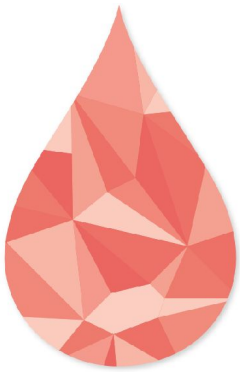


3- Haemoglobinopathies = Haematology 435 =

إن أحسنا فمن الله عزوجل, وإن أخطأنا فمن أنفسنا والشيطان.
نظراً لشمولية العمل لمحاضرات الطلاب والطالبات بإمكانكم اعتماده كمصدر للمذاكرة.

Objectives:

- To understand the normal structure and function of haemoglobin
- To understand how the globin components of haemoglobin 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.

➢ **Color Codes:**

- Pink: Girls' notes. Blue: Boys' notes. Red: Important Notes. Gray: Extra notes.
- Purple: Lecture notes & Pathoma notes.

➢ **Done by:** Samar AlOtaibi.

➢ **Members:** Raghda Alqassim - Abdulaziz Alshalan - Rifan Hashim - Nouf AlRushaid - Kowthar Almousa Lulu Yousef.

This Lectures is only 9 Slides the rest are Extra Just read it!

➢ **References:**

- Girls&Boys Doctors Slides and Notes.
- Lecture notes pathology (chapter 12)
- Pathoma (chapter 5)
- Team 434 & 433.

➢ **Correction file:** (HERE)

➢ **Check Your Understanding!** (HERE)

(Pathology Lecture Notes. P100) You can skip this!

- **Thalassemia Syndrome:** (are quantitative, not qualitative , abnormalities of Hemoglobin)

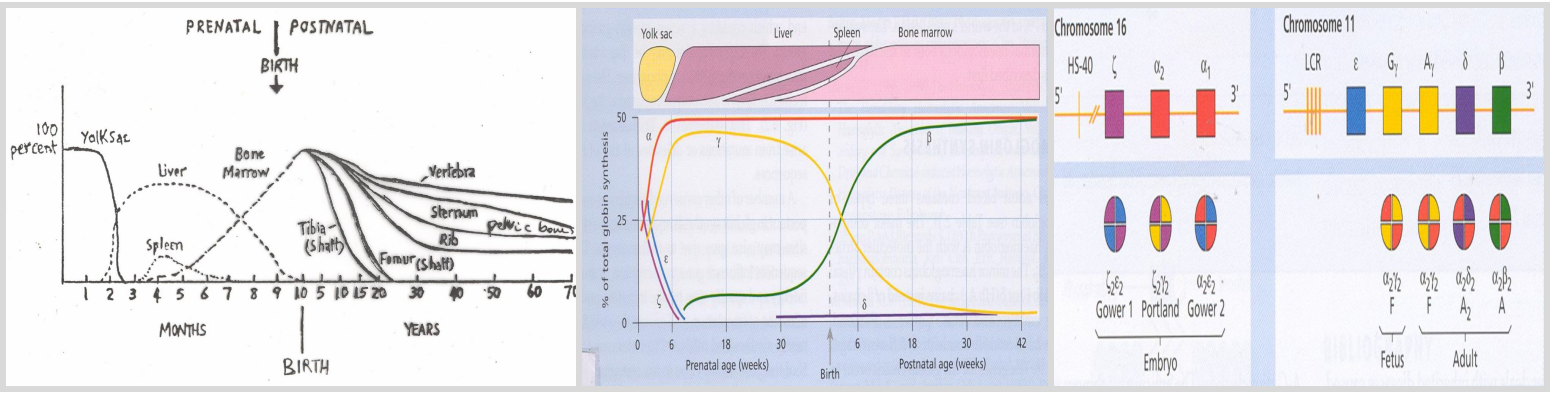
α - Thalassemia:

- There are a total of 4 **α-globin** chain genes, 2 from each parent.
- It depends upon the number of genes that affected.
- **α** chains are normally expressed Prenatally & Postnatally → (Prenatal & Postnatal diseases)
- In normal individuals 4 **α** genes (**αα/αα**) = 100% normal.

β - Thalassemia:

- **β** There are a total of 2 **β-globin** chain genes.
- **β** chains are normally expressed Postnatally → (Postnatal diseases - No Prenatal)
- The damage mainly by "Point mutation"
- Some **β** chains (**β** +) or None (**β**0)

