# Hematology


- Histology
- Biochemistry
- Pathology
- Pharmacology
- Physiology
- Microbiology

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lecture number : **3**

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Handout | Sheet | Slide

By: Anwer Abu Shanab

# Hemolytic anemias

**In the previous lecture we talked about 1. Hereditary Spherocytosis**

**2.G6PD deficiency**

**3.Paroxysmal Nocturnal Hematuria (PNH)**

**In this lecture We will talk about the 4th and 5th hemolytic anemia diseases : Thalassemia and Sickle Cell anemia.**

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## 4th: Thalassemia

## What is thalassemia?!

It's an **Inherited** disorder (autosomal recessive), and it means : Anemia due to Deficiency in synthesis of hemoglobin A.

Hemoglobin A is made up of 2 alpha chains and 2 beta chains. → Thalassemia result from decreased production or mutation in Alpha or Beta chains → Therefore we can divide thalassemia into α- and β-thalassemia based on decreased production or any deficiency of α and β globin chains.

Please note that : Normal types of Hemoglobin are

HbF($\alpha_2 \gamma_2$)

HbA ($\alpha_2 \beta_2$)

HbA2 ($\alpha_2\delta_{2)}$



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# Thalassemia Types

## Alpha thalassemia :

Is usually due to Gene Deletion ( part of it or all of it ..

- Normally there are 4 alpha genes on chromosome 16.

Alpha thalassemia molecular classification can be :

1. If one gene is deleted: there are three others working, the patient will be completely asymptomatic(silent carrier) →RBCs are slightly smaller than normal and pallor but their function is good → now what is the problem here? If they mate with another person like them, they will transfer the disease to the offspring and the new baby might have intermedia or severe thalassemia .
2. 2 genes are deleted →minor → mild anemia (mainly asymptomatic) and is associated with increased risk of severe thalassemia in offspring.
3. 3 genes are deleted → only one is remaining → and this functional gene produces very few amount of HbA →so hbA will drop significantly and it's called hemoglobin H disease! Resembles B-intermediate; do not need lifelong blood transfusions.

4. If 4 genes are deleted →there is no Hb at all and the baby will die in utero , during embryogenesis.. its incompatible with life..

## Etiology :

Since there are 4 genes encoding the alpha chain,this means there are more options for the mutation here :P or more mutations can happen.

→clinical classification for thalassemia …from asymptomatic to very severe ☺

## Beta thalassemia :

Is usually due to point mutation, one nucleotide is changed.

- Normally there are 2 beta genes on chromosome 11.

Beta Thalassemia molecular classification can be :

1. **Minor** →: 1 gene is mutant, mild thalassemia ,RBC slightly smaller and paler than normal →Asymptomatic, but problem is when they marry someone with this same mutation, that's why we do a premarital test.
2. **Major** : The 2 genes are mutant : patient will have the severe symptoms early in life during infancy ,HbA will drop significantly ,the patient will have chronic hypoxia and Lifelong anemia and he will require blood transfusion because of the severe anemia.

3.**Intermedia** :in the middle between the 2 previous types, one gene might be mutated or even two genes but the harm is not that severe compared to major , anemia milder than in major ( for example hg instead of 6 is 9 g/dl), the quantity of beta chains is somehow good so symptoms appear mildly. No need for regular blood transfusion.



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- ➔ HbH : which is a type of alpha thalassemia when three genes are mutated. And patients will have chronic persistent anemia anemia that is similar to the symptoms of beta thalassemia intermedia → when alpha chains are low → there are excess beta and gamma chains
- ➔ When beta chains combine to form a tetramer → HbH is formed
- ➔ When Gamma chains combine to form a tetramer -> Hb Bart is formed
- ➔ Both HbH and Hb Bart appear in alpha intermediate (3 genes deletion)
- ➔ Hb Barts is a diagnostic feature for this disease :D
- ➔ no need for blood transfusion

**Mode of inheritance :**

**The mutation is inherited in an Autosomal recessive mode.**

**Prevelance : Mediterranean region, Africa, middle east, India and South East Asia**

Symptoms of the major and intermedia thalassemia appear after six months of age because HbA is not mature yet to function → and the baby still have fetal Hb → HbF ☺

After age of six months HbF drops and the signs of HbA deficiency appear..

