2012

KING SAUD UNIVERSITY
COLLEGE OF MEDICINE
HAEMATOLOGY TEAM
GIT BLOCK

Haematology Block

Lecture 4

Thalassemia

Mohanned Al Essa

Thalassemia

Is an inherited (homozygous or heterozygous) blood disorder passed down through in which the body makes an abnormal form of hemoglobin. The disorder results in excessive destruction of red blood cells, which leads to anemia.

Occurs when there is a defect in a gene that helps control production of one of haemoglobin proteins.

It is one of the most common genetic haematological diseases all over the world.

α-Thalassemia

Are gene defects affecting the production of the α globin protein (α chain).

These are usually caused by gene deletions.

It's most commonly found in Jezan, Sharqeya, Khaiber, and Al Ula.

As there are normally four copies of the α-globin gene, the clinical severity can be classified according to the number of genes that are missing or inactive into four groups :

1) Silent Carrier

- Loss of one gene.
- No sign for illness.

2) α-Thalassemia Trait

- Loss of two genes.
- Have two types: Asian and African.
- Usually not associated with anemia, although (MCV) and (MCH) are low.
- Red blood cell count is over (5.5) - *Increased*.
- Hb A, Hb A2, Hb F will be slightly decreased.

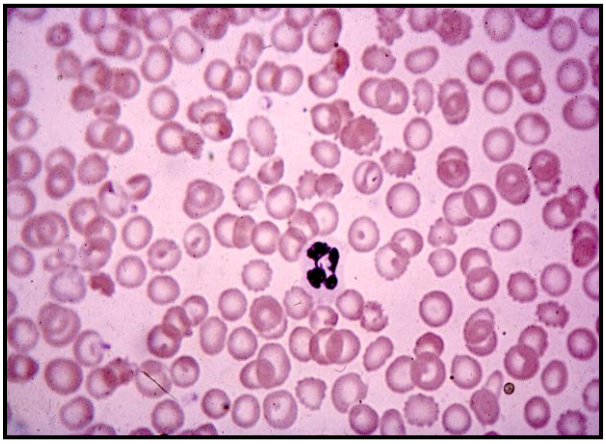
3) Haemoglobin H Disease (Hb H)

- The absence of 3 genes.
- A clinical disease that needs treatment and regular blood transfusion.
- Moderately severe (Hb 7-11) microcytic, hypochromic anemia.
- Patients have splenomegaly and sometimes mental retardation.
- Haemoglobin H (β4) can be detected in red blood cells.

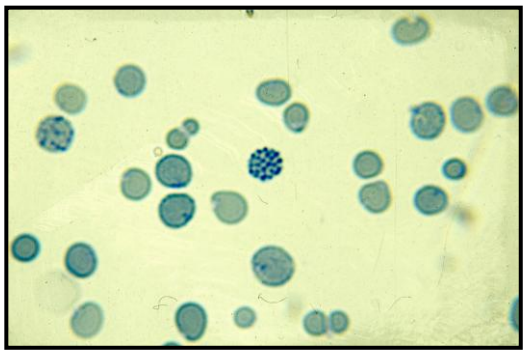
4) Hydrops Fetalis

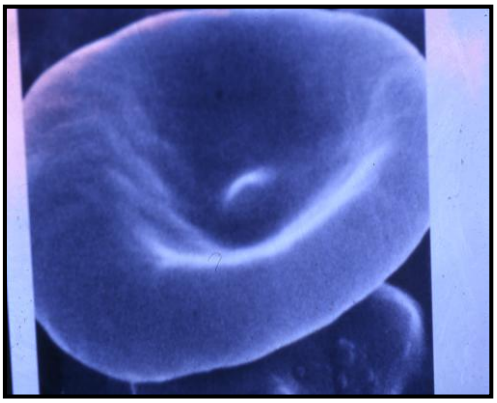
- Loss of all 4 genes.
- Severe anemia → leads to heart failure → edema and ascites.
- Hb Barts (γ4) will be present.

