Post test

Q1- Define anemia and number of species?

Q2- List at least four common signs or symptoms that might indicate a person has anemia?

Q3- Describe two different causes of anemia, providing a brief explanation for each?

Week 11: Examination

Week 12

Lecture title:

Study the abnormal Hb (Hb .S)

Learning objective:

By the end of this practical lecture, students will be able to :

1-Explain the molecular basis of Hemoglobin S (HbS).

2-Describe the physiological consequences of HbS polymerization, leading to sickling of red blood cells and subsequent vaso-occlusion

3-Differentiate between the clinical presentations of sickle cell trait (HbAS), sickle cell anemia (HbSS), and compound heterozygotes, relating each to their respective genetic makeups and HbS percentages.

4-Identify and explain the principles behind common laboratory methods used for HbS detection, including the solubility test, hemoglobin electrophoresis, and molecular testing.

5-Interpret the results of the aforementioned laboratory tests to determine the presence and type of HbS-related condition.

6-Discuss the clinical significance of early and accurate diagnosis of HbS for patient management and genetic counseling.

Pre test

Q1- Normal adult hemoglobin (HbA) is composed of how many alpha and beta globin chains?

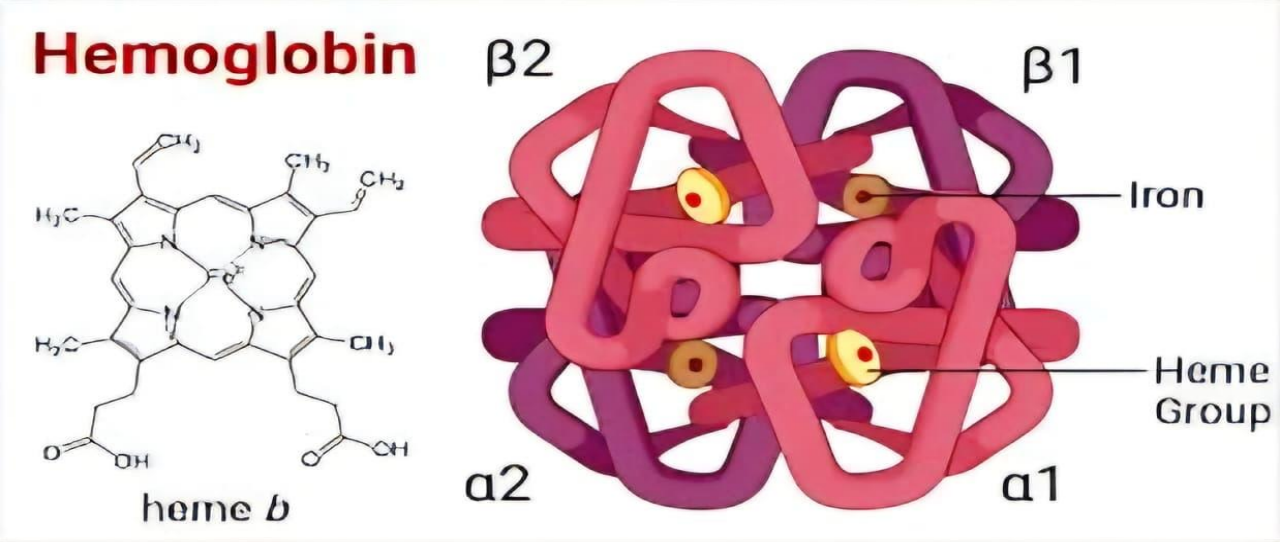
a) One alpha, one beta
b) Two alpha ,one beta
c) Two alpha, one beta
d) One alpha , two beta
d) One alpha, two beta

Q2- The specific point mutation in the beta-globin gene that leads to HbS involves a change from?

a) Cytosine to Guanine
b) Adenine to Thymine
c) Guanine to Adenine
c) Glutamic acid to Valine

