# Hematology

## PATHOLOGY

☑ Sheet ☐ Slide ☐ Handout

Number: 4

Subject: Intrinsic hemolysis

Done By: Majd Al Rawashdeh

Corrected by: Dr Ahmad

Doctor: Ahmad Mansour

Date:

Price:

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Last time we've talked about hemolytic anemia – the extrinsic part, which is due to trauma, infection, or immune mechanism

Today we'll talk about **the intrinsic defects.**

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*(Figure 1)*This is hemoglobin; it has 2 α chains and 2 β chains, each one of those has a heme ring and an iron atom on it.

If iron is decreased that's iron deficiency. If we have a problem in the level of the heme ring that's called sideroblastic anemia (you don't need to know it), if we have a problem in hemoglobin its sickle cell anemia or thalassemia; these are **hemoglobinopathies.**

*(Figure 2)* This is the RBC membrane; if we have a problem in the membrane we call it **membranopathies**. Among the membranopathies, is spherocytosis.

So we have **hemoglobinopathies** and **membranopathies.**

*(Figure 3)* G6PD is an enzyme that you know well, its deficiency is an example of **enzymopathies.**

*(Figure 4)*On RBC membrane there is a protein called GPI; is a kind of nocturnal ***protein that has proteins stuck to it that protect our cells from damage by complement.

*Figure 1*

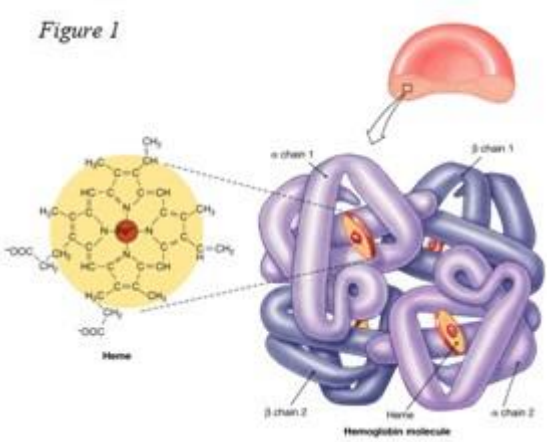



*Figure 2*

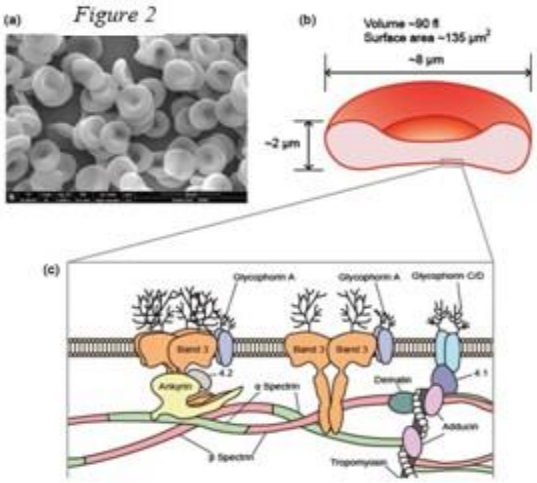



*Figure 3*

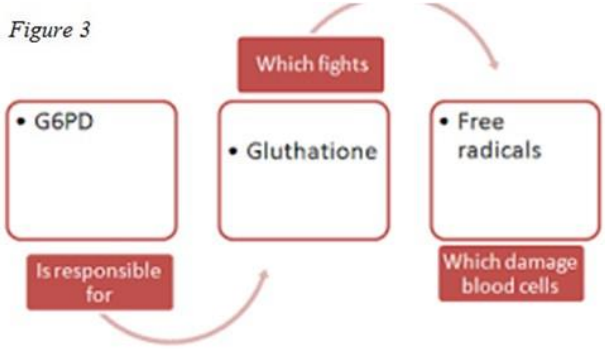
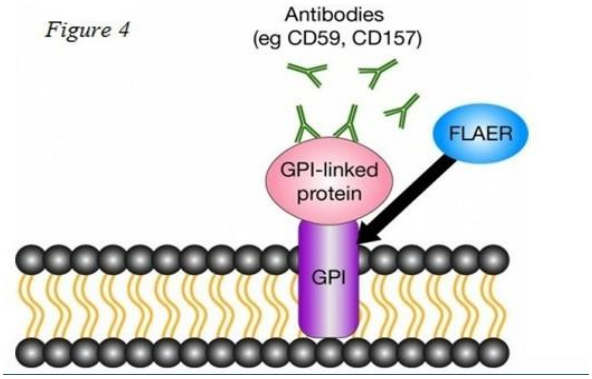