Laboratory Findings and Morphological Features



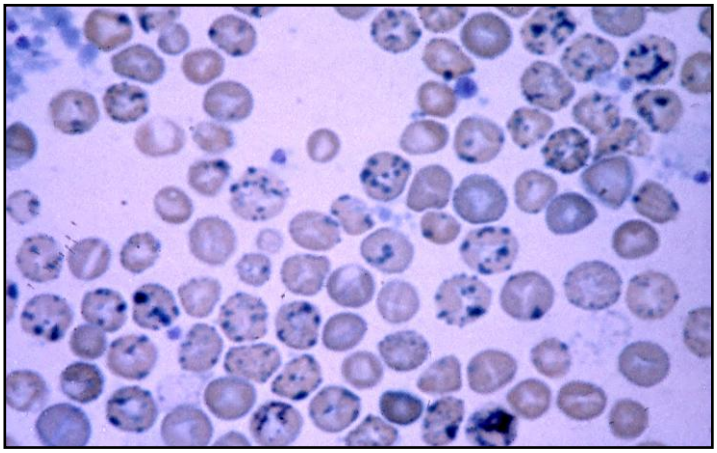
BLOOD FILM MORPHOLOGY:
- *Hypochromic microcytic RBCs*
- *α thalassemia*



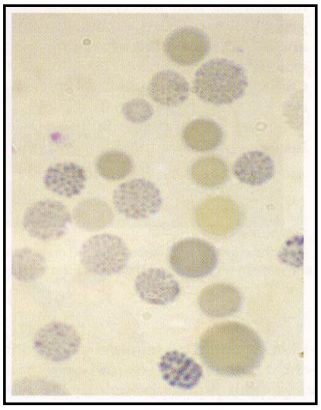


BLOOD FILM MORPHOLOGY:
- *Golf Ball Appearance*
- *Supravital stain*
- ***Indicates Hb H disease***

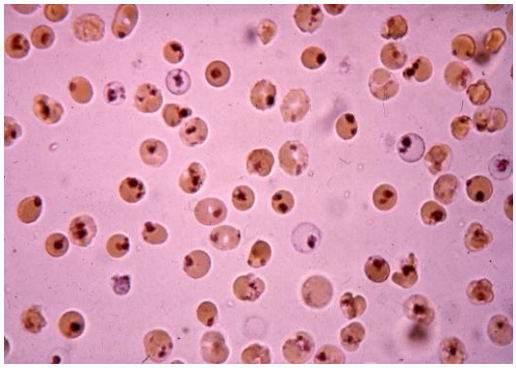
ELECTRON MICROSCOPE
- *Bull's-eye appearance of RBC*
- *Target Cells*



BLOOD FILM MORPHOLOGY:
- *Hb H Disease*
- *3 alpha deletion*



BLOOD FILM MORPHOLOGY:
- *Reticulocytes: immature RBCs that appear because of stress on the bone marrow* ***(hemolytic disease)***
- ***Supravital stain***



BLOOD FILM MORPHOLOGY:
- *Appearance of* ***Heinz bodies****.*
- *found by methyl v. stain*

β-Thalassemia

Are gene defects affecting the production of the β globin protein (β chain).

β-Thalassemia is generally divided into four major groups depending on the molecular defect :

1) **β^{+}-Thalassemia**: Small amounts of β chain are synthesized (β+).
2) **β^{0}-Thalassemia**: No β chain synthesis (β^{0}), has two types: Ferrara Variant & Indian Variant.
3) **δβ-Thalassemia**: The absence of δ gene & β gene.
4) **HPFH (Hereditary Presentence of Fetal Haemoglobin):** Characterized by life-long synthesis of γ gene (High Hb F) to compensate the absence of β gene.

