Reviewed by Mohammed AlEssa Abdulaziz AlFuhayd Lama AlMutairi

haematology Team 443

MED 443 King Saud University

Haemoglobinopathy

GNT Block

COLOR INDEX:

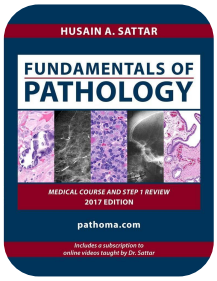
- Main text
- Dr. Notes
- Male's text
- Femal's text
- Important
- Extra

Editing file:

Objectives

- To understand/review the normal structure and function of hemoglobin and how the globin components of hemoglobin change during development and postnatally.
- To understand the mechanisms by which the thalassaemias arise
- To appreciate the contribution of haemolysis and ineffective erythropoiesis to the pathophysiology of thalassaemia
- To understand the pathophysiology of sickle cell anaemia
- To be able to describe the clinical presentation and complications of sickle cell anaemia
- To understand the role of haemoglobin electrophoresis and high performance liquid chromatography in the investigation of globin disorders
- To appreciate the many other haemoglobin variants associated with disease

Click on PATHOMA for a revision and more info!



Our YouTube's playlist for this lecture!

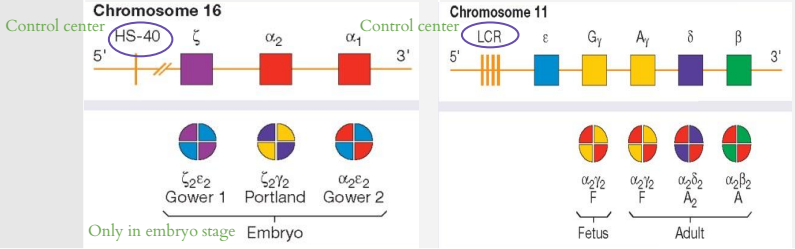
This lecture was given by: Dr.Osamah T.Khajoh and prof. Fatma Al Qahtani

Normal Structure and function of Hemoglobin

Hemoglobin is critical to the normal function of the red cell, the fundamental role of which is the **transport of oxygen** from the lungs to the tissues.

The normal tetramer Hb molecule comprises

each globin molecule is associated with a Hem group (Non protein)		Globin chain (protein)	
		Two 'alpha- like' globin polypeptide chains	Two 'beta-like' globin chains
Iron binding O2 (In its Ferrous form, Fe2+ in the center)	Porphyrin ring	encoded on **chromosome 16** (two α globin genes in each allele of chromosome 16 (total 4 genes))(you inherit 1 allele from each parent)	encoded on **chromosome 11** (one β globin gene in each allele of chromosome 11 (total 2 genes))(you inherit 1 allele from each parent)



Age	Hb	Chains		Feature
Adult	**HbA**	α2	β2	Major adult hemoglobin with a much smaller contribution from HbA2 96-98% of adult Hb
Adult	HbF	α2	γ2	Fetal Hb 0.5-0.8% of adult Hb
Adult	HbA2	α2	δ2	Usually 1.5-3.5% of adult Hb
Fetus	HbF	α2	γ2	HbF has a **higher oxygen affinity** than the adult hemoglobins facilitating transfer of oxygen from the maternal to the fetal circulation.
Embryo Found in the pic above	Gower 1	ζ2	ε2	Normally, the synthesis of α-like and β-like chains is balanced.
	Gower 2	α2	ε2	An imbalance between the production of α and β chains is the pathophysiological basis of the thalassemias (a quantitative issue).
	Portland	ζ2	γ2	

γ = Gamma, δ = Delta , ζ = Zeta , ε =Epsilon

Normal Structure and function of Hemoglobin

In chain, synthesis begins since: EXTRA

- α: Embryogenic life and continues until death (red line)
- β: Embryogenic life and continues until death (Green line)
- γ: Embryogenic life and decreases in adult life.
  - **Why**? Synthesized from liver and spleen where the site of Hb synthesis prenatally.
- δ: 30 weeks just before birth and continues until death (Dark purple)
- **Epsilon(ε)** (Blue line) & **Zeta(ζ)** (light purple): Embryogenic life and stops at 8 weeks. Synthesized in yolk sac.

Each type of Hb has a specific time for synthesis. EXTRA

Embryogenic Hb

Duraition: first 6 weeks of pregnancy,
Site :occurs in the yolk sac

Fetal Hb

Site: Occurs in the liver and spleen.

Postnatal Hb

Site: In the bone marrow.
Liver and **spleen** begin to shut down.
Hb can be synthesized by bone marrow **pre**natally as well.

Synthesis of globin chains in prenatal and postnatal life

After delivery: fully dependance on BM and shut down of all other

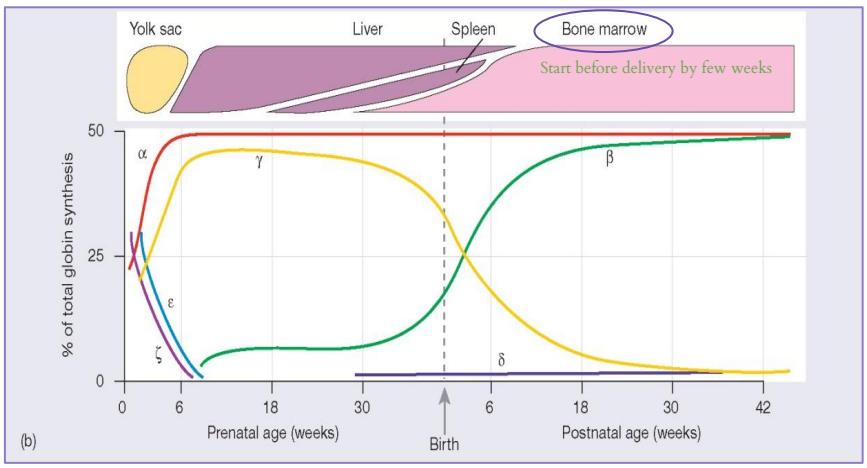


Figure 7.1 (a) The globin gene clusters on chromosomes 16 and 11. In embryonic, fetal and adult life different genes are activated or suppressed. The different globin chains are synthesized independently and then combine with each other to produce the different haemoglobins. The ygene may have two sequences, which codefor either aglutamic acid or alanine residue at position 136 (G, or A, respectively). LCR, locus control region, HS-40, see text. (b) Synthesis of individual globin chains in prenatal and postnatal life.
H

Table 6.1 Classification of haemolytic anaemias.

