BLEEDING TIME

DEFINITION: Is the time that elapses between the puncture of the skin and the stoppage of bleeding.

OBJECTIVES:

1. To evaluate platelet function and blood vessel integrity.
2. To screen patients for bleeding tendencies before surgery.

METHODS:

1. Duke test. Is the easiest to perform.
2. Ivy's test.

DUKE TEST

MATERIALS &INSTRUMENTS

1-Sterile disposable lancet.

2-Stopwatch.

3-Filter paper.

4-Cotton &alcohol.

PROCEDURE

1- Make the ear lobe worm either by rubbing or by hot water.

2- 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.

3- Start the watch as soon as blood appears.

4- Blot the drop of blood by filter paper every 10 – 15 sec. until blood stops.

5- Stop the watch immediately and this will be the bleeding time.

NORMAL RANGE: up to 5 minutes.

IVY'S TEST

This method is more reliable method because it is done under standard condition of pressure.

PROCEDURE

1- Apply sphygmomanometer cuff on the upper arm.
2- Inflate the cuff up to 40 mm Hg.
3- Clean the fore arm avoiding visible veins.
4- Make 2 punctures 3 cm apart.
5- Start 2 watches one for each puncture as soon as blood appears.
6- Blot the blood from the 2 punctures by filter paper every 10 – 15 sec. until blood stops.
7- Stop the watch immediately after stopping of bleeding from each puncture.
8- Take the bleeding time for the two punctures and divided by 2 to get the mean value and this means the bleeding time.

NORMAL RANGE: 2 – 4 minutes.

DIFFERENTIAL WHITE BLOOD CELL COUNT

DEFENITION It is the relative no. of each type of WBC present in the blood (Neutrophile, Basophile, Eosinophile, Lymphocyte and Monocyte).

It is also to determine if there is abnormal or primitive type of these cells in the blood.

REQUIREMENT

1- Blood sample.

2- Leishman stain and staining rack.

3- Slides & spreader with smooth edge.

4- Distilled water.

PREPERATION OF LEISHMAN STAIN

0.2 gm of leishman powder.

100 ml Methanol.

Mix and warm for 15 minutes with occasional shaking.

The solution is then filtered.

PROCEDURE

1- Spread a drop of the patient`s *blood* on the slide by the spreader of an angle of about *45°* to the slide and then moved back to make contact with the drop & spread quickly along the line of contact of the spreader with this slide. *The film must not be too thin and not too thick and the tail of the film should be smooth.*

2- Write the name of the patient on the beginning of smear (thick area).

3- Leave to dry in the air.

4- Put the slide on the rack.

5- Put 10 drops of *leishman`s stain* on the blood film & leave for (0.5-1) minute.

6- Add equal or double amount of *fresh distal water* , observe the appearance of a violet mirror on the top of the stain.

7- Leave for 10-15 minutes.

8- Wash with fresh D.W.

9- Clean the back of the slide by water.

10. Leave to dry in the air.
11. Examine under the microscope using *oil immersion lens* .

The cells should be counted in a strip running the whole length of the film the lateral edges of the film are avoided.

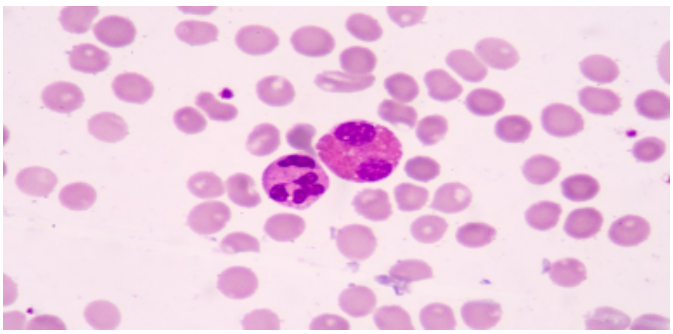
The film should be examined & if less than 200 cells are encountered in a single narrow strip.

One or more additional strip should be examined until at least 200 cells have been counted.

1- Neutrophile cell: - (12-14 µ in diameter)

Cytoplasm: - Acidophilic, contain fine pinkish red granules.