α - Thalassemia	β - Thalassemia
Silent carrier state: - 1 deletion is present. 3 (-α/αα) - 75% of the normal α chains - Asymptomatic. - Normal lab tests.	**β - Thalassemia minor:** - 1 β-globin chain is damaged. - Asymptomatic. - Lab tests (↑ Hb A1 - ↑ Hb F)
α - Thalassemia trait = Mild: - 2 deletion is present. - Cis 2 (- -/αα) seen in Asians. - Trans 2 (- α/- α) seen in African Americans. - 50% of the normal α chains - Offspring don't develop hemoglobin H disease or Hydrops fetalis)	**β - Thalassemia Intermedia:** - Has a Severe Anemia. - But, no transfusions are needed.
Hemoglobin H disease: **The only one Seek for treatment** - 3 deletion is present. 1 (- -/-α) - 25% of the normal α chains - Increase Hb H. - Forms Heins bodies (In crystal blue stain).	**β - Thalassemia major:** - Called (Cooley anemia) - Patients normal at birth, and symptoms develop at about 6 months as "Hemoglobin F levels decline"
Hydrops fetalis: - 4 deletion is present. 0 (--/--) - 0% of the normal α chains - Lethal in utero.	-



First image:

Each type of hemoglobin has a specific time of synthesis.

1- EMBRYONIC HB:

-Time :the first 6-8 weeks of pregnancy (prenatal)

-location :formation of hemoglobin occurs in the yolk sac

2- FETAL HB:

-location : liver and spleen

3-ADULT HB:

-location: bone marrow

-Note that sometime before birth ,the liver and spleen begin to shut down so that the HB synthesis occurs in bone marrow.

-This is the site of HB synthesis until death!

(so ,adult HB formation begins before birth until death)

Where exactly?

-Vertebra

-Sternum

-Pelvic bone

(these three until death)

-Ribs

-femur *(till 25)* and tibia *(till 20)*

Second image : (Helpfull Video)

-Note that **α** chain starts from the beginning of embryonic life till death(continuously synthesized)

-Epsin ε and Zeta :formed in yolk sac in embryonic life in the first 8 weeks only,then they stop.

If they continue =disease manifestation.

-Gamma: from liver and spleen ,decrease in adult life.

-Beta: starts in embryonic life and continues until death.

-Delta: starts from 30 weeks and continues till death.

Third image:

-It is important to know the types of embryonic HB:

(gower 1,portland,gower 2)

-these do not emerge except in embryonic life.

-Chromosome 16 codes for embryonic HB (for **α** and zeta globin chains)

-The normal adult person has :

Mainly HBA ,HBA2 ,and very little amount of fetal HB.

-fetus only has: FETAL HB.

-chromosome 11 codes for all other globin chains (beta ,gamma, epsin..)

Hemoglobinopathies = abnormal Hb chain. Thalassaemia = deficiency of Hb chain .

Thalassemia occurs due to abnormal ratio of globin chains.

Hemoglobin "Khan Academy 14:33" Hemoglobin function "14;57"

❖ Hemoglobin types:

NAME	CHAINS	
Haemoglobin A	α2	β2
Haemoglobin A2	α2	δ2
Haemoglobin F	α2	γ2
Haemoglobin H	-	β4
Haemoglobin Bart's	-	γ4

❖ The haemoglobins present at birth in normal newborn:

Name	%
HbA	15-40
HbA2	< 0.3
HbF	60-85
Hb Bart's	< 0.5

❖ The normal human haemoglobins:

EMBRYONIC (Upto 8 Weeks gestation)	Fetal	Adult "Doctor Fatma said only know the saudi community"	
ζ2 Ɛ 2 Hb Gower I ζ 2 γ2 Hb Portland α2 Ɛ2 Hb Gower II	α2 γ2 HbF 60 - 85% α2β2 HbA 15 - 40%	**Caucasian:** α2 β2 HbA → 97.0% α2δ2 HbA2 → 2.5% α2 γ2 HbF → 0.5%	**Saudi:** 95.0% 3.5% 1.5%

-Normal alpha chain is made up **141** amino acid
-Normal beta chain is made up of **146** amino acid

- Some Types of HB the doctor talked about:

-HB H: has four beta chains.
-HB bart's: four gamma.
-HB lepore: (2 alpha+2 (delta beta))

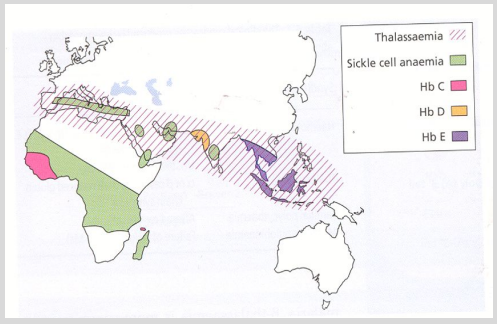
❖ α and β THALASSAEMIA "Overview":

-Decrease in the number of **alpha** chains leads to **alpha** thalassemia.
-Decrease in the number of **beta** chains leads to **beta** thalassemia.

- ➢ The thalassaemias are divided into two main groups, the α- thalassaemias and the β- thalassaemias, depending on whether the defect lies in the synthesis of α- or β-globin chains respectively.
- ➢ The pathophysiology reflects the impact of an imbalance in the expression of α and β globin chains.
- ➢ 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).
- ➢ The resulting anaemia **leads to an increased erythroid drive.**
- ➢ There is further expansion of the marrow into bones not typically used for haemopoiesis (for compensation), and into the spleen.
- ➢ **The long-term consequences of thalassaemia:** therefore include:

- Splenomegaly (also due to compensation,(the spleen tries to make up for the defective hematopoiesis).
- Bony deformities.
- Iron excess as well as chronic anaemia.

