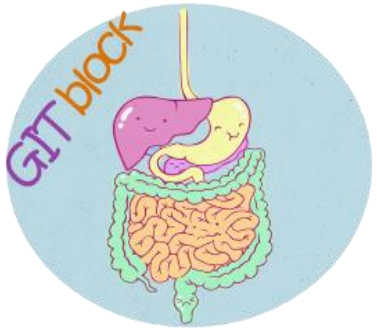




# Approach to haemolysis

NOTE : THIS TEAMWORK DON'T VIEW EVERYTHING IN THE SLIDES ONLY THE IMPORANT THINGS NOTED BY THE DOCTORS

**Color coding**

- important
- Extra info
- Notes from lecturer

دعاء قبل المذاكرة :

(اللهم أني أسالك فهم النبين و حفظ الملرسلين و الملائكة المقربين اللهم اجعل السنتنا عامرة بذكرك و قلوبنا بخشيتك، أنك على كل شيئا قدير و حسبنا الله نعم الوكيل )

DON'T FORGET to check our editing file : haematology edit

Please don't hesitate to contact us on:Haematology434@gmail.com

| | Male | Female |
|---|---|---|
| Hemoglobin(g/dL) | 13.5-17.5 | 11.5-15.5 |
| Hematocrit (PCV) (%) | 40-52 | 36-48 |
| Red Cell Count ($\times 10^{12}$) | 4.5-6.5 | 3.9-5.6 |
| Mean Cell Volume (MCV) (fL) | 80-95 | |
| Mean Cell Hemoglobin (MCH) (pg) | 30-35 | |
| MCHC % | 31 - 37 | |
| Platelet count | 140-450x$10^3$/L | |
| NORMAL PLATELET SIZE MPV | 7.2-11.1 fl | |
| NORMAL PLATELET DIAMETER | 1-2.5 µ | |
| WBC | 4000-11,000 /L | |
| Segmented (neutrophils) | 1.8-7.8 | |
| Eos | 0-0.45 | |
| Baso | 0-0.20 | |
| Lymphs | 1.0-4.8 | |
| Monos | 0-0.80 | |

A Good Doctor Knows normal CBC

# Hemolysis

Definition..? Premature destruction of RBCs.

- Hemolysis could be due to:

a.Defect in the RBCs (intra-corpuscular) as in congenital hemolytic Anaemia.

b.Defect in the surrounding environment (extra-corpuscular) as in acquired Anemia.

## Clinical Features of Hemolysis..?

- Pallor, lethargy
- Jaundice indirec
- Splenomegaly
- Gall stones (Pigment – bilirubin)
- Dark urine (urobilinogen)
- Bone deformity (In some types of haemolytic anaemia)
- Leg ulcers (in some types of haemolytic anaemia).sickle cell anemia

# Laboratory Features of Hemolysis

| 1.Features of increased red cell breakdown. | 2.Features of increased red cells production. | 3.Damaged red cells. |
|---|---|---|
| a.↑ serum bilirubin is raised (unconjugated and bound to albumin). | a.Reticulocytosis<br>Excess of reticulocytes in the peripheral blood. | a. Morphology (e.g.Microspherocytes, Elliptocytes, red cells fragmentation). (Elliptocytes are abnormally shaped RBCs that appear oval or elongated). |
| b.↑ urine urobilinogen. | | |
| c.↑ faecal stercobilinogen. | | |
| d.**Absent** serum haptoglobins.<br>Protein that bends to Hb. | b.Bone marrow erythroid hyperplasia | b.Increased osmotic fragility, Autohaemolysis, etc.<br>Measure erythrocyte resistance to hemolysis |
| e. ↑ lactate dehydrogenase (LDH)<br>Lactate dehydrogenase is an enzyme found (blood cells and heart muscle).It's released when there's a tissue damage which makes it a significant marker of common injuries and disease. | | c.Shortened red cell survival (This can be shown by 51Cr labeling with study of the sites of destruction). |

# Types of Hemolysis

## Intravascular haemolysis more dangerous

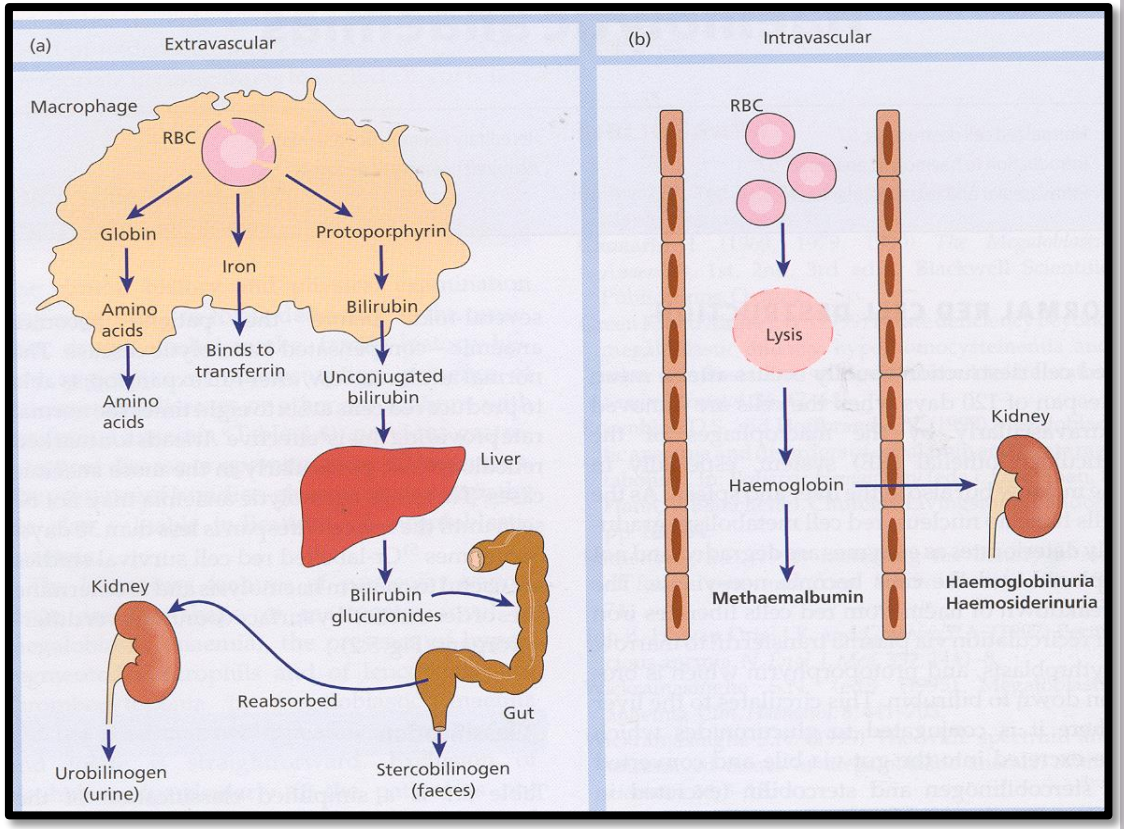
The process of breakdown of red cells directly in the **circulation**.

## Extravascular haemolysis

Excessive removal of red cells by cells of **RE system** in the spleen and liver.

