MED439
KING SAUD UNIVERSITY

Haemoglobinopathy

Objectives:

- To understand 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 clinical presentations and complications of thalassaemia
- 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

Dr's notes
Important
Extra notes
** Only in girls slide
** Only in boys slide

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Editing file

Overview of Haemoglobinopathies

Quantitative issue

Fewer or no a/b globin chains

E.g : **Thalassaemia**

Structural (qualitative) issue

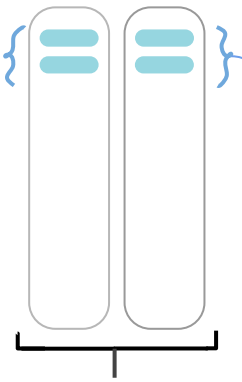
Structural Hemoglobin Variants

Change in Amino Acid itself

- **HbS: in sickle cell anemia**
  Normally **glutamic acid** is used, in this disease **valine** take place.
- **HbC** (lysine instead of Glu. At **6** position)
- **HbE** (lysine instead of Glu. At **26** position)

α-Thalassemia

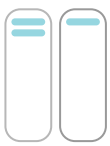
Loss of one gene or more of **alpha** globin synthesis



Chromosome **16**

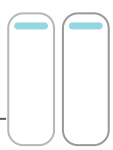
4 genes encode a-globin synthesis (2 from each parents)
دامهم اربعة فبنلاحظ إن فيه 4 احتمالات، ممكن يفقد جين واحد، جينين، وهكذا

Loss of **1** gene

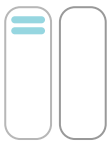


α +-Thalassemia
علامة الزائد معناها بيكون فيه الفا

Loss of **2** genes

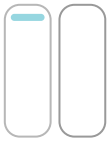


Homozygous

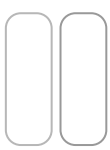


Heterozygous

Loss of **3** genes (Called: **HbH)**

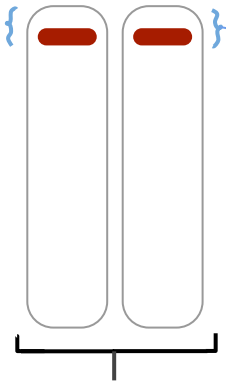


Loss of **4** genes (no alpha genes) Called: **Bart's hydrops fetalis** syndrome
الجنين بيموت



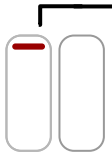
B-Thalassemia

Substitution of one gene or more of **Beta** globin synthesis

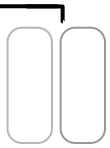


Chromosome **11**

2 genes encode B-globin synthesis (1 from each parents)
فعندنا حالتين، واحد من الوالدين ماعنده =hetero
كلهم ماعندهم =homo



Heterozygous



Homozygous

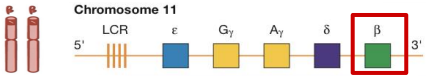
Normal Hemoglobin

Structure and Function

- 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 2 α globin polypeptide chains and 2 β globin polypeptide chains; each chain is associated with a heme group, which comprises a porphyrin ring with iron in its ferrous form (Fe2+) at the center.
- HbF has a higher oxygen affinity than the adult hemoglobins facilitating transfer of oxygen from the maternal to the fetal circulation

α globin chains are encoded on **chromosome 16 (4 α alleles)**
(as well as zeta)

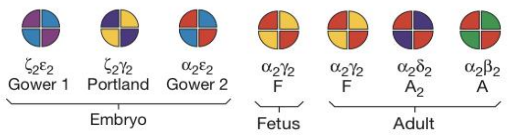
β globin chains are encoded on **chromosome 11 (2 β alleles)**
(as well as γ, δ, ε)



Age	Hb	Chains		Feature
Adult	HbA	α2	β2	Major adult hemoglobin (normally found as most common type) 96-98% of adult Hb
	HbF	α2	γ2	0.5-0.8% of adult Hb
	HbA2	α2	δ2	1.5-3.5% of adult Hb
Fetus	HbF	α2	γ2	**Higher oxygen affinity**, only one type of fetal Hb

Age	Hb	Chains	
Embryo	Gower 1	ζ2	ε2
	Gower 2	α2	ε2
	Portland	ζ2	γ2

Female Dr. said you should know embryo and adult hemoglobin.