Notes: Excess iron because of repeated blood transfusions given to a patient suffering from thalassemia ,so with each blood transfusion more iron is acquired!
-Where is thalassemia most commonly found :1-mediterranean region 2-ksa 3-far east(philippines ,indonesia ,taiwan)
-Thalassemias are the most common haemoglobinopathies in the world.



❖ α -THALASSAEMIA:

- HETEROZYGOUS "Not severe"
- HOMOZYGOUS "Very severe"

- **(α+)-Thalassaemia trait (deletion of one or two α globin genes):**

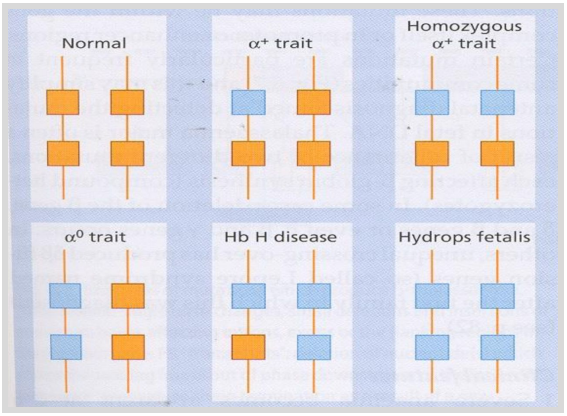
- This is seen when an individual inherits the (α+)-thalassaemia allele from one parent or two parents and a normal chromosome 16 from one or two parents (i.e. heterozygotes for the (α+) determinant or homozygous (α+) trait).
- Affected individuals are asymptomatic, although they have minor haematological changes such as slight reductions in **mean cell volume (MCV)** and **mean cell haemoglobin (MCH).**

Notes:

- Normal alpha chain should have two copies from mother and two from father, but normal beta chain only requires one copy from each parent!

Explanation of pic:

1- (Alpha+ trait) :only one copy is missing while other three are present (mild) ,so in this case ,only one parent is affected!
2- (Homozygous alpha + trait) : each parent has 1 absent copy (so both parents are affected)
3- (Alpha zero trait) : one parent gives 2 copies while the other gives zero.
4- (Hemoglobin H) : is moderate to severe has only one normal copy(deletion of other 3)
5- (Hydrops fetalis) : all 4 copies are absent.

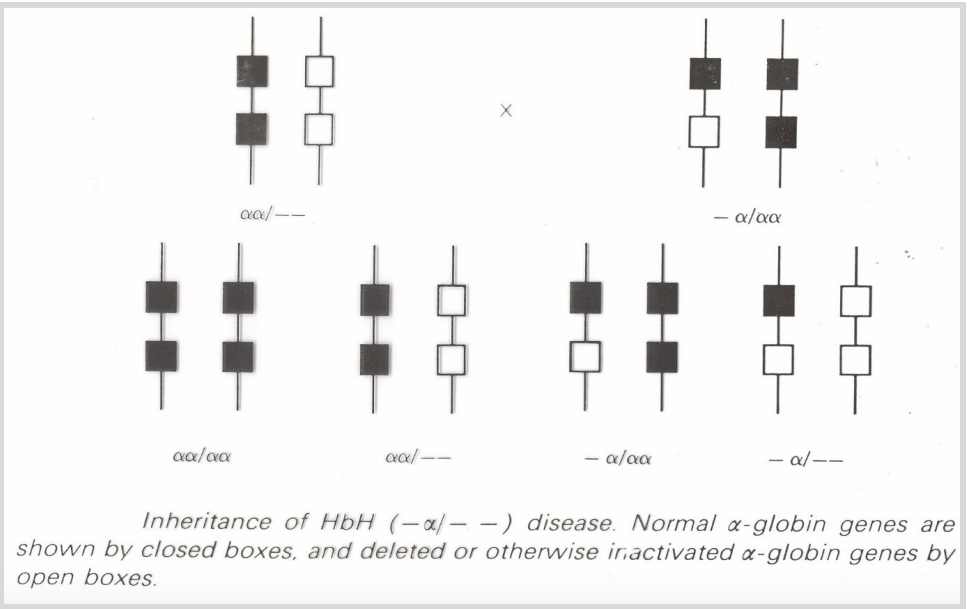


- **(α0)-Thalassaemia trait (deletion of both α-globin genes on one chromosome 16):**

- The **Hb** is either normal or slightly **reduced** and the **MCV and MCH are low.**

❖ Haemoglobin H disease (Deletion of Three α -globin genes):