Pathogenesis of Thalassemia:

You need to understand all the mechanisms of these symptoms..

We already mentioned that production of HbA is decreased,RBCs do not have enough amount of Hb so appear smaller in size and

pallor..(hypochromic microcytic anemia ) → hypoxia → life long and persistent.

***Now let's start with Beta thalassemia :

As we know in beta thalassemia there are decreased copies of Beta globin chains and relatively increased copies of Alpha globin with each DNA replication → so?

Decreased Beta → decreased HbA ( which is two alphas and two betas) ( normally HbA is the most abundant type → over 95%)

Now we have EXCESS alpha chains!! they bind other local chains → 1.gamma chains and results in excess fetal Hb ( HbF )( two alphas and two gammas )(normally it's not more than 1% !)

Or 2. Bind two delta chains → increased HbA2 ( two alphas and two deltas )( in normal situations it's not more that 3.5% of the total Hb in rbc !)

So HbA2 and HbF increase in beta thalassemia.. and this is for diagnostic and also for therapeutic purposes , where you find that HbA is decreased and HbF and HbA2 are increased

Now let's talk about how we can diagnose beta thalassemia?

By Hb electrophoresis : different globin chains have different electrical charges. Hg is separated on gel and an electrical current is applied. Each type of Hb migrate a specific distance and hence can be recognized ,they are separated according to..physical and chemical characteristics

------ normally HbA is the most common one( around 95%) then HbA2 (from 1 to 3.5%) then HbF (not more than 1% )------

<u>In beta thalassemia HbF and HbA2 increase secondary to alpha,gamma and delta chains excess.</u>

We already said that we have excess unpaired alpha chains that will cause damage→ they form solid masses and are called*** **hemichromes** ****→ they accumulate in the RBC → they cause RBC membrane damage → these RBC are identified in the spleen →

EXCTRAVASCULAR HEMOLYSIS!! → and if the amount of excess hemoglobin chains is very high we might have intravascular hemolysis! And even hemolysis in the Bone Marrow itself early in RBC production..

**In a nutshell :** Thalassemia is anemia due to decreased HbA production and also Intra and Extravascular hemolysis as mentioned in the mechanism above :D

in intermedia and major thalassemia, when more than one chain are mutant .. and as we said these patients have persistent anemia and **high erythropoietin levels** during all their lives!.--> persistent increase in erythropoietin causes many symptoms and complications and they are :

1. it inhibits hepcidin**→ which is an important hormone in iron metabolim →normally it prevent absorption of iron in GIT**→ hepcidin is inhibited by erythropoietin ..so the end result that there will be increased absorption of iron in GIT →so patients will have secondary hemisderosis ( which is accumulation of hemosiderin ) it damages every organ in the body from the heart to the kidneys and lungs to the skin.. it's a very bad complication; for example :

in the skin it causes melanocyte proliferation → skin pigmentation ! so patients with Thalassemia will have dark skin all over their body because of melanocytes not directly from hemosiderin☹ → so melanin is secondary to hemosiderosis!

Hemosiderosis is an infiltrative disease ( infiltrative means : condition caused by the diffusion or accumulation in cells or tissues of substances not normally found in those cells or tissues. →in this case masses of iron )

Any organ that will receive hemosiderosis will be damaged.. number one organ that will be damaged is the heart ☺

Again -.- chronic hypoxia in thalassemia major and intermedia stimulates erythropoietin this causes bone marrow activation( always active!) ! So RBC normoblast proliferation in bone marrow is persistent → Increased number of normoblasts, patients will end with erythrocytosis.