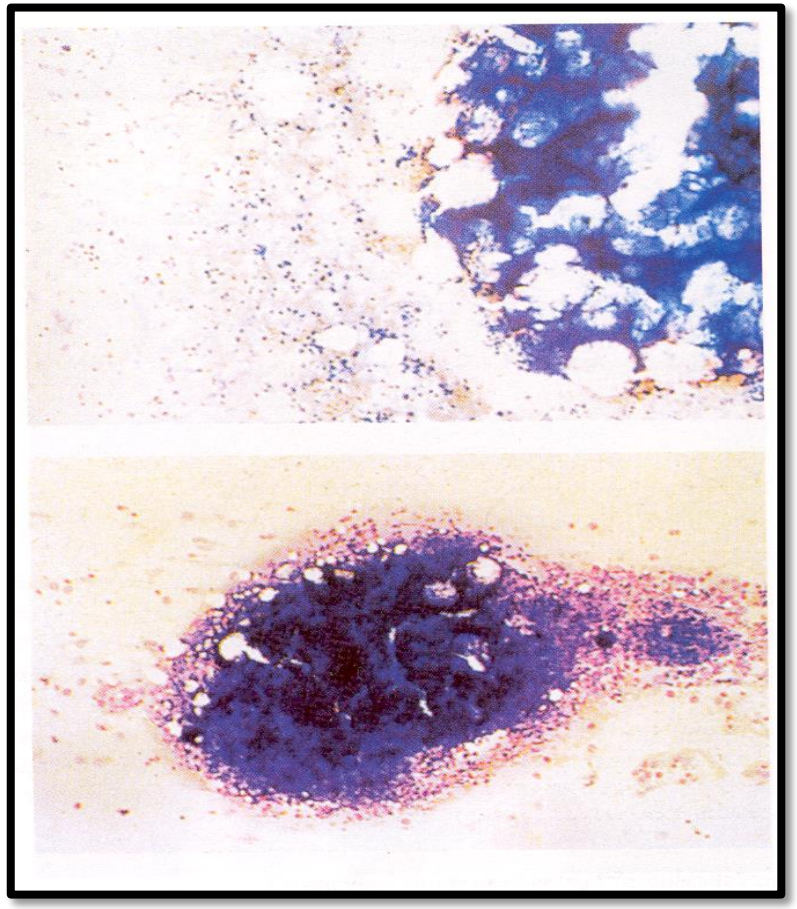
Laboratory features of **intavascular** haemolysis:

1. Haemoglobinaemia and haemoglobinuria.
2. Haemosiderinuria (Iron storage protein in the spun deposit of urine) "Brown urine".



# Causes of intravascular hemolysis

1. Mismatched blood transfusion (usually ABO).
2. G6PD deficiency with oxidant stress.
3. Red cell fragmentation syndromes.[1]
4. Some autoimmune Haemolytic Anaemia.
5. Some drug and infection-induced Haemolytic Anaemia.
6. Paroxysmal Nocturnal Haemoglobinuria.[2]
7. March Haemoglobinuria[3]
8. Unstable Haemoglobin.



[1]The red cell fragmentation syndrome can occur due to abnormalities of the heart or the blood vessels or vascular malformations.

[2]Activating of complement➔intravascular hemolytic anemia (during night)

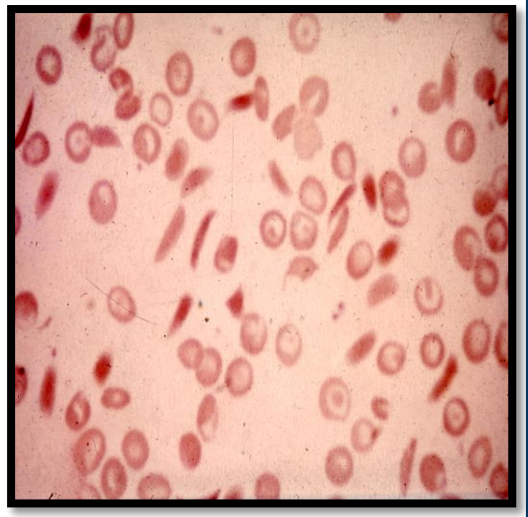
[3]Damage of RBCs between the small bones of the feet due to prolonged marching or running

# Hemolytic Anemia

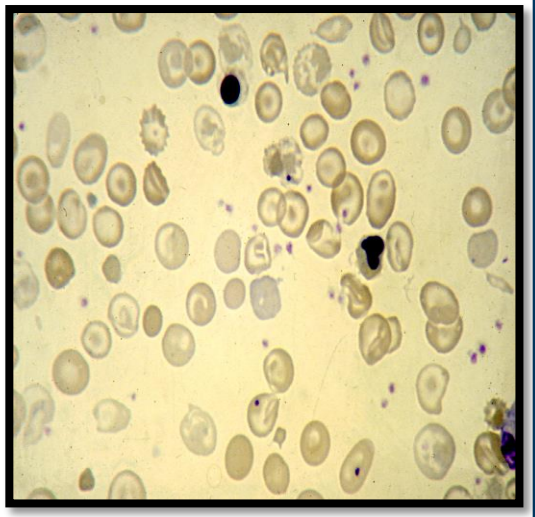
| 1. Congenital | 2.Acquired |
|---|---|
| o Sickle cell disease & other Haemoglobin disorders (Hb genetic abnormalities: HbS,HbC,unstable Hb).<br><br>o Thalassaemias.<br><br>o Enzymopathies<br>Eg.G6PD deficiency, PK deficiency.<br><br>o Membranopathies<br>Structural abnormalities of the RBCs membranes.<br><br>Eg. Hereditary spherocytosis, Elliptocytosis, Acanthocytosis. | o Allografts, especially marrow transplantation.<br>o Drug associated.<br>o Red cell fragmentation syndrome.<br>o Arterial grafts, cardiac valves.<br>o Microangiopathic.<br>o Thrombotic Thrombocytopenic Purpura, Haemolytic Uraemic syndrome.<br>o Meningococcal sepsis<br>o Pre-eclampsia<br>o Disseminated intravascular coagulation like AML M3<br>o March haemoglobinuria<br>haemolysis caused by repeated mechanical injury to red cells that travel through small vessels. |

| Infections: | Chemical and physical agents: | Secondary to: |
|---|---|---|
| o Malaria, clostridia. | o Especially drugs, inductrial/domestic substances, burns. | o Liver and renal disease.<br>o Paroxysmal nocturnal Haemoglobinuria.<br>o Autoimmune Haemolytic Anaemias. |