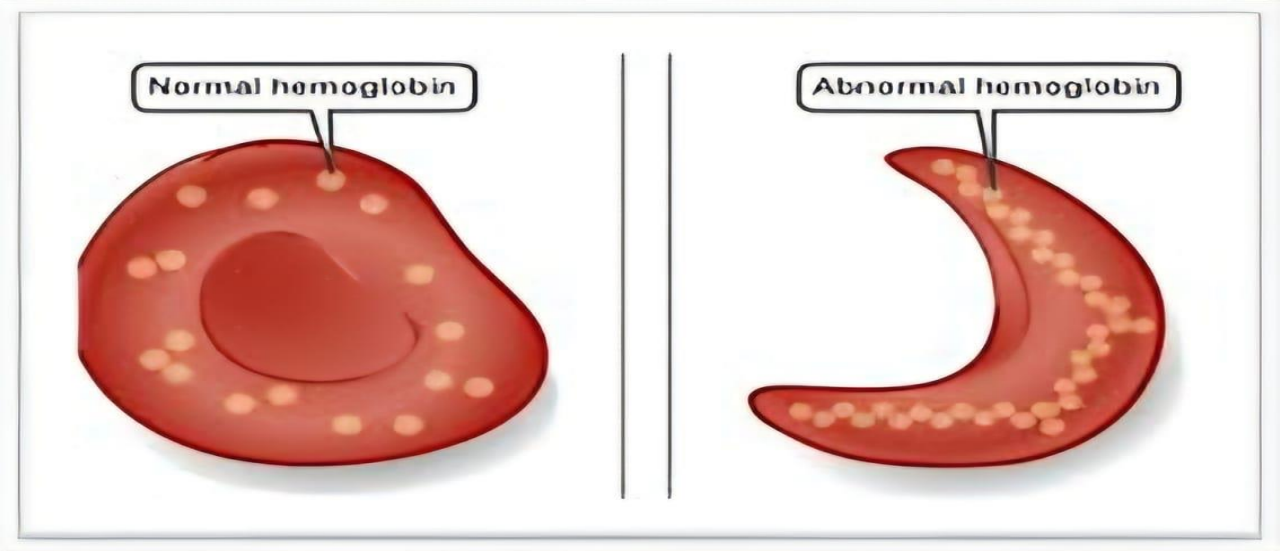
Study the abnormal Hb (Hb .S)

Hemoglobin is the protein in red blood cells (RBCs) that carries oxygen from the lungs to the rest of the body. Normal adult hemoglobin, **Hemoglobin A** (**HbA**), is composed of two alpha globin chains and two beta globin chains.



HbS arises from a single point mutation in the gene encoding the beta-globin chain, This mutation results in the substitution of a hydrophilic amino acid (glutamic acid) with a hydrophobic amino acid(valine) at the sixth position of the beta-globin chain.

This seemingly minor change has profound consequences. When deoxygenated, HbS molecules polymerize, forming rigid rods that distort the red blood cell into a characteristic sickle or crescent shape. These sickled cells are inflexible and can obstruct small blood vessels, leading to a variety of clinical manifestations known as **sickle cell crisis**.



Clinical Significance of HbS

The clinical presentation of HbS depends on the genetic structure of the individual:

1- **Sickle Cell Trait (HbAS):** Individuals who inherit one copy of the HbS gene and one copy of the HbA gene are typically asymptomatic carriers. They have about 35-40% HbS and usually do not experience sickling crises under normal physiological conditions.

2- **Sickle Cell Anemia (HbSS)**: Individuals who inherit two copies of the HbS gene have sickle cell anemia, the most severe form of the disease. They have primarily HbS (80-95%) and suffer from chronic hemolytic anemia and recurrent vaso-occlusive crises, leading to pain, organ damage, and reduced life expectancy if not properly managed.

3- Compound Heterozygotes (e.g., HbSC, HbS-beta thalassemia):

These individuals inherit HbS along with another abnormal hemoglobin gene (e.g., Hemoglobin C, or a beta-thalassemia mutation). The clinical severity varies depending on the specific combination.

Laboratory Methods for HbS Detection

Accurate diagnosis and monitoring of HbS are essential for patient management, the primary laboratory methods used:

1- Solubility Test (Sickle Solubility Test)

Principle:

This is a rapid, qualitative screening test. HbS, when deoxygenated, is relatively insoluble and will precipitate, forming a turbid solution. Other hemoglobins remain in solution.

Procedure:

A blood sample is mixed with a reducing agent (e.g., sodium dithionite) in a phosphate buffer.

Result:

-Turbid solution: Positive result, indicating the presence of HbS .

- Clear solution: Negative result .

2- Hemoglobin Electrophoresis (Cellulose Acetate and Citrate Agar)

Principle:

Hemoglobins have different electrical charges due to their amino acid composition. In an electrical field, they migrate at different rates towards an anode or cathode.

Procedure:

Hemolysate is applied to a cellulose acetate membrane, which is then placed in an electrophoresis chamber with an alkaline buffer.

3- Molecular Testing (DNA Analysis)

Principle:

Directly detects the specific gene mutation responsible for HbS (GAA to GTA substitution in the \beta-globin gene)

Methods:

Polymerase Chain Reaction (PCR), DNA sequencing.