*Figure 4*



So the 5 diseases we'll talk about today are:

1. Spherocytosis
2. Sickle cell anemia
3. Thalassemia
4. G6PD deficiency
5. Paroxysmal nocturnal hemoglobinuria.

All of these diseases are under the umbrella of intrinsic defects that will lead to anemia of blood loss or hemolytic anemia.

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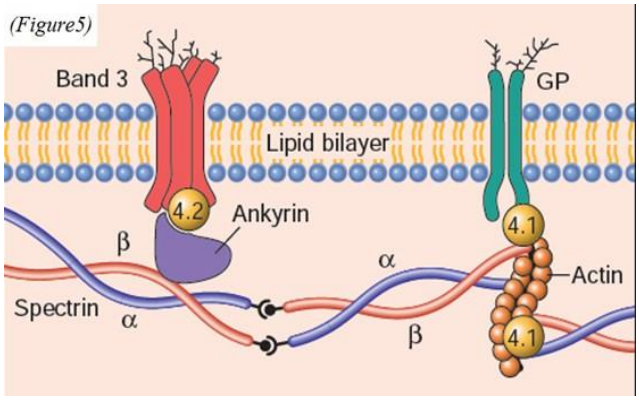
## ❖ Spherocytosis

What is the shape of RBCs? It's a biconcave disc, why so? They work deformable when they're biconcave so they pass through the capillaries easily andto increase the surface area for oxygen exchange.

*(Figure5)*For that to happen we have a cytoskeletal structure; start with **spectrin**(the intertwined protein) it makes a 2D carpet under the membrane, this carpet will be attached to the membrane by other proteins: **Ankyrin**, **band 4.2**, **band 3**(you can also notice **glycophorin** and **band 4.1**).

*(Figure5)*

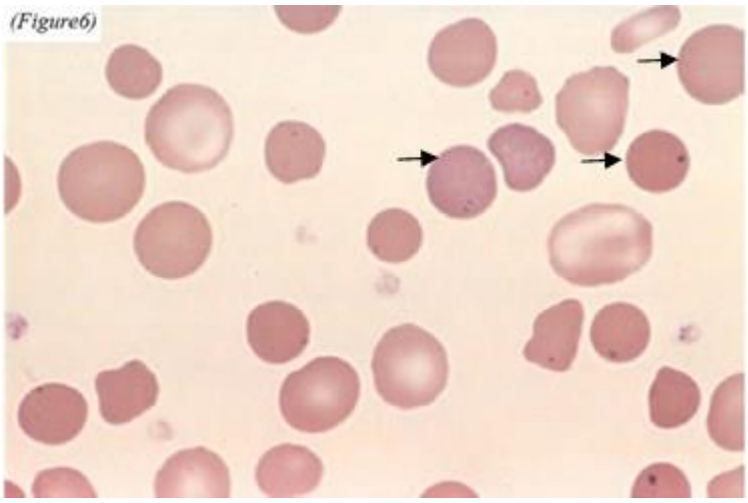



A deficiency in any of these proteins will result in unstable membrane structure and that's what happens in hereditary spherocytosis.

The membrane will be lost bit by bit because it's not stable, when we lose the surface; the smallest size for a given mass is the **sphere**→ it will not pass easily through the capillary bed (it will last longer) → the PH will decrease and the glucose content on the RBCs will also decrease, both of these factors (PH and glucose) will result in further hemolysis.