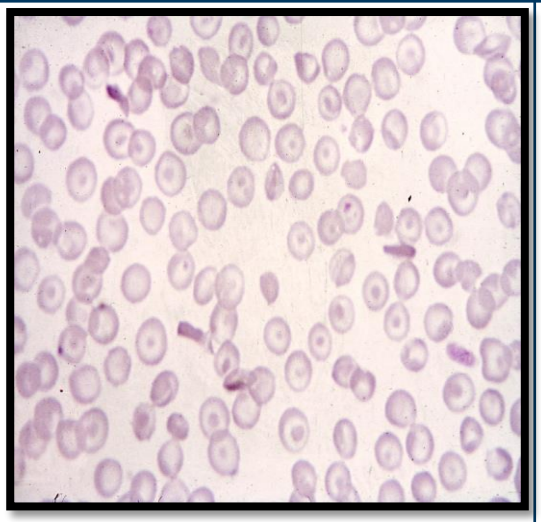
# Under the Microscope



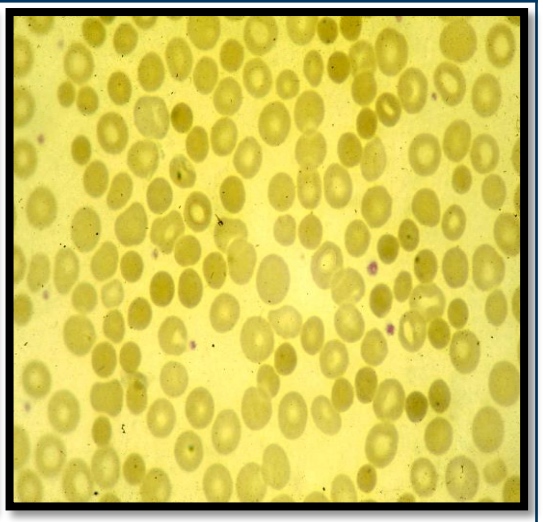
SICKLE CELL ANAEMIA



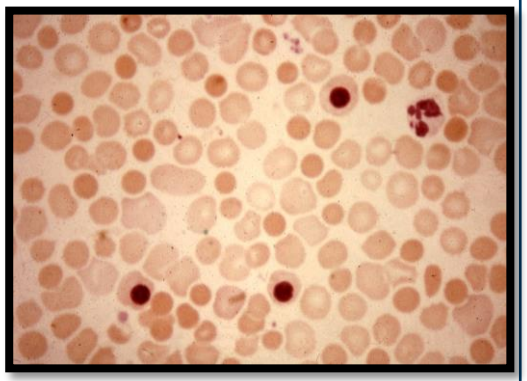
THALASSAEMIA MAJOR



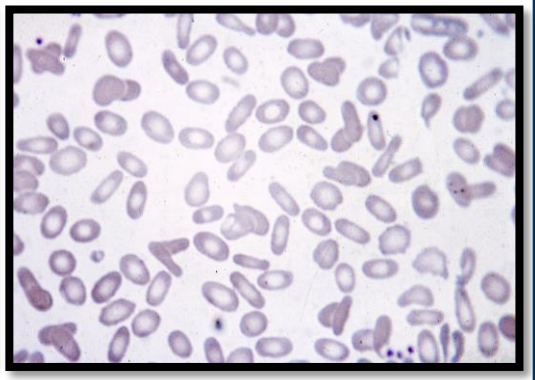
SICKLE BETA-
THALASSAEMIA



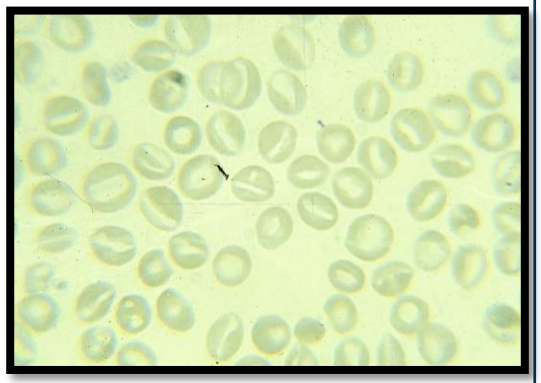
SPHEROCYTOSIS
6GPD defect



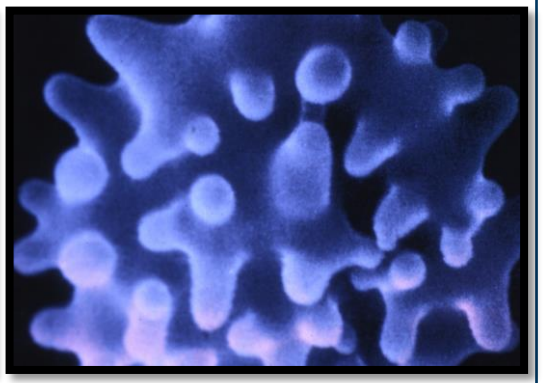
SPEROCYTOSIS
NEW BORN



ELLIPTOCYTOSIS
(Cigar shape)

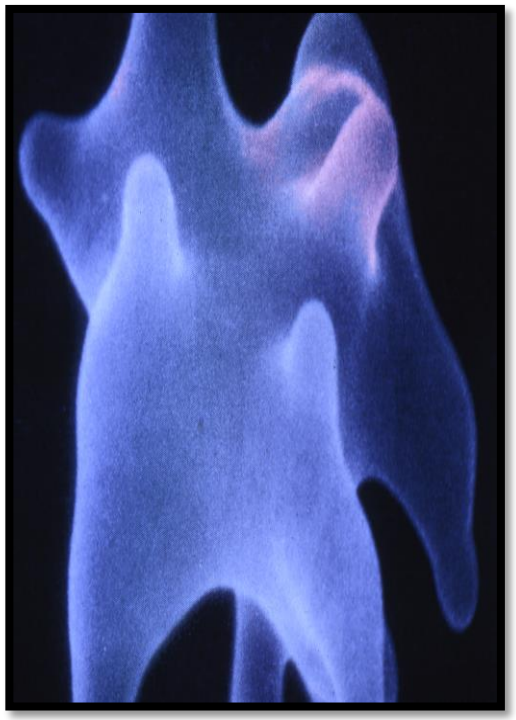


STOMATOCYTOSIS
(Open mouth shape)

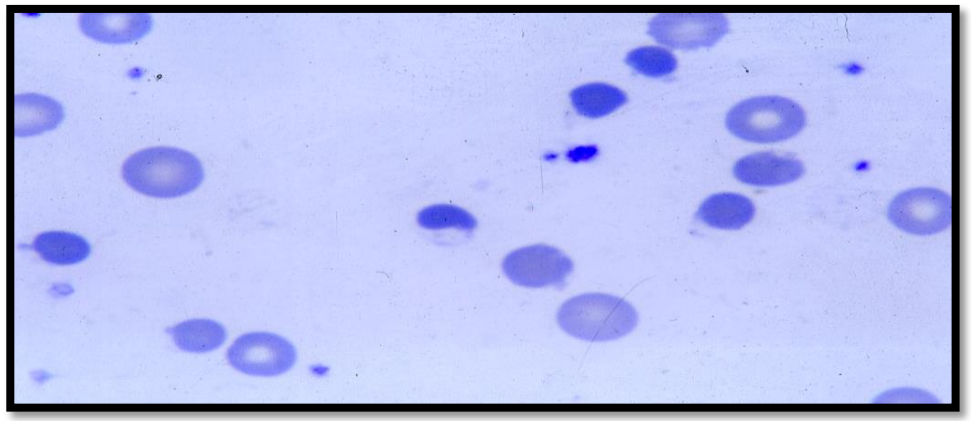


ACANTHOCYTOSIS
(fingers like projections)