	β-Globin synthesis	β^{-} mRNA	β-Globin Gene	δ-Globin Synthesis	γ-Globin Synthesis
β^{+}-Thalassemia β^{0}-Thalassemia	Decreased Absent	Decreased Absent	Present Present	Present Present	Present Present
Ferrara Variant Indian Variant	Absent Absent	Inactive Absent	Present Partially Deleted	Present Present	Present Present
δβ -Thalassemia HPFH	Absent Absent	Absent Absent	Deleted Deleted	Absent Absent	Increased Increased

NORMAL: *We have two normal β genes, one each chromatid from the mother and father.*

We also can divide β-Thalassemia in four groups according to the severity of the clinical manifestations:

1) Thalassemia Major

- Occurs in one of four offspring if both parents are carriers of the β Thalassemia trait.
- The majority of genetic lesions are point mutations rather than gene deletions.
- Either no β chain (β^{0}) or small amounts (β+) are synthesized, thus, Hb A is absent.
- There is an excess α chains production precipitate in red blood cells causing haemolysis.
- Production of (γ) chains helps to 'mop up' excess α chains and to decrease the severity of this condition, thus, Hb F is increased.

2) Thalassemia Intermedia

- This is a syndrome that is characterized by moderate anemia (Hb 7.0-10.0).
- Patients don't need regular blood transfusion.
- Caused by a variety of genetic defects:
  - Homozygous B thalassemia with increased Hb F.
  - Haemoglobin lepore.

LEPORE: *Abnormal hemoglobin caused by the crossing over and fusion of beta (β) and delta (δ) genes, result in partial deletion of both genes.*

3) Thalassemia Minor (Trait)

- Patients will have one normal β gene and the other one is defected.
- Common, usually symptomless.
- Hypochromic, microcytic blood cells (MCV and MCH are very low).
- High red blood cell count (>5.5) and mild anemia (Hb 10-12).
- Increased Hb A2 (>3.5%).
- If both parents carry β thalassemia trait there is a 25% risk of a thalassemia major child.

4) Thalassemia Minima

- There is a normal β gene and the other one is slightly deleted.
- Very mild or no anemia.

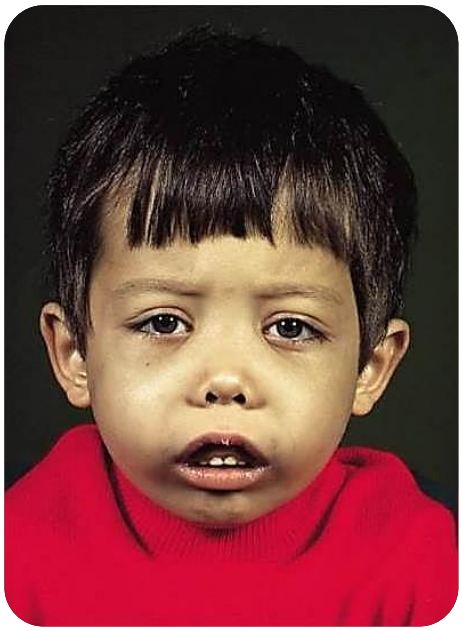
Clinical Manifestations

- These clinical manifestations are found in β-Thalassemia Major and Inermedia.
- Usually β-Thalassemia Minor patients are asymptomatic unless they are under stress : Infections, Pregnancy, Surgery.

- **Anemia:** Appears in 3-6 months after birth (After switching from γ chain to β chain).
- **Pallor**
- **Jaundice:** Due to hemolysis.
- **Apathy & Anorexia**
- **Failure to Thrive**
- **Hepatomegaly – Splenomegaly:** Due to hemolysis.
- **Skeletal Deformity & Bone Expansion:** Due to intense marrow hyperplasia leads to a thalassemic facies, board maxillary bone, frontal boozing of the skull, very thin bones, hair on end appearance on X-Ray.
- **Iron Overload:** Because of the regular blood transfusion.