Hereditary	Acquired
Membrane	**Immune**
Hereditary spherocytosis, hereditary elliptocytosis	*Autoimmune*
Metabolism	Warm antibody type (see Table 6.5)
G6PD deficiency, pyruvate kinase deficiency	Cold antibody type
Haemoglobin	*Alloimmune*
Genetic abnormalities (Hb S, Hb C, unstable); see Chapter 7	Haemolytic transfusion reactions
	Haemolytic disease of the newborn
	Allografts, especially marrow transplantation
	Drug associated
	Red cell fragmentation syndromes
	See Table 6.6
	March haemoglobinuria
	Infections
	Malaria, clostridia
	Chemical and physical agents
	Especially drugs, industrial/domestic substances, burns
	Secondary
	Liver and renal disease
	Paroxysmal nocturnal haemoglobinuria (see Chapter 22)

G6PD, glucose-6-phosphate dehydrogenase; Hb, haemoglobin.

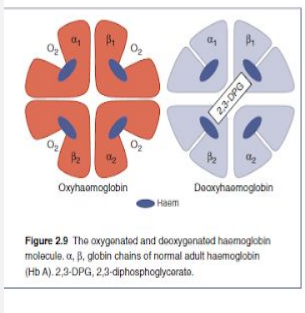


Figure 2.9 The oxygenated and deoxygenated haemoglobin molecule. α, β, globin chains of normal adult haemoglobin (Hb A). 2,3-DPG, 2,3-diphosphoglycerate.

A tetramer of four globin chains each with its own haem group in a 'pocket' is then formed to make up a haemoglobin molecule (Fig. 2.9).

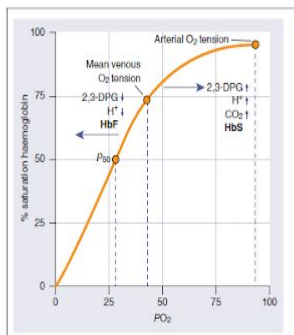


Figure 2.10 The haemoglobin oxygen (O_2) dissociation curve. 2,3-DPG, 2,3-diphosphoglycerate.

Male Slides

Overview of Haemoglobinopathies

EXTRA

Quantitative issue
Fewer or no α/B globin chains

E.g : **Thalassaemia**

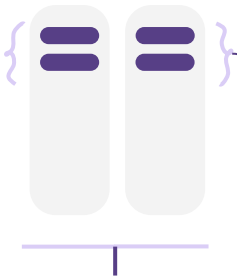
Structural (qualitative) issue
Structural Hemoglobin Variants
Due to Change in Amino Acid itself

HbE
lysine instead of Glu.
At **26** position

HbC
lysine instead of Glu.
At **6** position

HbS
valine instead of Glu.
At **6** position

α-Thalassemia
Loss of one gene or more of **alpha** globin synthesis
(alpha globin production decreased)



Chromosome 16

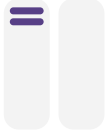
4 genes encode a-globin synthesis
(2 from each parent)
Since there are four genes, there **will be 4 possible Outcome**

Loss of **1** gene
1 alpha is lost so we have 3 alpha now.

α +-Thalassemia

Loss of **2** genes

Homozygous



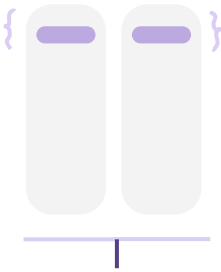
Heterozygous

α^0-Thalassaemia

Loss of **3** genes
Called: **HbH**
4 beta globins
why? because there is no enough alpha , so beta will interact with itself and producing 4 beta

Loss of **4** genes (no alpha genes)
Called: Bart's hydrops fetalis syndrome
The fetus will die.
4 gamma globulins

B-Thalassemia
Substitution of one gene or more of **Beta** globin synthesis
(beta globin production decreased



Chromosome 11

2 genes encode B-globin synthesis
(1 from each parents)
there **will be 2 possible Outcome**

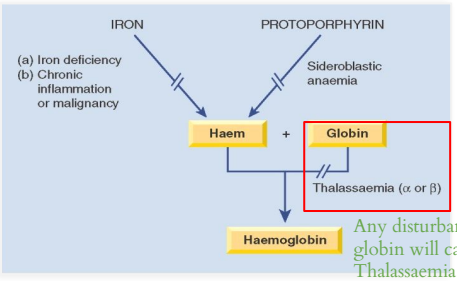
Heterozygous

Homozygous

Thalassemia انيميا البحر الأبيض المتوسط

Thalassemia is an important disease in paediatric but it's benign

Figure 3.1 The causes of a hypochromic microcytic anaemia. These include lack of iron (iron deficiency) or of iron release from macrophages to serum (anaemia of chronic inflammation or malignancy), failure of protoporphyrin synthesis (sideroblastic anaemia) or of globin synthesis (a- or B-thalassaemia). Lead also inhibits haem and globin synthesis.



Any disturbance of globin will cause Thalassaemia

Thalassemia

Thalassemias are a **heterogeneous** group of hereditary blood disorders characterized by faulty globin chain synthesis resulting in defective hemoglobin, which can lead to anemia.

The Thalassemias are divided into two main groups depending on whether the defect lies in the synthesis

β-thalassemias

gene **mutation** of beta chains of hemoglobin

α-thalassemias

gene **deletion** of alpha chains of hemoglobin

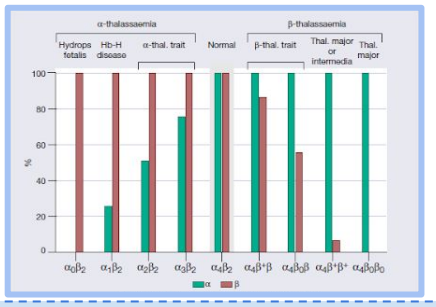


Figure 7.3 The ratio of a : B globin chain synthesis (y axis) depending on the number of functioning a and ß chain genes (x axis).
Source: Mehta A.B. & Hoffbrand A.V. (2014) Haematology at a Glance, 4th edn. Reproduced with permission of John Wiley & Sons.