*(Figure6)* this is what we see in spherocytosis, the pointed cells are spherocytes: <u>circular RBCs that have no central pallor.</u>

*(Figure6)*



What is the most common disease to cause spherocytes in peripheral blood?

It's not hereditary spherocytosis; it is **immune hemolytic anemia** most of the time, or any cause of extra vascular hemolysis.

At the level of us as medical students and the exams that we'll have; spherocyte is always hereditary spherocytosis.
But in practice (in real life); any form of extra vascular hemolysis causes spherocytosis.

Intra vascular hemolysis causes **schistocytes**; because the cells are being literally ruptured within the blood.
In extra vascular hemolysis, immune hemolytic anemia specifically, there is **spherocytes**.

Back to our subject, hereditary spherocytosis:

- Mostly autosomal dominant, it can be autosomal recessive (but if it's autosomal recessive it's usually a very sever disease).
- It's more prevalent in north Europe, so if you work in these countries in the future, or in the united state; you might see it. It's really unlikely to see spherocytosis in Jordan.
- Mutations are in the proteins that we just discussed; ankyrin, band 3, and spectrin.
- The patient will come usually with moderate clinical course.
- It can be complicated by aplastic crisis which is infection with parvo virus (B19 specifically).It preferentially affects erythroid precursor cells within the bone marrow; it suppresses them.

In normal people who have normal RBCs survival (for 120 days); usually that doesn't cause a problem. But people with spherocytosis who have decreased RBCs survival (90-100 days) this can cause a problem.

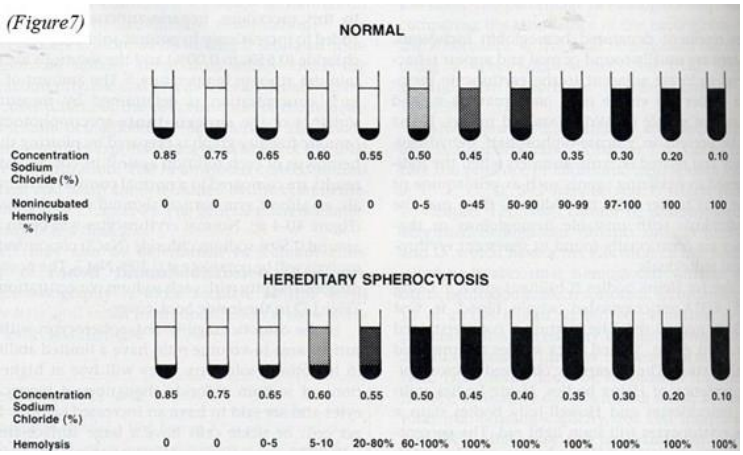
- They will have Anemia, jaundice, gallbladder stones, and splenomegaly. (Any form of chronic hemolytic anemia will cause these).
- MCHC is high, it's probably the only anemia in which MCHC is high because there is a decrease in size (MCHC is hemoglobin concentration within the cell); cell lost only the membrane but hemoglobin content within that cell is still intact.
- Diagnosis involves osmotic fragility test

Normally, osmolarity is around 0.9. When you put RBCs in normal (**saline**) solution **nothing** will happen. If you put the RBCs in a 0.8 (lower), water will move freely from the solution into the RBCs (water moves from lower concentration to higher concentration).

What happen in normal people is that RBC is expandable because it has this biconcave disc there is a little bit of room to accommodate more water, while spherocytes don’t have that. Just decrease the saline concentration and see where hemolysis begins. **Normally** it should start at **0.5, 0.4**. In **spherocytosis** it starts at **0.6 or above** (depends on the degree of mutation or the severity of the disease).

*(Figure7&8)* what happens in spherocytosis is that hemolysis starts at a **higher concentration** than the normal people, because of the inability of the RBC to accommodate more free water.
Above is normal, below is spherocytosis.
Above, at 0.55 there is nothing, at 0.5 there is hemolysis. Hemolysis shows as pink due to the dissolved hemoglobin in serum in the tube.
At 0.6: the normal is almost clear, the abnormal is not.

