2012

KING SAUD UNIVERSITY
COLLEGE OF MEDICINE
HAEMATOLOGY TEAM
GIT BLOCK

Haematology Block

Haematology: Lecture 3

Haemolysis & Haemoglobinopathies

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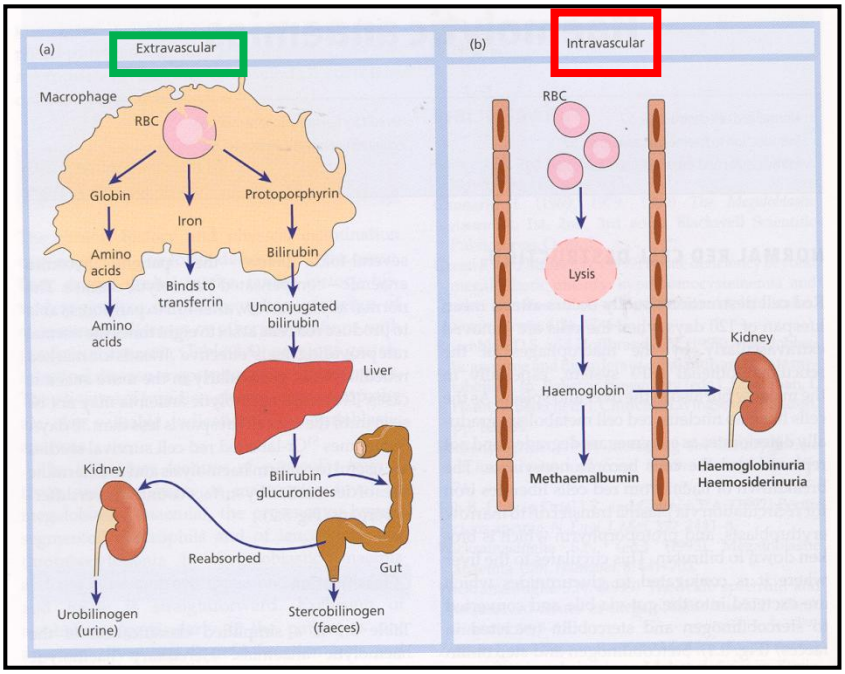
❖ Definitions:

- Hemolysis: Premature destruction of RBCs
- Hemolytic Anemia: Anemias that result from an increase in the rate of RBCs destruction (destruction may occur after only **30 days of life span**)
- Hemoglobinopathies: The presence of abnormal types of Hb in the blood (which lead to hemolysis)



Normal RBCs destruction:

- Normally occurs after a mean life span of **120 days**.
- RBCs are removed extravascularly by the Macrophages of the Reticuloednothelial system, esp. in Bone marrow and in liver and spleen.
- Normally occurs extravascularly (outside vessels)
- Intravascular haemolysis occurs in some pathological disorders.

RBCs destruction:

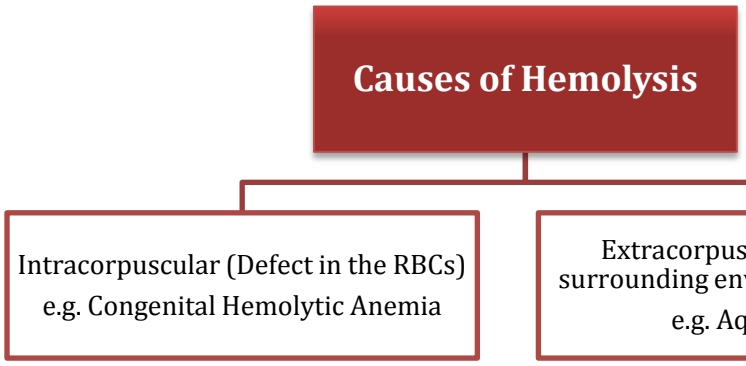
Extravascular: (Normal RBCs destruction)

- Macrophages remove RBCs extravascularly after 120 days.
- The normal breakdown of RBCs in the macrophages will give enzymes: Globin, iron & Protoporphyrin.
- Globin -> Amino Acids
- Iron (it's liberated from the breakdown of the heam of the RBC) -> binds to Transferrin -> for reticulation to Bone marrow erythrobalsts.
- **Protoporphyrin** -> **Bilirubin** -> Liver -> conjugates with glucuronides -> **bile** secretion (in duodenum) -> converted then into **stercobilinogen** >> which is excreted in the faeces & also partly reabsorbed and excreted by the kidney as **Urobilinogen**

Intravascular: (Abnormal RBCs destruction)

- RBCs lysis occurs in the blood vessel.
- Product of lysis: **Methaemalbumin** <<Abnormal product present in the blood.
- It will then go to the Kidney, and will be excreted as hemoglobinurea or hemosiderinurea (the iron in the Hb will be excreted)

❖ Causes of Hemolysis (Hemolytic Anemia)