Post test

Q1- Which laboratory method directly detects the specific genetic mutation responsible for HbS?

a) Solubility Test .
b) Hemoglobin Electrophoresis .
c) Molecular Testing (DNA) Analysis .
d) Complete Blood Count (CBC).

Q2- Why is accurate diagnosis and monitoring of HbS essential for patient management?

a) To determine the patient's blood type .
b) To assess the risk of infectious diseases .
c) To guide treatment strategies and genetic counseling .
d) To predict the patient's life expectancy without intervention .

Week 13
Lecture title:

Study the hemostasis disorders

Learning objective:

By the end of this lecture, university students should be able to:

1-Define hemostasis and differentiate between hemorrhage and thrombosis as disorders of hemostasis.

2-Identify and describe the main components of hemostasis.

3-Classify common hemostasis disorders .

4-Explain the underlying causes and characteristics of key platelet disorders.

5-Describe the main coagulation factor disorders.

6-List and explain the common inherited and acquired thrombophilias.

7-Identify and interpret the common laboratory tests used to diagnose hemostasis disorders.

Pre test

Q1- Hemostasis is defined as a physiological process that?

a) Promotes blood clotting indiscriminately
b) Stops bleeding while maintaining blood fluidity
c) Dissolves all existing blood clots
d) Primarily involves red blood cell aggregation

Q2- What is the primary function of the vascular wall in hemostasis immediately following an injury?

a) To produce coagulation factors
b) To initiate platelet aggregation
c) To undergo vasoconstriction
d) To synthesize fibrinogen

Study the hemostasis disorders

Hemostasis is a complex physiological process that stops bleeding while maintaining blood fluidity. Disorders of hemostasis, can manifest as either excessive bleeding (**hemorrhage**) or excessive clotting (**thrombosis**).

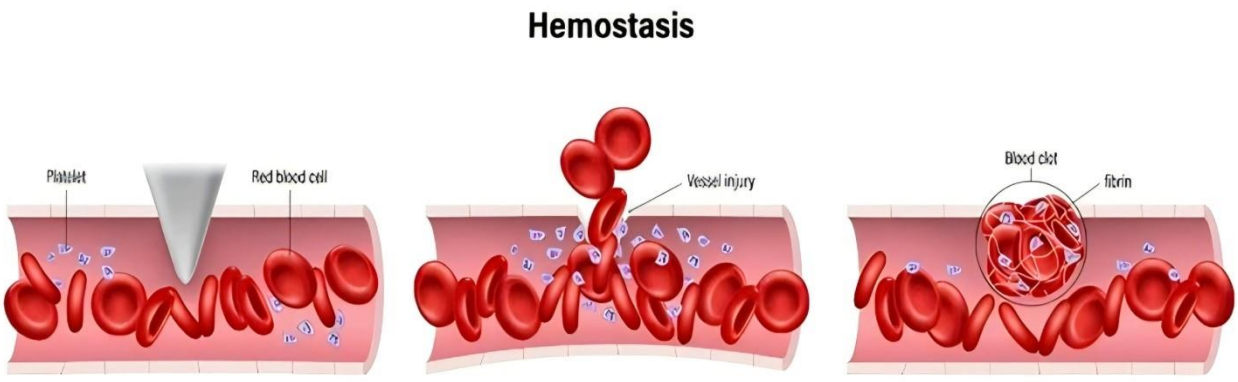
Components of Hemostasis:

Hemostasis involves the intricate interplay of three main components:

1-Vascular Wall: Provides the initial vasoconstrictive response to injury.

2-Platelets: Form the primary hemostatic plug at the site of injury.

3-Coagulation Factors: Form a stable fibrin clot to reinforce the platelet plug .



Damage blood vessel **Platelet plug formation** **Clot formation**

Platelets or thrombocytes are a nucleated cells derived from the megakaryocytic cells in the bone marrow .
Platelets are the cells that circulate in the blood and bind together when they recognize damaged blood vessels. They help form blood clots to slow or stop bleeding and to help wounds heal.
Platelets, the smallest of blood cells, can only be seen under a microscope. They're literally shaped like small plates in their non-active form.
Platelets have a short lifespan in the blood about (5 to 10) days. This means the platelet count can rise and fall quickly in response to disease or trauma.

