431 Teams
medical students

Haematology 431 Team

4/4

HAEMOLYSIS & HAEMOGLOBINOPATHIES

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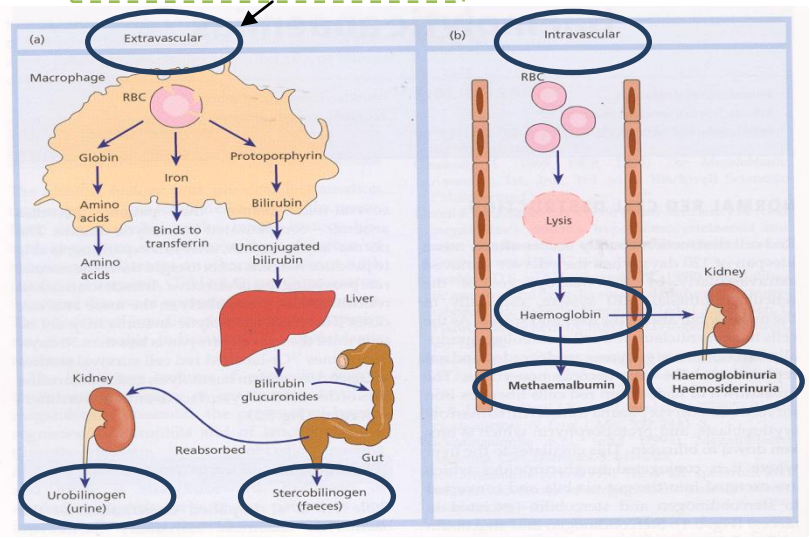
Green Words or frame is the team notes

important

Hemolysis:

Whether in the liver spleen or bone (Normal RBC destruction)



- **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 (extracorpuscular) as in acquired Anaemia.**

Clinical Features of Hemolysis:

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

Laboratory Features of Hemolysis :

1. Features of increased red cell breakdown.

a. **↑ serum bilirubin is raised (unconjugated and bound to albumin).**
b. **↑ urine urobilinogen.**
c. **↑ faecal stercobilinogen.**
d. **Absent serum haptoglobins.**
e. **↑ lactate dehydrogenase (LDH)**

2. Features of increased red cells production.

a. **Reticulocytosis**
b. **Bone marrow erythroid hyperplasia.**

3. Damaged red cells.

c. **Morphology (e.g. microspherocytes, elliptocytes, red cells fragmentation).**
d. **Increased osmotic fragility, autohaemolysis, etc).**
e. **Shortened red cell survival (This can be shown by 51Cr labeling with study of the sites of destruction.**

Normally the age of the RBCs is 120 days but it become around 30 to 60 days in hemolysis

Intravascular and extravascular haemolysis:

a. **Intravascular haemolysis, the process of breakdown of red cells directly in the circulation.**
b. **Extravascular haemolysis excessive removal of red cells by cells of RE system in the spleen and liver.**

The main laboratory features of intravascular haemolysis are as follows:

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**
- **Some drug-and infection-induced haemolytic anaemias**
- **Proxysmal nocturnal haemoglobinuria** >> type of anemia
- **March haemoglobinuria**
- **Unstable haemoglobin**

>> Types of hemoglobin disorder

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. (From 430 team)

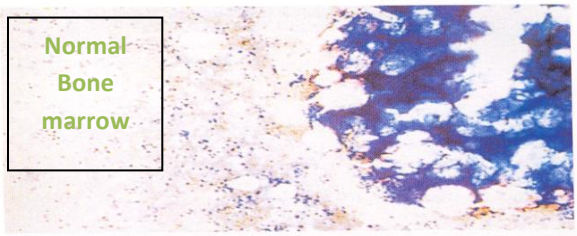
Increased destruction:

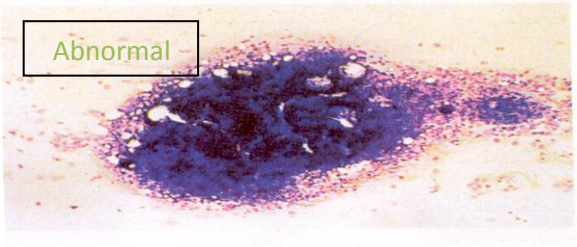
- **Biochemical consequences of extravascular haemolysis**
  - Hyperbilirubinaemia (unconjugated) >> Increased destruction
  - Reduced serum haptoglobin
- **Biochemical consequences of intravascular haemolysis**
  - Reduced serum haptoglobin
  - Haemoglobinaemia
  - Haemoglobinuria
  - Methaemalbuminaemia *
  - Reduced haemopexin levels *
- **Morphological evidence of damage of red cells**
  - Microspherocytes, red cell fragments, sickle cells
- **Reduced red cell life-span**
  - * Now rarely used in investigating a patient.

Increased production:

The presence of the erythroid precursors

- **Peripheral blood**
  - Reticulocytosis and erythroblastqaemia;
  - Macrocytosis
- **Bone marrow**
  - Erythroid hyperplasia; reduced
  - Myeloid/erythroid ratio
- **Bone**





Haemolytic anaemia:

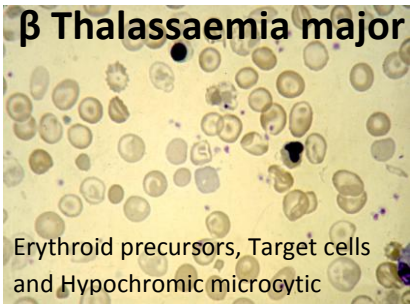
A. **Congenital**
   - Sickle cell disease & other haemoglobin disorders
   - thalassaemias
   - enzymopathies >> Eg. G6PD deficiency
   - membranopathies >> Deficiencies in the proteins of the RBCs membranes

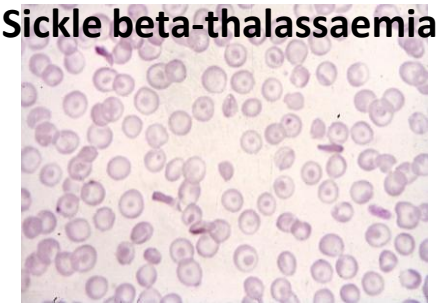
B. **Aquired**

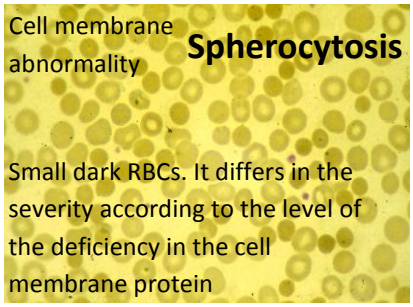
Classification of Haemolytic Anaemias

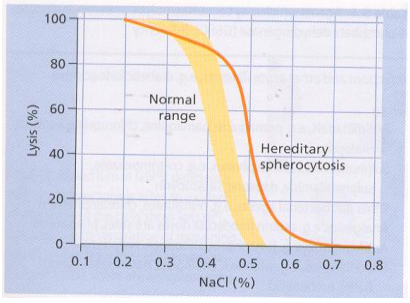
Hereditary	Acquired
Haemoglobin	**Allografts, especially marrow transplantation**
Abnormal (Hb S, Hb C, unstable)	**drug associated**
Thalassaemia	Red cell fragmentation syndrome
Membranopathy	**Arterial grafts, cardiac valves**
Enzymopathy	**Microangiopathic**
	Thrombotic thrombocytopenic purpura Haemolytic uraemic syndrome
	Meningococcal sepsis
	Pre-eclampsia
	Disseminated intravascular coagulation
	March haemoglobinuria
	Infections
	Malaria, clostridia
	Chemical and physical agents
	Especially drugs, inductrial/domestic substances, burns
	Secondary
	Liver and renal disease
	Paroxysmal nocturnal haemoglobinuria