For your Knowledge:
Paroxysmal nocturnal heamoglobinuria (PNH) is an exception for acquired anemia, for although it's acquired but the RBCs here have an intrinsic defect

❖ Clinical Features of Hemolytic Anemia:

- Pallor, Lethargy
- Jaundice **(Because of high bilirubin)**
- Splenomegaly
- Dark urine (urobilinogen)
- Gall stones (Pigment – bilirubin) (May complicate the condition)
- Bone deformity (In some types of haemolytic anaemia)
- Leg ulcers (around the ankle, esp. in sickle cell anemia)
- Aplastic crises (Precipitated by infection)

❖ Laboratory Findings of Hemolytic Anemia:

1. Features of increased red cell breakdown:
   - ✓ ↑ **serum bilirubin** (**unconjugated** and bound to albumin).
   - ✓ ↑ urobilinogen in urine
   - ✓ ↑ faecal stercobilinogen
   - ✓ Absent serum haptoglobins. (indirect way)
   - ✓ **↑ lactate dehydrogenase (LDH) [Always first to be seen when blood work up for Hemolysis is done].**

- **Haptoglobins** are proteins present in normal plasma. It binds to Hb.
- In hemotyic anemia it will be absent because they will become saturated Hb (Because of the large number of Hbs produced from RBCs lysis) and the Haptoglobin – Hb complex will be removed from plasma by RE cells.

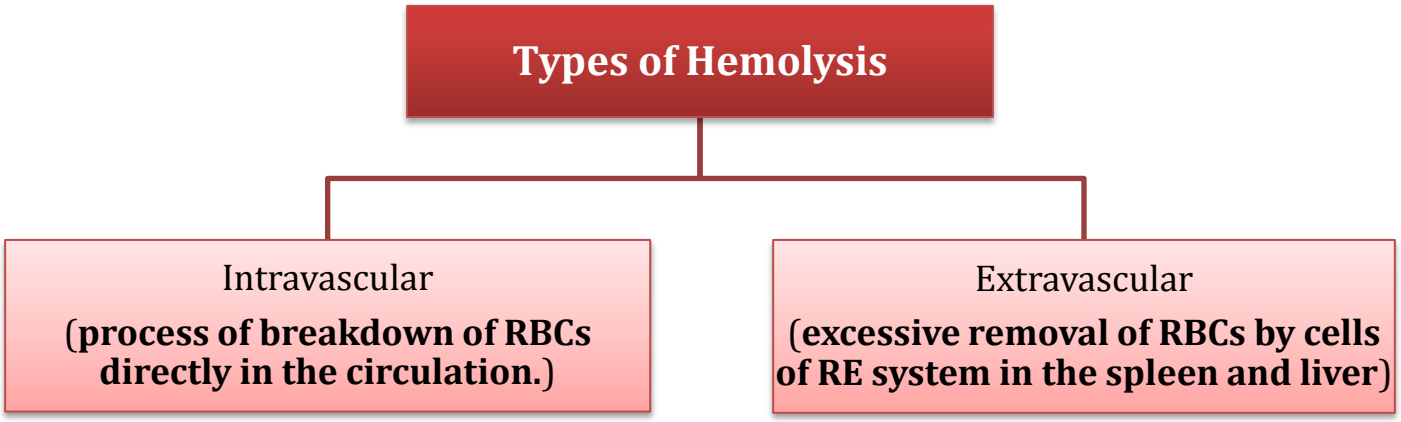
2. Features of increased red cells production:
   - ✓ **Reticulocytosis (If high> normal bone marrow. If it is low> there is a problem in the Bone marrow)**
   - ✓ Bone marrow erythroid hyperplasia

Response of Bone marrow

3. Damaged red cells:
   - ✓ **Morphology** (e.g. microspherocytes, elliptocytes, red cells fragmentation).
   - ✓ Increased osmotic fragility, autohaemolysis ,etc.
   - ✓ Shortened red cell survival (This can be shown by ^{51}Cr labeling with study of the sites of destruction).

^{51}CR (radiochromium) >> substance used in a radioactive lable tecqnique done to demonstrate decreased RBC survival, by seeing how long it can stay in the circulation **(Extra Knowledge)**

❖ Types of Hemolysis:



❖ The Main Laboratory Features Of Intravascular Haemolysis:

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

❖ Causes of intravascular haemolysis :

- Mismatched blood transfusion (usually ABO)
- G6PD deficiency with oxidant stress
- Red cell fragmentation syndromes
- **Some autoimmune haemolytic anaemias** (Very Imp)
- Some drug-and infection-induced haemolytic anaemias
- PNH (rare)
- March haemoglobinuria
- Unstable haemoglobin (Hb Changes after birth)

March Haemoglobinuria is a condition in which a patient will have hypersensitive RBCs, so if he/she walks on a smooth surface, the RBCs will be destructed.