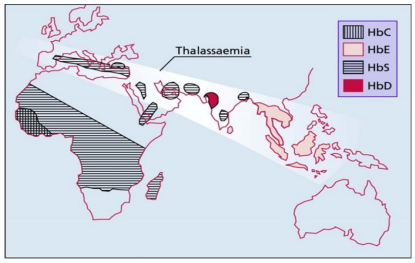


Figure 4.2 Distribution of the genes for the major Hb variants (S, E, C, D) and thalassaemia.

Pathophysiology:

- The chains which are present in **excess** will precipitate in the precursor red cells, leading to their **premature death** prior to release from the bone marrow (**ineffective erythropoiesis**)(there is an increase the pressure on the bone marrow to produce more red blood cells that are not effective) resulting to an ↑ **erythroid drive** and further expansion of the marrow into bones not typically used for hematopoiesis, and into the **spleen**. Will cause splenomegaly

Thus, the **long-term consequences** of thalassemia therefore include:

1. **Splenomegaly**
2. **bony deformities**
3. **iron overload due to transfusion**
4. ineffective erythropoiesis
5. chronic anemia.

Excess **globin chains** will precipitate in the precursor red cells → accumulate→ intracellular inclusion → damage RBCs cell membrane → **hemolysis** in **bone marrow** (Premature death) → hypoxia → sends signals to spleen and bone marrow to increase RBCs production → spleen enlargement + bony deformities

When there is ineffective erythropoiesis, the bone marrow produces a higher number of immature or abnormal red blood cell precursors. These cells are often destroyed prematurely, leading to an increased workload on the spleen as it tries to filter out the damaged or abnormal cells from the circulation.

The continuous activity of the spleen in removing these abnormal red blood cell precursors can lead to an enlargement of the spleen, a condition known as splenomegaly.

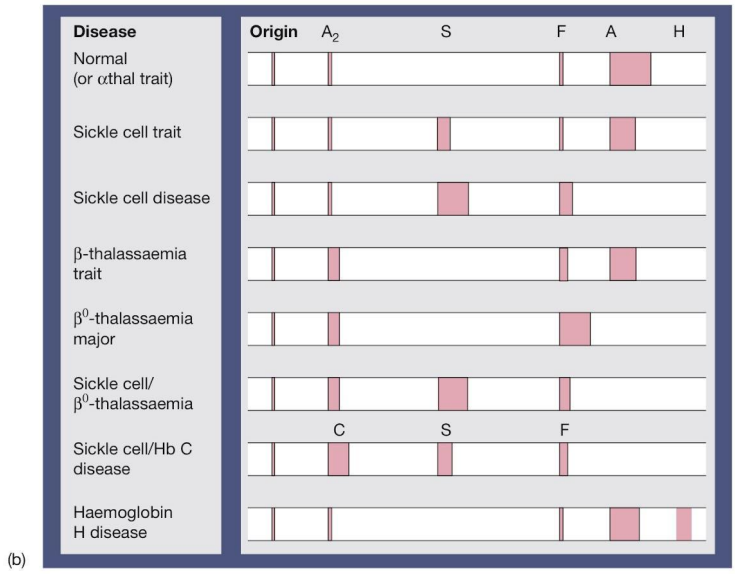
Thalassemia

Male Slides

Table 7.2 Classification of thalassaemia*.

Clinical			
Hydrops fetalis			*Thalassaemia minor*
Four gene deletion α-thalassaemia			β^0-Thalassaemia trait
Thalassaemia major			β^+-Thalassaemia trait
Transfusion dependent, homozygous			
β^0-thalassaemia or other combinations of β-thalassaemia trait			
Thalassaemia intermedia (non-transfusion dependent thalassaemia)			α^0-Thalassaemia trait
See Table 7.3			α^+-Thalassaemia trait
Genetic			
Type	**Haplotype**	**Heterozygous thalassaemia trait (minor)***	**Homozygous**
α-*Thalassaemias*†			
α^0	– –/	MCV, MCH low	Hydrops fetalis
α^+	–α/	MCV, MCH minimally reduced	As heterozygous α^0-thalassaemia† Compound heterozygote $\alpha^0\alpha^+$ (– –/–α) is haemoglobin H disease
β-*Thalassaemias*			
β^0		MCV, MCH low (Hb A_2 >3.5%)	Thalassaemia major (Hb F 98%, Hb A_2 2%)
β^+		MCV, MCH low (Hb A_2 >3.5%)	Thalassaemia major or intermedia (Hb F 70–80%, Hb A 10–20%, Hb A_2 variable)

α^0 = 2 α genes deleted or mutated, α^+ =one α gene deleted or mutated
*See text for the less common diseases: δβ-thalassaemia, haemoglobin Lepore and dominant β-thalassaemia trait



(b)

Figure 7.12 **(a)** High performance liquid chromatography. The different haemoglobins elute at different times from the column and their concentrations are read automatically. In this example, the patient is a carrier of sickle cell disease. **(b)** Haemoglobin electrophoretic patterns in normal adult human blood and in subjects with sickle cell (Hb S) trait or disease, β-thalassaemia trait, β-thalassaemia major, Hb S/β-thalassaemia or Hb S/Hb C disease and Hb H disease.

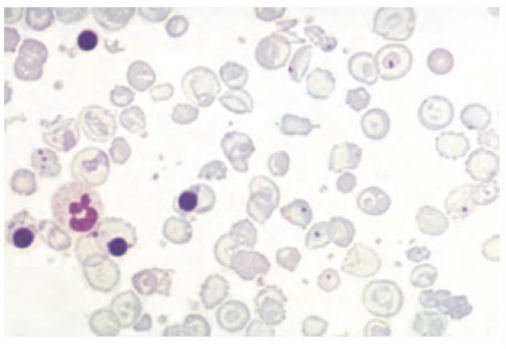


Figure 7.11 Blood film in β-thalassaemia major post-splenectomy. There are hypochromic cells, target cells and many nucleated red cells (normoblasts). Howell–Jolly bodies are seen in same red cells.

Table 7.3 Non-transfusion dependent thalassaemia (thalassaemia intermedia).