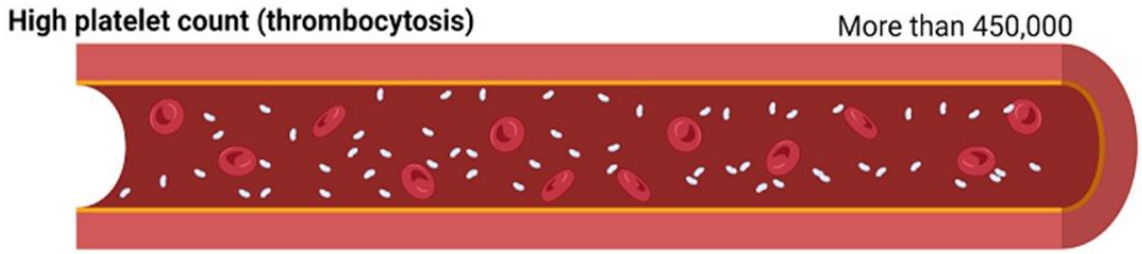
Normal range:-
(150,000 – 450,000) per mm .

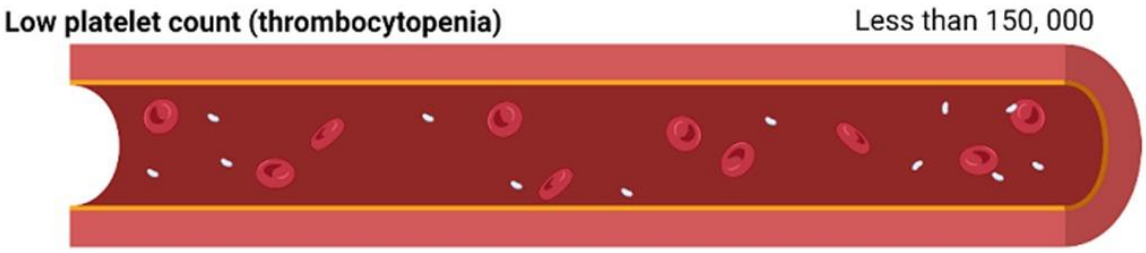
Thrombocytosis:- is the presence of HIGH platelets counts in the blood .
Increase in :-
1- Anemia
2- Inflammation .
3- Splenactomy .
4- Physiological hemorrhagia :-
a-Labor .
b-After surgery operation .
c-Extensive damage tissue.

5- Pathological hemorrhagia .

Thrombocytopenia :- is the presence of low platelets counts in the blood.
Decrease in :-
1- Viral infection .
2- Idiopathic thrombocytopenic purpura (ITP) .
3- Liver disease .
4- Autoimmune disease .
5- Hypersplenism

6- HIV infection .
7- Pregnancy .
8- Bone marrow causes .
9- Leukemia .





Platelets Count

Materials Required :-

1- Blood sample .
2- Haemocytometer .
3- (Micropipette)using in R.B.C count .
4- Diluent solution .
5- Microscope .
6- Cotton , filter paper .
7- petridish .

The diluting solution :

there are two type of diluting solution could be used :

1- Formal citrate : this fluid does not cause lysis for RBC and WBC .

Disadvantage :-

some platelets may be lie on RBS or beneath them so they will not be counted and give error to total count .

2- (1%) Ammonium oxalate :-

1 gm Ammonium oxalate in 100 mL Distil water function lyse all cells but not platelets.

Disadvantage :-

the fragments of the cells that are lysed (broken) may be counted as platelets and give error to total count.

Procedure :-

1- Prepare fingertip for puncture .

2- Wipe off the 1 drop of blood .

3- Fill end pipet (using in R.B.C count) blood up to 1 mark , than diluent up to 101

mark to mark 1 : 100 dilution .

4- Mix blood & dilution fluid using the pipet shaker .