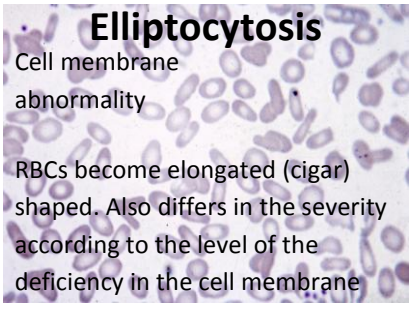
Also autoimmune

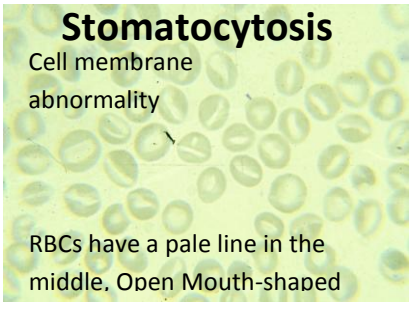


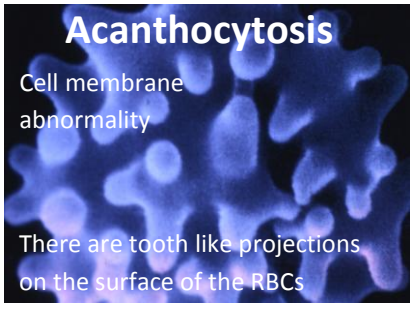


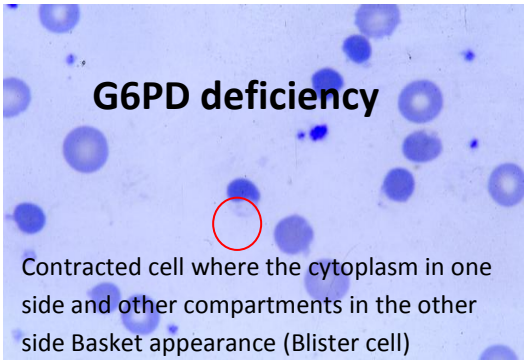


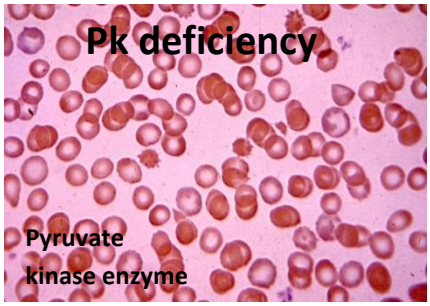












Agents which may cause haemolytic anaemia in glucose-6-phosphate dehydrogenase (G6PD) deficiency:

Infections and other acute illness, e.g. diabetic ketoacidosis

Drugs

- **Antimalarials, e.g. primaquine, pamaquine, chloroquine, Fansidar, maloprim**
- **Sulphonamides and sulphone, e.g. co-trimoxazole, sulphanilamide, dapsone, salazopyrin**
- **Other antibacterial agents, e.g. nitrofurans, chloramphenicol.**
- **Analgesics, e.g. aspirin (moderate doses are safe), phenacetin**
- **Antihelminths, e.g. β–napthol, stibophen, nitrodazole**
- **Miscellaneous, e.g. vitamin K analogues, naphthalene (moth balls), probenecid**

Fava beans (Possible other vegetable)

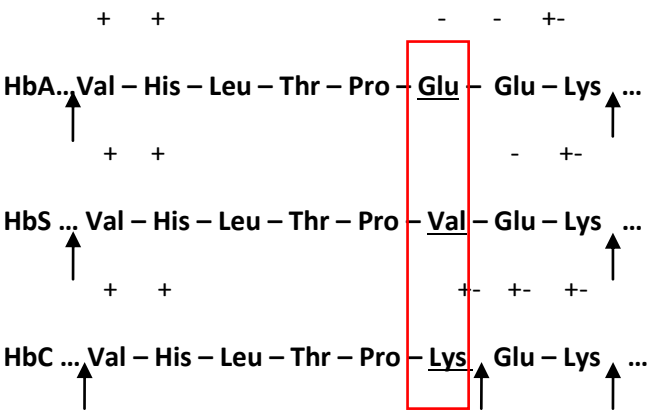
Abnormal haemoglobins (Haemoglobinopathies)

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

The doctor said only the first five are important. And she mentioned the location of the next five only.