Homozygous β-thalassaemia
Homozygous or compound heterozygotes with mild β^+-thalassaemia
Coinheritance of α-thalassaemia
Enhanced ability to make fetal haemoglobin (γ-chain production)
Heterozygous β-thalassaemia
Coinheritance of additional α-globin genes (ααα/αα or ααα/ααα)
Dominant β-thalassaemia trait
δβ-Thalassaemia and hereditary persistence of fetal haemoglobin
Homozygous δβ-thalassaemia
Heterozygous δβ-thalassaemia/β-thalassaemia
Homozygous Hb Lepore (some cases)
Haemoglobin H disease

α-Thalassemia

α-thalassaemia typically arises from gene deletions.

Epidemiology: α-Thalassemia is seen with the greatest frequency in

1. south-east Asia (Thailand, the Malay Peninsula and Indonesia)
2. west Africa
3. the Middle East..

Each chromosome 16 has an α-globin locus consisting of two α-globin (i.e; 4 genes) genes plus the regulatory sequences essential for their normal expression.

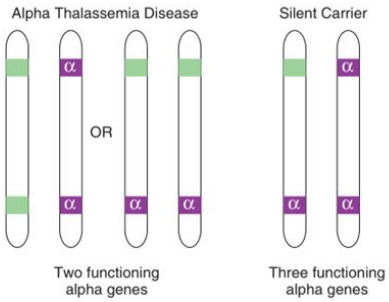
In most patients with α-thalassemia, there is a **deletion** of one or more of the α-globin genes; there are occasional cases that are the consequence of non-deletional defects.

It's mostly due to deletion, and rarely due do non-deletional defects.

Gene deletion

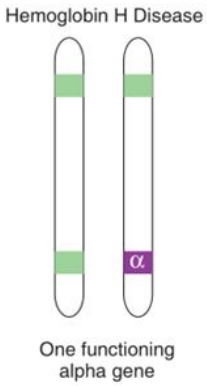
Deletion of 1 or 2 genes

Causa an Asymptomatic condition with minor hematological features



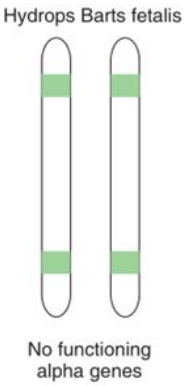
Deletion of 3 of the 4 α-globin genes

Cause a More severe imbalance of α:β-globin chains and results in **hemoglobin H,** consist of **four beta globins disease.**



Loss of all 4 α-globin genes

Causes **Hb Bart's**, Consist of **four gamma globins** (seen in **hydrops fetalis** syndrome)



0.4% of deliveries are stillbirths due to Hb Bart's hydrops fetalis syndrome and HbH disease is found in about 1% of the population.

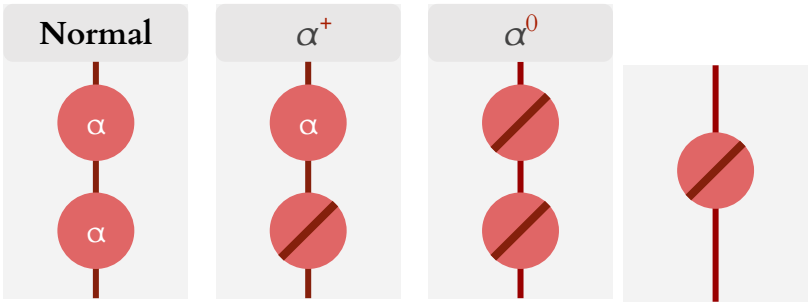
α-Thalassemia (Cont.)

α-Thalassemias Subtypes

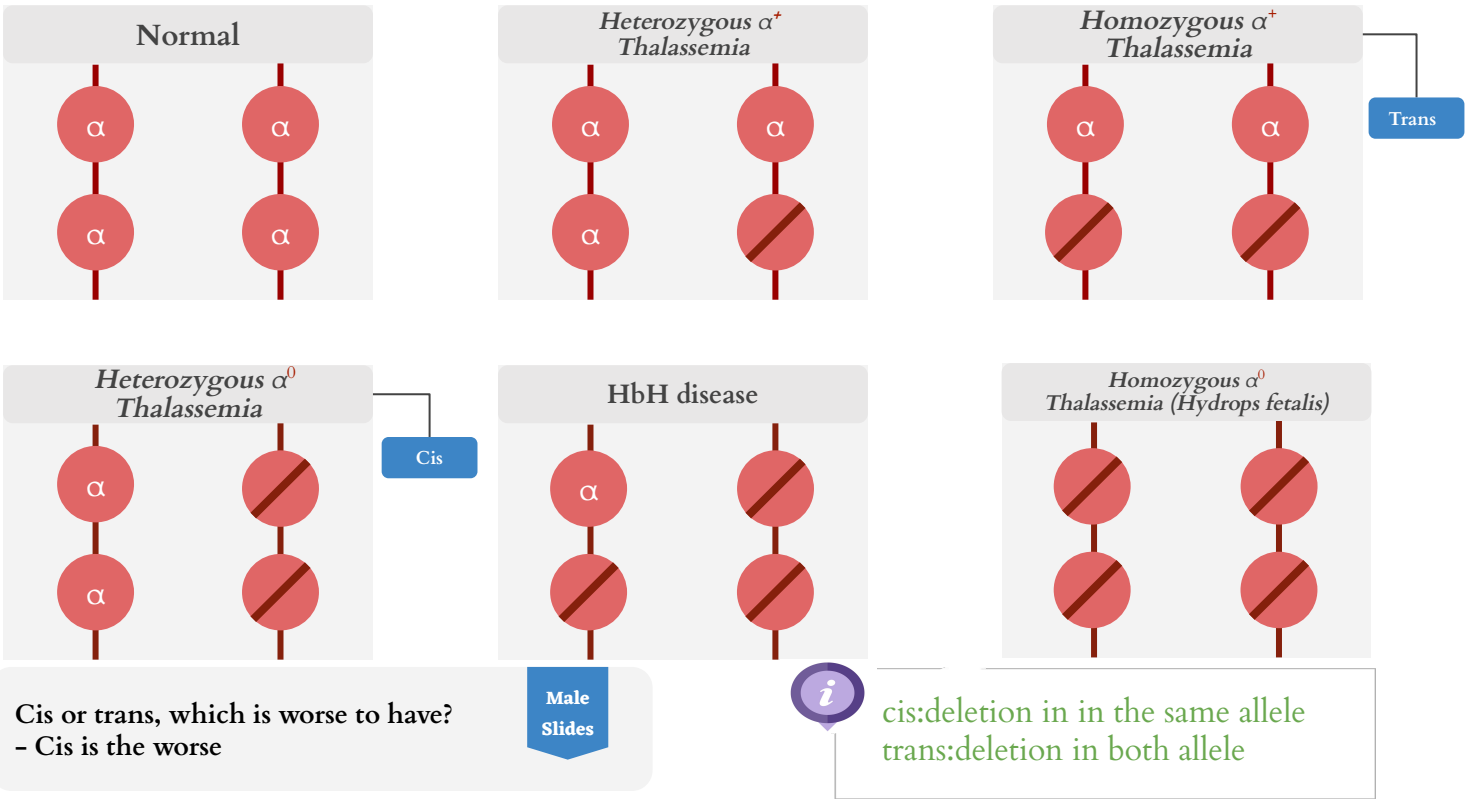
pay attention that the difference between trait and disease is the number of alpha globin genes that been deleted