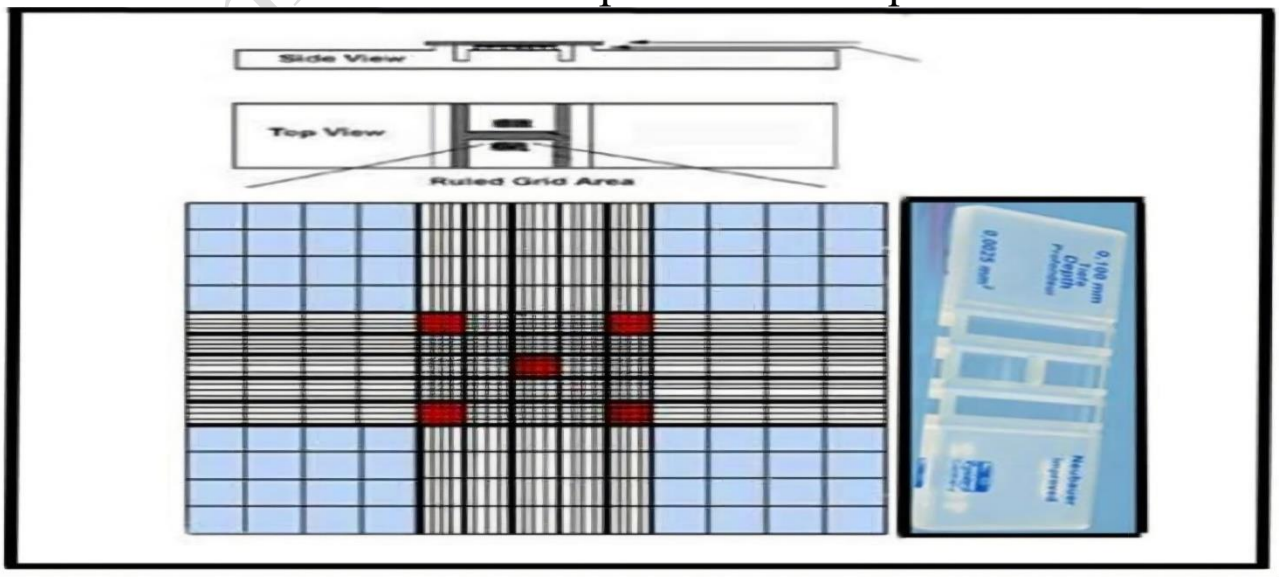
5- Discard few & charge the hemacytometer using a different pipet for each chamber.

6- Place the hemacytometer in a petri dish / wet cotton ball or wet filter paper (to prevent evaporation) & allow to stand for 15 – 20 min and 15 – 25 c° .

7- Examination under microscope .

Calculation

Platelets count = total number platelets in 5 square x1000



Common Hemostasis Disorders

hemostasis disorders into two main groups :

1- Bleeding Disorders

These disorders result from defects in one or more components of the hemostatic system, leading to an increased tendency to bleed.

A- Platelet Disorders

- Thrombocytopenia: Low platelet count. Can be due to decreased production (e.g., bone marrow failure, chemotherapy), increased destruction (e.g., Immune Thrombocytopenic Purpura - (ITP, Thrombotic Thrombocytopenic Purpura - TTP).
- Platelet Function Disorders (Thrombocytopathies): Normal platelet count but impaired platelet function.

B- Coagulation Factor Disorders

1- Inherited **Coagulation Disorders:**

- Von Willebrand Disease (VWD): The most common inherited bleeding disorder, affecting von Willebrand factor (vWF), which is crucial for platelet adhesion and stabilizes Factor VIII.

- Hemophilia A (Factor VIII deficiency) .

- Hemophilia B (Factor IX deficiency) .

2- **Acquired Coagulation Disorders**:

Often due to liver disease (impaired synthesis of most factors), Vitamin K deficiency (factors II, VII, IX, X) .

- Rare Factor Deficiencies :

Deficiencies of factors I (fibrinogen), II (prothrombin), V, VII, X, XI, and XIII.

2-Thrombotic Disorders (Hypercoagulable States / Thrombophilias)

These disorders involve an increased propensity to form blood clots, leading to conditions like deep vein thrombosis (DVT) and pulmonary embolism (PE). They can be inherited or acquired.

- Inherited Thrombophilias :

1-Factor V Leiden Mutation: Most common inherited thrombophilia, making Factor V resistant to inactivation by activated protein C.
2-Prothrombin Gene Mutation: Leads to elevated prothrombin levels.
3-Antithrombin Deficiency: Impairs the body's natural anticoagulant system.
4- Protein C Deficiency .
5- Protein S Deficiency .

- Acquired Thrombophilias

1-**Malignancy**: Many cancers are associated with an increased risk of thrombosis.
2-**Pregnancy**: Hormonal changes and physical factors increase thrombotic risk.
3-**Oral Contraceptives/Hormone Replacement Therapy**: Estrogen-containing preparations increase risk
4-**Surgery and Trauma**: Inflammatory response and immobilization.

Laboratory Tests for Hemostasis Disorders

1- Complete Blood Count (CBC) with Platelet Count.

2- Prothrombin Time (PT) / International Normalized Ratio (INR)

Evaluates the extrinsic and common pathways of coagulation (Factors VII, X, V, II, I).

3- Thrombin Time (TT)

Measures the time it takes for fibrinogen to be converted to fibrin in the presence of thrombin. Directly assesses fibrinogen function.

4- Fibrinogen Level

Quantifies the amount of fibrinogen (Factor I), a key protein in clot formation.

5- D- dimer

Measures fibrin degradation products, indicating recent or ongoing fibrinolysis (clot breakdown).