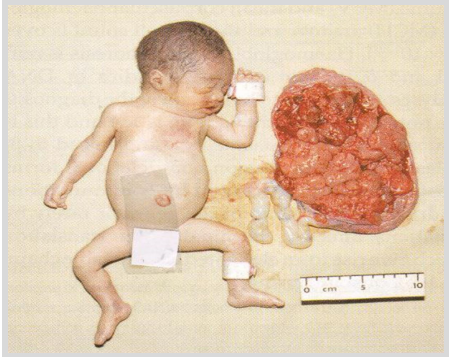
- This **chronic haemolytic anaemia** results from the inheritance of both the *(α+)- and (αo)-thalassaemia alleles*, leaving one functioning α-globin gene per cell.
- α-globin chains are produced at very low rates, leaving a considerable excess of β-chains, which combine to form **tetramers (β4).**
- This tetramer is known as **Haemoglobin H. Seek for treatment .**
- *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.*
- Most patients are moderately affected, **with anaemia of 7-11g/dl and markedly hypochromic, microcytic indices.**
- Supravital staining 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.



Inheritance of HbH (−α/− −) disease. Normal α-globin genes are shown by closed boxes, and deleted or otherwise inactivated α-globin genes by open boxes.

❖ Hb Bart's hydrops fetalis syndrome (deletion of all four α-globin genes):

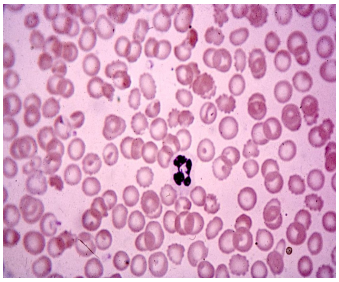
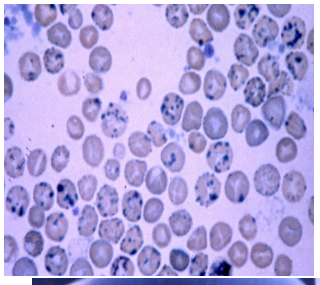
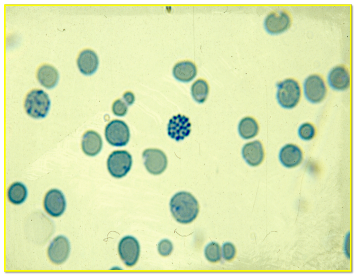
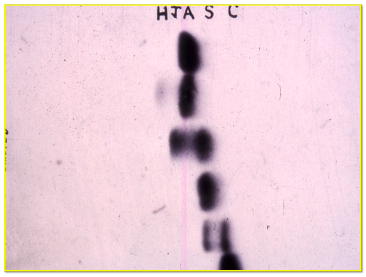
- **No α-chains** can be formed, and the **fetal β- like chain γ-globin forms tetramers known as Hb Bart's** (So bart's =4 gamma).
- This haemoglobin is *not useful for oxygen transport* and, despite the persistence of the embryonic haemoglobin Hb Portland (ζ2γ2), there is intrauterine or neonatal death due to **hydrops.**

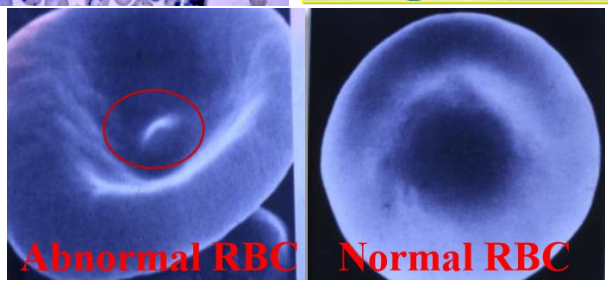


Patient will not seek for treatment because either die before birth or immediately after.

❖ LABORATORY DIAGNOSIS OF ALPHA THALASSEMIA SYNDROME:

- High red cell count in the trait.
- **Hypochromic microcytic red cells** & **target cells.**
- Normal serum iron or low in children.
- Normal total iron binding capacity or high in children.
- Positive Hb H inclusion bodies in the blood film preparation & positive Heinz bodies with vital stains.
- Hemoglobin electrophoresis show presence of hemoglobin H (Hb H disease).
- Hemoglobin electrophoresis show low Hb A2 level.
- Genetic study to confirm the diagnosis.



❖ β-Thalassaemia:

- The World Health Organization estimates that **1.5% of the world's population are carriers of β-thalassaemia**.
- The prevalence of the β-thalassaemia trait is particularly high in southern Europe (10-30%) and southeast Asia (5%), common in Africa, the Middle East, India, Pakistan and southern China.[1]

- **α-thalassaemia** typically arises from gene deletions.
- **β-thalassaemia** usually results from a multiplicity of different single nucleotide substitutions, insertions or small deletions affecting the β-gene itself.