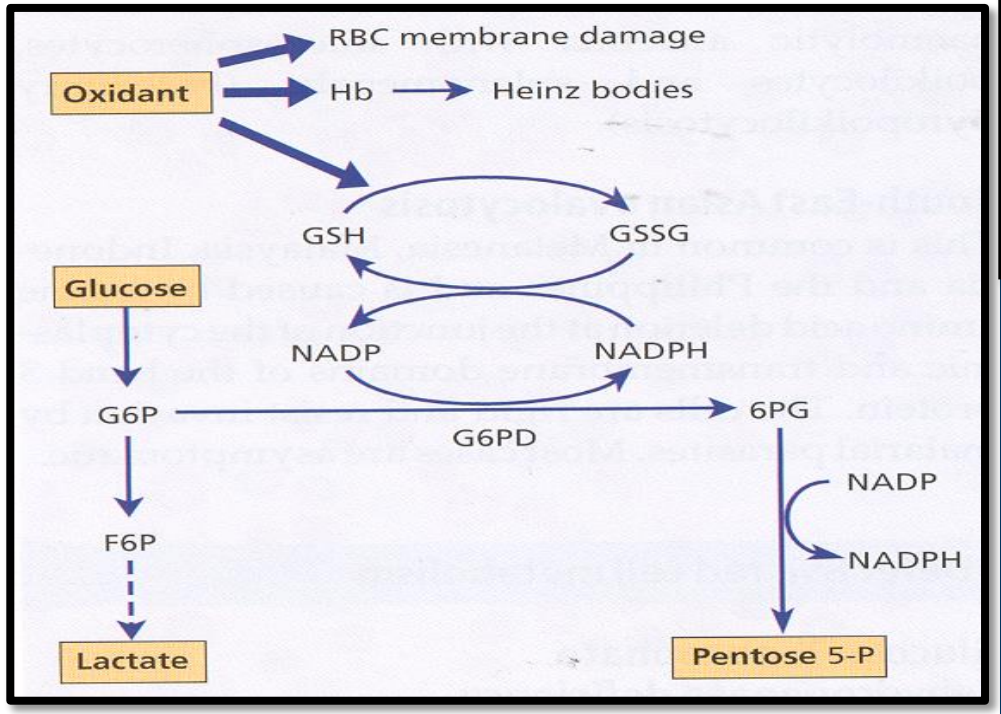
# Under the Microscope

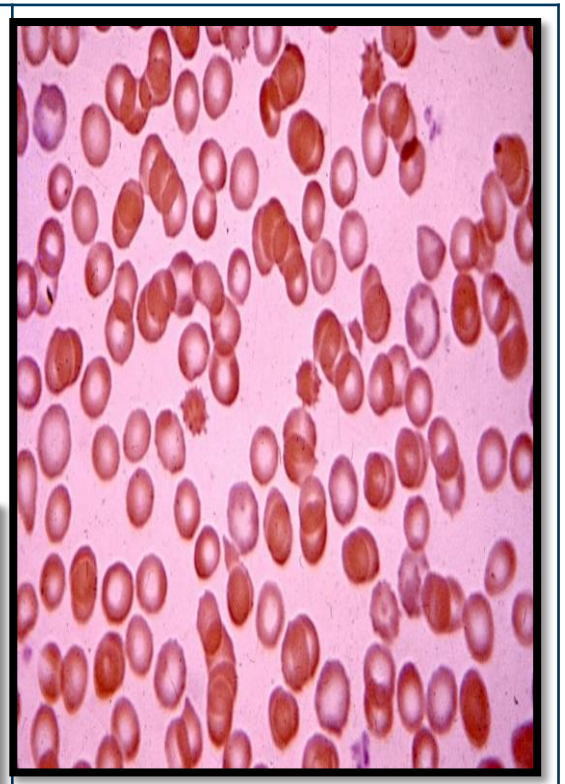


**ACANTHOCYTOSIS**
**(fingers like projections)**



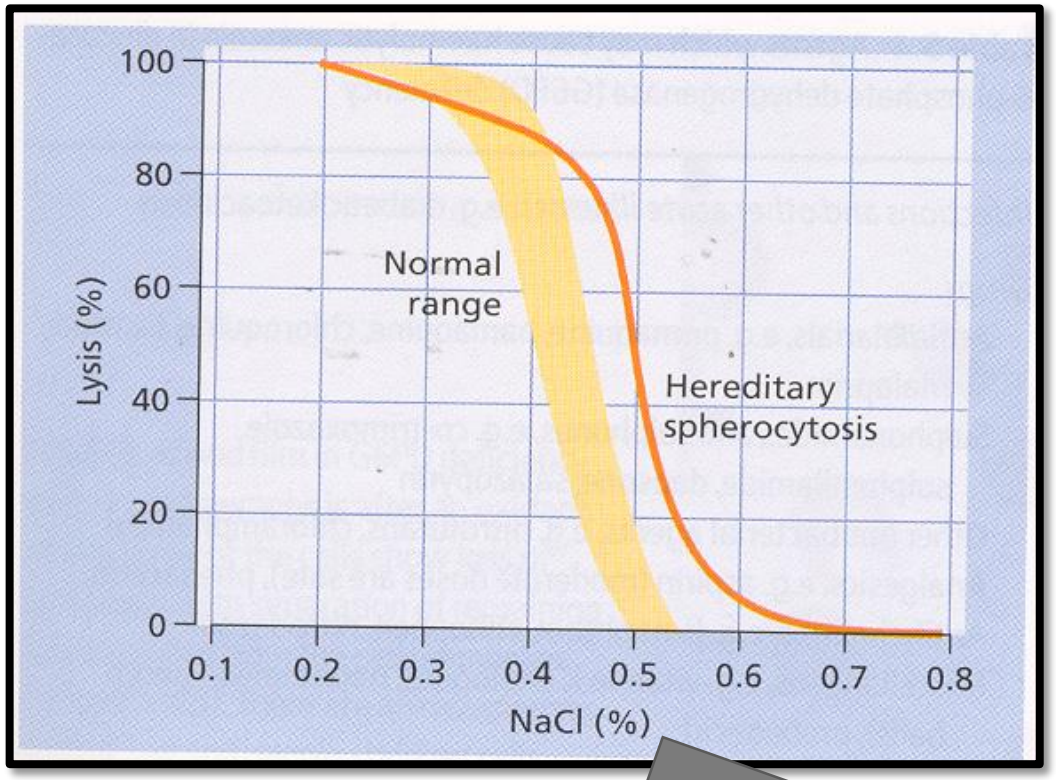
**G6PD DEFICIENCY**



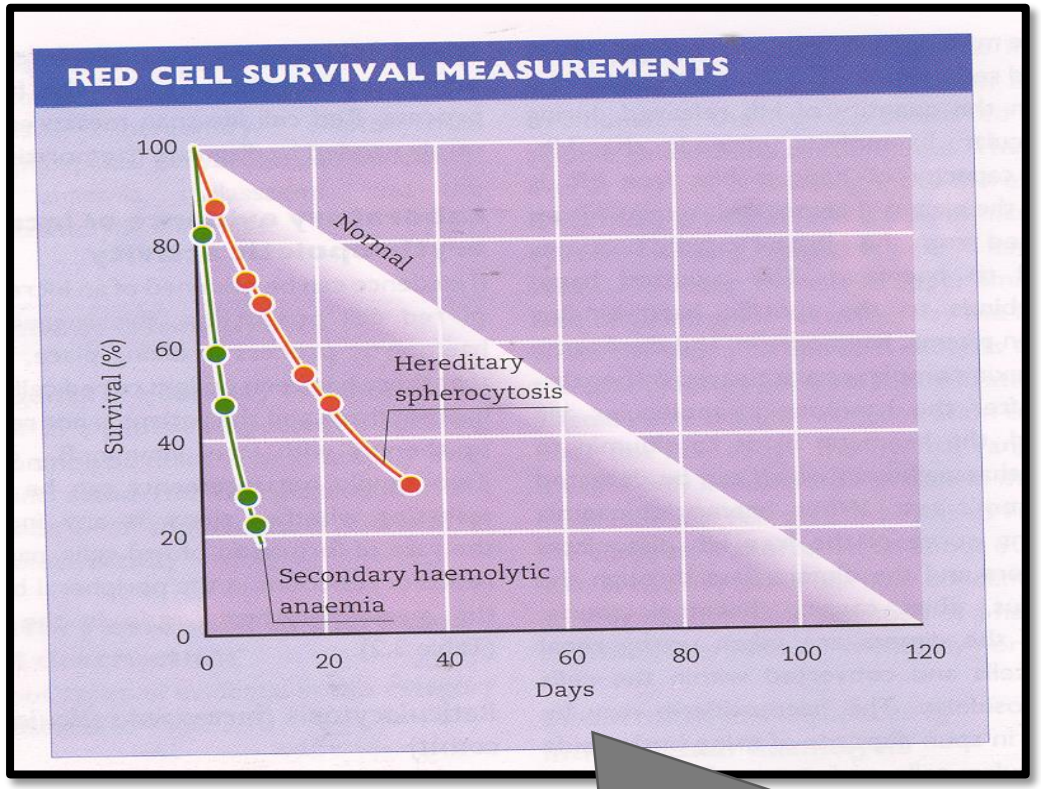



**PK DEFICIENCY**
Pyruvate kinase deficiency

# Some Illustrations




Osmotic fragility test measures red blood cell (RBC) resistance to hemolysis when exposed to a series of increasingly dilute saline solutions. The sooner hemolysis occurs, the greater . here it the osmotic fragility of the cellserocytosis shows that in Hereditary sphit's highly increased