6- Factor Assays

Quantifies the activity of specific coagulation factors (e.g., Factor VIII, Factor IX, Factor VII, Factor XI).

Post test

Q1- Which laboratory test is used to quantify the activity of specific coagulation factors like Factor VIII or Factor IX?

a) Prothrombin Time (PT)
b) D-dimer
c) Thrombin Time (TT)
d) Factor Assays

Q2- Which inherited bleeding disorder is characterized by a deficiency in Factor VIII ?

a) Hemophilia B
b) Von Willebrand Disease
c) Hemophilia A
d) Factor VII Deficiency

Week 14
Week 15
Lecture titl

Study the bleeding time

Study the Clotting time

Learning objective:

By the end of this practical lecture, students will be able to :
1-Define and differentiate between coagulation, clotting, and hemostasis .
2-Identify and list the thirteen primary coagulation (clotting) factors, recognizing their alternative names where applicable.
3- Explain the key factors that influence bleeding time .
4-Describe the three main components involved in the hemostatic mechanism .
5- Outline the four main steps of hemostasis .
6-Define clotting time and prothrombin time, differentiating between their clinical significance and measurement methods.
7- Identify common causes of abnormal prothrombin time.
8- List common methods for assessing hemostatic function.
9- Describe the principle of the PT test.
10-State the normal reference range.

Pre test

Q1-What is the primary role of coagulation factors ?
a) To fight infections b) To transport oxygen
c) To form blood clots and stop bleeding .
d) To maintain blood pressure .

Q2-Which step is the FIRST in the mechanism of hemostasis ?
a) Vasoconstriction b) Platelet plug formation
c) Blood coagulation d) Clot retraction.

Study the bleeding time
Study the Clotting time

Blood Coagulation / Clotting

Coagulation or clotting (**Hemostasis**) is the process of forming clots in the walls of damaged blood vessels and preventing blood loss while maintaining blood in a fluid state within the vascular system.

occurs through a series of reaction due to activation of a group of substance, the substance necessary for clotting are called **(Coagulation factors).**

Coagulation factor :-

Are proteins in the blood **,**They help form blood clots to stop bleeding when have an injury. These proteins are also called **(clotting factors).**

The blood have several different types of clotting factors that are all important for making blood clots, this factors is :

1- Fibrinogen (Factor 1)
2- Prothrombin (Factor II)
3- Thromboplastin (Factor III)
4- Ionized Calcium (Factor IV)
5- Proaccelerin (Factor V)
6- Factor VI
7- Proconvertin (Factor VII)
8- Antihemophilic factor (Factor VIII)
9- Chrismats factor (Factor IX)
10- Stuart factor (Factor X)
11- Plasma thromboplastin antecedent (Factor XI)
12- Hageman's factor (Factor XII)
13- Fibrin-stabilizing factor (Factor XIII)

The factors which affect the bleeding time are

1- Size and nature of the injury.
2- Condition of the vessel wall.
3- Number of platelets.

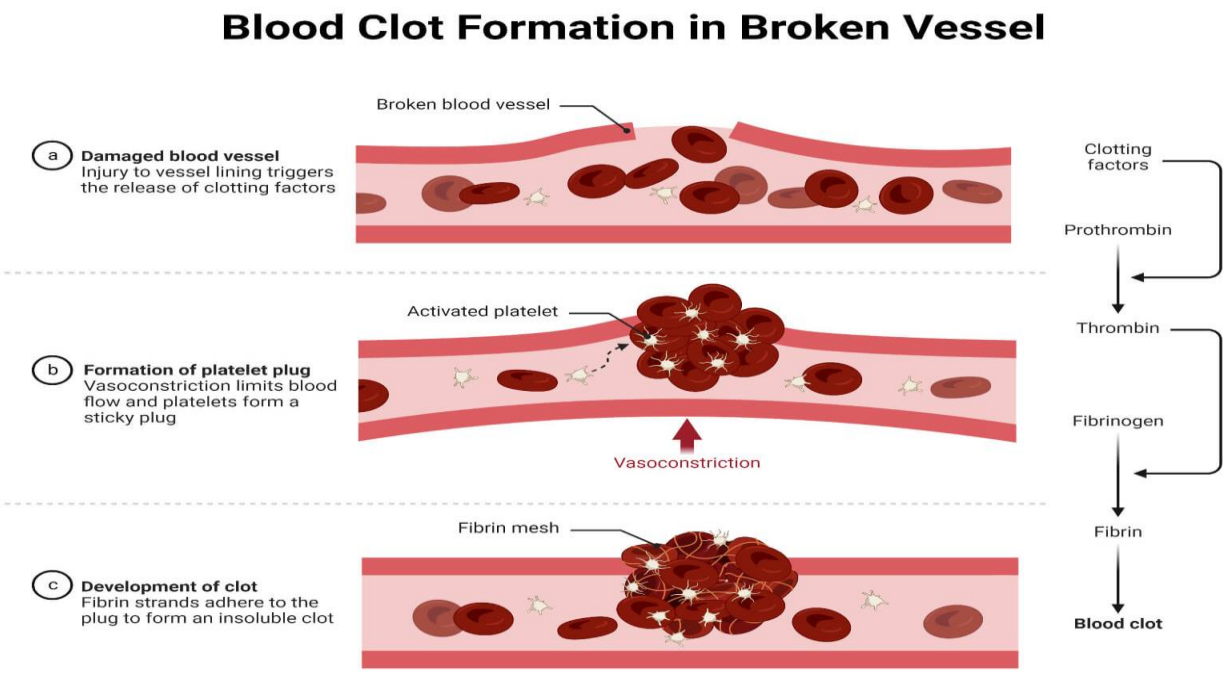
The hemostatic mechanism involves the following:-