❖ Heterozygous & Homozygous β-Thalassaemia:

Heterozygous β-thalassaemia (Beta-thalassaemia trait):	Homozygous β-Thalassaemia:
• Most affected subjects with beta thalassaemia 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**. • HbA2 levels will be raised above the normal range to 3.5-7.0% (classical finding). (The most important) • Slightly increased HbF levels, in the range of 1-5%. • **Electrophoresis** can be used to diagnose beta thalassemia.	• Defects of β-globin on both copies of chromosome 11 • **Marked anaemia.** • **Transfusion dependent.**

[1] **الثلاسيميا** وتسمى أيضاً **فقر دم حوض البحر الأبيض المتوسط** مرض وراثي يؤثر على كريات الدم الحمراء وينتشر في منطقة حوض البحر الأبيض المتوسط.

❖ Clinical classification of the thalassaemias:

1)Thalassaemia minima:

- Describes the presence of α -thalassaemia mutation that is **without clinical consequences.**

2)Thalassaemia minor:

- Describes patients with microcytosis and hypochromic red cells secondary to thalassaemia mutations, but with only mild anaemia or a normal haemoglobin.
- Patients who inherit a single affected allele are usually in this category. (Heterozygous β-thalassaemia).

3)Thalassaemia intermedia:

- Patients will also have a microcytic hypochromic anaemia.
- **Increased erythroid drive**[2] to maintain their haemoglobin
- **Packed bone marrow** with a decreased myeloid:erythroid ratio.
- Extramedullary haematopoiesis, giving splenomegaly[3].
- **Transfusion may** be required to maintain the haemoglobin at times of additional physiological stress.
- (In thalassemia intermedia both HBA2 and HbF are increased)

4)Thalassaemia major:

- Have severe anaemia 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 chromosome 11. (Homozygous β-Thalassaemia)
-

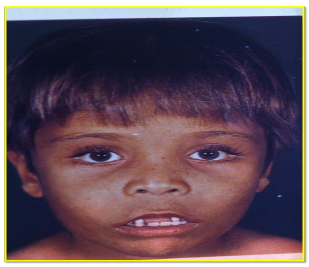
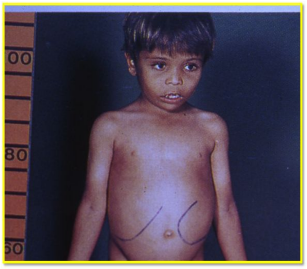
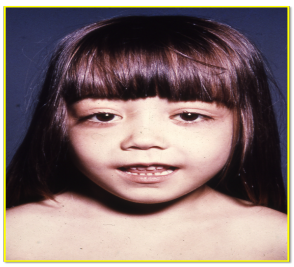
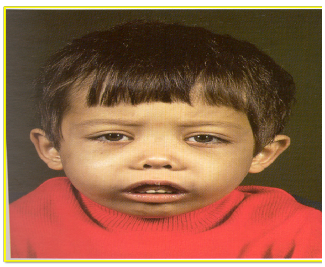
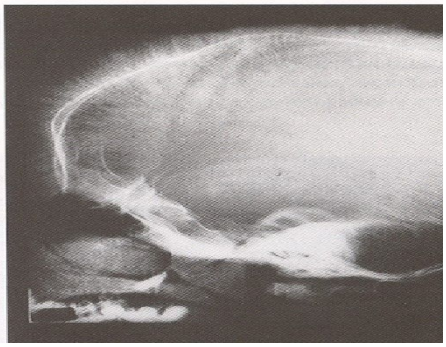
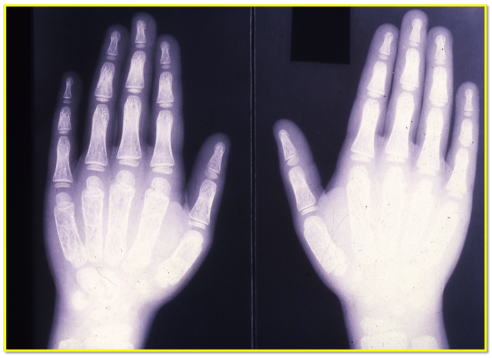
- **High HbF because there is no β . why not HbA2? because HbA2 can not exceed 8% either prenatal or postnatal.**
- **The presenting symptoms are most severe in (Major) and least severe in minima.**

---

[2] **Erythropoiesis** is the process which produces red blood cells (erythrocytes)
[3] hematopoiesis occurring outside of the medulla of the bone (bone marrow), e.g. spleen.