*(Figure7)*

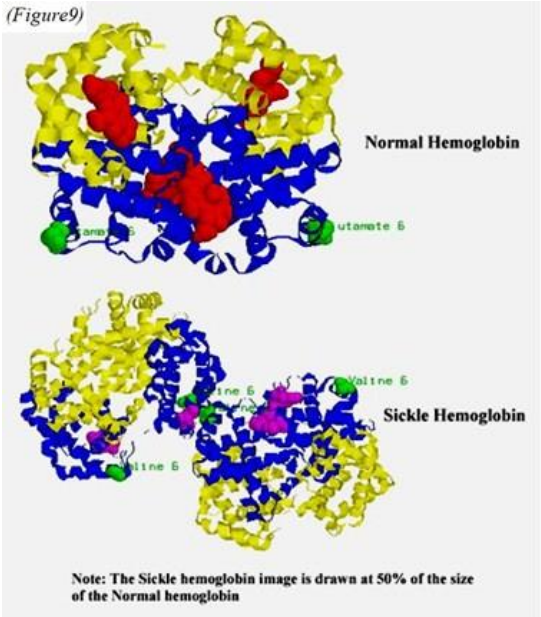



*(Figure8)*


Let's move on to **hemoglobinopathies**.

- Sickle cell anemia

*(Figure9)*




*(Figure9)*Again, hemoglobin has 2 α& 2 β chains, in the β chain, we have a glutamic acid at the sixth position, which is hydrophilic amino acid. In Sickle cell disease it's being replaced by valine, which is hydrophobic; leading to conformational change within the hemoglobin.

Sickle cell disease is the most common hemoglobinopathy. In homozygotes when we have two affected gene; all HB is replaced by HbS, while in heterozygotes only half of it will be replaced.

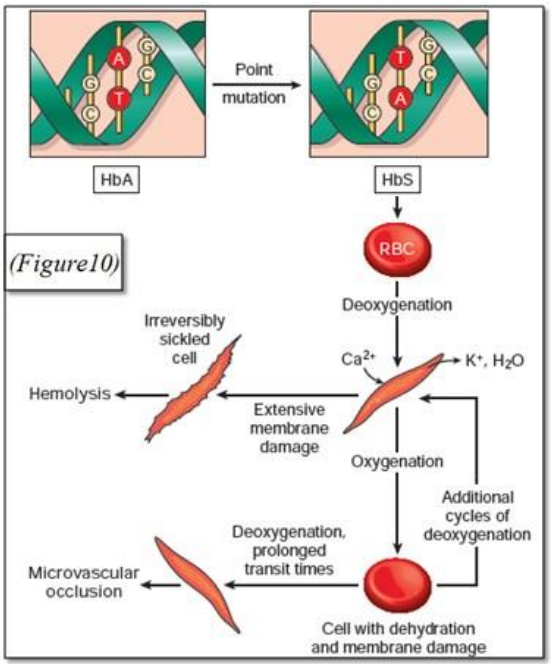
It's more common in African Americans because it's believed that sickle trait (this gene mutation) will protect against malarial infection. Malaria is very common in West Africa and Nigeria (the countries from which the slaves were taken) that's why African Americans have a high gene frequency of sickle cell disease.

*(Figure10)* What will happen when we have a sickle cell? When Hemoglobin is deoxygenized, conformational changes will occur followed by precipitation; it will attach to the membrane & cause damage to it.

Then oxygenation will occur, that will be reversible only at first. With repeated cycles of polymerization→ permanent damage and sickling to the cell → sickles are blood deformable; will be eaten by the spleen, and most importantly they will cause occlusion of the small blood vessels causing ischemia.