Red Cell Survival Measurements:
to confirm hemolysis. Anything before the white line indicates hemolysis and in Hereditary spherocytosis (lifespan of RBC 30d) and in 2ndry hemolytic anaemia (15d)

# **Extra info** to understand the clinical manifestations

- The fate of sickled RBC's:

1. irreversible sickled cell, which undergoes hemolysis that causes : chronic hemolytic anemia with Pallor, Jaundice, Apathy & Anorexia.
2. reversible sickled cell that causes microvascular occlusion that leads to -> Tissue hypoxia

(how? the damaged membrane of the sickled cell has more tendency to ***adhere*** ***(gets sticky)*** to the endothelial cells, making an occlusion that causes tissue hypoxia.) Those occlusions, the reversible sickled cells, happen in area where blood flow is "sluggish", like the spleen, bone marrow and in case of an inflammation.

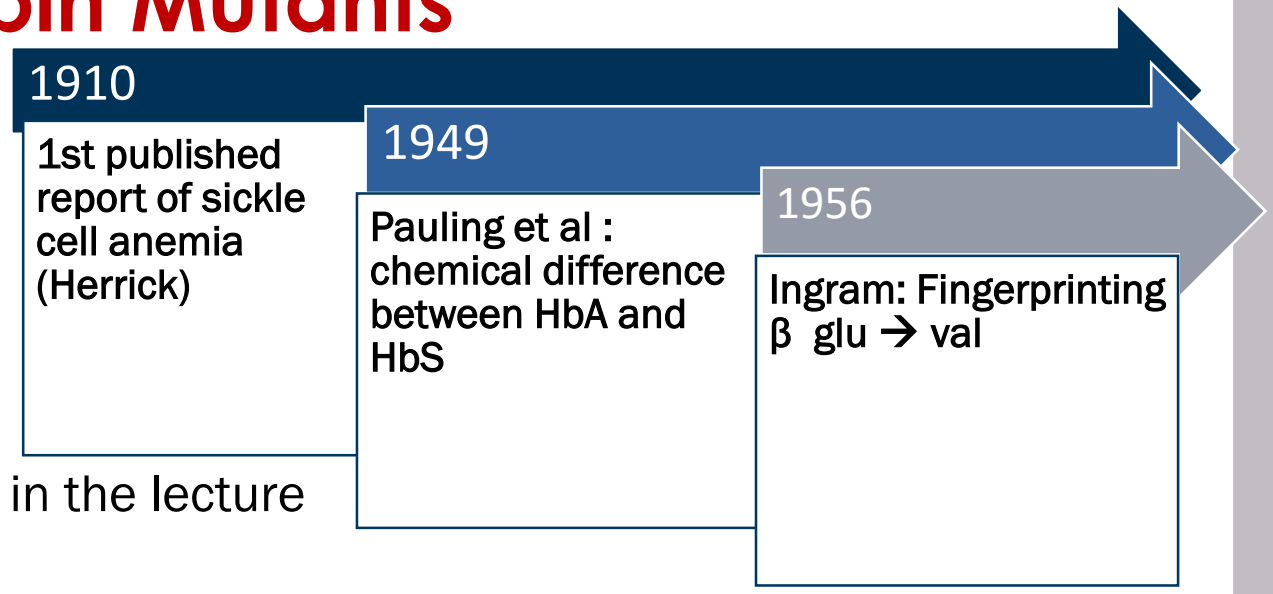
- The increased RBC's destruction will result in hyperbilirubinemia.
- The red pulps of the spleen become congested from the entrapment of sickled RBC'S -> splenomegaly
- Splenic enlargement/congestion will interfere with bacterial killing, making SC patients more susceptible to infections.
- A serious complication of pulmonary infections is acute chest syndrome.
- The body will undergo a compensatory mechanism to replace the destroyed RBC's. This include

1) erythroid hyperplasia (bone marrow expansion) that will appear as a bone deformity on x-ray
2) extramedullary hematopoiesis (production of RBC's outside the bone marrow) that occurs in liver and spleen -> hepatosplenomegay

# Some known Hemoglobin Mutants

1. Hb. S ---> α2 β2 6 GLU → VAL
2. Hb. C ---> α2 β2 6 GLU → LYS
3. Hb. E ---> α2 β2 26 GLU → LYS

These are the most important 3 Mutants in the lecture that the doctor mentioned.



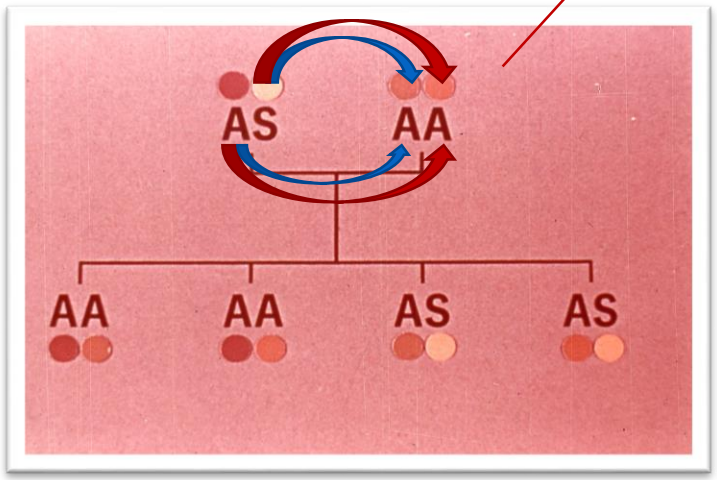

## DNA Coding for the Amino-Acid in the sixth position in the β-chain

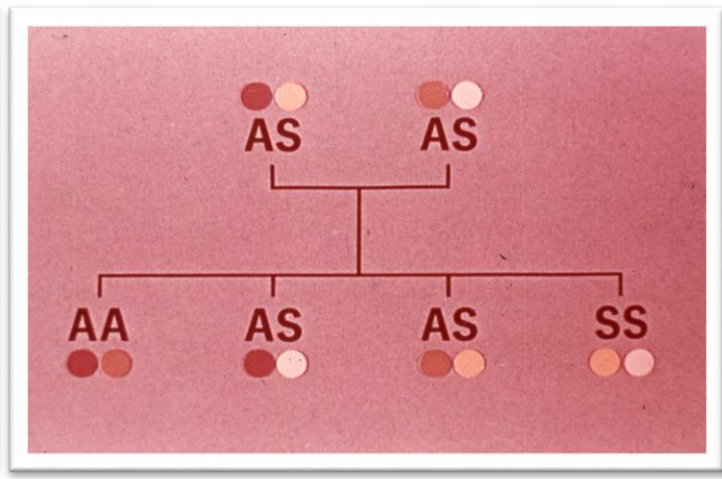
| Normal Amino Acid DNA Base Composition | 5 | 6 | 7 |
|---|---|---|---|
| | Pro | glu | glu |
| | CCT | GAG | GAG |
| Sickle Amino Acid DNA Composition | 5 | 6 | 7 |
| | pro | val | glu |
| | CCT | GTG | GAG |