Check chromosome picture to understand color code

Dr's note: we like to ask about it in OSPE

Each type of Hb has a specific time for synthesis

Embryogenic Hb: accounts for the first 6 weeks of pregnancy, and occurs in the yolk sac
Fetal Hb: Occurs in the liver and spleen
Postnatal Hb: In the **bone marrow**. Liver and spleen begin to shut down. Hb can be synthesized by bone marrow **pre**natally as well.

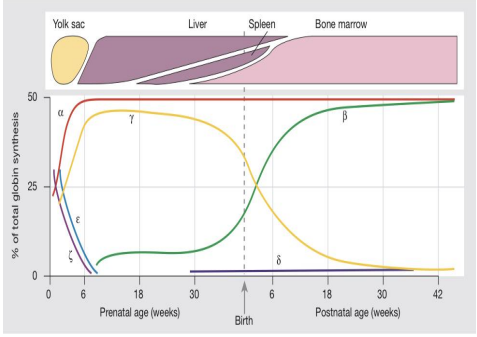


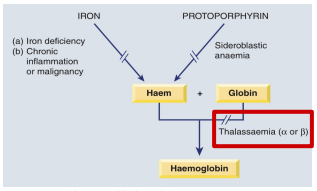
Fig (A): Synthesis of globin chains in prenatal and postnatal life

In ... chain, synthesis begins since:
α: Embryogenic life and continues until death
β: Embryogenic life and continues until death
γ: Embryogenic life and decreases in adult life. Why? Synthesized from liver and spleen.
δ: 30 weeks just before birth and continues until death
Epsilon(ε) & Zeta(ζ): Embryogenic life and stops at 8 weeks. Synthesized in yolk sac.

Thalassemia

Hb & Thalassemia

- Normally, the synthesis of α-like and β-like chains is balanced.
- An imbalance between the production of α and β chains is the pathophysiological basis of the thalassemias (**a quantitative issue**).



Where will the changes occur?
In thalassemia, in **globin** synthesis

Introduction

Thalassemias are a heterogeneous group of **hereditary blood disorders** characterized by **faulty globin chain synthesis** resulting in defective hemoglobin, which can lead to anemia. Depending on where the defect lies in synthesis, they are divided into two main groups

- α-Thalassemias - due to gene **deletion** of alpha chains of hemoglobin
- β-Thalassemias - due to gene **mutation** of beta chains of hemoglobin

Pathophysiology

Excess globin chains will precipitate in the precursor red cells

Leading to their **premature death** prior to release from the bone marrow **(ineffective erythropoiesis)** it dies in bone marrow before it enters circulation

Resulting in ↑ **erythroid drive** and further expansion of the marrow into bones not typically used for hematopoiesis (like long bones, only activated in ↑ demand), and into spleen.

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

- **Splenomegaly**
  (436: Since bone marrow is not enough/ not working, body will request from the spleen which will increase the demand on the organ and cause: splenomegaly)
- **Bony deformities** (expansion of bone)
  (Anemia → ↑ erythropoietin → bone marrow hyperplasia and skeletal deformities)
- **Iron overload** due to: **transfusion** and ineffective erythropoiesis as well as **chronic anemia**.

Epidemiology

- α-Thalassemias: most commonly seen in Asian and African descent.
- β-Thalassemias: most commonly seen in people of Mediterranean descent

Thalassemia provides partial **resistance against malaria**.

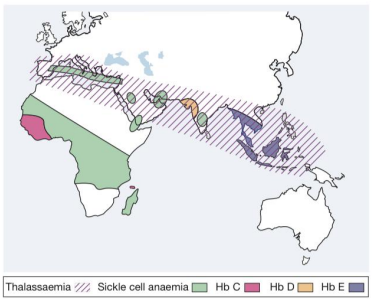


Figure 7.4 The geographical distribution of the thalassaemias and the more common, inherited, structural haemoglobin abnormalities.

α- Thalassemia

Osmosis

Epidemiology

Most commonly seen in south-east **Asia** (Thailand, the Malay Peninsula, Indonesia) and west **African descent.** Thalassemia provides partial **resistance against malaria**.