❖ Causes of increased destruction of RBCs:

Biochemical consequences of extravascular haemolysis
- Hyperbilirubinaemia (unconjugated) - Reduced serum haptoglobin
Biochemical consequences of intravascular haemolysis
- Reduced serum haptoglobin - Haemoglobinaemia - Haemoglobinuria - **Methaemalbuminaemia * (Can't carry O_2 normally)** - **Reduced haemopexin levels** *

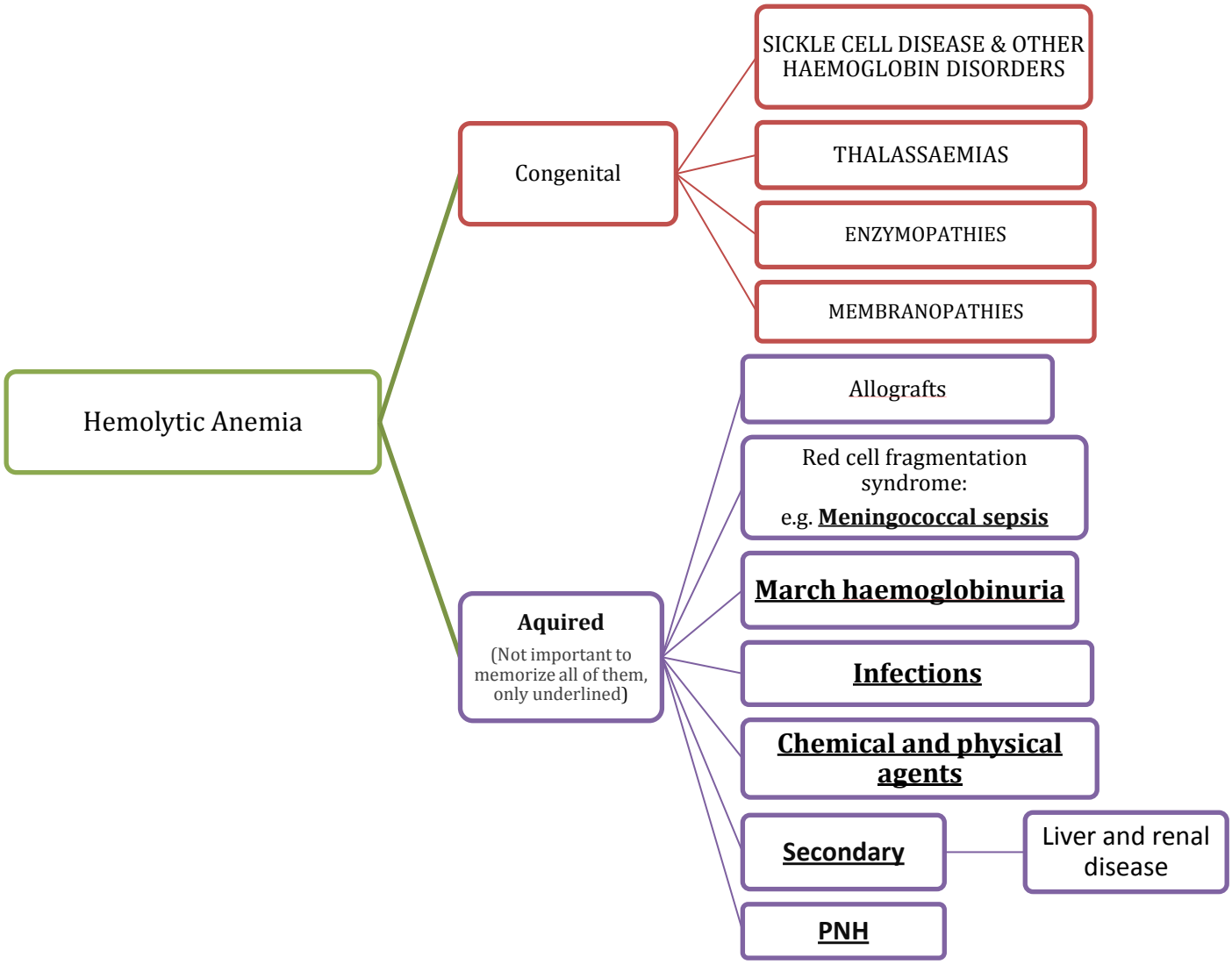
Morphological evidence of damage of red cells
- Microspherocytes, red cell fragments, sickle cells
Reduced red cell life-span

Not Imp

❖ Causes of increased Production of RBCs:

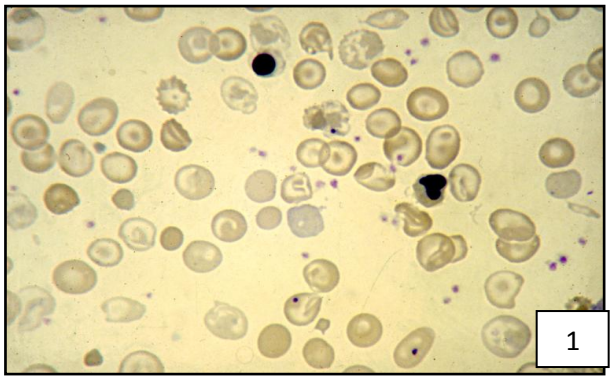
Peripheral blood
- Increased Reticulocytosis and erythroblastqaemia; - Macrocytosis
Bone marrow
- **Erythroid hyperplasia; reduced (we check this by bone marrow biopsy)** - Myeloid/erythroid ratio
Bone
- Changes in the skull and tubular bones