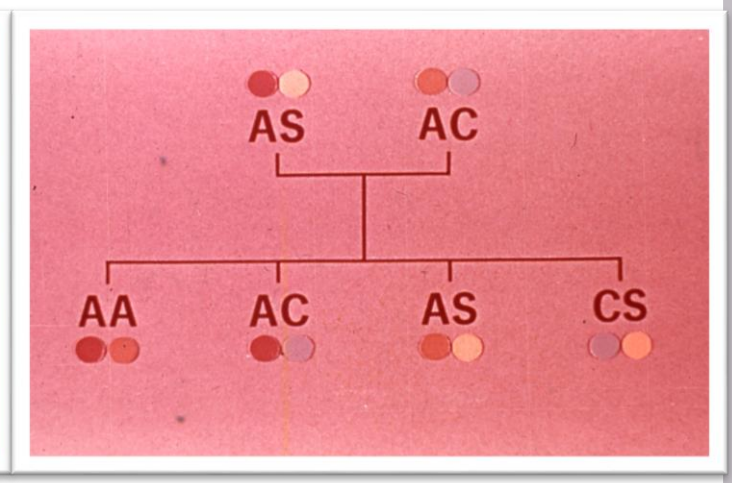
**Molecular change in sickle cell anaemia**

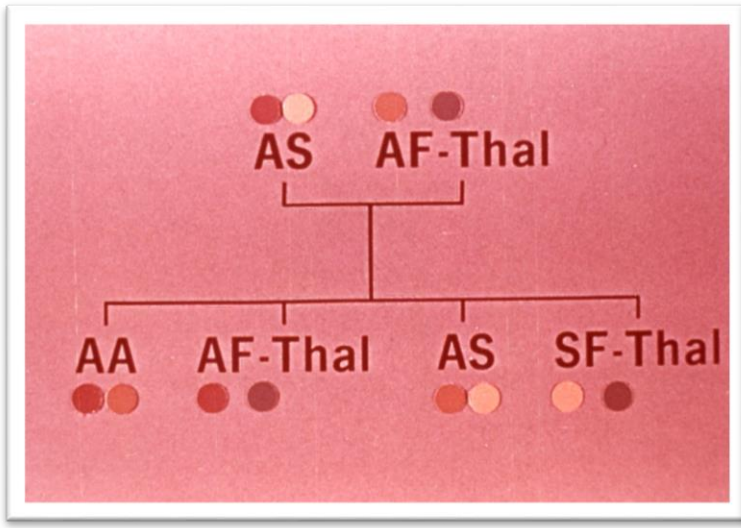
There is a single point mutation in the DNA coding for the amino acid in the sixth position in the β- globin chain (adenine is replaced by thymine). This leads to an amino acid change from glutamic acid to Valine .

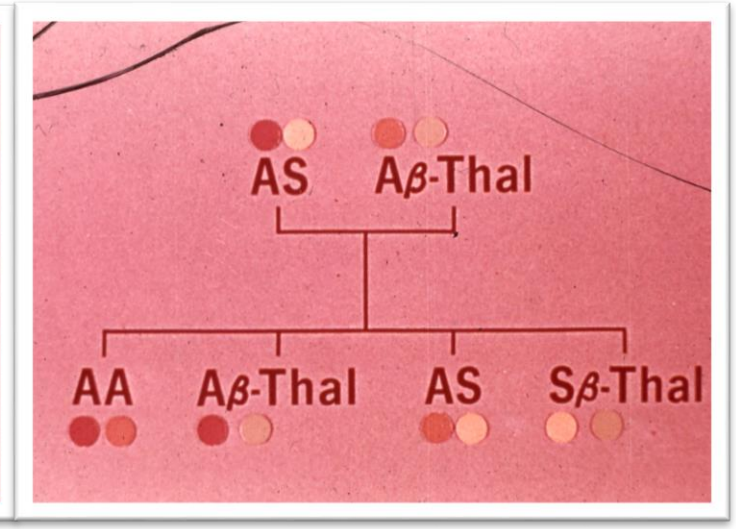
Case 1 If one parent was a carrier (trait) of an abnormal allele (of HbS) 50% of their children will be carriers (trait)




سهلة هي نفس قاعدة مندل للوراثة اللي اخذناها بالمدرسة

# Sickle cell diasease

Go back to the previous slide to understand this.

## The sickle cell trait:

| 1. Homozygous sickle cell disease | 2. Doubly Heterozygous sickle cell disease |
|---|---|
| SS → sickle cell anemia | CS → Sickle cell / Haemoglobin C disease |
| | Sβ-Thal → Sickle cell / Thalassaemia |

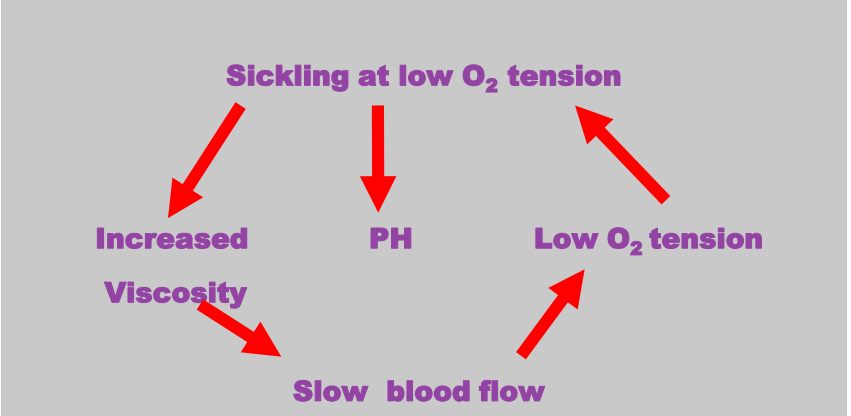
## Properties of Hb S:

1. Low Solubility
2. Conformational changes – when exposed to low oxygen → "Tactoid formation" → Sickled cells → Irreversibly sickled cells.
3. Increased mechanical fragility → Haemolysis
4. Increased viscosity → Organ infarction (Caused by obstruction of small blood vessels by the Abnormal Hb S)

## Factors affecting sickling:

1. Oxygen tension : (50-60 mmHg for SS, 20-30 mmHg for AS)
2. pH : inhibited at alkaline pH, Exacerbated at acidification
3. Concentration of Hb S
4. Presence of other Haemoglobins ; it was found that presence of other haemoglobins with Hb S decrease the severity of Hb S
5. Polymerization: S > D > C > J = A > F

✓Note: Up to 45% of Hb S in Haemoglobin Electrophoresis indicate sickle cell trait. More than 45% of Hb S in Haemoglobin Electrophoresis indicate sickle cell disease (sickle cell anemia).



# CRISES IN SICKLE CELL DISEASE

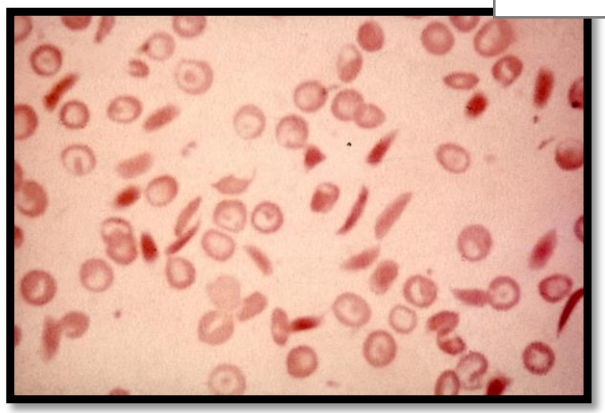
- **Crises in sickle cell disease:**

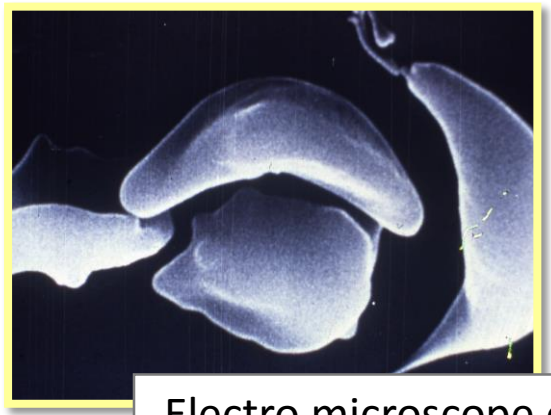
1. Hyperhaemolytic
2. Aregenerative or aplastic
3. Small vessel occlusion