Etiology

- **Overview: Usually due to deletion of at least one out of the four existing alleles on chromosome 16 (2 alleles per chromosome). Thus, there are four forms of the disease.**
  - 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.

Subtypes

The severity of alpha thalassemia depends on the **number** of defective α-globin alleles. **α Thalassemia subtypes classified based on** deletion of:

<table>
<tr><th>1 α allele</th><th>2 α allele</th><th>3 α allele</th><th>4 α allele</th></tr>
<tr><td colspan="2">Asymptomatic condition
with minor hematological features</td><td>Severe anemia, no problem in fetus</td><td>Lethal in utero</td></tr>
<tr><td>α +-Thalassemia trait

(-α/αα) instead of (αα/αα)</td><td>Alpha thalassemia trait (minor form).

(-α/-α or --/αα) instead of (αα/αα)</td><td>Deletion of 3 of the four alpha globin genes causes a more severe imbalance of α:β globin chains.
Hemoglobin H disease, four beta globins</td><td>Loss of all four α-globin genes causes Hb Bart's, four gamma globins, (hydrops fetalis syndrome)</td></tr>
<tr><td></td><td rowspan="2">If (--/αα), it's known as α^0-Thalassaemia trait (deletion of both α-globin genes in same allele on ch 16)</td><td>Found in about 1% of the population</td><td>Due to Bart's hydrops fetalis syndrome, 0.4% of deliveries are stillbirths (babies can't survive)</td></tr>
<tr><td></td><td colspan="2">Both HbH and Hb Bart's can be seen on electrophoresis

To read more about electrophoresis click here</td></tr>
</table>

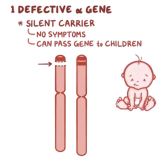
α- Thalassemia

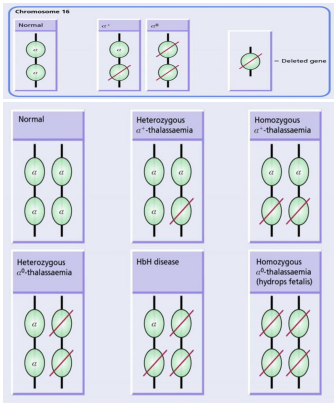
Osmosis

Subtypes

Reminder: Thalassemias are **hereditary blood disorders** characterized by **faulty globin chain synthesis. Their Inheritance pattern** is autosomal recessive, meaning a patient **must have** inherited mutated genes from both parents to get the disease.

α +- Thalassemia trait (alpha plus): deletion of 1 α globin gene





This is seen when an individual inherits:

- α+-thalassemia allele from one parent
- a normal ch 16 from the other parent

(i.e. heterozygotes for the α+ determinant).

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

α^0-Thalassaemia trait (alpha 0): 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. **Why?**

- Because thalassemia is often accompanied by the destruction of a large number of RBCs. This causes your spleen to **enlarge** and work harder than normal. An enlarged spleen can make anemia worse, and it can reduce the life of transfused red blood cells.
- **But how can an enlarged spleen make anemia worse?** An enlarged spleen can reduce the number of healthy RBCs, platelets, and WBCs in your bloodstream, leading to more frequent infections. Anemia, and increased bleeding also are possible.

Hemoglobin H disease: deletion of three α-globin genes

Details in the next slide...

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 (ζ2γ2), there is **intrauterine or neonatal death due to hydrops.** Cannot survive, dies either before or after birth

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)

α- Thalassemia

Osmosis

Subtypes

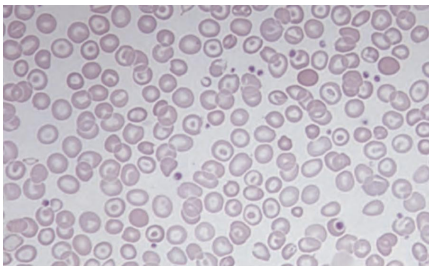
Hemoglobin H disease: deletion of three α-globin genes

- This chronic hemolytic anemia results from the inheritance of both the α+- and α0-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 (β4). This tetramer is known as HbH.
only β chains exist, α chains are almost absent.
- 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.
- 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**.