THALASSEMIA MAJOR: *Severe Anemia*
THALASSEMIA INTERMEDIA: *Moderate Anemia*
THALASSEMIA MINOR: *Mild Anemia*
THALASSEMIA MINIMA: *Very Mild Anemia*

BONE HYPERPLASIA: *Occurs as a result of the increase demand on RBCs, so the whole bone morrow will become hyper-active.*



β-THALASSEMIA MAJOR FACIAL APPEARANCE:
Enlarged maxilla, Frontal boozing of the skull

Clinical Features According to the Severity of the Condition and Types

	Major	Intermedia	Minor	Minima
Severity of Manifestations	++++	++	+, ±	±, 0
Genetics	**Homozygotes, Double heterozygotes**	**Homozygotes, Double heterozygotes, Rarely heterozygotes**	**Heterozygotes**	**Heterozygotes**
Splenomegaly	++++	++,+++	+,0	0
Jaundice	+++	++,+	0	0
Skeletal changes	++++,++	+,0	+,0	0
Anemia (Hb, g/dl)	**<7**	**7 – 10**	**>10**	**Normal**
±, little or no abnormality; +, mild abnormality; ++++, prominent abnormality				

Laboratory Diagnosis

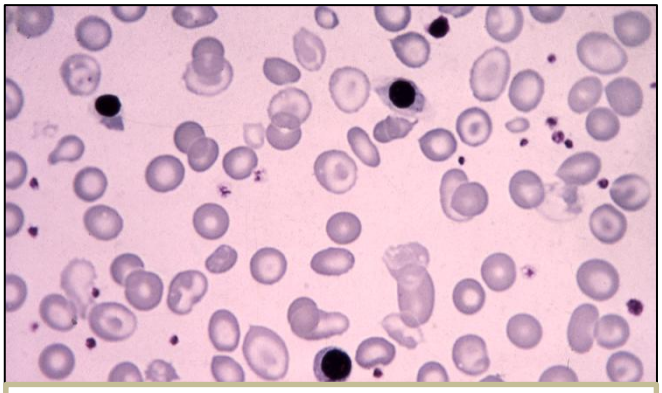
- These laboratory findings are mostly found in β-Thalassemia major.

1) Blood Film :

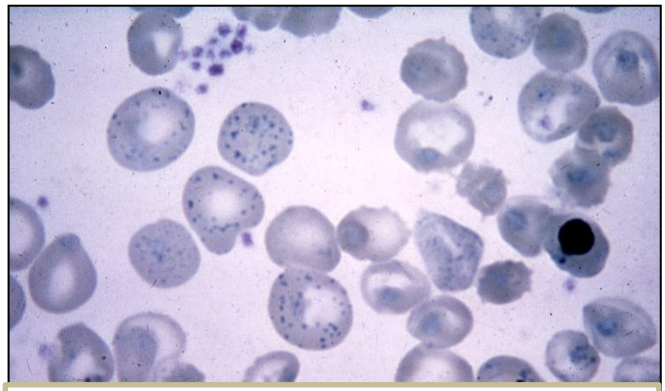
- There is a severe hypochromic, microcytic anemia.
- Raised reticulocyte percentage.
- Target cells and basophilic stippling (due to accumulation of RNA).
- Nucleated red blood cells.

2) Haemoglobin Electrophoresis

- Is now used as first line method to diagnose haemoglobin disorders.
- It's used to indicate the levels of different types of Hb and the presence of abnormal Hemoglobins to identify different types of diseases.
- Genetic study (DNA analysis) is used also to identify the defect on each allele.