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
Hb. G. PHILADELPHIA	α2 68 ASN → LYS β2
Hb. ZAMBIA	α2 60 LYS → ASN β2
Hb. G. CHINESE	α2 30 GLU → GLN β2
Hb. HASHARON	α2 47 ASP → HIS β2
Hb. J. TONGARIKI	α2 115 ALA → ASP β2
Hb. J. OXFORD	α2 15 GLY → ASP β2
Hb. NORFOLK	α2 57 GLY → ASP β2

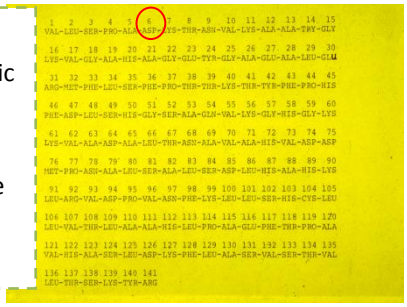


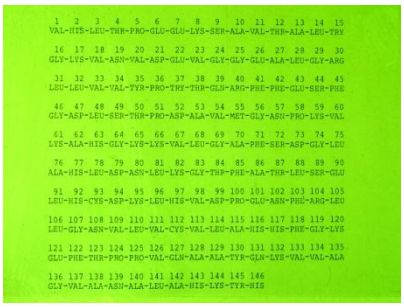
You should only know the change that happens to the 6th amino acid and the result Hb of that change

Amino acid sequences of the peptides 4 in haemoglobins A, S and C.

SICKLE CELL DISEASE:

Defect in the glutamic acid in β globin causes the sickle cell anemia





How to differentiate between the trait and the disease:

Trait is the mild >> Hb s 45% or less

The disease >> Hb s above 45

From hemoglobin electrophoresis

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

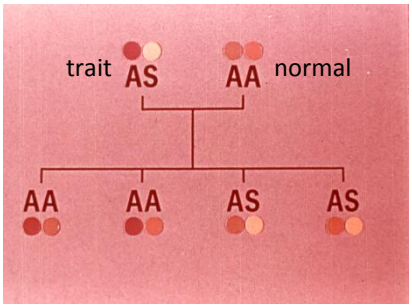
Molecular pathology of sickle cell anaemia. There is a single base change in the DNA coding for the amino acid in the sixth position in the β-globin chain (adenine is replaced by thymine). This leads to an amino acid change from glutamic acid to valine. A, adenine; C, cytosine; G, guanine; glu, glutamic acid; pro, proline; T, thymine; val, valine.

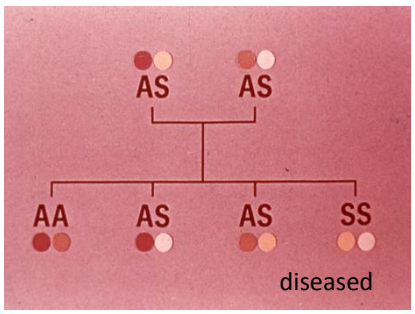
1910 1st published report of sickle cell anaemia (Herrick)

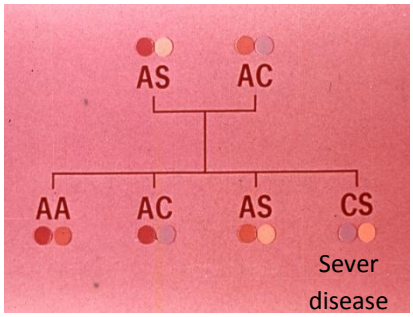
1949 Pauling et al : chemical difference between HbA and HbS