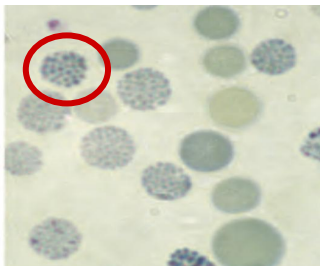
Findings

- Most patients are moderately affected, with a **mild anemia of 7-11g/dl** and markedly **hypochromic, microcytic** indices.
- Supravital staining used for diagnosis of the blood film demonstrates cells with many **HbH inclusions**, giving a characteristic '**golf-ball**' appearance.
- Most patients will be transfusion independent.
- **Splenomegaly** is seen in most patients.

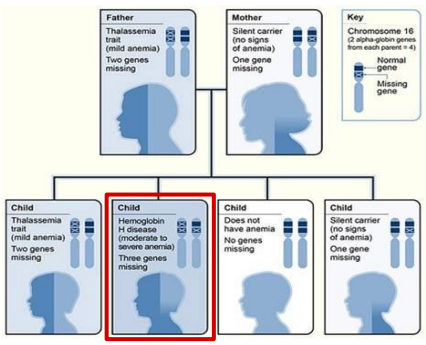
Dr's note : we like to ask about it in OSPE



Blood film shows hypochromic microcytic cells with target cells and poikilocytosis (shape variations)



Supravital staining shows "Golf-Ball" appearance



Inheritance of HbH

β- Thalassemia

Osmosis

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. most commonly seen in people of Mediterranean descent

Etiology

In a normal cell, the β-globin chains are coded by a total of two alleles
Thus, there are two forms of the disease. 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.

Subtypes

Heterozygous β-thalassemia (Beta-thalassemia trait) (diminished beta production)

- Most affected subjects with beta thalassemia trait are **asymptomatic**.
- The Hb concentration is either normal or slightly reduced, hypochromic and microcytic red cell indices are seen.
- ★ Examination of peripheral blood film may show red cell abnormalities such as **target cells** and **poikilocytes** (variation in shape)
- ★ β-thalassemia **HbA2 levels will be raised** 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 (severe form) (absent beta production)

- Defects of β-globin on **both** copies of **chromosome 11**
- Marked anemia
- **Transfusion dependent**

Clinical Classification of Thalassemias

Osmosis

Classified according to symptoms and severity:

Thalassemia Minima

Describes the presence of a thalassemia mutation that is **without** clinical consequences. Meaning, no symptoms will be seen.

Thalassemia Minor

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.
a-thalassemia trait مثل اللي يخسر فيها جين واحد

Thalassemia Intermedia

Patients will also have a **microcytic hypochromic anemia**,
increased **erythroid drive**[1] to maintain their hemoglobin, packed bone marrow with a decreased myeloid to erythroid ratio[2], and **extramedullary hematopoiesis splenomegaly.**[3] Transfusion may be required to maintain the hemoglobin at times of additional physiological stress.

Thalassemia Major

- Have **severe anemia** and are **transfusion dependent.**(lifelong transfusions)
- 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. Most dangerous.

---

1 : Formation of abnormal cells will lead to **hemolysis >** low RBCs lead to **hypoxia >** The rate of **erythropoiesis** is sensitive to the oxygen tension of the arterial blood. So When oxygen tension falls, more RBCs are produced from

A-bone marrow in the bones like (skull, spine and hip)
B-extramedullary **(liver + spleen)**

2: Myeloid to erythroid ratio=

$$\frac{\text{Myeloid}}{\uparrow\ \text{Erythroid}}$$

Erythroid فمعناها لما نقول الريشيو نقصت معناها زادت

3: Splenomegaly due to A-excessive destruction of abnormal RBCs, B-extramedullary hematopoiesis, and C-transfusional overload.

Clinical Course and Complications of Thalassemia Major

Osmosis

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

- Splenomegaly
- Bony deformities (Bone marrow found in bones > with increased erythropoiesis > enlarge bones)
- Growth retardation
- **Iron** absorption from the gut is increased
- Marked **iron overload**

Why iron overload happens?