❖ Classification of Hemolytic Anemia:



❖ Different Types of Hemolytic Anemias:

1. Sickle Cell Anemia (Detailed explanation later)
2. Thalassemia Major
3. SICKLE BETA-THALASSAEMIA
4. Spherocytosis
5. Spherocytosis (New Born)
6. ELLIPTOCYTOSIS
7. STOMATOCYTOSIS
8. ACANTHOCYTOSI
9. G6PD DEFICIENCY
10. PK DEFICIENCY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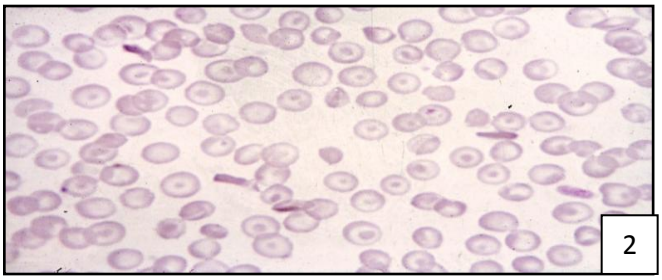
1. **Thalassemia Major** (Explained in lecture 4)
   - Hb Defect Amemia
   - Blood Film Morphology:
     - ✓ Hypochromic microcytic
     - ✓ Target cells
     - ✓ Nucleated RBCs
2. **Sickle Beta – Thalassemia**: (Not very Imp)
   - Blood Film Morphology:
     - ✓ Thalassema characteristics
     - ✓ Sickle shaped RBCs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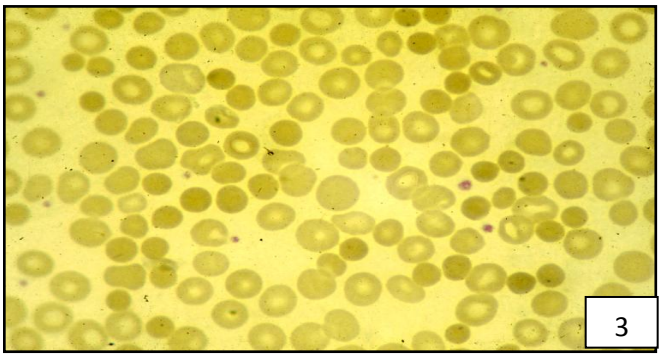
3. **Spherocytosis**:

➢ Membrane defect anemia

➢ RBCs with defected membrane will be: (as shown in blood film)

- ✓ RBCs Increasingly spherical in shape
- ✓ Dark & reduced in size< because of membrane defect.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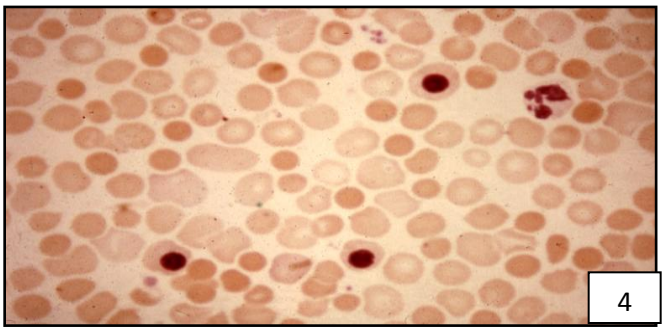
4. **Spherocytosis (New Born)**:

➢ The reason we said new born, is because the blood film shows **Nucleated RBCs**.

➢ Blood Film morphology will show:

- ✓ RBCs Increasingly spherical in shape
- ✓ Dark & reduced in size RBCs
- ✓ Nucleated RBCs