❖ Clinical Manifestations in Thalassaemias: (Important)

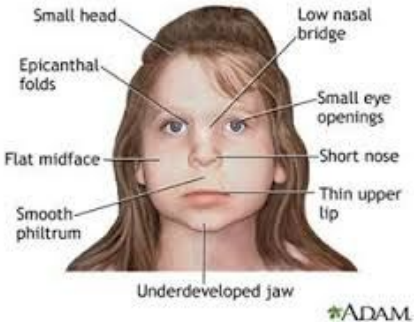
- Pallor.
- Jaundice.
- Apathy[4] and Anorexia.
- Failure to Thrive[5]
- Hepato-splenomegaly. (due to extramedullary haematopoiesis)
  -abdominal swelling-
- Skeletal Deformity. (secondary to unchecked marrow expansion)
- Iron Overload manifestations. (because multiple transfusion)

Thalassemic face: in β-thalassemia major They have the same facial characteristics:

- Forehead bossing (protrusion)
- Prominent maxilla
- Widen space between eyes Because of increasing the demand on bone marrow, facial bones become expande.
- Nose depression

X-Ray: Hair-on-ends appearance. (as a result of skeletal deformity) very clearly.



[4] lack of interest, enthusiasm, or concern.
[5] Slow growth.

(Pathoma Bridge)EXTRA!

❖ Thalassemia:

➢ Anemia due to decreased synthesis of the globin chains of hemoglobin.
(↓ Globin → ↓ Hemoglobin → microcytic anemia)
➢ Inherited mutation; carriers are protected against *Plasmodium Falciparum malaria*.

❖ α-Thalassemia:

➢ Is usually due to gene deletion.
➢ Normally, 4 alpha genes are present on chromosome 16.

1. One gene deleted-**asymptomatic.**
2. Two genes deleted- mild anemia with ↑ RBC count; cis deletion is associated with an increased risk of **severe thalassemia** in offspring.

Cis deletion	Trans deletion
Is when both deletions occur on the same chromosome; seen in Asians.	Is when one deletion occurs on each chromosome; seen in Africans, including African Americans.

3. Three genes deleted - severe anemia; β chains form tetramers (HbH) that damage RBCs; HbH is seen on **electrophoresis.**
4. Four genes deleted- lethal in utero (hydrops fetalis); y chains form tetramers (Hb Barts) that damage RBCs; Hb Barts is seen on **electrophoresis.**

❖ β-Thalassemia:

➢ Is usually due to gene mutations (point mutations in promoter or splicing sites); seen in individuals of African and Mediterranean descent.

1. Two β genes are present on chromosome ll; mutations result in absent (β0) or diminished (β+) production of the β-globin chain.

2. β-Thalassemia minor(β/β+): is the mildest form of disease and is usually asymptomatic with an increased RBC count.

- Microcytic, hypochromic RBCs and target cells are seen on blood smear (Pig.5.3).
- Hemoglobin electrophoresis shows slightly decreased HbA with increased HbA2 (5% normal 2.5%) and HbF (2% normal!%).

3. β-Thalassemia major (β0/β0): is the most severe form of disease and presents with severe anemia a few months after birth is, high HbF at birth is temporarily protective.

- A tetramers aggregate and damage RBCs, resulting in ineffective erythropoiesis and extravascular hemolysis (removal of circulating RBCs by the spleen).
- **Massive erythroid hyperplasia ensues resulting in:**
  - expansion of hematopoiesis into the skull (reactive bone formation leads to 'crewcut' appearance on x-ray, Fig. 5.4) and facial bones ('chipmunk facies')
  - extramedullary hematopoiesis with hepatosplenomegaly.
  - risk of aplastic crisis with parvovirus B19 infection of erythroid precursors.
- Chronic transfusions are often necessary; leads to risk for secondary hemochromatosis.
- Smear shows microcytic, hypochromic RBCs with **target cells** and **nucleated red blood cells.**
- Electrophoresis shows little or no HbA with increased HbA2 and HbF

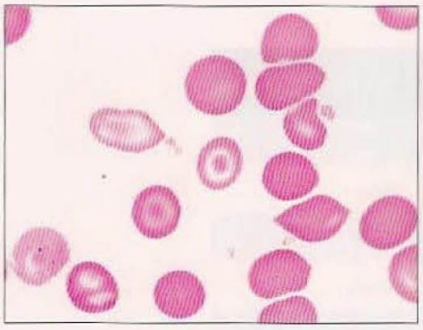


Fig. 5.3 Target cells.

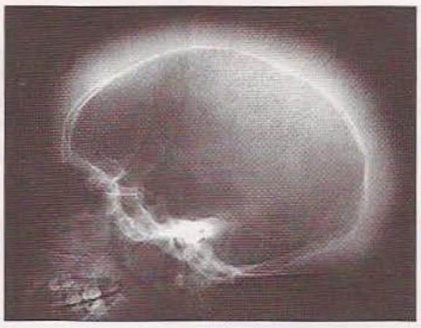


Fig. 5.4 'Crewcut' appearance. (Reproduced with permission, www.orthopaedia.com/x/xgGvAQ)

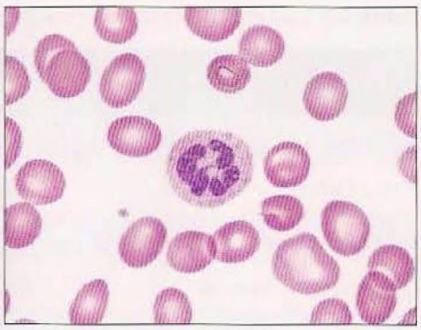


Fig. 5.5 Hypersegmented neutrophil in macrocytic anemia.