- **Factors precipitating crises in sickle cell disease:**

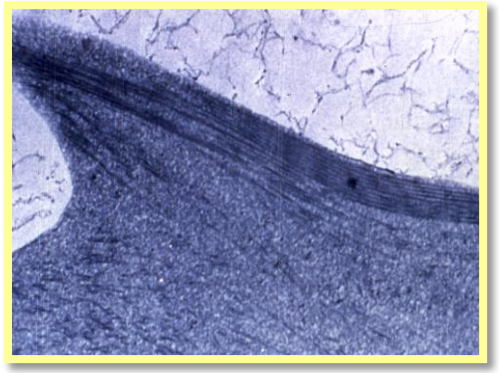
1. INFECTIONS (especially malaria)
2. Pyrexia
3. Exposure to cold
4. Dehydration
5. Pregnancy

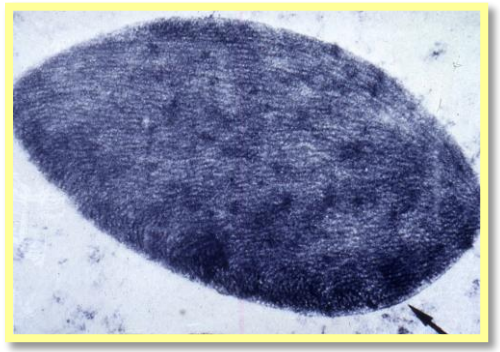
Blood film of sickle cell disease



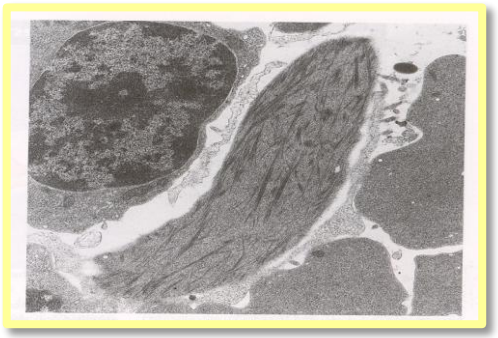


Electro microscope of sickle cell disease





(Tactoid formation )



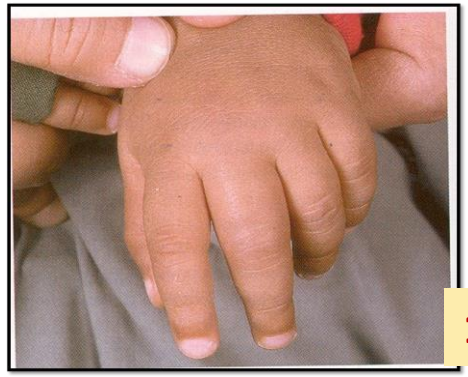
# Clinical manifestations of Sickle Cell disease

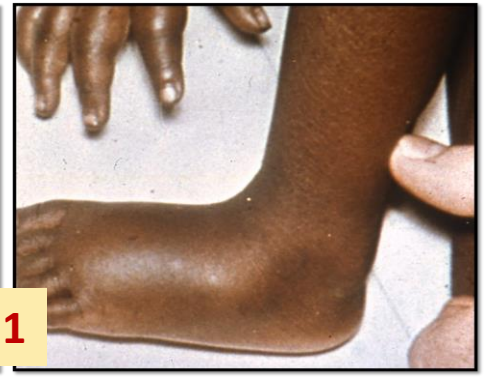
- ✓Hemolytic anemia
- ✓ Tissue infraction

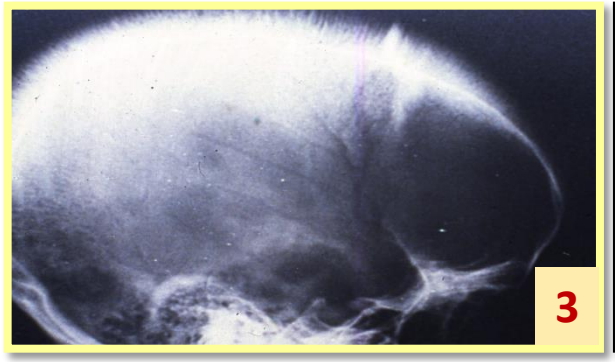
# Clinical manifestations of Sickle Cell Anemia

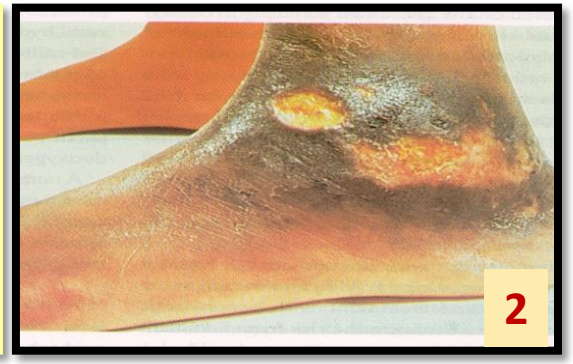
- ✓Pallor (Anemia)
- ✓Jaundice & Dark Urine
- ✓Apathy & Anorexia
- ✓ Hand-Foot Syndrome **1** (Young Children)
- ✓ Splenic sequestration (Young children)
- ✓ Hepatic Sequestration
- ✓Bones and Joints Pain
- ✓Abdominal Pain

- ✓Recurrent Infections & Chest Symptoms (Acute Chest Syndrome)
- ✓Hepato-Splenomegaly
  - I. Early Childhood
  - II. Association with Thalassemia
- ✓CNS Presentations
- ✓Leg Ulceration **2**
- ✓Skeletal Deformity **3**(due to bone marrow expansion -> compensatory mechanism)



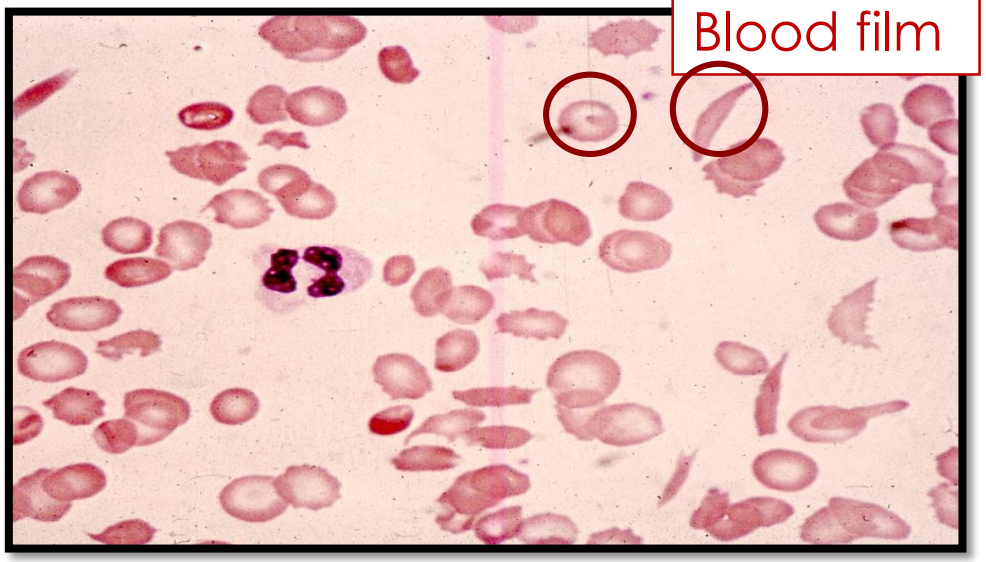





# Laboratory Diagnosis of Sickle Cell Disease

I. CBC
II. Blood Film -> shows sickled cells + target cells
III. Sickle Solubility Test
IV. Hb Electrophoresis -> shows HbS
V. Genetic Study