α^+-Thalassemia trait Very mild	α^0-Thalassaemia trait Mild	Hemoglobin H disease Moderate disease	Hb Bart's hydrops fetalis syndrome Very severe
Deletion of one α globin gene	deletion of both α-globin genes in same allele on chromosome 16	deletion of three α-globin genes	deletion of all four α-globin genes ينتفخ الطفل

Chromosome 16



- **HbH Disease:** faulty α-globin chain synthesis → ↓ α-chains → impaired pairing of α-chains with β-chains→ ↑ free β → ↑ **HbH (tetramers of β4)**
  - **HbH** has an extremely high affinity for oxygen and therefore is not useful for oxygen delivery, leading to tissue hypoxia.
- **Hemoglobin Bart disease (major form):** faulty α-globin chain synthesis, four defective alleles (--/--) → ↓ α-chains → impaired pairing of α-chains with γ-chains→ ↑ free γ-chains → ↑ **Hb-Bart's** (consists of four γ-chains, γ-tetramers)



Cis or trans, which is worse to have?
- Cis is the worse

Male Slides

cis:deletion in in the same allele
trans:deletion in both allele

Figure 4.3 A diagram to show how the two forms of abnormal chromosome 16 (a' and a) are arranged to give the different forms of a-thalassaemia. Homozygotes for a-thalassaemia die from Hb Bart's hydrops fetalis syndrome

α-Thalassemia (Cont.)

α-Thalassemias Subtypes (Detailed)

α^+-Thalassemia trait

Deletion of **one** α globin gene

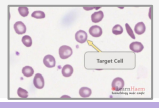
This is seen when an individual inherits the
- α^+-thalassemia allele from one parent. and
- normal **chromosome 16** from the other parent (i.e. heterozygotes for the α^+ determinant).

Affected individuals are **asymptomatic**, although they have minor hematological changes such as **slight reductions** in
1. mean cell volume (MCV)
2. mean cell hemoglobin (MCH).

α^0-Thalassaemia trait

deletion of **both** α-globin genes in **same allele** on chromosome 16

The **Hb** is either normal or slightly reduced and the **MCV and MCH are low**. However, **RBC count is elevated and RDW is not affected** (Thalassemic picture) and frequently form target cells **(why?).**(The target cell appearance is a consequence of the altered membrane structure and increased rigidity caused by the imbalanced globin chain production in thalassemia alpha, because there is a decrease in α-globin production **and an increase in**β-globin production, but not as much as HbH disease)



Hemoglobin H disease

deletion of **three** α-globin genes

- This chronic hemolytic anemia results from the inheritance of
  - both the α^+- and α^0-thalassaemia alleles, **leaving one functioning α-globin gene per cell.**
- α-globin chains are produced at very low rates, leaving a considerable excess of β-chains, which combine to **form tetramers** (β_4).(4 beta globin comes together)
- This tetramer is known **as HbH.**
- **HbH** is unstable and precipitates as the erythrocytes age, forming rigid membrane-bound inclusions that are removed during the passage of affected red cells through the spleen.
- The damage to the membrane brought about by this removal results in a shortened red cell lifespan.

Hb Bart's hydrops fetalis syndrome

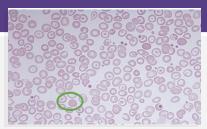
deletion of all **four** α-globin genes

- **No** α-chains can be formed, and the fetal β-like chain, **γ-globin** forms tetramers known as **Hb Bart's.**
- This hemoglobin is not useful for oxygen transport and despite the persistence of the embryonic **hemoglobin Hb Portland** ($\zeta_2\gamma_2$)
- There is intrauterine or neonatal **death** due to **hydrops**.

Finding of Hemoglobin H disease

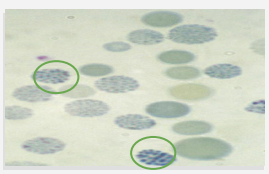
Most patients are moderately affected, with a mild anemia of 7-11g/dl and markedly **hypochromic, microcytic indices.**

The blood film shows marked hypochromic, microcytic cells **with target cells** and poikilocytosis



Supravital staining of the blood film demonstrates cells with many HbH inclusions, giving a characteristic '**golf-ball** appearance.

Supravital staining with brilliant cresyl blue reveals multiple fine, deeply stained deposits (**'golf ball'** cells) caused by precipitation of **aggregates of ß-globin chains.** HbH can also be detected as a fast-moving band on haemoglobin electrophoresis



Most patients will be transfusion independent.

Splenomegaly is seen in most patients.

β-Thalassemia

Epidemiology: The World Health Organization estimates that 1.5% of the world's population are carriers of β-thalassemia. The prevalence of the β-thalassemia trait is particularly high in **southern Europe** (10-30%) and **south-east Asia** (5%), common in Africa, the **Middle East**, India, Pakistan and southern China.

β-thalassaemia usually results from a **multiplicity** of different single nucleotide substitutions, **insertions** or **small deletions** affecting the β-gene itself or occasionally in promoting regions (**mutations**).