It's recommended to watch Pathoma Videos .. VERY HELPFULL!!

(All theses slides are Extra .. Not IMPORTANT .. JUST READ THEM)

❖ Diagnosis of Haemoglobinopathies, including Thalassaemias:

A. Personal & Family History.
B. Physical Examination.
C. Laboratory Investigation:

1. Haematological Tests:

CBC, Red cell indices, blood film, Morphology, reticulocyte count.

2. Sickling Tests: Sickle cell[6] test, Sickle cell solubility test.

3. Hb Electrophoresis[7] at alkaline/acidic pH and quantitation.

4. Quantitation of HbA2 and HbF

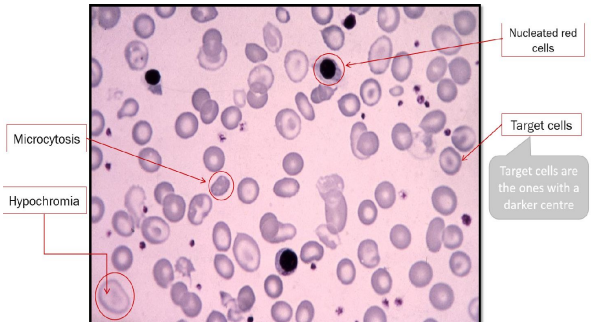
5. Serum iron/total iron binding capacity and ferritin level

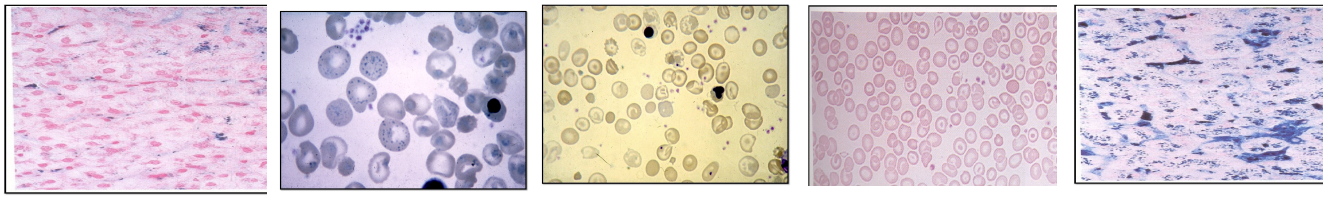
6. Biochemical tests:

Liver functions tests, renal function tests, blood gases and acid-base status, bone profile and urinalysis.

7. Special Tests:

- Family studies (Laboratory Investigations)
- Measurement of Alpha/Non-Alpha chain ratio
- Gene Studies.





[6] Video. Very helpful.
[7] Video.

❖ Prenatal diagnosis of the haemoglobinopathies (Including thalassaemia):

DNA Analysis:

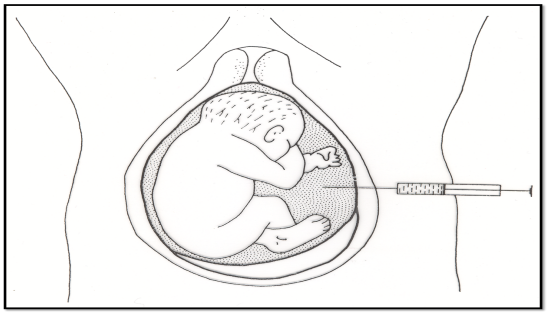
A. Chorionic villus[8] sampling[9]:

- Transcervical approach (9 – 11 weeks of pregnancy)
- Transabdominal approach (up to 15 weeks of pregnancy)

B. Amniotic fluid cell analysis (16 – 20 weeks gestation)

C. Fetal blood sampling (> 20 weeks gestation):

- DNA analysis
- Haematological parameters
- Biochemical analysis
- Globin chain synthesis:
  - α/β Ratio
  - α/ɣ Ratio
  - α/δ Ratio



❖ DNA ANALYSIS:

1. Gene mapping
2. RFLPs linkage analysis (Restriction fragment length polymorphisms)
3. Oligonucleotide probes (Using short gene probes 17 – 19 Nucleotide)
4. Gene amplification: (Enzymatic amplification of DNA sequences)
5. DNA polymerase chain reaction technique.

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[8] **Chorionic villi** are **villi** that sprout from the **chorion**"المشيمة" to provide maximum contact area with maternal blood.