➢ Sometimes it is normal for newborn babies to have dark and small RBCs because of the stress of being born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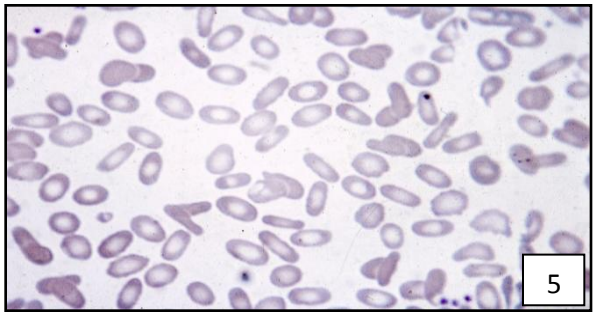
5. **Elliptocytosis**:
- Similar to Spherocytosis in Lab findings & clinical presentation, but different in RBC appearance.
- Membrane Defect Anemia
- Blood Film Morphology:
  - ✓ Large number of cigar shaped RBCs (elliptocytosis)
- Small numbers Elliptocytes may be present in normal blood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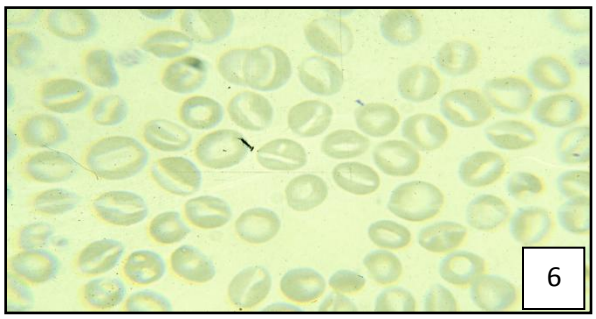
6. **Stomatocytosis:**
- Membrane Defect Anemia
- Blood Film:
  - ✓ Mouth appearance on RBCs
- Associated with Liver disease & Alcoholism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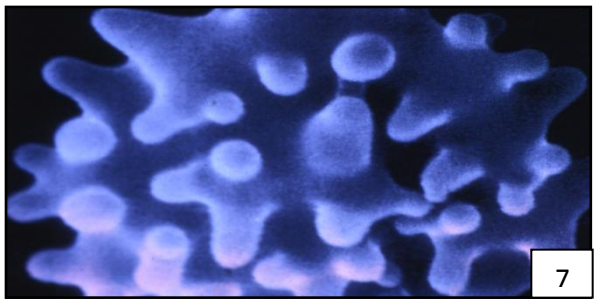
7. **Acanthocytosis:**
- Electron Microscope:
  - ✓ Finger-like projection on RBCs (Acanthocytes)
- Indicates Liver & Renal Diseases.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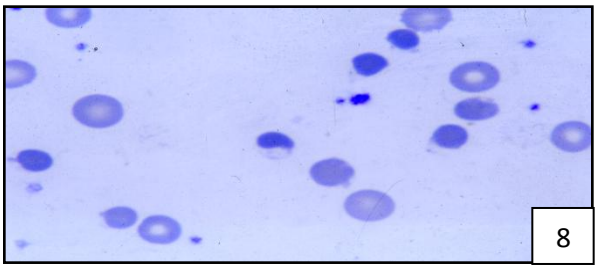
8. **G6PD Deficiency:** (Explained in Lec 1)
- Metabolic deffect Anemia
- Blood film Morphology:
  - ✓ Blistter cells.
  - ✓ Heinz Bodies

➢Agents causing Hemloytic Anemia in G6PD deficiency:
- Infections
- Drugs
- **Fava beans (most important)**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❖ Abnormal haemoglobins (Haemoglobinopathies):

Some Known Haemoglobin Mutants

NAME	SUBSTITUTION
Hb. S	α2 β2 6 GLU → VAL
Hb. C	α2 β2 6 GLU → LYS
Hb. E	α2 β2 26 GLU → LYS
Hb. O ARAB	α2 β2 121 GLU → LYS
Hb. D PUNJAB	α2 β2 121 GLU →GLN
Hb RIYADH	α2 β2 120 LYS → ASN
Hb. HAMMERSMITH	α2 β2 42 PHE → SER
Hb. N. BALTIMORE	α2 β2 95 LYS → GLU
Hb. KORLE-BU	α2 β2 73 ASP → ASN
Hb. K. WOOLWICH	α2 β2 132 LYS → GLN
Hb. K. IBADAN	α2 β2 46 GLY → GLU
Hb. KÖ LN	α2 β2 98 VAL → MET
Hb. J. BALTIMORE	α2 β2 16 GLY → ASP

Important to know Name and Substitution

HbS (6 Glu >> Val)
HbC (6 Glu >> Lys)
HbE (26 Glu >> Lys)

Know location only

Important

EFFECTS OF HAEMOGLOBIN VARIANTS

Variant	Clinical and haematological abnormalities
HbS	Recurrent painful crises (in adults) and chronic haemolytic anaemia; both related to sickling of red cells on deoxygenation*
HbC	Chronic haemolytic anaemia due to reduced red cell deformability on deoxygenation, * deoxygenated HbC is less soluble than deoxygenated HbA.
Hb Köln, Hb Hammersmith	Spontaneous or drug-induced haemolytic anaemia due to instability of the Hb and consequent intracellular precipitation.
HbM Boston, HbM Saskatoon	Cyanosis due to congenital methaemoglobinaemia as a consequence of a substitution near or in the haem pocket.
Hb Chesapeake	Hereditary polycythaemia due to increased O_2 affinity.
Hb Constant Spring, Hb Lepore, HbE	Thalassaemia-like syndrome due to decreased rate of synthesis of normal chains.
Hb Indianapolis	Thalassaemia-like syndrome due to marked instability of Hb