1- Properties of the vessel wall.
2- The platelets.
3- The coagulation mechanism.

Mechanism of clotting blood

Hemostasis involves 4 main steps :-

1 -Vasoconstriction .
2 - Platelets plug formation .
3 - Blood coagulation or clotting
4 - Clot retraction.



Stages the blood clotting:-

- Formation of prothrombin activator .
- Conversion of **prothrombin** into **thrombin .**
- Conversion of **fibrinogen** into **fibrin** .

Methods of hemostatic function tests:-

1 - Bleeding time .
2 - Clotting time .
3 - Prothrombin time.

Bleeding time determination :-

Is the time taken from the onset of blood appears from of wound (puncture) until stopping of bleeding (Hemostasis).
Long time of bleeding are found in patients with disorder of **platelets function** and in some patient with **intrinsic vascular defects**.

Methods of bleeding time:-

There are two methods for measuring Bleeding time

1-Duke`s method.

2-Ivey`s method.

1-Duke`s method

Materials:-

1- Filter paper.
2- Timed watch.
3- Disposable lancet.
4- Cotton and 70% alcohol.

Procedure

1- Make the ear lobe warm either by rubbing or by hot water.
2- Clean and sterile the lobe of your subjects ear with 70% alcohol
3- Make a good puncture (by lancet) at the bottom of the ear lobe by inserting the whole pointed end of the lancet at the ear lobe .
4- Star the watch as soon as blood appears.
5- Remove the blood oozing from the wound every 30 second on a clean piece of filter paper , using a different area of the paper each time .
6- Continue until the bleeding stops.
7- Count the spots of the blood.
8- their number will divided by **two** ,the result will be the bleeding time in **minutes** by this method.

The normal bleeding time is :-

(2 __ 5 MINUTES)

The bleeding time is prolonged in(purpura)

Is the spontaneous hemorrhages , usually beneath the skin from mucous membrane , and internal organs.

Clotting or coagulation time estimation

Clotting time:- The time taken from the onset of the wound until the formation of the clot without the addition any substance .

Clotting time estimation methods :-

1- Capillary tubes method .

2- two slide method .

Capillary tubes method

Materials :-

1- Capillary tubes.
2- Timed watch .
3- Cotton , 70% alcohol.
4- Disposable lancet .

Procedure :-

1- Clean the finger with 70% alcohol.
2- Allow the finger to dry.
3- Make a puncture noting the time at which it is do done .
4- Fill the capillary tube with blood .
5- Break of a small pieces of the capillary tube every (30 second) until you notice that the blood has clotted.

6- When the blood clot you will see a thread of clotted blood to each end of the break.
7- Notice the time when clotting is first seen.
reference range of clotting time: (**4 _ 9 min**)

Prothrombin time (PT)

Is a blood test that measures how long it takes blood to clot. **prothrombin time or (factor II)** is one of clotting factors made by the liver.

A prothrombin time test can be used to check for:-
1- bleeding problems.
2-To check whether medicine to prevent blood clots is working.
3- Check for a low level of vitamin K ,its needed to make prothrombin and other clotting factors.
4- It checks to see if five different blood clotting factors (factors I, II, V, VII, and X) are present.