## Indications for Blood Transfusion in Sickle Cell Anemia

I. Splenic sequestration
II. Hepatic sequestration
III. Aplastic crisis
IV. Overwhelming infections
V. Elective or emergency surgical operation
VI. Painful crisis associated with severe hemolysis
VII. Pregnancy

## Indications for exchange transfusion*

I. Strokes
II. Pulmonary infarcts with infection
III. Pregnancy (Severe persistent painful crisis)
IV. Priapism
V. Preparation for major surgery

*The removal of most of a patient's blood followed by introduction of an equal amount from donors

Q1 A patient's Hemoglobin Electrophoresis showed 45% of Hb S the patient has..... and his symptoms may worsen when.....:

A. Sickle cell anemia, Oxygen tension is 27 mmHg
B. Thalassemia , He bleeds
C. Sickle cell trait, Oxygen tension is 35 mmHg
D. Sickle cell disease, Dehydrated

Q2 What is the mutation in Hb C :

A. β2 6 GLU → VAL
B. β2 6 GLU → LYS
C. β2 26 GLU → LYS
D. β2 6 VAL → LYS

Q3 Premature destruction of RBCs is:

A. Jaundice
B. Polycythemia
C. Hemolytic anemia
D. Thalacemia

Q4 .Infections that can cause hemolytic anemia is:

A. sallmoella
B. HIV
C. E.coli
D. Malaria

Q5 Homozygous sickle cell disease (Hb ss) can usually be differentiated from sickle cell trait (Hb AS) by

A. Sickle cell test.
B. Hb electrophoresis.
C. Osmatic fragility test.
D. Reticulocyte count.

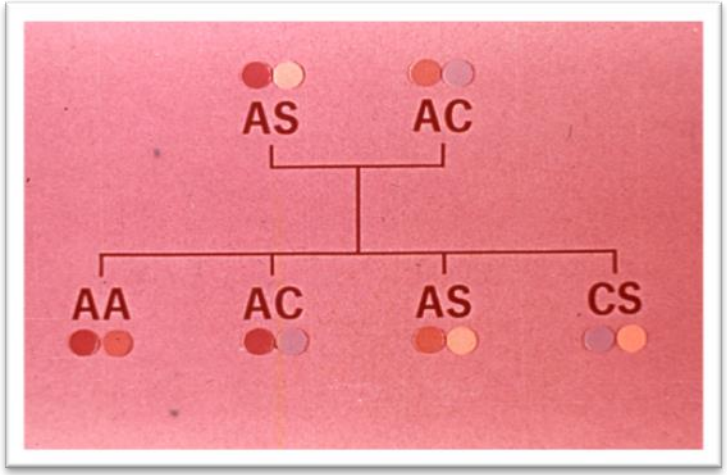
Q6 Intravascular hemolysis is characterized by:

A. Haemoglobinaemia and Haemoglobinuria
B. Increase Serum unconjugated bilirubin
C. Spherocytosis anemia
D. Thalassemia major

1- C
2- B
3-C
4-D
5- B
6- A

## 1-If both parents were carriers of an abnormal allele of different types (one Hb S the other Hb C) what is the outcome if they had children ?

- 25% of their children will be severe sickle cell patients or diseased. Termed as double heterozygous sickle cell disease (sickle cell anemia) (CS)
- 25% of their children will be Normal
- 50% of their children will be carriers of the Abnormal allele.(25% carrier for S 25 % carries for C)




## 2-What is the most characteristic feature of Hb S?

- Tactoid formation

## 3.What are the features of Features of increase red cell breakdown:

- ↑ serum bilirubin is raised (unconjugated and bound to albumin).
- ↑ urine urobilinogen.
- ↑ faecal stercobilinogen.
- Absent serum haptoglobins Protein that bends to Hb.

## 4. A) what are the laboratory tests that can be done to diagnosis Sickle Cell Disease?

- Ans: Blood Film, Hb Electrophoresis

## B)Describe the laboratory findings in those tests?

- Ans: blood film (sickled cells) Hb Electrophoresis (presence of HbS)*

*Note: sickle cell Disease—90% HbS, 8% HbF, 2% HbA, (no HbA)
Sickle cell Trait—55% HbA, 43% HbS, 2% HbA,

# Thank you for checking our work

Now you can check a lecture out :D


Done by:

Reema F. Al-Rasheed

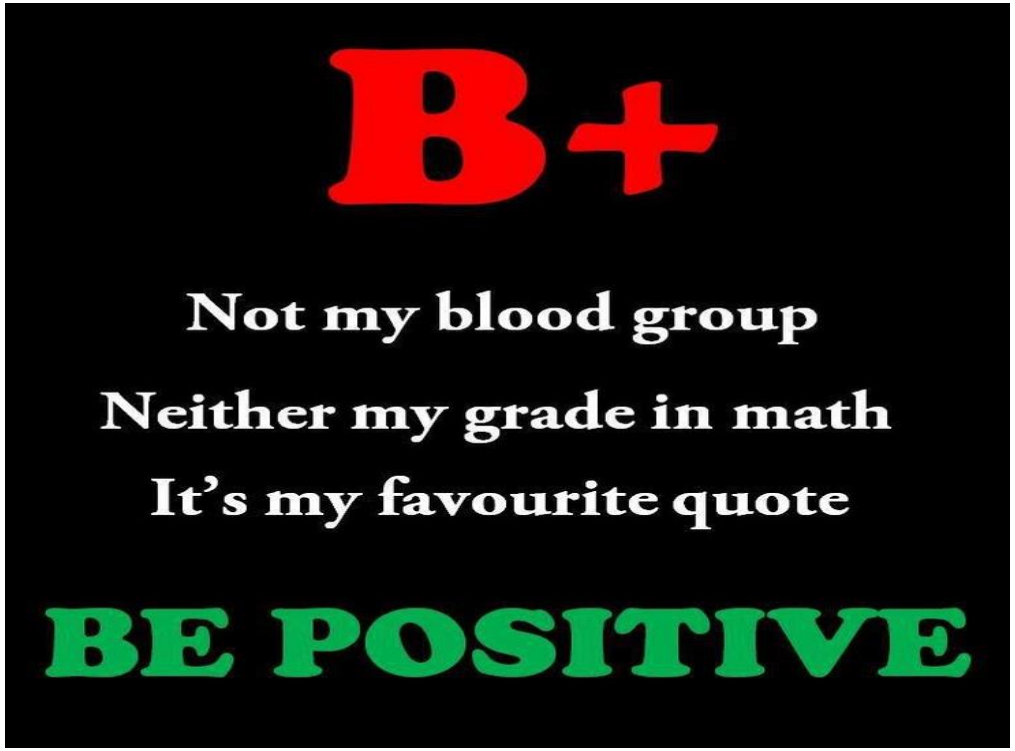
Ashwag R. Almutairi

Ahad Awadh

Reviewed by:

Hadeel B.Alsulami

Abdullah M. Albasha


دعاء بعد المذاكرة :

(اللهم اني أستودعتك ما قرأت وما حفظت وما تعلمت، فرده لي عند حاجتي اليه أنك على كل شيء قدير، وحسبنا الله ونعم الوكيل)