β-Thalassemias Subtypes

Heterozygous β-thalassemia (Beta-thalassemia trait) mild

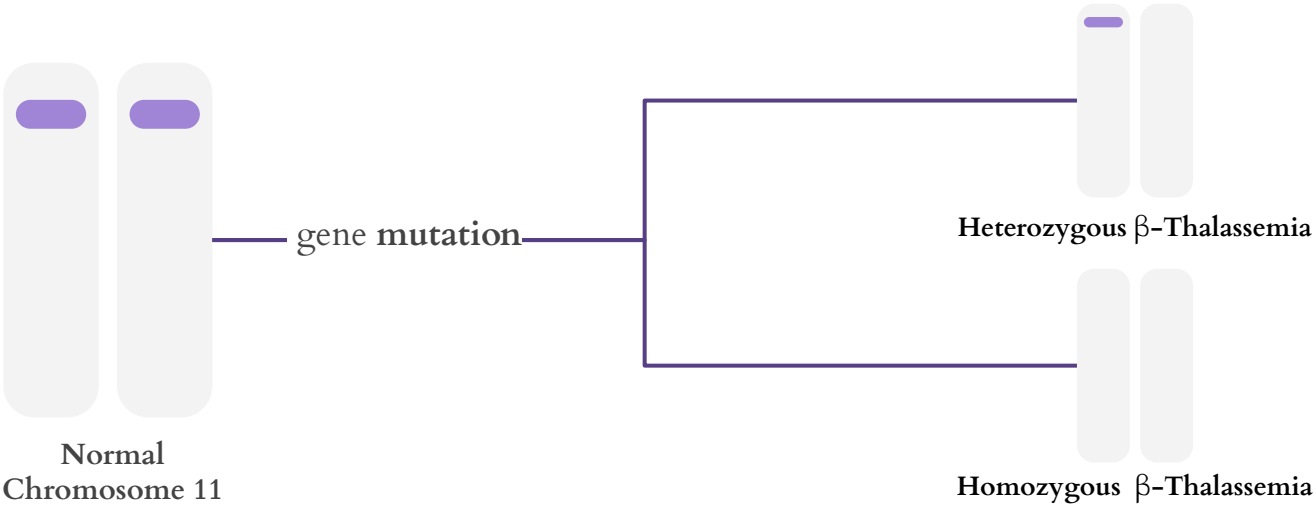
1. Most affected subjects with beta thalassemia trait are **asymptomatic**.
1. The **Hb concentration** is either normal or slightly reduced, **hypochromic and microcytic red cell indices are seen.**
1. Examination of peripheral blood film may show red cell abnormalities such as **target cells** and poikilocytes.

β-thalassemia **HbA2 levels will be raised** most of the time(this is how you differentiate it with α-Thalassemias) ** above the normal range to 3.5-7.0%. Sometimes, a slightly increased Hb F levels, in the range of 1-5%.

Homozygous β-Thalassemia

Defects of β-globin on **both** copies of **chromosome 11.**

- Marked anemia.
- **Transfusion dependent.**



Classification of the Thalassemias

Clinical classification of the Thalassemias

Thalassemia major	*Thalassemia intermedia*	*Thalassemia minor*	*Thalassemia minima*
• Have severe **anemia** and are **transfusion** dependent. • Their increased erythroid drive leads to a packed erythroid marrow and splenomegaly • development of bony abnormalities secondary to unchecked marrow expansion. • Patients in this category are those with complete loss of **β-globin** expression from both copies of **Ch11**.\ • beta thalassemia major is associated with high levels of fetal hemoglobin, this is like compensatory mechanism	• patients will also have a **marked microcytic hypochromic anemia** • increased **erythroid drive** to maintain their hemoglobin • packed bone marrow with a decreased myeloid to erythroid ratio, **and extramedullary hematopoiesis, giving splenomegaly.** • Transfusion may be required to maintain the hemoglobin at times of additional physiological stress.	• describes patients with **microcytosis and hypochromic red cells** secondary to thalassemia mutations • but with only **mild** anemia or a **normal** hemoglobin. **Patients who inherit a single affected allele are usually in this category.**	• describes the presence of a thalassemia mutation that is **without clinical consequences.**

This classification can be applied for both α & β thalassemia in general.

Genetic counselling and antenatal diagnosis of β-thalassemia major:

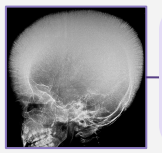
- Antenatal diagnosis can be made early during pregnancy (If the fetus has β thalassemia major, mother can have an abortion.) from an analysis of:
  - **Chorionic villous** DNA (at 9-12 weeks)
  - **Amniocyte DNA** (at 13-16 weeks),
  - Later using DNA from blood obtained from an 18-20-week-old fetus.
- Newer techniques focus on the non-invasive analysis of **fetal DNA** in the maternal circulation.
- A premarital screening (الفحص قبل الزواج) national program, is one of the major intervention leading to **reduced** incidence of beta thalassemia major.

β-Thalassemia major

The clinical course and complications of thalassemia major:

Anemia - is the principal feature of thalassemia **major**,
the massive expansion of erythroid activity results in **several complications** :

1. **Splenomegaly**
2. Bony deformities
3. Growth retardation
4. Iron absorption from the gut is **increased**. → Marked iron overload



hair on end appearance is commonly seen with beta thalassemia due to iron overload

- Why iron overload happens?
  - hemolysis of RBCs > free Hb in circulation > free heme > heme will recycle to : unconjugated bilirubin + iron
- What is the cause of hemolysis?
  For example in a-Thalassaemia (few or no alpha globin chain) B-globin chains will join together > result in abnormal Hb >
- Why individuals with thalassemia have hyperbilirubinemia> jaundice ?
  Secondary to ongoing hemolysis and ineffective erythropoiesis
- There is raised levels of HbA2 and HbF with beta thalassemia major

Iron **deposition** due to **overload** occurs in

In the Liver → leading to cirrhosis	**Myocardium** → resulting in Congestive cardiac failure, and potentially fatal arrhythmia
Other endocrine organ → delayed puberty	**Pancreas** → causing Diabetes mellitus

Treatment of β-thalassemia major:

1. **Transfusion** — Transfusion are planned to maintain the pre-transfusion Hb concentration at **9-10g/dL or above.**
2. **Iron chelator** — required subcutaneous infusion treatment over several hours on five days of the week.
3. Best treatment — **HSCT** — Hematopoietic stem cell transplantation (HSCT) is curative.
4. **Splenectomy** — If massive enlarged spleen
5. **Gene therapy** — (see the NEJM quick tack, https://www.nejm.org/do/10.1056/NEJMdo005273/full/).
   Recently

Structural Hemoglobin Variant

Over 1000 abnormal hemoglobin variants have been reported. The majority of structural Hb variants are the consequence of a **single-point mutation** with a **single amino acid substitution** in the affected globin chain (e.g. **HbS**, **HbE**, **HbC** and **HbD**).