A PT test may also be called an **INR test**. **(International Normalized Ratio)** stands for a way of standardizing the results of prothrombin time tests, no matter the testing method.
An abnormal prothrombin time is often caused by **liver disease or injury or by treatment with blood thinners .**

Prothrombin deficiency can be caused by :-
1- Lack of vitamin K (some babies are born with vitamin K deficiency) .
2- Liver disease .
3- Use of medicines that prevent clotting (anticoagulants such as Warfarin)

Principles
When reagent is add to normal anticoagulated plasma, the clotting mechanism initiated forming solid gel clot within a specified period of time.

Material:-

- Blood sample.
- Test tube contains anticoagulant (Sodium Citrate).
- PT reagent.
- Centrifuge.
- Water path.
- Stopwatch.

Procedure :-

1-Mix nine parts of freshly collected blood with one part of sodium citrate then centrifuge immediately for 10 min at 2500 rpm.

2-Add 0,1 ml of plasma In test tube and place tube in water bath for 3-5 min at37 c.

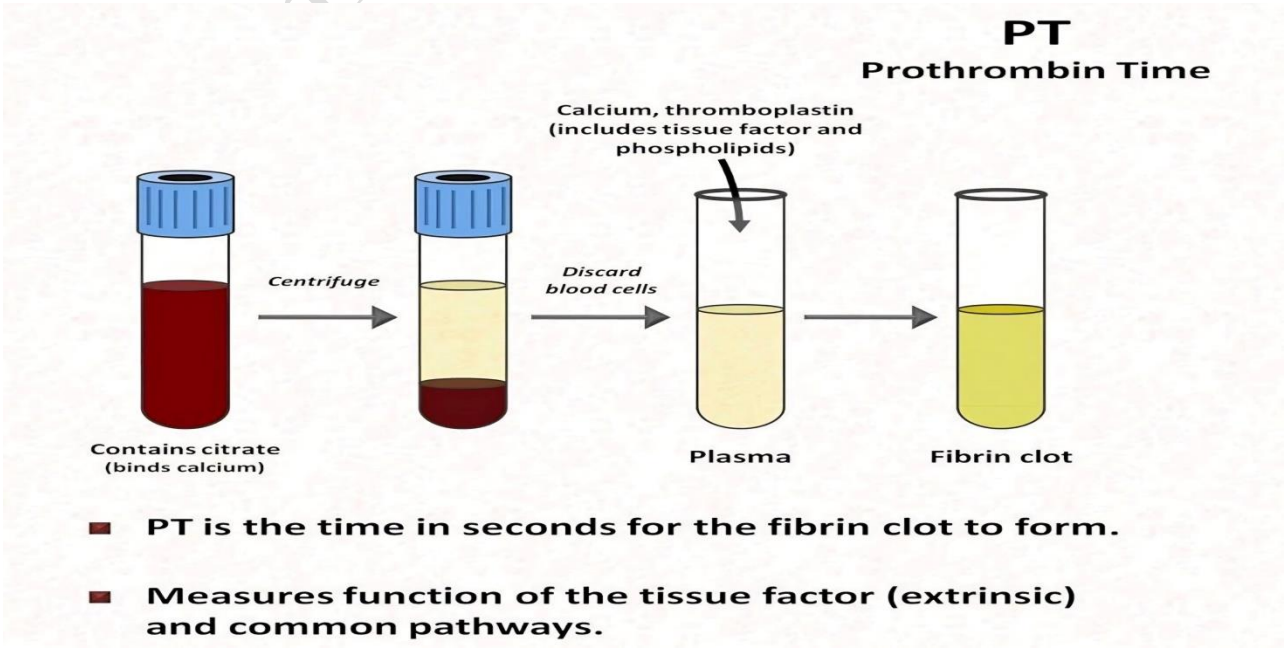
3-Add 0,2ml of reagent, simultaneously start stopwatch.

4-Gently tilt the tube back, forth and stop the stopwatch as soon as.

5-The first fibrin strand is visible and the gel clot formation begins.

6-Record the time in seconds.

The reference range for Prothrombin time is usually around **(11-16) seconds**.



Post test

Q1- Coagulation factors are primarily?
a) Carbohydrates.
b) Lipids .
c) Proteins.
d) Nucleic acids.

Q2-Which of these factors affects bleeding time?
a) Blood group.
b) Size and nature of the injury.
c) Age of the individual.
d) Body temperature.

Q3- A normal bleeding time using the Duke's method typically falls within the range of ?
a) 0-1 minute.
b) 2-5 minutes.
c) 6-10 minutes.
d) 11-15 minutes.

Q4- Compare and contrast "clotting time" and "Prothrombin Time (PT)," highlighting their definitions, the factors they assess, and their primary clinical applications?

Q5- List at least three different conditions or situations that can lead to an abnormal Prothrombin Time (PT) result?

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