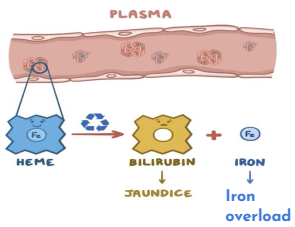
hemolysis of RBCs > free Hb in circulation > free heme > heme will recycle to : unconjugated bilirubin + iron

- **What is the cause of hemolysis?**

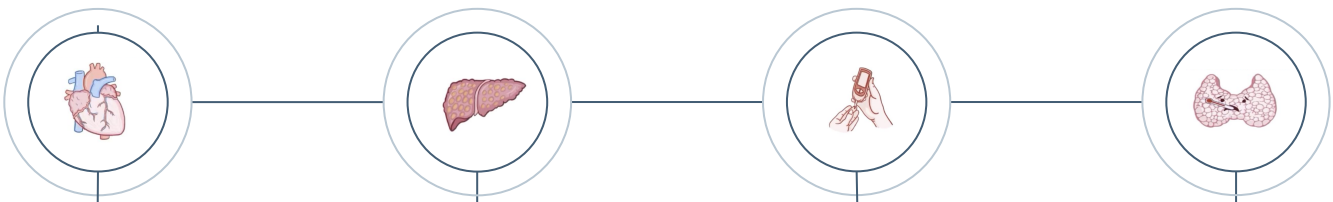
For example in a-Thalassaemia (few or no alpha globin chain) B-globin chains will join together > result in abnormal Hb >

- **Why individuals with thalassemia have hyperbilirubinemia> jaundice ?**

Secondary to ongoing hemolysis and ineffective erythropoiesis



Iron deposition due to overload



Iron deposition in myocardium:

- **Congestive cardiac failure**
- Potentially fatal **Arrhythmias**

(chronic iron overload reduces Ca2+ currents)

Liver cirrhosis

This excess iron is stored in major organs, especially your liver. Over a period of years, the stored iron cause > damage > cirrhosis

Damage to the pancreas leads to DM

Insulin's effect

Damage endocrine organs

>delayed puberty

β-Thalassemia Treatment & Diagnosis

Osmosis

β-Thalassemia treatment

- Transfusion are planned to maintain the pre-transfusion Hb concentration at **9-10g/dL** or above.
- Splenectomy can be performed.
- **Iron chelator**[1], required subcutaneous infusion treatment over several hours on five days of the week.
- Hematopoietic stem cell transplantation (HSCT) is curative.
- Gene therapy **(under study)** *click here to read more (mentioned in the slides)

Genetic counselling & antenatal diagnosis of β-Thalassemia Major

- Antenatal diagnosis can be made early during pregnancy from an analysis of:
  - **Chorionic villous DNA**[2] (at 9-12 weeks)
  - Amniocyte DNA[3] (at 13-16 weeks),
  - Later using DNA from blood obtained from an 18-20-week-old fetus.

    If the fetus has β thalessemia major, mother can have an abortion.
- Newer techniques focus on the non-invasive analysis of fetal DNA in the maternal circulation.
- A pre-marital screening الفحص الطبي قبل الزواج, national program, is one of the major intervention leading to **reduced incidence** of beta thalassemia major.

1- Iron chelation therapy is the removal of excess iron from the body with special drug
مثل فيه دواء يمسك بالحديد ويخليه يطلع عبر Stool

2- This is an optional test for pregnant. They do a biopsy for part of the placenta (called: chorionic villi وهذا سبب اسم الفحص)
طيب وش دخل المشيمة بالثالاسيميا؟ قالوا إن المشيمة تكون مطابقة تقريبًا لجينات الجنين > يفحصون الحمض النووي > يشوفون الكروموسوم إذا كامل او فيه جينات مفقودة مثل حقات الالفا والبيتا فيعرفون مسبقًا عن المرض

3- Another test for pregnant > amniotic fluid is used to analyze DNA from the cells
يدخلون أبرة عشان يأخذون جزء من السائل ثم يفحصون الخلايا اللي فيه عشان يتأكدون من الكروموسومات والجينات اللي جواها بحثًا عن اختلالات جينية

Structural Hemoglobin Variant

Osmosis

- 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**).
- 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).**