[9] Picture

❖ MANAGEMENT OF THE THALASSEMIAS:

- ➢ **Blood Transfusion**
- ➢ **Iron chelation therapy**[10]
- ➢ **Splenectomy**[11]
- ➢ Hormone replacement.
- ➢ Bone marrow transplantation.
- ➢ Gene therapy.[12]

❖ Summary Of Recommendations For The Treatment Of Thalassemia Major:

➢ TRANSFUSION:

In the absence of cardiopathy: (when the Hb remains consistently **below 8 g/dL,** or earlier if there are other indications)	in the presence of cardiopathy, or when the Hb is less than 5 g/dL:
• **Blood-type** the patient completely. • **Vaccinate** hepatitis B negative patients against hepatitis. • Keep the **pretransfusion** Hb between **10.5 and 11 g/dL**. • Do not raise the **posttransfusion** Hb a**bove 16 g/dL**.	• Inject **furosemide** 1-2 mg/kg; • Preferably use **fresh blood**. • Do **not** transfuse **more than 5 mL/kg of blood**. • If necessary, divide the blood among 2 or more bags.
• Give **10-15 mL/kg** of blood preparation in **2 h**.	• Do not transfuse faster than **2 mL/kg**, or for **more than 4 h**.
• **Choose a 3-4 week transfusion interval.**	• Use very short inter-transfusion intervals.

[10] **Iron chelation therapy** is the removal of excess **iron** from the body with special drugs.
[11] **Splenectomy** is a surgical procedure to remove your spleen. The spleen helps fight infection and filters unneeded material, such as old or damaged blood cells.
[12] Video

❖ IRON CHELATION THERAPY:

1) **Desferrioxamine S.C.** 20-60 mg/kg/day in 8 h (average 40 mg/kg/day, or 280 mg/kg/ week). Desferroxamine is the best iron chelating therapy.
2) In selected subjects[13], give **desferrioxamine i.v. in high dose**, maximum 100 mg/kg over 8 h, only on the days of transfusion.

❖ SPLENECTOMY:

1) Is indicated when the blood consumption is more than 1.5 times normal.
2) Give **anti-pneumococcal vaccine** to children more than 2 years old prior to splenectomy.
3) Inform the patients and their family doctors of increased risk of serious **infections**.
4) Give prophylactic **penicillin**, and a platelet **anti-aggregant** when there is thrombocytosis.

❖ Oral Iron Chelation Therapy:

- Deferiprone [Ferriprox]
- Oral Tablet or Syrup 5 to 10 mg /kg /day divided in 3 doses.
- More effective than desferoxamine in chelating cardiac iron.
- Total iron excretion with deferiprone is less than with desferoxamine.
- Major adverse effect especially in children include - Gastrointestinal symptoms, joint pain, liver disfunction, neuropenia in 27% of patients.
- Deferasirox (EXJADE, NOVARTIS)
- The dose is 20-30 mg/kg/day once daily.
- Approved by FDA.
- Reduction of liver iron to 50%, reduction of serum ferritin to 70% after 1 year treatment.

Side effects:

- Nausea, vomiting, diarrhea, abdominal pain, skin rash.
- Mid increase in serum cratinine in 30% of patients as wit Desferoxamine ocular and auditory disturbance have been reported.
- Increase in serum transaminases in 10% of patients.
- Reduction of the dose in steps 5-10mg/kg/day every 3-6 months depending on serum ferritin level.

[13] If the patient is hypotensive or in shock. Read

❖ Assessment of Iron Stores:

- Serum ferritin.
- Serum iron and percentage saturation of transferrin (iron-binding capacity)
- Bone marrow biopsy (Perl's stain) for reticuloendothelial stores
- DNA test for mutation resulting in Cys282 Tyr in the HFE gene.
- Liver biopsy (parenchymal and reticuloendothelial stores)
- Liver CT scan or MRI
- Cardiac MRI
- Desferrioxamine iron excretion test (chelatable iron)
- Repeated phlebotomy until iron deficiency occurs.

❖ Assessment of tissue damage caused by iron overload:

➢ **Cardiac:** Clinical; chest X-ray; ECG; 24-h monitor; echocardiography; radionuclide (MUGA scan to check left ventricular ejection fraction at rest and with stress.

➢ **Liver:** Liver function tests; liver biopsy; CT scan.

➢ **Endocrine**: Clinical examination (growth and sexual development) glucose tolerance test; pituitary gonadotrophin release tests; thyroid, parathyroid, gonadal, adrenal function, growth hormone assays; radiology for bone age; isotopic bone density study

❖ Investigations And Follow-up:

Prior to treatment:	Study the case, and do complete red cell typing.
Before each transfusion:	Hb, cross-match and red cell antibody detection, serum transaminases (in areas with a high incidence of hepatitis). Record the date of transfusion, net weight and mean hematocrit of the blood preparation, and the Hb of the patient
After each transfusion:	Measure the post-tansfusion Hb.
Every 3 months:	Measure height and weight.
Every 6 months:	Ferritin estimation.
Every year:	Evaluate growth and development. Calculate the transfusion indices. Evaluate iron balance. Complete evaluation of the case.
Variable intervals:	Cardiac and endocrinological investigations according to the clinical state of the patient.