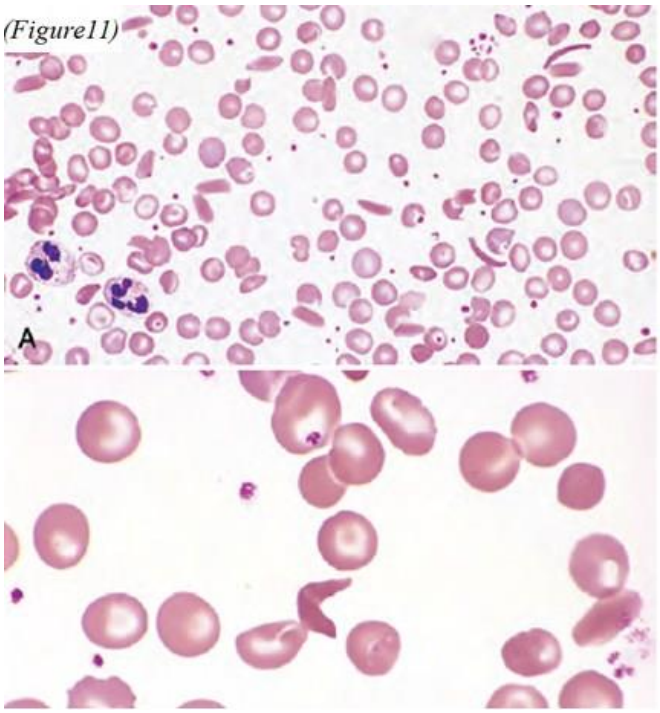
So, irreversibly sickled cells leading to hemolysis and deoxygenation secondary to microvascular occlusion

This is the pathophysiology of sickle cell disease.



*(Figure10)*

*(Figure11)* In Sickle cell disease we will see sickle cells that have pointy edges. We don't see these cells in heterozygous, we only see it in sickle cell disease (homozygous).



*(Figure11)*

What are the factors that affect sickling of hemoglobin? (Important)

1. Presence of hemoglobins other than HbS
2. Intracellular concentration of hemoglobin
3. Transit time for RBCs within the vessels

Let's talk about each one of them:

<u>1. Presence of hemoglobins other than HbS:</u>

- In heterozygote half of HB is **HbS**, and the other half is **HbA**, the binding between HbS and HbA is very <u>weak</u>, which means NO sickling.

- **HbF** ($\alpha 2\gamma 2$) has a <u>weak</u> binding capacity with HbS, that's why sickle cell symptoms and signs appear after 6-9 month, because this is when the body switches from HbF to HbA (beta chain synthesis starts after this period). If we find a way to increase HbF within our bodies the sickling will decrease.

-**HbC** (instead of valine there is lysine at the sixth position) it's also common in African Americans. If we have one gene that has **HbS** and the other gene has **HbC**, the binding between them is strong, so despite the fact that the patient has heterozygote sickle , he will act as if he have sickle disease (meaning he's not asymptomatic; the patient have symptoms and they are severe).

2. Intracellular concentration of hemoglobin:

How much hemoglobin we have within the RBC? If it's high sickling will be easy and if it's lower sickling will be harder. So if we find a way to decrease the hemoglobin within the cell sickling will decrease.

**Alpha thalassemia** is a disease that results in decrease production of hemoglobin, so the concentration of hemoglobin will be low → lower probability of sickling. (If a patient have sickle cell gene with alpha thalassemia; the probability of sickling is low).

One of the things that cause sickling is **dehydration** (the same concept applies here).

3.Transit time for RBCs within the vessels

Transit time is the time the RBCs need to pass through vascular bed. When the passage time within the vessels is **long** like in spleen and bone marrow (there is a lot of anastomosis and complex net of capillaries); the ability to sickle is **high**. When the circulation is fast the sickling is low. That's why most common symptoms happen in the bone, because bone marrow is one of the most commonly affected organs because of the long passage time.

Among all hemolytic anemias; splenomegaly is most prominent in **spherocytosis** (spleen is the biggest here).