Normoblast : nucleated precursors



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→ Now this sounds weird → thalassemia is anemia.. why do we have High RBC count in thalassemia?!!!!! this is secondary to increased erythropoietin stimulation!

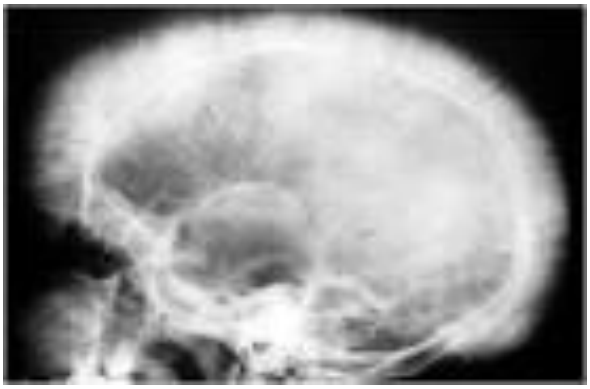
→Now if you remember what we said about anemia : its reduction in the Hb mass in the cell..we said that RBC count is not accurate! So this is what occurs in thalassemia we have increased RBC count but they are very pale and small (so count is not something to rely on for diagnosis) →this process is called ineffective erythropoiesis ☺

3. we have high normoblast proliferation and this large number of cells causes high metabolism so the cells are always active and replicating so hematopoiesis expands into the skull → reactive bone formation and high normoblast count leads to **crewcut appearance** → it appears as small spikes on the skull on X-ray ( as a shadow )

3. extra medullary hematopoiesis ( production of RBC outside the bone marrow ) →during embryo life, the production of RBC is the highest in LIVER then SPLEEN then bone marrow but later on in life, only bone marrow produces RBC's ,spleen and liver stop producing RBCs..

What happens after stimulation of erythropoietin? Back stimulation of stem cells that became normal and stopped producing RBCs!! They are activated secondary to stimulation of erythropoietin and now we have enlargement of spleen and liver!--> ! **Hepatomegaly and splenomegaly.**

**4.**abnormal bone growth due to hypoxia ( the increased number of normoblast, these cells steal oxygen and this causes abnormal bone growth so patients who have thalassemia major and intermedia usualy have short stature! ,also hematopoiesis in skull, facial bones ( keep in mind the chipmunks facial bones!!! → prominent) and also chin shows abnormal bone growth..



5. Heart failure is common to secondary thalassemia major

Because of three things:

1)its anemia → one of its signs if you remember the first lecture tachycardia (increase function of heart to compensate for aneia)→ heart failure!!

2)also transfusion of blood causes increase in the volume of blood→ it goes straight to the heart → heart failure

3)also hemisiderosis → we said it destroys any organ in its way :P like heart for example!

6. Thrombosis its common in patients with thalassemia → explanation → because of deformed cell membrane ( due to excess globin chains ) and this will attract platelets and also it injures blood cells.

As you can see thalassemia is a very complicated disease and you need to understand all the complications we mentioned and the pathogenesis we discussed☺

Morphology :

**RBCs are hypochromic and microcytic (central pallor is more than one third, MCV is lower than 80)

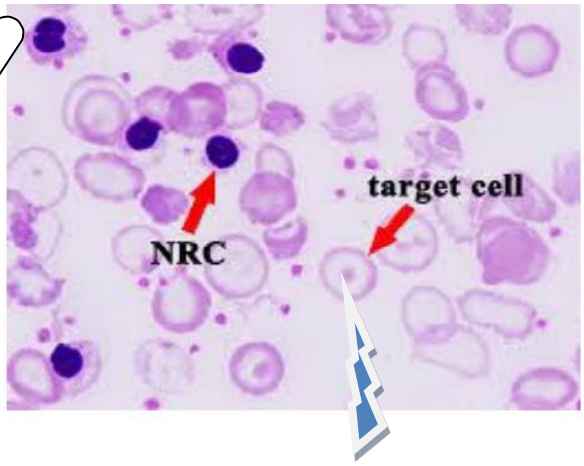
****Another feature is **target cells** → In the middle of RBC there is a red dot, it happens in thalassemia and other diseases, usually its from abnormal hemoglobin synthesis.( بالنص في نقطة زي لعبة الاسهم)