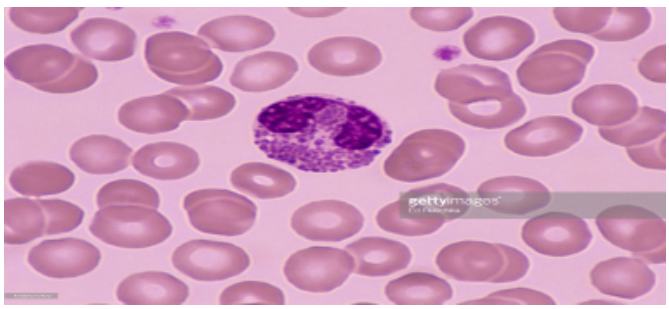
Nucleus: - Usually lobulated consist of 2-5 lobes.



2- Eosinophile cell: - (16µ in diameter)

Cytoplasm: - Acidophilic, contain coarse bright red granules.

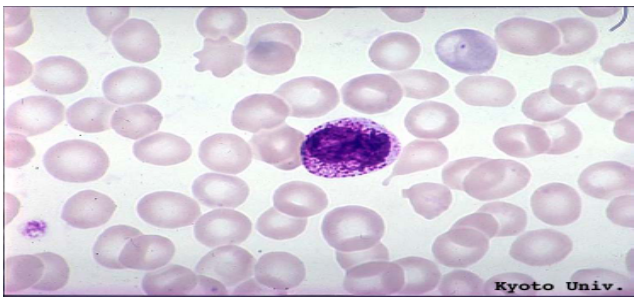
Nucleus: - usually consist of 2 lobes.



3- Basophile: - (14-16 µ in diameter)

Cytoplasm: - acidophilic, contain coarse black blue granules and cover most of the cell even the nucleus.

Nucleus: - Usually lobulated consist of 2-5 lobes.

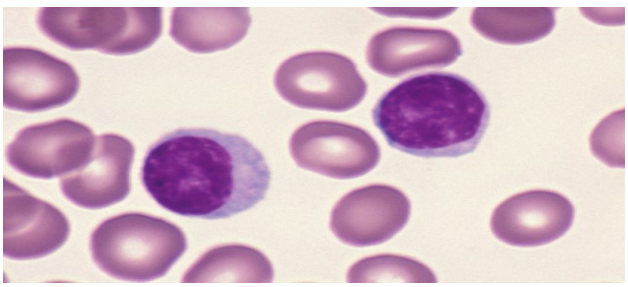


4- Lymphocyte: - (9-12µ in diameter) small lymphocyte.

(12-16 µ in diameter) large lymphocyte.

Cytoplasm: - usually sky blue in the color with no granules.

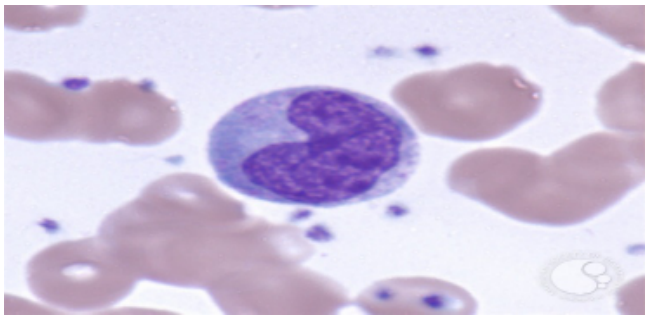
Nucleus: - occupying most of the cell and it is a round, dark violet in the color. The larger lymphocyte may contain a kidney shape nucleus.



5- Monocyte: - (15-18µ in diameter) the largest cells.

Cytoplasm: - cloudy blue in color (grayish blue).

Nucleus: - is usually kidney shape with pale violet color.



NORMAL RANGE

1- N= 40-75%.

2- E= 1-6%.

3- B= 0-1%.

4- L=20-50%.

5- M= 2-10%.

ABNORMALITIES OF ERYTHROCYTE

Red blood cells are the major cellular component of blood. Mature red blood cells are biconcave discs that lack nucleus.They have a typical diameter 7.2µ.