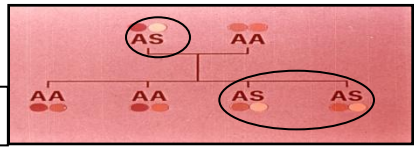
* Only in homozygotes

❖ Sickle Cell Disease:

Normal β-chain	Amino acid	pro	glu	glu
	Base composition	CCT	GAG	GAG
Sickle β-chain	Base composition	CCT	GTG	GAG
	Amino acid	pro	val	glu

Molecular pathology of sickle cell anaemia: Single base change amino acid in the 6th position in the β-globin chain (adenine is replaced by thymine) >> **Glutamic acid is replaced by Valine**.

❖ Sickle Cell Disease hereditary properties :

1


1. If one parent is a carrier (AS) >> 50% of the children will be carriers

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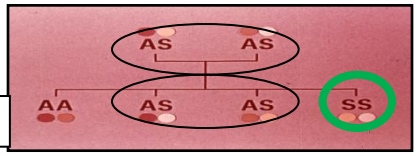

2. If both parents are carriers (AS):
   - 50% of the children will be carriers (AS)
   - 25% of the children will have the disease (SS)

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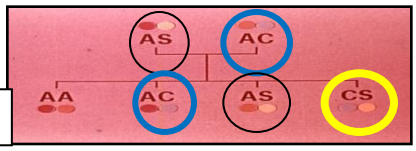

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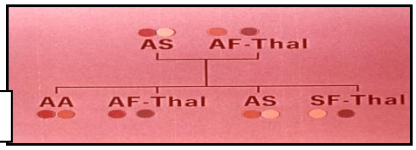


3. If both parents are carriers, but for different abnormal genes (AS & AC):
   - 25% Carriers of first gene (AC)
   - 25% Carriers of second gene (AS)
   - 25% Diseased child (both abnormal Hbs (HbS & HbC) [CS]
4. (Same as 3 but instead of AC it is AF-Thal)

❖ The Sickle Cell Trait:

- Homozygous Sickle Cell Disease (SS) = Sickle Cell Anemia
- Doubly Heterozygous Sickle Cell Disease (2 different diseased genes in 1 patient > the patient will have both diseases)
  - Sickle cell / Hemoglobin C disease (SC)
  - Sickle Cell / Thalassemia

❖ Properties of HbS (Sickle cell Hemoglobin):

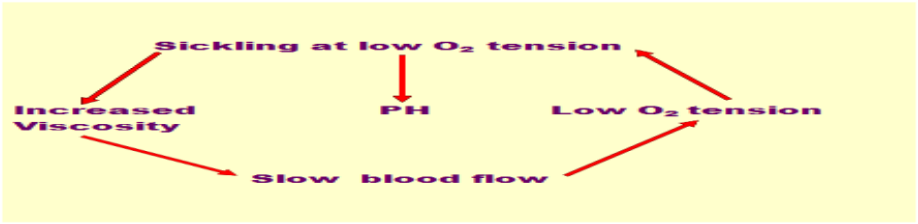
- Decreased Solubility (Crystal-like formation of RBCs)
- Conformational Changes "Tactoid formation"
  - ➔ Sickled Cells
  - ➔ Irreversibly sickled cells.
- Increased Mechanical Fragility -> Hemolysis
- Increased Viscosity -> Organ Infarction.

❖ Factors Affecting Sickling:

- O_2 tension: 50 – 60 mmHg for SS
  20 – 30 mmHg for AS
- pH: Inhibited at alkaline pH exacerbated by acidification.
- Concentration of Hb
- Presence of other abnormal Hbs

SS: Hemolysis will occur as soon O_2 is reduced to 50-60 mmHg

AS: Hemolysis will not occur & RBCs will survive unless O_2 was severely reduced to (20-30) mmHg

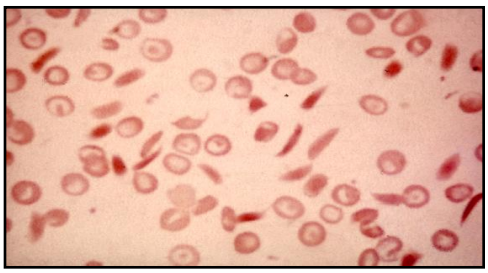


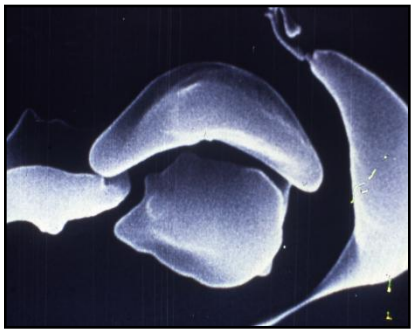
❖ Factors Precipitating Crises:

- Infections (Especially Malaria)
- Pyrexia
- Exposure to cold.
- Dehydration
- Pregnancy

❖ Lab Findings of Sickle Cell Anemia:

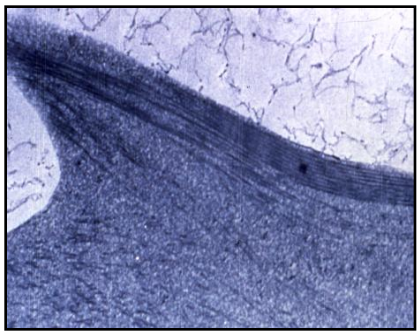
- Blood Film Morphology:
  - ✓ Hypochromic Microcytic
  - ✓ Target Cells
  - ✓ Sickle shaped RBCs (Banana shaped)
- To say anemia or disease, the percentage of sickled cells should be high, otherwise, it might be a trait.





Electron Microscope:

- Sickle Shaped RBCS
- Long Fibers

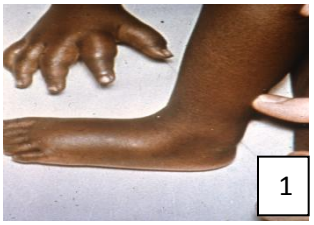


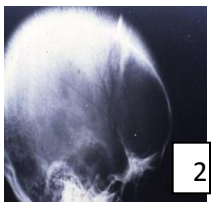
❖ Crises in Sickle cell disease:

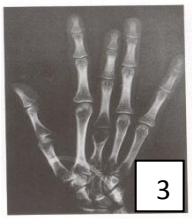
- HyperHemoylsis
- Agenerative or Aplastic **(Shut down of bone marrow, due to strong infection)**
- Small Vessel Occlusion **(due to infarction)**

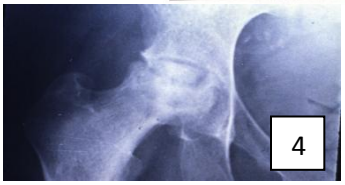
❖ Clinical Manifestation of Sickle Cell Disease:

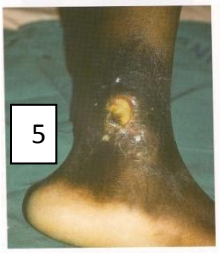
- Hemolytic Anemia
- Tissue Infarction
- **Pallor** (Anaemia)
- **Jaundice & Dark Urine** (due to Increased Billirubin)
- Apathy & Anorexia
- **Hand-Foot Syndrome** (Young Children) [due to infarction]
- Splenic sequestration (Young Children)
- Hepatic Sequestration
- Bones, Joints Pain (Due to infarction)
- Abdominal Pain
- Recurrent Infections & Chest Symptoms (Acute Chest Syndrome)
- Hepato-Splenomegaly
  - ➔ (Early Childhood)
  - ➔ (Association with Thalassaemias)
- CNS Presentations (Due to infarction)
- **Leg Ulceration** (Infection)
- Skeletal Deformity

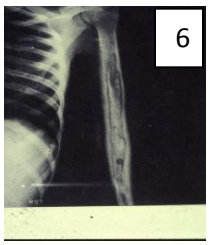


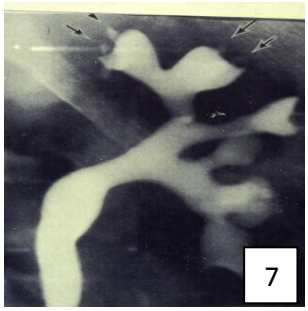








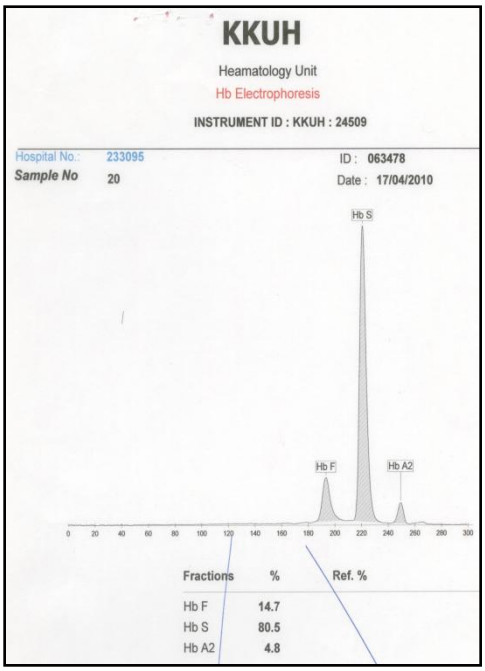




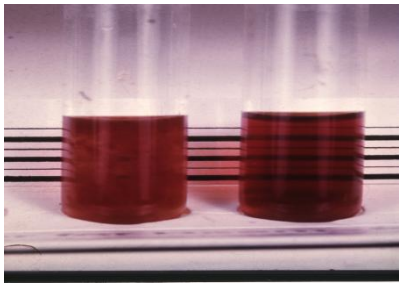
1. Hand – Foot Syndrome; Dactylitis (Painful Swelling of fingers) & Absence of normal features of hand and foot
2. Hair on End appearance (X-ray) [Due to Bone Marrow expansion] >Differential Diagnosis: Thalassemia , Sickle cell anemia
3. Hand-foot syndrome (X-ray shows shortening of the fingers, Mostly the middle finger)
4. Flat head of femor < Due to avascular necrosis (X-ray) [Also seen by MRI]
5. Leg ulcer
6. Osteomalaytis >> infectious agent = Salmonella
7. Papillary Necrosis of Kidny