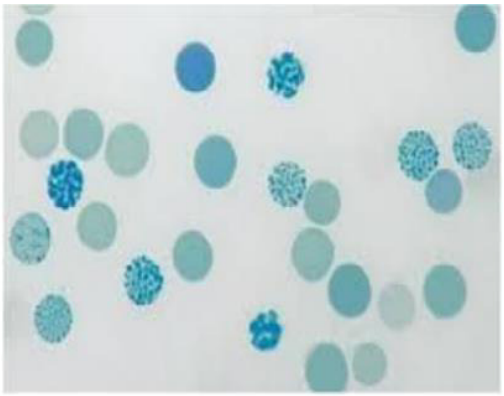
*** also you can see Basophilic stippling: blue small dots all over the cell→.. they are rememant of RNA and mitochondria and they are

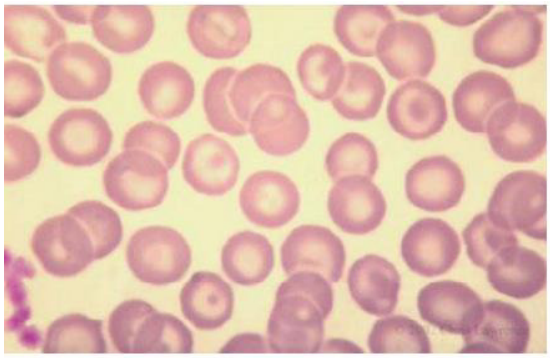
condensed in abnormal bodies→ they happen due to increased number of normoblast → and their persistent ,we call it basophilic stiplling and it appears on the blood film

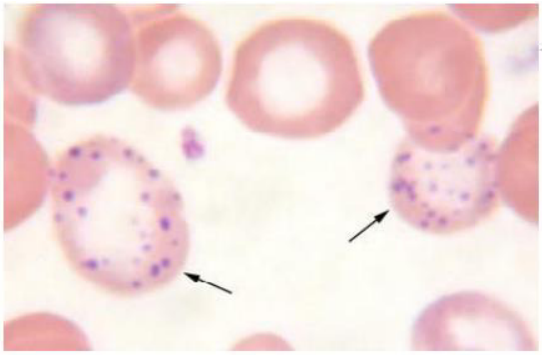
Also you can see Normoblast( due to the large number ) they appear in peripheral blood **nucleated** !! → because of increased number in bone marrow.

We talked about hemoglobin H disease already now in the blood film using supravital stain -> we can see golf Ball shaped cells full of small dots which resemble tetra beta (occurring only on HbH disease)..( a lot of dots ) if its deposited as small dots all over the cells like golf ball →these deposited things are **hemichromes** → if they appear then its HbH disease , (pay attention that Hemichromes are seen in Thalassemia, while Heinz bodies in G6PD deficiency)







## 5th : sickle cell anemia:

Sickle cell anemia is also a **hemoglobinopathy**, caused by a mutation in Beta chain of the glutamate; when one of the codons(6th AA) is mutant it will give rise to Valine instead of the normal Glutamate.

Valine is **hydrophobic** while glutamate is **hydrophilic**, so it changes the physical characteristics of the beta chain.

Symptoms of sickle cell anemia begin early in life, after the drop of the feta hemoglobin at age of 6 months. Similarly to thalassemia.

**Mode of inheritance** : Autosomal recessive

**prevalence** :

similar to thalassemia, common in middle east, south Arabia also in Africa and India.