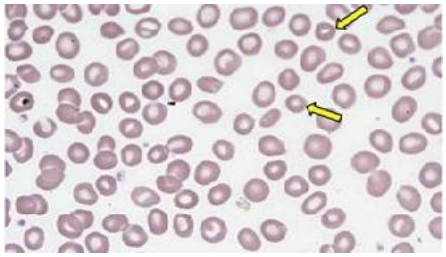
Variable abnormal erythrocyte morphology is found in various pathological conditions:

1. Variation in size
2. Variation in shape
3. Variation in color
4. Presence of inclusion bodies

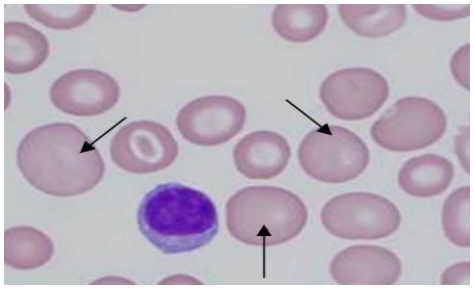
I. Variation in size

Variation in size of RBC is called anisocytosis. Anisocytosis is divided into Macrocytosis and Microcytosis.

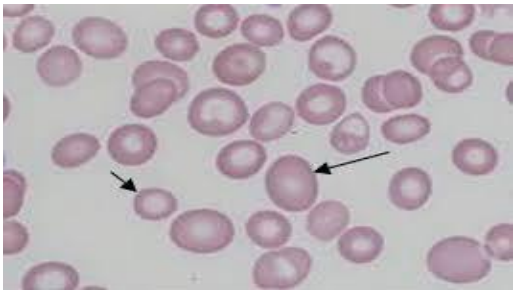
1. **Microcytosis**: RBCs smaller than the normal size are considered as microcytes.
   Ex: Iron deficiency anemia.



2. **Macrocytosis**: RBCs larger than the normal size are considered as macrocytes.Ex: Liver diseases andmegaloblastic anemia.



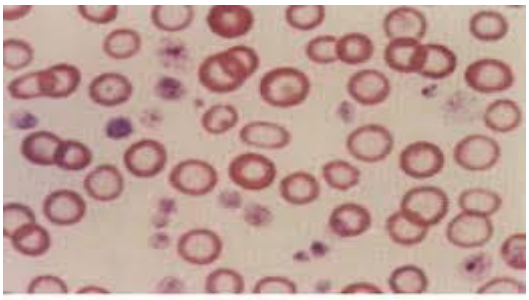
3. **Anisocytosis** : Presence of both macro and microcytes with normal erythrocyte in one field.



II. Variation in color

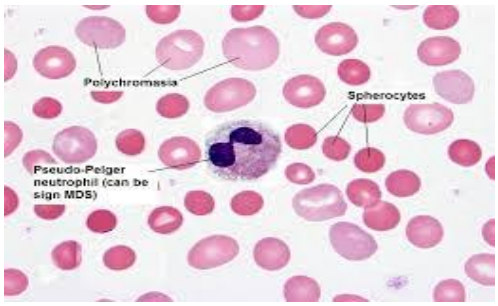
RBCs that appear disc shaped and having an area of central pallor that occupies approximately one-third of the cell's diameter (containing normal amount of hemoglobin) are considered as normochromic RBCs.

1. **Hypochromasia**:Hypochromasia indicates that the red blood cells have less hemoglobin amount than normalRBCs. ex: Iron deficiency anemia.