More explanation: when the transit time is high (cells are very slow) that will give the time to the local macrophages to cause problems, also the PH will decrease causing more sickling and more vascular occlusions (decrease in PH causes more deoxygenating and more amino acids will bind to each other).

Patients with this disease (homozygous) will have:

- Chronic hemolytic anemia
- Fatty change in the heart, liver and renal tubules secondary to ischemia
- Reticulocytosis and erythroid hyperplasia in bone marrow
- Bone changes; when there is anemia there is increase erythropoietin → induction of bone marrow production from areas that do not normally do that such as the skull
- Extramedullary hematopoiesis in liver and spleen.

In hemolytic anemias there is splenomegaly because of macrophages hyperplasia (like spherocytosis).

- In adults the spleen is being replaced by fibrosis, the reason is repeated occlusions and repeated ischemia result in necrosis, this is referred to as "autosplenectomy" (spleen will be converted into fibrous band with no splenic tissue). No splenomegaly is noted in adults with sickle cell disease.
- In children there is mild enlargement secondary to congestion.
- In both cases (adult & kids) we have functional asplenia (the spleen is not working) even in kids when it's a little bit enlarged because o f the macrovascular occlusion.
- What are the problems that might arise from functional asplenia? Increased risk of infection by encapsulated bacteria, specifically **pneumococci** with increased risk of **salmonella osteomyelitis.**
- Vessel occlusion, bone pain, chest pain when the pulmonary bed is affected and stroke.
- Aplastic crisis (as in spherocytosis).

- Diagnosis with electrophoresis, or fetal DNA via chorionic villus sample.
- Treatment with HYDROXYUREA or BONE MARROW TRANSPLANTION.

  - Hydroxyurea Increases HbF, it has Anti inflammatory effect secondary to decrease WBC production, increases MCV so HB concentration decreases, and production of NO for vasodilation and inhibition of platelet aggregation.

- One of the things that you do to your sickle cell disease patient when he presents to the ER is **profuse hydration**.

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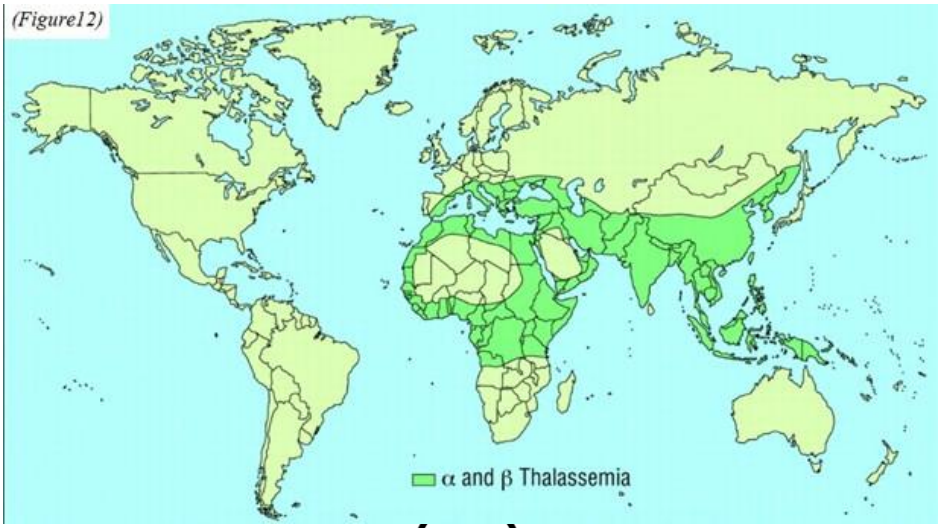
## ❖ Thalassemia

Unlike sickle cell disease, in thalassemia amino acids sequence is completely normal. But there is decrease in the production → low hemoglobin, low globin chain synthesis &when it's produced its completely normal.

Thalassemia is of 2 types: alpha thalassemia & beta thalassemia.
Normally we have **4** alpha genes and **2** beta genes.