Hemoglobin	Original Geographic Association	Amino Acid Substitution	% Present in Heterozygotes
S	Africa	β6 Glu —> Val	40%
C	Africa	β6 Glu —> Lys	40%
E	Asia	β26 Glu —> Lys	30%
G Philadelphia*	Africa	α68 Asn —> Lys	20, 30, 40% **
D Los Angeles***	Europe/India	β121 Glu —> Gln	40%
O Arab	Africa	β121 Glu —> Lys	40%
Lepore#	Mediterranian	δ(1-87) β(116-146)#	10-15%

When **the amino acid substitution** results in an overall change in the charge of the hemoglobin molecule, **its migration in a voltage gradient** is altered and this can be demonstrated by standard **electrophoretic techniques**. The speed of migration is characteristic for each abnormal hemoglobin.

Abnormal hemoglobin variants are now usually detected by **high-performance liquid chromatography** (**HPLC**). **The most common structural Hb variant is hemoglobin S (HbS).**

Different clinical and haematological abnormalities associated with some structural haemoglobin variants

Variant	Clinical and haematological abnormalities
HbS	**Recurrent painful crises (in adults) and chronic haemolytic anaemia, both related to sickling or 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, Hb Radcliffe	Hereditary polycythaemia due to increased O_2 affinity
Hb Kansas	Anaemia and cyanosis due to decreased O_2 affinity
Hb Constant Spring, Hb Lepore, HbE	Thalassaemia-like syndrome due to decreased rate of synthesis of abnormal globin chain
Hb Indianapolis	Thalassaemia-like syndrome due to marked instability of Hb

HbS

HbS - Found in Sickle cell pts:

A mutation in **the β-globin gene** results in the **charged glutamic acid** residue in **position 6** of the normal β-chain being replaced by an **uncharged valine molecule.**

↓

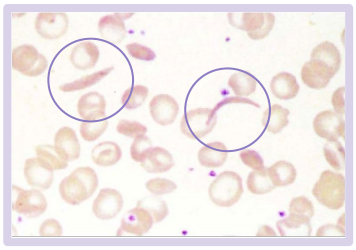
The interaction of sickle β-globin chains with normal α-globin chains forms **HbS**

↓

When **deoxygenated**, **HbS is much less soluble than deoxygenated HbA**, and HbS molecules **polymerize**, eventually forming long fibers.

↓

These result in the deformation of the cell into the well-recognized sickle shape.



sickle-shaped red cells with pointed ends and some partially sickled red cells from the blood film of a patient with sickle cell anaemia (homozygote for HbS).

Sickle Cell Trait (Mild)	Sickle Cell Anemia
Heterozygotes (one gene for normal β-globin and one for βS)	It is a descriptive name when patient **have at least a copy of beta globin being S and another beta harbor any mutations** (beta that/S, S/S, S/D, etc.).
Their red cells contain between 20% and 45% HbS **the rest being mainly HbA.**	**Homozygotes for sickle β-globin** are described as having sickle-cell anemia.Their red cells contain almost **exclusively HbS and NO HbA**; there is a small but variable percentage of fetal hemoglobin.
Individuals with sickle cell trait are usually **asymptomatic**. However, **spontaneous hematuria** may occur occasionally due to microvascular infarctions in the renal medulla. **Renal papillary necrosis** may rarely occur. **The red cells do not sickle until the O2 saturation falls below 40%.**	Sickled red cells then **occlude** the **microvasculature**, with **poor downstream perfusion** and oxygenation. They may be **lysed** directly in the circulation, where the resulting free hemoglobin scavenges nitric oxide. HbS are **less deformable** than normal red cells and this results in a chronic, extravascular, hemolytic anaemia. The Hb usually varies between **6-9 g/dL.** Very low

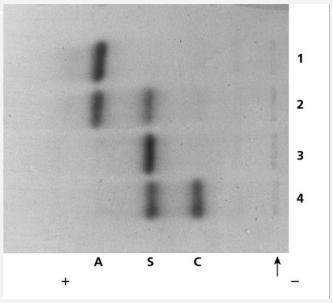


Figure 4.5 **Electrophoresis** of haemolysates on cellulose acetate (pH 8.5). The arrow marks the site of application of the haemolysate.
(1) Normal adult.
(2) Individual with sickle cell trait; 35% of the Hb consists of HbS and most of the remainder is HbA.
(3) Patient with sickle cell anaemia; most of the Hb is S and there is no A.
(4) Double heterozygous for HbS and HbC. This results in a disease that is usually milder than that in homozygotes for HbS.

Sickle Cell Anemia

Diagnosis :

Sickled cells are invariably present on the blood films of patients with HbSS. HbSS is made by finding:

1. A positive result with a screening test for HbS (**Sickle solubility test**). And
2. A peak at an appropriate position on an **HPLC trace**, confirmed by isoelectric focusing or hemoglobin electrophoresis(this is how you can differentiate between SCD and SC trait)

In young children

A classic acute painful presentation is with dactylitis (digits inflammation), or the 'hand-foot syndrome', in which there is occlusion of the nutrient arteries to the metacarpals and metatarsals (see next slide) and painful swelling of the hands and feet. Very severe pain and swelling

In the central nervous system

Cerebral infarction occurs in approximately 10% of patients under the age of 20, and is a cause of significant morbidity in sickle cell patients. It has been found that children with an increased velocity of blood flow in the major cerebral vessels are at particular risk of stroke.