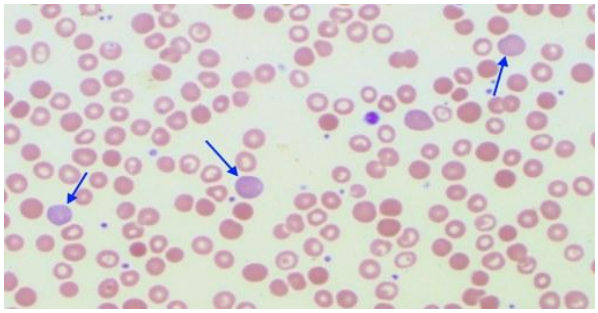


hypochromic cells

2. **Hyperchromasia:** RBC is darker in color than normal; this may be due to larger amount of heamoglobin than normal RBCs. Ex: spherocytosis



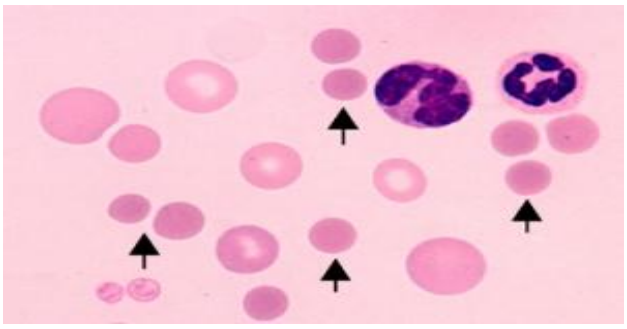
3. **Polychromasia**: Is a variation in staining of erythrocytes with the Wright's stain because of the presence of young erythrocytes.blue-staining RBCs, indicating that they are immature due to early release from bone marrow.Ex: Reticulocyte.



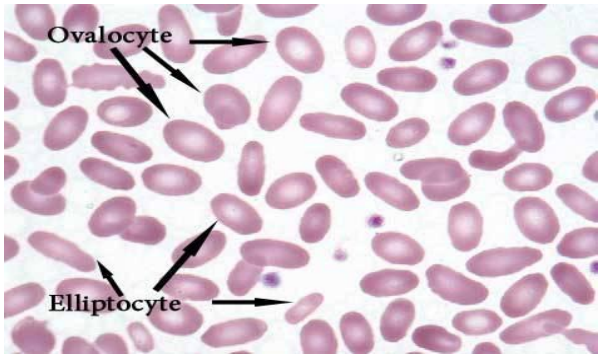
III. Variation in shape

Variation in the shape of RBC is called poikilocytosis.
Following are some abnormal RBC shapes :

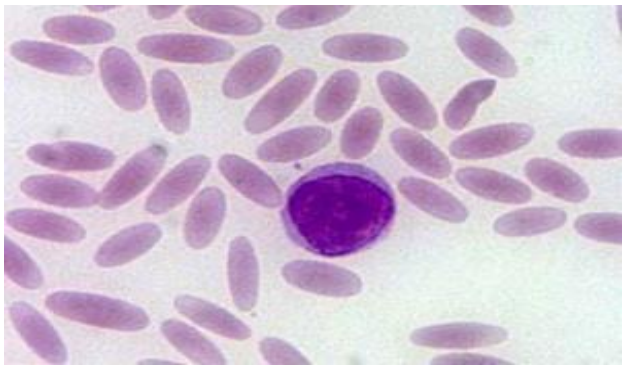
1. **Spherocytes**: RBCs lacks the biconcave shape and becomes more spherical, no central pallor is present with increased hemoglobin content.EX: Hereditary spherocytosis, hemolytic anemia and post transfusion reaction.



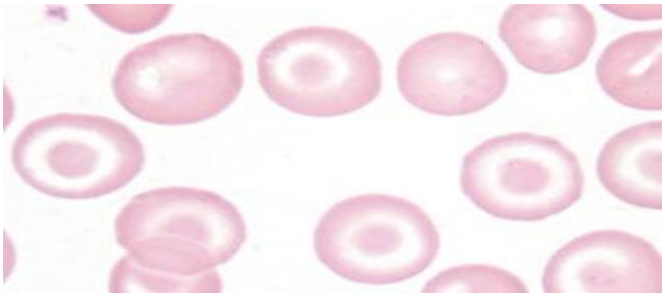
2. **Ovalocytes**: Oval shaped RBCs.ex :Hereditary ovalocytosis.