BLOOD FILM PICTURE OF β-THALASSEMIA MAJOR:
Nucleated red blood cells and many target cells



BLOOD FILM PICTURE OF β-THALASSEMIA MAJOR:
Dots inside the cell participate extra α chain production

Laboratory Findings and Morphological Features

	Major	Intermedia	Minor	Minima
Hypochromia	++++	+++	++	+
Microcytosis	+++	++	+	**0**
Target cells	**10 – 35%**	++	+	±
Basophilic stippling	++	+	+	**0, +**
Reticulocytes (%)	**5 – 15**	**3 – 10**	**2 – 5**	**1 – 2**
Nucleated red cells	+++	**+, 0**	**0**	**0**
±, little or no abnormality; +, mild abnormality; ++++, prominent abnormality				

β-Thalassemia is more common than α-Thalassemia in KSA, especially in Najran, Sharqeya, and Al Ula.

Prenatal Diagnosis of the Haemoglobinopathies (Including Thalassemia)

- Important when both partners show an abnormality and there is a risk of a serious defect in their offspring.
- Several techniques are available, the choice depending on the stage of pregnancy and the potential nature of the defect :

If both partners carry β-Thalassemia trait, they shouldn't marry in KSA.

DNA Analysis

- Samples to be analyzed are obtained by different ways :
  1. Chorionic Villus Sampling: By Transcervical approach (9 – 11 weeks of pregnancy) or Transabdominal approach (up to 15 weeks)
  2. Amniotic fluid cell analysis (16 – 20 weeks gestation)
  3. Fetal blood sampling (> 20 weeks gestation): Include DNA analysis, Haematological parameters, and Biochemical & globin chain analysis.
- **DNA analysis** is done by using one of the following methods :
  - Gene mapping
  - Restriction fragment length polymorphisms (RFLPs) linkage analysis
  - Oligonucleotide probes
  - Gene amplification: DNA polymerase chain reaction technique (PCR).

Summary

- Thalassemia is one of the most common genetic haematological diseases all over the world.

α-Thalassemia

- α-Thalassemia: are gene deletions related to α globin protein (α chain).
- α-Thalassemia is most commonly found in Jezan and Sharqeya.
- **α-Thalassemia trait:** No anemia, MCV & MCH are low and RBCs are high.
- **Haemoglobin H Disease:** Moderate microcytic hypochromic anemia, patients suffer from splenomegaly, Hb H (β4) can be detected.
- **Hb H Disease:** When stained *Supravital Stain* → Golf Ball Appearance.
  When stained *Mythl V. Stain* → Appearnce of Heinz bodies.
- **Hydrops Fetalis:** Loss of all 4 genes → death in utero, Hb Barts (γ4) is found.

β-Thalassemia

- β-Thalassemia is a gene defect related to β globin protein (β chain).
- Is divided into four types: **β^{+}-Thalassemia, β^{0}-Thalassemia, δβ-Thalassemia, HPFH.**
- **HPFH:** High Hb F.
- **β-Thalassemia Major:** Point mutations, absence of Hb A, excess a chain production, Increased Hb F.
- **β-Thalassemia Intermedia:** Moderate anemia, no need for regular blood transfusion, caused by genetic defects (e.g. Haemoglobin Lepore).
- **β-Thalassemia Minor:** Common, asymptomatic, hypochromic (↓ MCH) microcytic (↓ MCV) mild anemia, increased RBCs, A2 is high.
- Clinical Manifestations are found in β-Thalassemia Major and sometimes Intermedia.
- **Symptoms:** Anemia, Skeletal Deformity, Hepatomegaly, Splenomegaly, Iron Overload.
- **Skeletal Deformity:** Board maxilla, Frontal boozing, Hair on end appearance on X-Ray.
- **In Blood Film:** Target cells and Nucleated red cells are found in β-Thalassemia Major**.**
- Haemoglobin Electrophoresis is now used as first line method for diagnosis.
- β-Thalassemia is more common than α-Thalassemia in KSA, especially in Najran, Sharqeya.
- Best way to obtain a sample: Chorionic Villus Sampling.
- Best method of DNA analysis: DNA polymerase chain reaction technique (PCR).