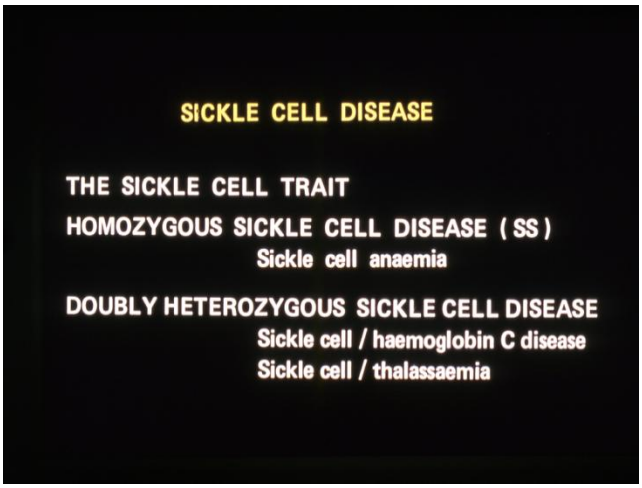
1956 Ingram: Fingerprinting

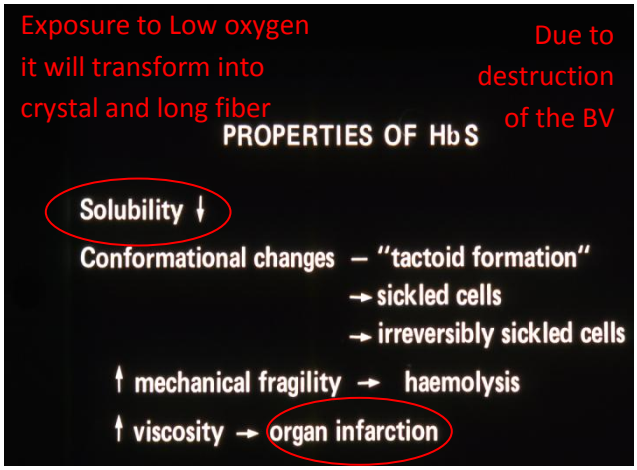
βglu → val

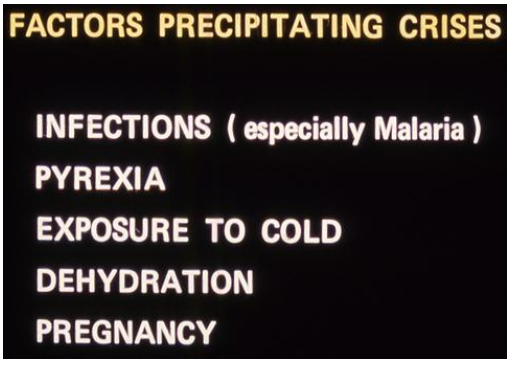


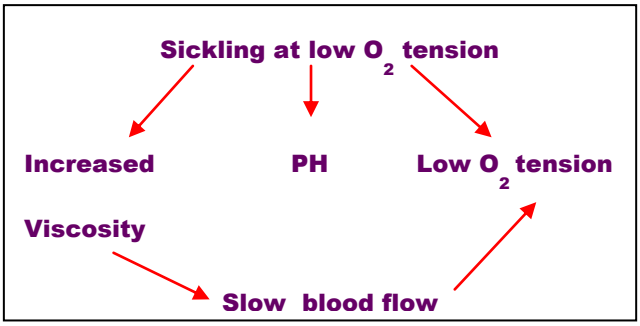












Clinical Manifestations in Sickle Anaemia:

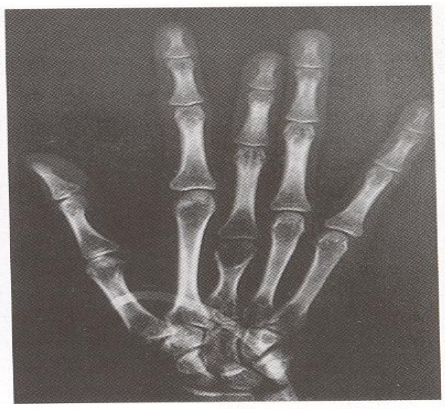
- Pallor (Anaemia)
- Jaundice & Dark Urine
- Apathy & Anorexia
- Hand-Foot Syndrome (Young Children)
- Splenic sequestration (Young Children)Hepatic Sequestration
- Bones, Joints Pain >> very sever
- Abdominal Pain

clinical manifestations of sickle cell disease:

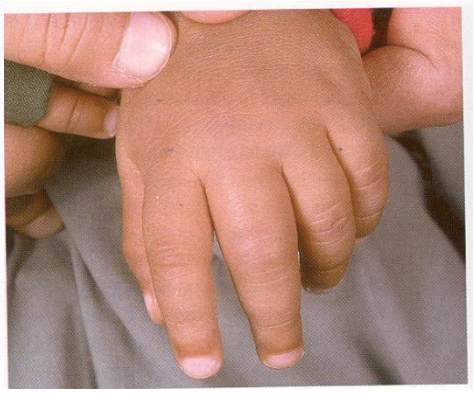
-Haemolytic anemia
-tissue infarction

Clinical Manifestations in Sickle Anaemia:

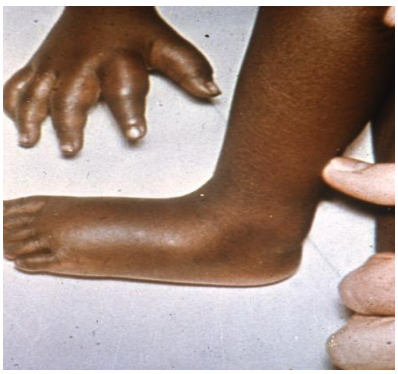
- Recurrent Infections & Chest Symptoms (Acute Chest Syndrome)
- Hepato-Splenomegaly
  - → (Early Childhood)
  - → (Association with Thalassaemias)
- CNS Presentations
- Leg Ulceration >> very common
- Skeletal Deformity



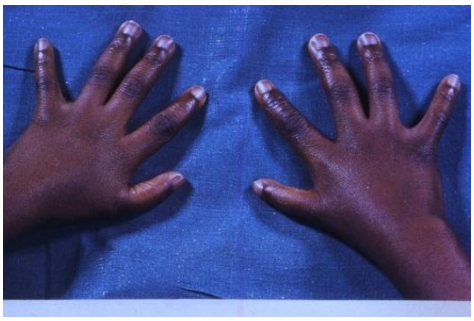
There is marked shortening of the right middle finger because of dactylitis in childhood affecting the growth of the epiphysis.



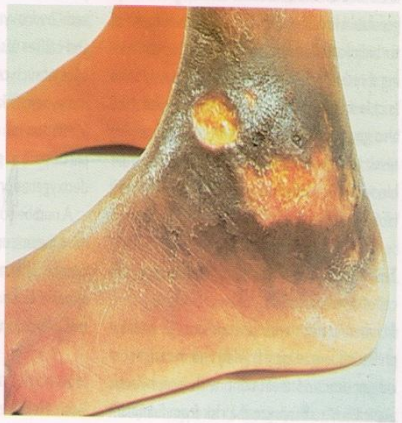
painful swollen fingers (dactylitis) in a child



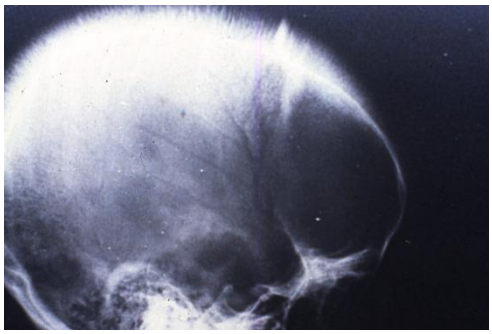
Foot and hand syndrome >> foot and hand swollen and sever pain



The fingers are at the same length



Leg ulcer very common in sickle cell anemia



Hair on end appearance due to Expansion of the bone marrow

Laboratory Diagnosis:

- **CBC** >> Low Hb and hypochromic microcytic anemia Target cell
- **Blood Film** >> sickle cells
- **Sickle Solubility Test**
- **Hb Electrophoresis** >> It is the most important and confirmative test for the sickle cell anemia
- **Genetic Study**

We add deoxygenated substance to the blood sample:

- Normally the RBCs handle it

- But in the sickle cell anemia there will be breaking down of the RBCs and produce turbid appearance

The sickle solubility test

Treatment of Sickle Cell Anaemia:

1. **Management of Painful Crisis (Analgestic)**
   - Paracetamol (Acaetaminophen)
   - Aspirin
   - Codeine
   - Other analgestic which is recommended is piracetam – 300 mg/kg/day in 3 divided doses for 3 days. I.V. Each dose is mixed with 300ml of dextrose 5% every 8 hrs.
2. **Hydration – intravenous fluid and oral fluids**
   - I.V. Children - 5% Dextrose + 0.225 Normal Saline
   - Adult - 5% Dextrose + 0.450 Normal Saline
3. **Bacteriological Investigations.**
4. **Treatment of Infection.**
5. **Patient / or family reassurance.**
6. **Oxygen therapy.** → Because when the oxygen decreases crisis develop more
7. **Blood transfusion.**
8. **Special treatment for certain crises & certain complications.**
9. **Anti-Sickling agents.**
   - 1 – Desamino-8-D- Arginine Vasopressin (DDAVP)
   - Oral Zinc Therapy
   - Vitamin E

Drugs which can raise the level of HbF:

- **Hydroxy Urea, Erythropoietin.**
- **Bone marrow transplantation & gene therapy**

Sickle Cell Anaemia Steady Management:

1. **Age of 4 – 6 Months**
   - **Routine Haematology**
   - **Hb Electrophoresis**
   - Regular **Check-up** (2-4 months)
   - Folic Acid Supplementation
   - **Start Penicillin Prophylaxis**
   - **Screen Parents**
   - Genetic Counselling
   - Parent and Family Education
2. **Age of 1 Year**
   - **Haemophilus influenzae vaccination**, meningococcal vacination (and continue every 2 years)
   - Regular check-up
3. **Age of 2 – 4 Years**
   - Start **pneumococcal vaccination** (and continue every 2 years)
4. **Age of 5 - 6 Years**
   - Abdominal ultrasound (Baseline)
5. **Age of 7 - 10 Years**
   - Abdominal ultrasound and later 6 monthly (Gall bladder and Gall stones)
   - Eye examination (Opthalmologist) yearly
6. **Above 10 Years**
   - **Continued patient education**
   - Prevention of complication
   - Early recognition and treatment of complications
   - Regular **check-up** (4-6 months)

- Blood count with reticulocyte count
- Routine biochemistry
- **Organ function tests** >> liver and kidneys
- Urine analysis

Indications for Blood Transfusion in Sickle Cell Anaemia:

- Splenic sequestration
- Hepatic sequestration
- Aplastic crisis
- Overwhelming infections
- Elective or emergency surgical operation >> e.g. for the removal of gallstones
- Severe painful crisis associated with severe haemolysis
- Pregnancy if they are at labor

Indications for exchange transfusion:

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

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**	

→ Looks like the sickle cell anemia

Abnormal Haemoglobin Variants:

Hb C:-

* Is due to replacement of glutamic acid in position 6 of the beta chain by lysine ($\alpha_2\beta_2$ 6-GLU→ LYS).

* About 7-22% of people of West Africa ar hetrozygotes especially Nigeria and North Ghana

* Homozygotes are rare and have mild to moderate hemolytic anaemia with many thick target RBCs in the blood film and mild to moderate splenomegaly.

* The chronic hemolytic anaemia is due to reduced red cell deformability on deoxygenation.

Deoxygenated HbC is less soluble than deoxygenated HbA.

* Double heterozygotes with sickle Hb S/C give moderate to sever anaemia with symptoms of sickle cell disease.

Hb E:

* ($\alpha_2\beta_2$ 26 GLU → LYS) is one of the most common beta-chain variants.

* It is very prevalent in South East Asia (50%) of the population are heterozygotes.

* Patients who are homozygous generally have mild haemolytic anaemia, microcytic hypochromic red cells and mild enlargement of the spleen.

* Carriers are symptomless unless they have combined other mutations such as the one for alpha thalassemia, or beta-thalassemia trait.

High Oxygen affinity haemoglobins

Hb Chesapeake:

* (α_2-92 ARG → LEU β_2).

* Carriers are without clinical symptoms.

* Homozygous of erythrocytosis (polychemia) due to increased O_2 affinity.

* The patients have no splenomegaly. (except for patients with concomitant β-thalassemia).

* They have normal WBC, and normal platelets.