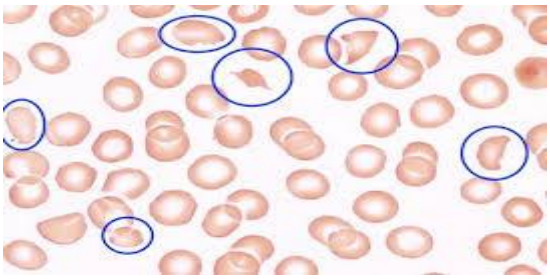
3. **Elliptocytes**: The RBCs are oval or elliptical in shape.ex: Hereditary elliptocytosis,



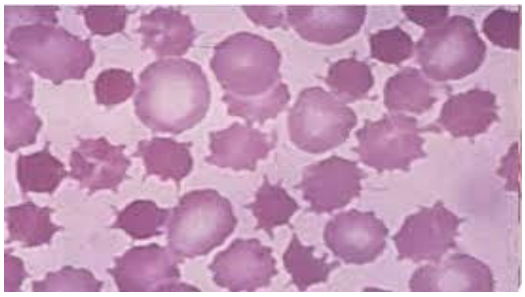
4. **Target cells**: Red cells have an area of increased staining which appears in the area of central pallor.ex: Thalassemia.



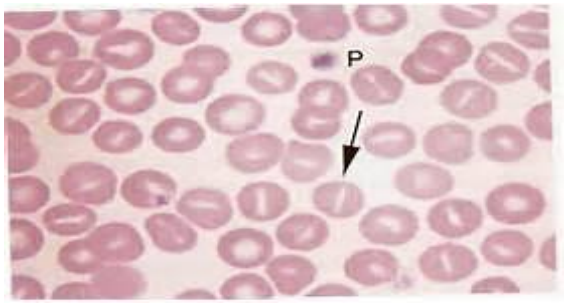
5. **Schistocytes**: These are fragmented RBCs smaller than normal size.ex: hemolytic anemia, uremia, artificial heart valves.



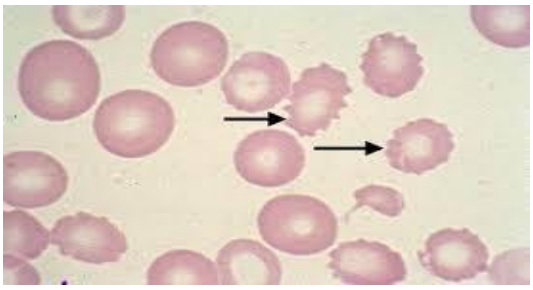
6. **Acanthocytes**: RBCs with irregularly spaced projections. Projections vary in width but usually contain a rounded end. Ex: liver diseases and alcoholism.



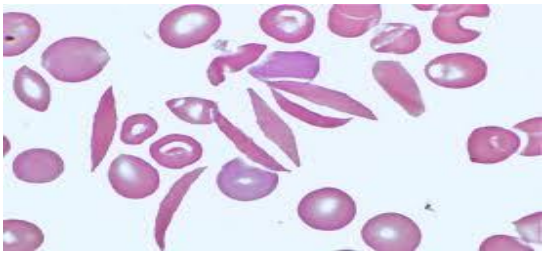
7. **Stomatocytes**: Red cells with a central linear slit or stoma. Seen as mouth shaped form in peripheral smear. EX: excess alcoholism



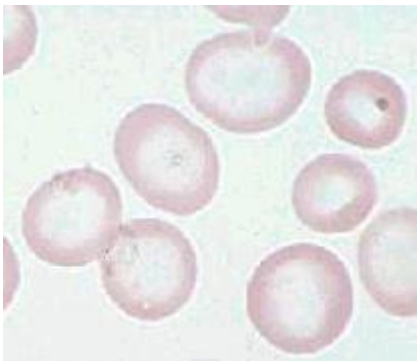
8. **Burr cells**: Red cells with uniformly spaced pointed projections on their surface.Ex: uremia.



9. **Sickle cells**: These are sickle-shaped Red Blood Cells.ex: sickle cell anemia.