Family History and Molecular testing

Male Slides

Treatment:

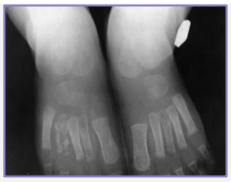
The principles of the management of sickle cell anemia include:

- Management of the increased infection risk by immunization
- Administration of folic acid daily to prevent secondary folate deficiency.
- Avoidance of factors precipitating painful crises such as dehydration, hypoxia, circulatory stasis.
- Treatment of painful crises with oral or intravenous fluids and analgesics, including opiates when necessary.
- Early detection of the acute chest syndrome (blood gas measurements and chest X-ray). Exchange transfusions are often needed to lower the patient's HbS levels and limit ongoing sickling.
- Blood transfusion when necessary,
- SCT and gene therapy

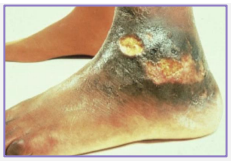
Sickle Cell Anemia

Clinical manifestations of sickle-cell anaemia.

- Chronic haemolytic anaemia and consequent cholelithiasis
- Splenic sequestration syndrome; rarely, hepatic Sequestration
- Acute chest syndrome
- Cerebral infarction, TIA, intracranial haemorrhage
- **Widespread painful vaso-occlusive crises** *
- **Bone infarction (osteonecrosis)**
- Osteomyelitis (Salmonella, Staphylococcus)
- Chronic leg ulcers
- **Priapism** *
- Chronic pulmonary disease and pulmonary
- hypertension
- Haematuria, proteinuria, chronic renal failure
- Pregnancy: increased peripartum fetal loss, preterm births, babies small for gestational age
- Aplastic crises due to parvovirus infection
- Proliferative sickle retinopathy (more common in HbSC disease)



X-ray of the feet of a child with sickle cell anaemia two weeks after the onset of **hand-foot syndrome** [1], showing **necrosis** of the right fourth metatarsal.



chronic leg ulcer with increased pigmentation of the surrounding skin in a woman with sickle cell anaemia.

Hemoglobin E&C

Among the commonest are HbE and HbC, both of which result from single amino acid substitutions in the β-chains.

Hemoglobin E

Is very common in south-east Asia (being found in about 50% of the population in some parts of Thailand). It gives thalassemic picture of CBC.

Hemoglobin C

- Is the consequence of a **glutamine to lysine** substitution in the β-globin chain. HbC is also seen in **homozygosity**;
- here the hemoglobin does not polymerize as with HbSS, but **can crystallize**(diamond like shape), with a resulting reduction in the flexibility of the red cell and a reduction in its survival.
- Homozygotes have a mild anemia, low MCV, splenomegaly and many target cells in their blood film. HbC is found in patients of West African origin.

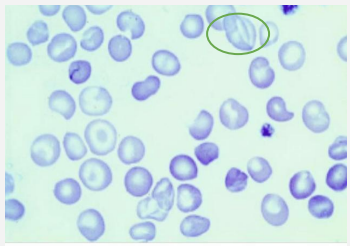


Figure 4.10 Target cells and irregularly contracted cells in the blood film of a homozygote for HbC.

When one allele being S and other being C or E, it is an example of a sickle cell disease (the most benign form is S/E).

Hb Types

		Hb	Chains	
Adult		HbA	α2	β2
		HbF	α2	γ2
		HbA2	α2	δ2
Fetus		HbF	α2	γ2
Embryo		Gower 1	ζ2	ε2
		Gower 2	α2	ε2
		Portland	ζ2	γ2
Diseased	Alpha Thalassemia	HbH	-	β4
		Hemoglobin Bart's	-	γ4
	Beta Mutations Abnormal beta chains, a single point mutation	Hb S	α2	β2
		Hb C	α2	β2
		Hb E	α2	β2

MCQs

From Slides

Q1. Which ONE of the following statements is TRUE about sickle cell anemia?			
A. The oxygen dissociation curve is shifted to the left.	B. It may cause ankle ulcers.	C It is NOT associated with stroke. .	D. It is NOT associated with atrophy of the spleen
Q2. Which ONE of the following statements is TRUE about β- thalassemia trait?			
A. It is associated with a raised hemoglobin A2 with normal CBC indices.	B. It is associated with iron overload.	C. It is associated with a normal reticulocyte index.	D. It is associated with splenomegaly.
Q3.Which ONE of the following statements is TRUE about beta-thalassemia?			
A. It may cause hemoglobin H disease	B. It causes a microcytic hypochromic blood picture	C. It is frequently cause a hydrops fetalis.	D. It is very common in the Far East.
Q4. Which ONE of these statements is TRUE about β- thalassemia major?			
A. It presents at birth.	B. It is usually caused by deletion of β globin genes.	C. It is associated with an increased risk of bone infarction	D. It is associated with stunted growth.
Q5. Which ONE of the following is a feature of thalassemia intermedia?			
A. It may be due to homozygous βo thalassemia without coinheritance	B. It does NOT associated with extramedullary hemopoiesis.	C. It is usually associated with splenomegaly.	D. It can NOT cause iron overload.
Q6. Which ONE of these statements is TRUE concerning sickle cell **trait**?			
A. It is a cause of anemia	B. It protects against malaria.	C. It is usually associated with splenomegaly.	D. It is a cause of frequent sickle cells in the peripheral blood

Click here for an explanation :)

A1. B A2. C A3. B A4. D A5. C A6. B

Members board

Team Leaders:

Aleen AlKulyah **Remaz Almahmoud** **Sultan albaqami**

Team Members:

- Milaf alotaibi
- Reuf Alahmari
- Deema almadi
- huda bin jadaan
- Elaf moatabi
- Aseel Alsaif
- Razan alsoteehi
- Maryam Alghannam
- Raghad Alqhatani
- Lama Alotaibi
- AlJoharah Alwohaibi
- Aroub Almahmoud
- Dana Almuhaisen
- Ryan alghizzi
- Feras Mazen
- Mishal Aldakhail
- Abdullah Alzamil
- Khalid Alanezi
- Mohammed Manee
- Ziad Alhabardi
- Zeyad Alotaibi
- Omar Alamri
- Moath Alhudaif
- Faris Alzahrani
- Abdullah Alkodari

Special thanks to 442 team

HEMATO.TEAM43@GMAIL.COM

443

haematology Team