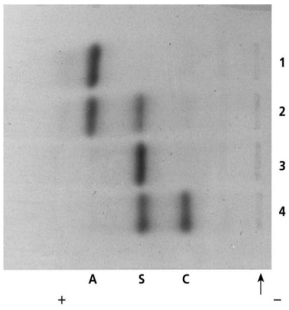


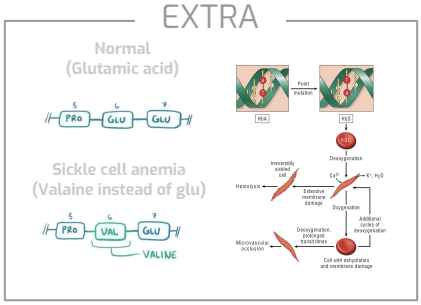
Figure 4.5 Electrophoresis of haemolysates on cellulose acetate (pH 8.5). The arrow marks the site of application of the haemolysate. (1) Normal adult. (2) Individual with sickle-cell trait; 35% of the Hb consists of HbS and most of the remainder is HbA. (3) Patient with sickle cell anaemia; most of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

1) HbS - Found in Sickel 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.

Uncharged (valaine) > hydrophobic AA. | Charged (Glu.) > hydrophilic AA

EXTRA



- 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.**
- Recurrent painful crises (in adult) and chronic haemolytic anemia, both related to sickling or red cells on deoxygenation.
- **Sickle Cell Trait (Mild):** once you see Trait = Mild
  - Heterozygotes (one gene for normal β-globin and one for βS) are described as having sickle cell trait. Their red cells contain between 20% and **45%** HbS, the **rest being mainly HbA.**
  - 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%.

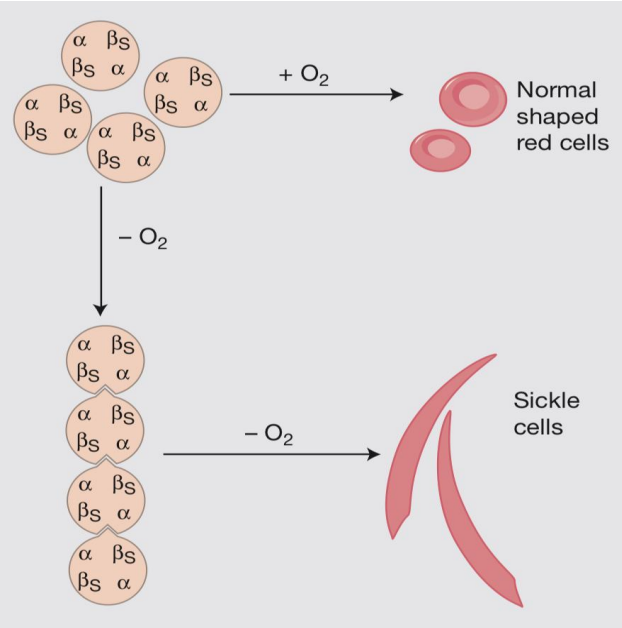
Structural Hemoglobin Variant

Osmosis

1) HbS - Found in Sickel Cell pts

Sickel Cell Anemia or Disease

- 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, ect).
- Homozygous 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.
- 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 and 9g/dL.**



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

Figure 7.14 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.

EXTRA Pictures from book

Structural Hemoglobin Variant

Osmosis

Sickel Cell Anemia or Disease

Diagnosis

- Sickled cells are invariably present on the blood films of patients with **HbSS**.
  HbSS is made by finding:
  - ❏ A positive result with a screening test for HbS (Sickle solubility test)
  - ❏ A peak at an appropriate position on an HPLC trace, confirmed by isoelectric focusing hemoglobin electrophoresis
- 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 (Figure 4.8) and painful swelling of the hands and feet.
- 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**.

Hand-foot syndrome

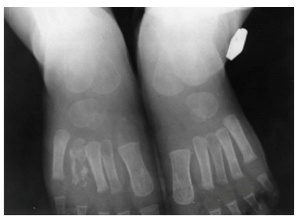


Figure 4.8 An X-ray of the feet of a child with sickle cell anaemia two weeks after the onset of hand-foot syndrome, showing necrosis of the right fourth metatarsal.