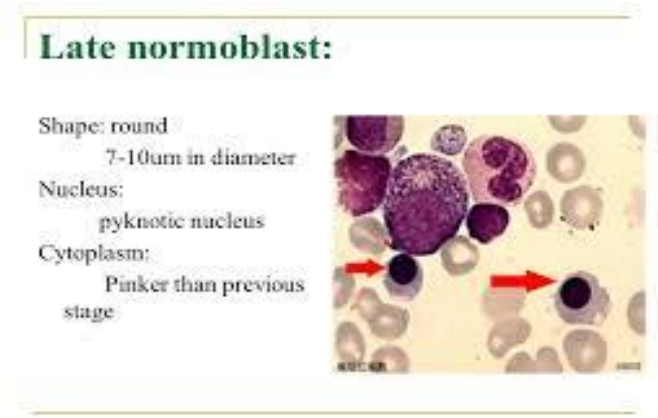


10. **Leptocyte. Ex: IDA.**

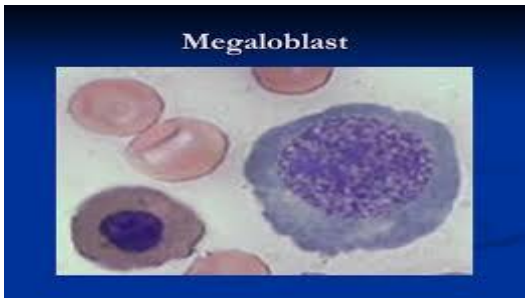


IV. Presence of inclusion bodies

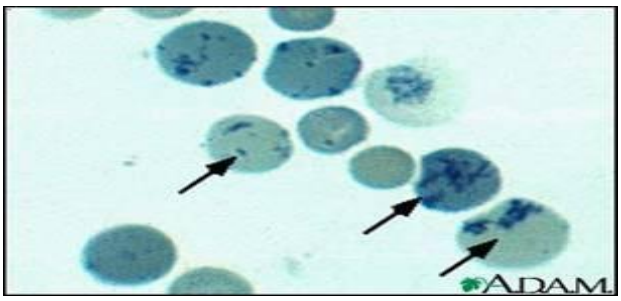
1. **Normoblast:**an immature red blood cell containing hemoglobin and a pyknotic nucleus and normally present in bone marrow but appearing in the blood in many anemias.



2. **Megaloblast:**An abnormally large nucleated red blood cell found especially in pernicious anemia or in certain vitamin deficiencies.



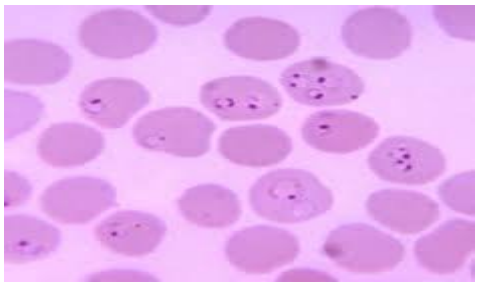
3. **Reticulocyte:** are immature red blood cells, typically composing about 1% of the red blood cells in the human body. They are reticular network of ribosomal RNA that becomes visible under a microscope with certain stains such as new methylene blue .



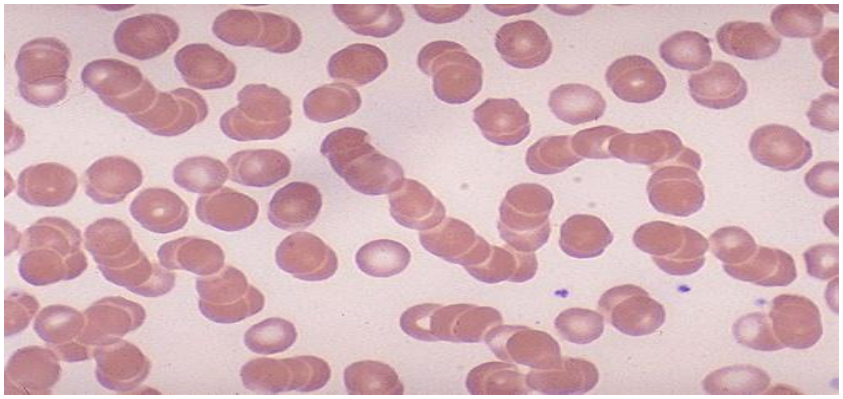
4. **Howell-Jolly bodies**: Small round cytoplasmic red cell inclusion with fragments of DNA. ex:hemolytic anemia

5. **Basophilic stippling**: These are considerable amount of small basophilic inclusions in red cells which represent precipitated RNA.ex : thalassemia, , liver damage and heavy metal poisoning.