❖ Laboratory diagnosis of Sickle Cell Disease:

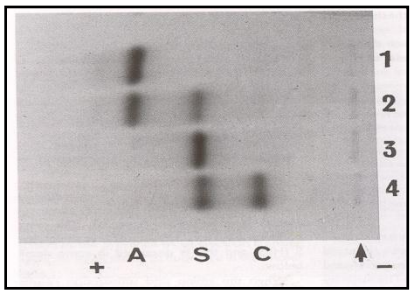
- CBC
- Blood Film
- Sickle Solubility Test
- **Hb Electrophoresis** (2 types)
- Genetic Study



HPLC (Hb Electophoresis – type 2):
<< In the picture:
HbF = 14.7%
HbS = 80.5%
HbA2 = 4.8%
Patient has Sickle cell anemia & beta-thalassemia



Sickle Solubility test



Gel Electrophersis:
1= Normal (A)
2= Carrier (AS)
3= Sickle cell anemia (S)
4= Diseases (SC)

❖ Indications for blood transfusion in Sickle Cell Disease:

- Splenic sequestration
- Hepatic sequestration
- Aplastic crisis
- Overwhelming infections
- Elective or emergency surgical operation
- Severe painful crisis associated with severe haemolysis
- Pregnancy

❖ Indications for exchange transfusion in Sickle Cell Disease:

- Strokes
- Pulmonary infarcts with infection
- Pregnancy (Severe persistent painful crisis)
- Priapism
- Preparation for major surgery

Pt with sickle cell anemia who is going into surgery must be prepared by giving O2 & fresh blood

Summary

- Normally RBCs destruction occurs after a mean life span of **120 days**
- RBCs destruction occurs extravascular (normal at a certain limit) & intravascular (abnormal)
- **Methaemalbumin**: Abnormal product present in the blood as a result of intravascular (abnormal) RBC lysis.
- **Laboratory Findings of Hemolytic Anemia**: **↑ serum bilirubin**, Absent serum haptoglobins, **↑ lactate dehydrogenase (LDH)** & **Reticulocytosis**.
- **The Main Laboratory Features Of Intravascular Haemolysis**: Haemoglobinaemia and haemoglobinuria, & Hemosidrinurea.
- **Causes of intravascular haemolysis : autoimmune** (most important), & others.
- **Causes of increased destruction of RBCs** :
- **Methaemalbuminaemia**
- **Reduced haemopexin levels**
- **Causes of increased Production of RBCs: Erythroid hyperplasia; reduced**.
- **Spherocytosis**: Dark & reduced in size RBCs
- **Spherocytosis (New Born)**: same features as Spherocytosis, in addition to the appearance of nucleated RBCs.
- **Elliptocytosis**: Large number of cigar shaped RBCs (elliptocytosis)
- **Stomatocytosis**: Mouth appearance on RBCs, Associated with Liver disease & Alcoholism
- **Acanthocytosis**: Finger-like projection on RBCs, Indicates Liver & Renal Diseases
- **G6PD Deficiency** hemolytic anemia is caused by:
  - Infections
  - Drugs
  - **Fava beans (most important)**
- Some Abnormal Hemoglobins:
  - **HbS (6 Glu >> Val)**
  - **HbC (6 Glu >> Lys)**
  - **HbE (26 Glu >> Lys)**
- Sickle cell disease happens due to replacement of **Glutamic acid by Valine at the 6th position.**
- **Properties of HbS (Sickle cell Hemoglobin):** Decreased Solubility, irreversible sickling of RBCs,increased mechanical fragility & increased viscosity.
- **Factors Affecting Sickling:** O_2 tension, pH, Concentration of Hb & Presence of other abnormal Hbs
- **Factors Precipitating Crises**: infections (esp. malaria), pyrexia, exposure to cold, dehydration & pregnancy.
- **Crises in Sickle cell disease:** HyperHemoylsis, Agenerative or Aplastic, Small Vessel Occlusion.
- **Clinical Manifestation of Sickle Cell Disease**: Pallor, Jaundice, Hand-foot syndrome, leg ulceration, etc.
- **Lab Diagnosis of sickle cell disease**: CBC, **Hb Electrophoresis**, Blood Film, Sickle solubility test, Genetic study.