**Sickle cell trait**: in case of ***heterozygous mutant gene***; so we have a normal one and the other is abnormal**, the normal one masks the abnormal one therefore the patient is only an asymptomatic carrier**, these carriers have **50% hemoglobin S, and 50% hemoglobin A, or we say 40% HbS because we have HbA2 and HbF.**

**Sickle cell anemia**: in case ***of homozygous mutant genes***, giving an abnormal sickle beta chain, these are the anemic symptomatic patients early in life after the age of 6 months, they have about 90% hemoglobin S and no HbA.

### Sickle cell anemia and Malaria

Sickle cell anemia patients are historically known to be resistant to malarial infection , both the carrier and the diseased, there is no fully explanation theory. One theory says it might be because the receptors on the RBCs are changed and the malaria doesn't like hemoglobin S.

## Pathogenesis:

Different from thalassemia.

Hemoglobin S is hydrophobic, it doesn't like the cytosol so→ it polymerizes and gets condensed longitudinally in **a needle shape**, in the RBC therefore changes the shape of the RBC resulting in the sickle cell shape, and at the end it will tear the cell membrane and causing **intravascular hemolysis**. And since it's abnormal in shape and the spleen will engulf it leading to **extravascular hemolysis.**

therefore the patient will have lifelong hypoxia **so erythropoietin will be increased** -similar to thalassemia- increased erythropoietin will inhibit hepcidin which will increase iron absorption, so patients will have secondary hemosidrosis. also persistent high erythropoietin will activate the normoblasts in the bone marrow so the patient will have **erythrocytosis** , so the patients will have abnormal bone growth as a result of hypoxia and increased RBCs production …,,, and **crewcut** appearance on x-ray ☺
Also it will activate stem cells in the spleen and the liver resulting in early **hepatosplenomegaly but later they have absent spleen (autosplenomegaly)**

## The main conditions that cause the RBCs to sickle :

hemoglobin S doesn't always polymerize resulting in the sickle cell shaped RBC, **certain conditions cause this polymerization like hypoxia, dehydration and acidosis** .

*even if the patient is an asymptomatic carrier, if any of these conditions happen he will have a sickle cell crisis.*

## Medical treatment:

This disease is a chronic irreversible condition, and there are only few ways which we can manage it through to decrease they symptoms and prevent complications, such as **Blood transfusion, and increasing fetal hemoglobin and hbA2 ; there are some drugs that increase fetal hemoglobin.**

*Bone marrow transplant is still under experiments, it might be the future revolution in treating such diseses.*

# Clinical complications:

**Vaso-occlusive crisis**:

sickle cell anemia is worse than thalassemia ,its patients are in risk of dying early. In sickle cell anemia the RBCs stick to each other because of their shape leading to **thrombosis** which may happen in any blood vessel at any site in the body- heart, brain, kidney….etc-
**so the patients might have myocardial infarction early in life at 20s or in adolescence.**

In lung acute chest syndrome→ patients might come with very severe chest, lungs, or ribs pain, caused by infarction in one of them, patient cannot breath.

**Aplastic crisis**

**It means that there is no hematopoiesis**, its secondary to either bone marrow infarction or infection by parvovirus B19; it attacks the earliest cells in the erythroid line = the pronormoblast?

For example, these patients might have chronic anemia, but suddenly it worsens and drops to 3 from 9, this is the aplastic crisis caused by either bone marrow ischemia or parvovirus.

Parvovirus can be visualized in bone marrow biopsy, the parvo particles are present in the nucleus of the normoblast.

This virus can cause aplastic crisis in any other chronic anemia not only sickle cell anemia also maybe in thalassemia, but it doesn't cause this crisis in normal –not anemic- people.

**Sequestration crisis:**

**Sequestration** means engulfment .

The spleen **is hyper functioning** leading to increased engulfment of all blood cells; RBCs and WBCs. The spleen becomes **massively enlarged**, it will be full of blood causing a **hypovolemic shock**, exactly like losing

a lot of blood outside the body, but here its stuck in the spleen, the patient might die.