6. **Malaria parasites**



7. **Rouleax formation**

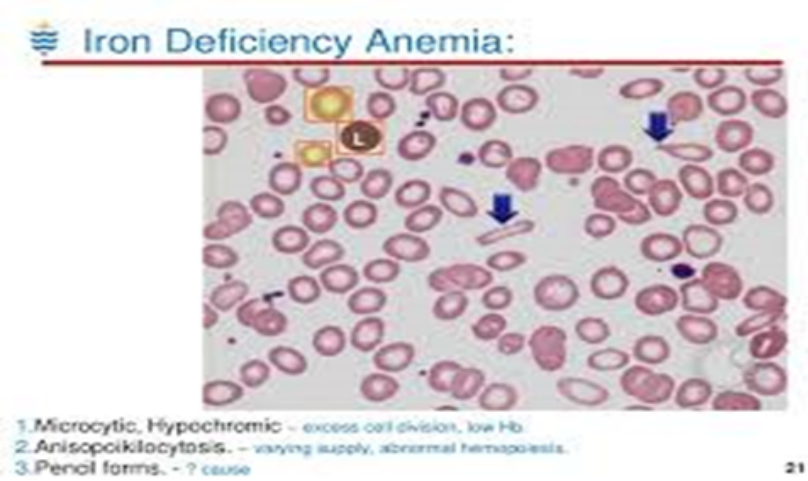


ANEMIA

DEFENITION: Anemia is a condition characterized by a deficiency of red blood cells, or of hemoglobin, in the blood.
The main causes of anemia are bleeding, hemolysis (excessive destruction of red blood cells), underproduction of red blood cells, and underproduction of normal hemoglobin._
The types of anemia by blood film which stain by lieshman`s stain depend on the RBCs abnormal shape, size & color.

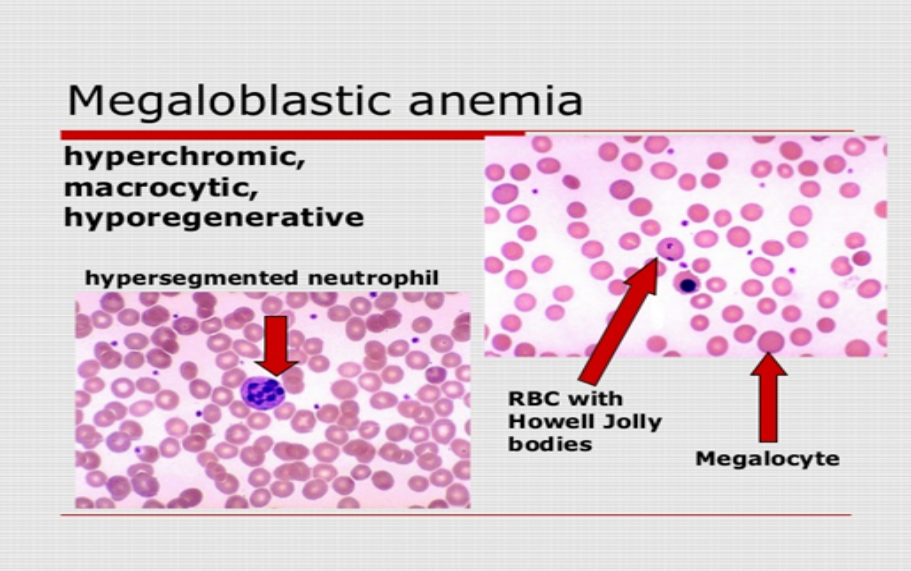
1- Iron deficiency anemia:-

Iron deficiency anemia is the most common form of anemia. It occurs when iron levels are too low. RBCs are microcytic, hypochromic with cigar cells could be present in sever anemia & there is target cell. MCV, MCH, MCHC are all decreased.