*(Figure12)* Thalassemia is most common in Africa, south East Asia and Middle East.



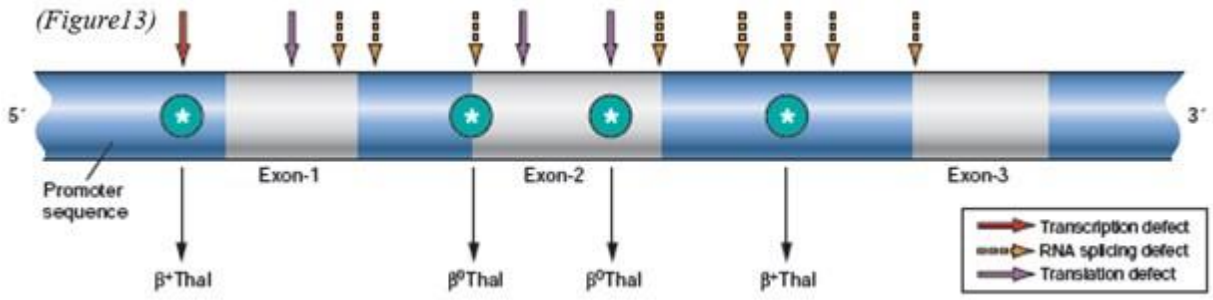
### ✓ β thalassemia

Beta thalassemia results from mutation at β globin gene on chromosome 11, the mutation has 2 types:

1) **$\beta^0$**in which there is completely absent production.
2) **$\beta^+$** there is some production but its reduced and amino acid sequence is completely normal.

*(Figure13)*What are the **mutation types** that happen in thalassemia?

- **Promoter region mutation** (promoter is the region in the DNA that's proximal to the gene itself). This promoter region will affect how much this gene is being expressed; if it has mutation, production will be decreased.
- **Splicing mutations**. before translation of mRNA, there is certain segments that need to be spliced (introns), if a mutation occur in these areas splicing will be abnormal resulting in either decrease production or completely absent production
- **Chain termination mutations** (stop codons); A change of amino acid resulting in a stop codon, synthesis will be stopped prematurely so there is no production of β globin chain.




After we had the mutation, how the anemia will happen? By **two mechanisms**:

- Decreased production of hemoglobin (Underhemoglobinization).
- Hemolysis; because we have excess precipitation of the alpha chain

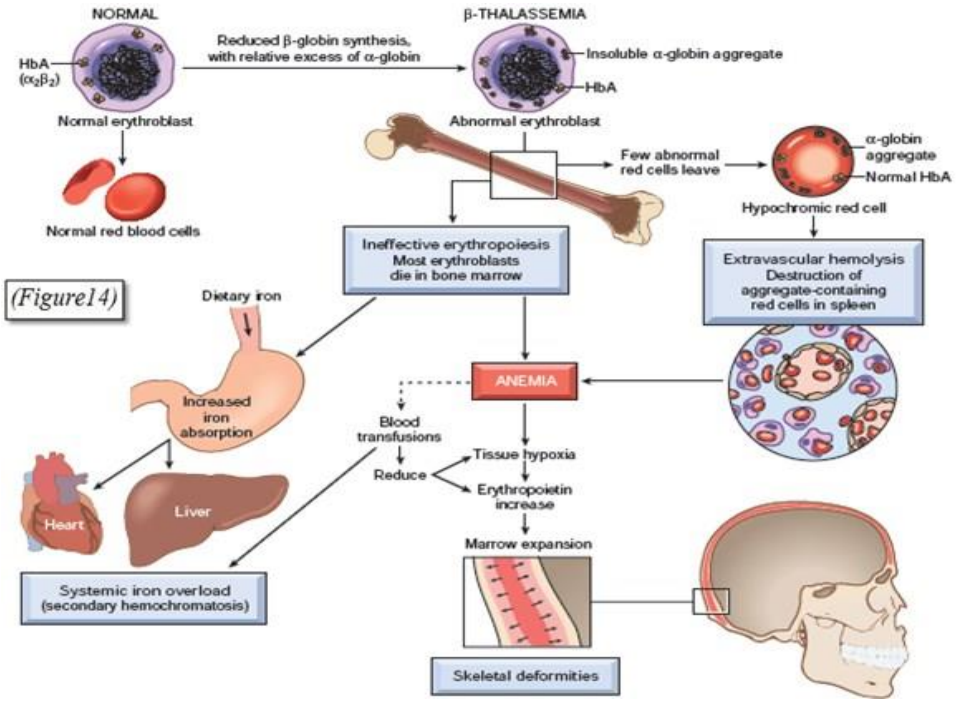
So anemia here is not exclusively anemia of **decreased production** or **peripheral consumption**; it has both mechanisms working side by side.