Chronic Ulcer

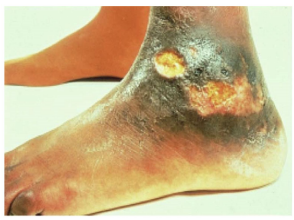


Figure 4.9 A chronic leg ulcer with increased pigmentation of the surrounding skin in a woman with sickle cell anaemia.

Treatment

1. Management of the increased infection risk by **immunization**.
2. Administration of Folic acid daily to prevent secondary folate deficiency.
3. Avoidance of factors precipitating painful crises such as dehydration,hypoxia, and circulatory stasis
4. Blood transfusion when necessary
5. 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.
6. Treatment of painful crises with oral or intravenous fluids and analgesics, including opiates and NSAID when necessary

Structural Hemoglobin Variant

Osmosis

2) HbE & HbC

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

HbE

Very common in south-east Asia (being found in about 50% of the population in some parts of Thailand). Caused by single point mutation in the β chain. At **position 26** there is a change in the amino acid, from **glutamic acid to lysine**

HbC

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**, with a resulting reduction in the flexibility of the red cell and a reduction in its survival. (120 days is normal)

- 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)**

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).

Sickle cell anemia

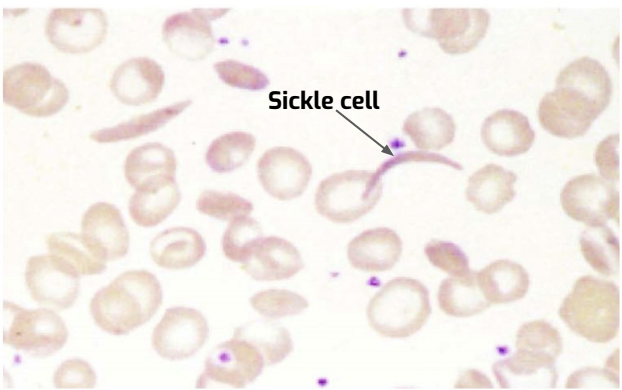


Figure 4.7 Two sickle-shaped red cells with pointed ends and some partially sickled red cells from the blood film of a patient with sickle cell anaemia (homozygote for HbS).

Target cell in HbC

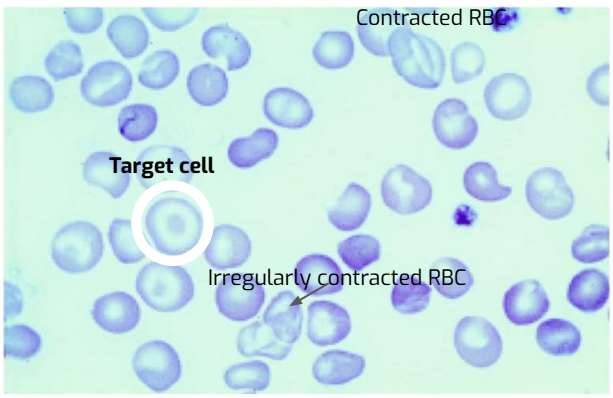


Figure 4.10 Target cells and irregularly contracted cells in the blood film of a homozygote for HbC.

Target cell distincts all hemoglobin abnormalities

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**	Hemoglobin H	-	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

You have to know first two types only

Table 4.1 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

Summary

Normal Structure of Hemoglobin

- **α globin chains encoded on on chromosome 16 (4 alleles)**
- **β globin chains encoded chromosome 11 (2 alleles)**
- HbF has a higher oxygen affinity than the adult hemoglobins
- An imbalance between the production of α and β chains is the pathophysiological basis of the thalassemias (a quantitative issue).