2- Megaloplastic anemia (Vitamin deficiency anemia):-

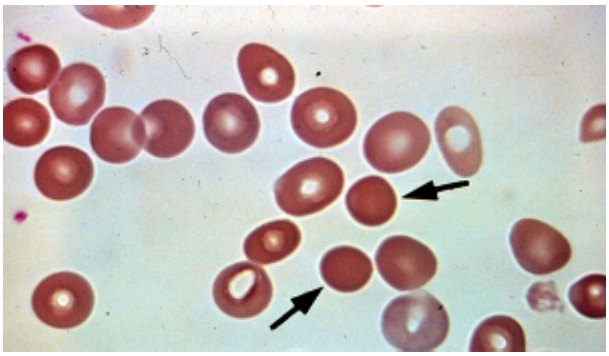
This type of anemia is caused by deficiency of vitamin B12 or folic acid which is necessary to produce enough red blood cells .RBCs are macrocytic, normochromic **& hypersegmented neutrophile as specific diagnosis** for megaloplastic anemia. MCV increase, MCHC normal.



3- Spherocytic anemia:-

Is an hemolytic anemia (a disease of the blood) characterized by the production of spherocytes (red blood cells (RBCs)) . This is caused by a molecular defect in one or more of the proteins of the red blood cell which result in abnormal red cells.

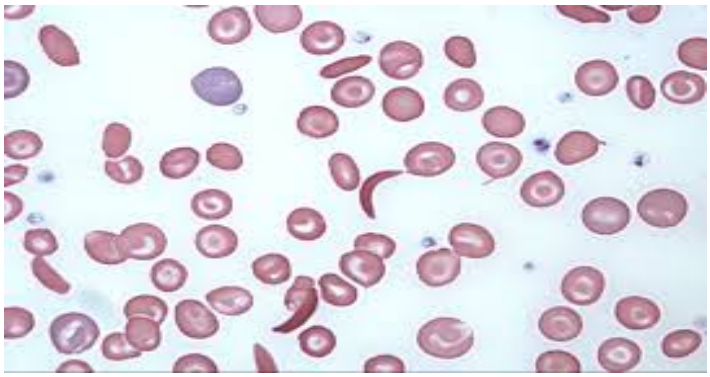
Normocytic, hyperchromic cell, spherocytic cell. MCV normal, MCH increased, osmotic fragility increased.



4- Sickle cell anemia:-

Sickle cell anemia is an inherited form of anemia — a condition in which have abnormal hemoglobin, called *hemoglobin S* . Sickle hemoglobin is not like normal hemoglobin. It can form stiff rods within the red cell, changing it into a crescent, *sickle* shape.

There are sickle cell and cigar shape & schistocyte because its type of haemolytic anemia.

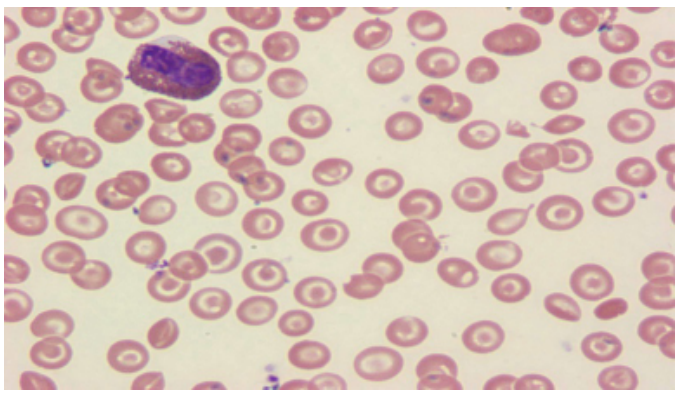


5- Thalassemia

Thalassemia is an inherited blood disorder in which the body makes an abnormal form of hemoglobin.

Thalassemia occurs when there's an abnormality or mutation in one of the genes involved in hemoglobin production.

There are target cell with schistocyte and acanthocyte.



6- Elliptocytic anemia

There are elliptocyte cell heavy appearance with normal RBCs, its explain that is hereditary disease haemolytic anemia.