## Autospleenectomy

Increased extramedullary hematopisesis will lead to **splenomegaly**; the spleen is enlarged because of all the RBCs, but these RBC wills destroy the spleen because of **repetitive spleen infarction** leading to fibrosis and at the end it will disappear, after a while, older patients will have **Autospleenectomy** , it will become smaller and we can't even palpate it, as if it was removed by its own.(different from thalassemia)

## Pariapism

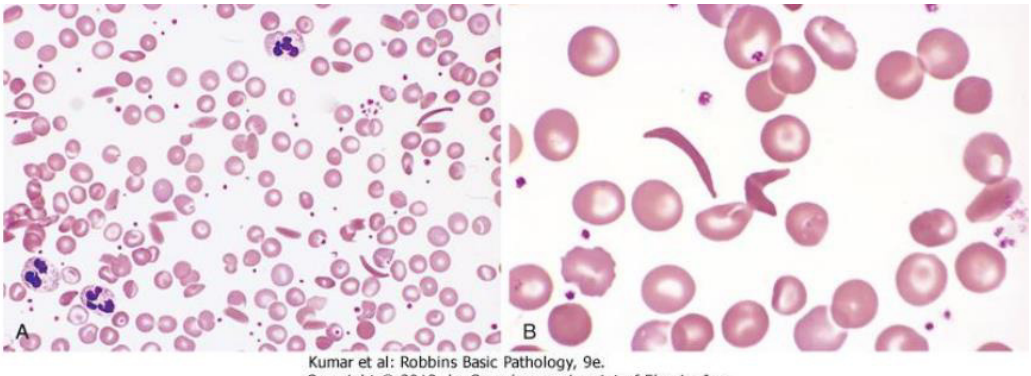
Persistent erection of the penis in men, its secondary to **thrombosis** in the veins of the penis, if the circulation stops during erection, the patient will have persistent priapism, if not treated, the patient will end up with infarction of penis.

## Skin ulcers

Common in lower limbs where circulation is slower, so it's more prone to **thrombosis** leading to skin ulcers.

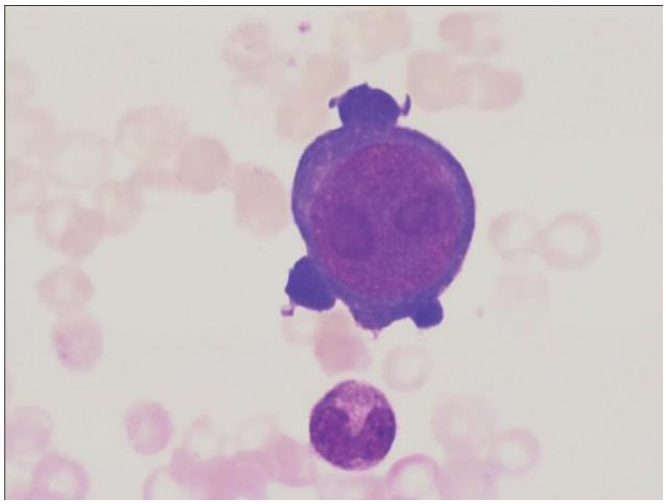
## Blood film

Sickle cell anemia and target cells are seen on blood smear in sickle cell disease but not sickle cell trait!




Kumar et al: Robbins Basic Pathology, 9e.

In Hemoglobin electrophoresis→ we can see HbS( this technique confirms the presence and amount of HbS)



You can see pronormoblast which is the earliest stage in the erythroid line!

➔ Para particles are seen in aplastic crisis secondary to parvovirus infection ☺

بالتوفيق جميعا في الامتحان و كان بوّدي ازبط الشيت اكتر بس الامتحان قرب و اذا ضلت معي كمان وقت زيادة الدفعة بتطخني و الامتحان بروح من بين ايدي:P

الله يبارك بوقتكم ☺

**الشكر الجزيل لدعاء دحبور لبدايه شرحها الخرافي للثلاسيميا و الي اضطريت اعيده نفسه**

***</3***



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