* High Hb, High RBCs count and high haematocrit. (HCT).

Hb D Punjab

($\alpha_2\beta_2$-121 GLU → GLN)

Prevalent in Indian and Pakistani in every 100 persons about 1 trait (1% of the population).

Trait are usually health.

Homozygous D/D have mild to moderate anaemia.

Combined double heterozygotes Hb S/D can give rise to moderate to a severe anaemia and symptoms of sickle cell disease.

Hb O Arab

($\alpha_2\beta_2$-121 GLU → LYS)

Heterozygotes are not symptomatic.

Double heterozygous with sickle S/O are clinically severe.

Hb O- Arab enhance the polymerization of HbS.

Unstable Haemoglobins

Hb koln ($\alpha_2\beta_2$-98 VAL → MET)

Hb Hammersmith ($\alpha_2\beta_2$ 42 PHE → SER)

Hb Hasharon (α_2-47 ASP → HIS β_2).

These abnormal haemoglobin cause haemolysis in the newborn (congenital non-spherocytic haemolytic anaemia).

Heinz body hemolytic anaemia with sensitivity to oxidant drugs, such as sulfonamides.

Reticulocytosis out of proportion to the level of Hb.

Increased formation of methemoglobin.

Spontaneous or drug induced haemolytic anaemia due to instability of the haemoglobin and consequent intracellular precipitation.

Thalassaemia – like peripheral blood picture.

Clinically: The patient have anemia, jaundice, splenomegaly / hepatomegaly and gall stones.

Low oxygen affinity haemoglobins

More than 50 variants with reduced oxygen affinity have been identified.

Hb kansas ($\alpha_2\beta_2$102 ASN → THR)

Hb Aukland ($\alpha_2\beta_2$ 25 GLY → ASP)

Rare as homozygotes.

Patients have anaemia and congenital cynosis due to reduced oxygen affinity.

Congenital methaemoglobinaemia

Hb M Boston (α_2 58 HIS → TYR - β_2)

Hb M Saskatoon (α_2-β_2-63 HIS → TYR)

Hb M Hyde park ($\alpha_2\beta_2$ 92 HIS → TYR)

Hb M IWATE ($\alpha_2$87 HIS → TYR-β_2)

Cynosis in homozygotes due to congenital methaemoglobinaemia as a consequences of substitution of amonoacids near or in haem pocket.

Hb iIndianapolis

(α2-β2112 CYS – ARG)

Is a rare and slightly unstable beta-globin variant.

Carriers are clinically normal with only mild reticulocytosis.

Homozygons have haemolytic anaemia and renal failure in severe cases.

Thalassaemia-like syndrome due to marked instability of the Hb.

Notes

1- Leg ulcers happen in two types: sickle cell anemia and congenital spherocytosis.

2-There is increase in LDH because this enzyme is rich in the RBC, so breaking down of the RBCs will lead to increase of this enzyme (which is a good sign of hemolysis)

3- The most common cause of intravascular hemolysis is mismatch blood transfusion.

4- Stomatocytosis: Open mouth Like cell. In patient with RH null disease, these patients have no Rh group in their blood (Hereditary disease)

5- If the glutamic acids in beta chain were changed by different amino acids we will call it single point mutation. (There will be abnormal haemoglobin)

6- Sickle cell patient can develop infarction of the brain, lung, kidney, liver and by the time the spleen will be very small from the infarction, and it's called autosplenectomy (because of increase viscosity)

7- If there is no spleen, the patient will be more susceptible to infections especially pnumococcal infection (capsulated microorganism)

8- We have to give these patients vaccines and prophylactic antibiotic for the whole life.

Questions

1-What is the main cause of intravascular hemolysis?

A- Mismatched blood transfusion.
B- Liver or renal disease.
C- Hypersensitive RBCs.
D- Infections.

2-Where can we find open mouth cell appearance?

A- Elliptocytosis.
B- Stomatocytosis.
C- Spherocytosis
D- Acanthocytosis.

3-Elliptocytosis:

A- Ball-like cells.
B- Cigar-like cells.
C- Open-mouth cells.

1-A 2-B 3-B