Adult			Embryo		
HbA	**2α**	**2β**	Gower 1	2ζ	2ε
HbF	2α	2γ	Gower 2	**2α**	2ε
HbA2	2α	2δ	Portland	2ζ	2γ

Thalassemias

α-Thalassemia

due to gene **deletion** of alpha chains encoded on **chromosome 16**

1.α+-Thalassemia trait deletion of 1 α globin gene	**Asymptomatic** individuals with minor hematological changes (slight reduction in MCV + MCH) **Individual inherits:** • α+-thalassemia allele from one parent • a normal ch 16 from the other parent (i.e. heterozygotes for the α+ determinant).
2. α0-Thalassaemia trait: deletion of 2 α-globin genes in same allele	Hb: normal or slightly reduced, MCV and MCH are low. However, RBC count is elevated and RDW is not affected.
3.Hemoglobin H disease: deletion of 3 α-globin genes	• Very low α-chain but high β-chains → impaired pairing of α-chains with β-chains→ free β-chains combine to form tetramers (β4). This tetramer is known as HbH. • **Findings**: 1. moderate, mild anemia of 7-11g/dl and markely **hpochromic, microcytic** indices. 2. **Supravital staining used for diagnosis of the blood film demonstrates cells with many HbH inclusions, giving a characteristic 'golf-ball' appearance.** 3. Most patients will be transfusion independent 4. Splenomegaly is seen in most patients.
4. Hb Bart's Loss of all 4 α-globin genes	**Known as Hydrops fetalis syndrome.** No α-chains can be formed, impaired pairing of alpha and gamma chains, so γ- globin forms tetramers known as Hb Bart's. Impaired oxygen transport, there is intrauterine or neonatal death due to hydrops.

Summary cont.

Thalassemias

β-Thalassemia
due to gene **mutation** of beta chains encoded on **chromosome 11**

Results from a **multiplicity** of different single nucleotide substitutions, insertions or small deletions affecting the β-gene itself or occasionally in promoting regions

Heterozygous β-thalassemia (Beta-thalassemia trait)	Homozygous β-Thalassemia
• **asymptomatic** • Hb concentration is either normal or slightly reduced, hypochromic and microcytic RBCs. • **target cells and poikilocytes** on peripheral blood film • **HbA2** levels will be **raised** above the normal range to 3.5- 7.0%. • Slightly increased **HbF** levels, in the range of 1-5%.	• Severe form • Defects of β-globin on both copies of chromosome 11 • Marked anemia • **Transfusion dependent**

Structural Hemoglobin Variant

Hemoglobin S Found in Sickle Cell pts	• Sickle β-globin chains: 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. • HbS: interaction of sickle β-globin chains with normal α-globin chains forms HbS. HbS causes deformation of RBC biconcave shape to sickle shape.
Sickle Cell Trait (AS)	Heterozygotes (one gene for normal β-globin and one for βS), usually asymptomatic. Contain between 20% and 45% HbS, the rest being mainly HbA. HB electrophoresis differentiates between sickle cell trait and sickle cell disease.
Sickle Cell Anemia (Disease) (SS)	• Copy of beta globin being S and another beta harbor any mutations. • (Homozygotes) • almost exclusively HbS and NO HbA more than 50% HbS • Sickled RBC ps occlude the micovasculature with poor downstream perfusion and oxygenation > they mey be lysed directly in the circulation • The Hb usually varies between 6 and 9g/dL. • Associated with ankle ulcers.
Hemoglobin E and C	• Both result from single amino acid substitutions in the β-chains. • 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).

Quiz

***The questions which are written by the red, were mentioned in Dr's 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 level 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 **NOT** a feature of thalassemia intermedia ?							
A	It may be due to homozygous βo thalassemia without coinheritance alpha thalassemia.	B	It may be associated with extramedullary hemopoiesis	C	It is usually associated with splenomegaly	D	It may 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
Q7) Sickle cell **trait** contains : IF the questions says **Trait** (A) will be correct . **BUT** IF the question says **Disease** (C) is correct . **Why ?** Because **Disease** means a percentage more than **50%**							
A	20%-45% HBS	B	20%-45% HBC	C	70% HBS	D	70% HBC
Q8) Which of the following gene deletion lead to hemoglobin H disease ?							
A	3 α genes deletion in chromosome **11**	B	3 α genes deletion in chromosome **16**	C	2 β genes deletion in chromosome **11**	D	2 β genes deletion in chromosome **16**
Q9) Which ONE of the following conditions has the highest HbF levels ?							
A	Hemoglobin H disease	B	Alpha thalassemia trait	C	Beta thalassemia minor	D	Beta thalassemia major

Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9
B	C	B	D	A	B	A	B	D

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Summary

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