*(Figure14)* Sequences of anemia:

-The body will sense anemia and absorb more iron that will deposit in the heart → this is what kills in β thalassemia major (heart disease).

-Expansion in the bone marrow of the skull and other bones

-Anemia here is hypochromic microcytic.




3 clinical Types of thalassemia:

1. β thalassemia major two genes are affected
2. β thalassemia minor only one gene is affected
3. β thalassemia intermedia (in between); either one gene is normal and the other is affected with sever mutation or both of the genes are affected by mild or moderate mutations.

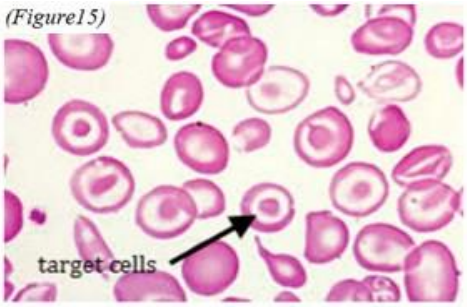
*** β thalassemia major** is common in Jordan, we try to prevent it by doing a screening test for thalassemia, its **MCV test**; people with β thalassemia minor have low MCV so they are advised not to marry their cousins.

It's done before marriage because here in Jordan we have what's called "Consanguineous Marriage"; if you have β thalassemia gene your cousin might have it, so there is a risk to have a children with homozygous gene mutation (β thalassemia major).

**Hemoglobin** is low, **MCV** is very low and **HbA2**(α2δ2)is increased for compensation; alpha is normal while beta have a problem so compensation will occur by producing delta.

*(Figure15)* **Morphology of thalassemia (Target cells specifically)**

We have **anisopoikilocytosis** which is variation in size and shape, and **Heinz bodies**.




These patients will have:

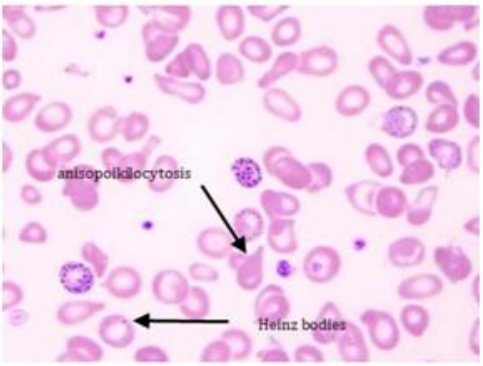
- Hepatosplenomegaly

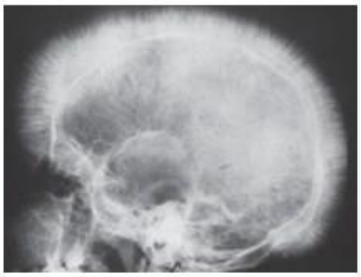
- Cardiac disease secondary to iron deposition.

- β thalassemia major are Transfusion dependent and they die early

- Guarded prognosis.

- The only hope for cure is stem cell transplantation.



*Crew- cut appearance*

*** β thalassemia minor:**

Same ethnic groups as β major, it's usually asymptomatic, CBC will show microcytosis.
They have elevated HbA2 and also have erythroid cell hyperplasia but they live normally.

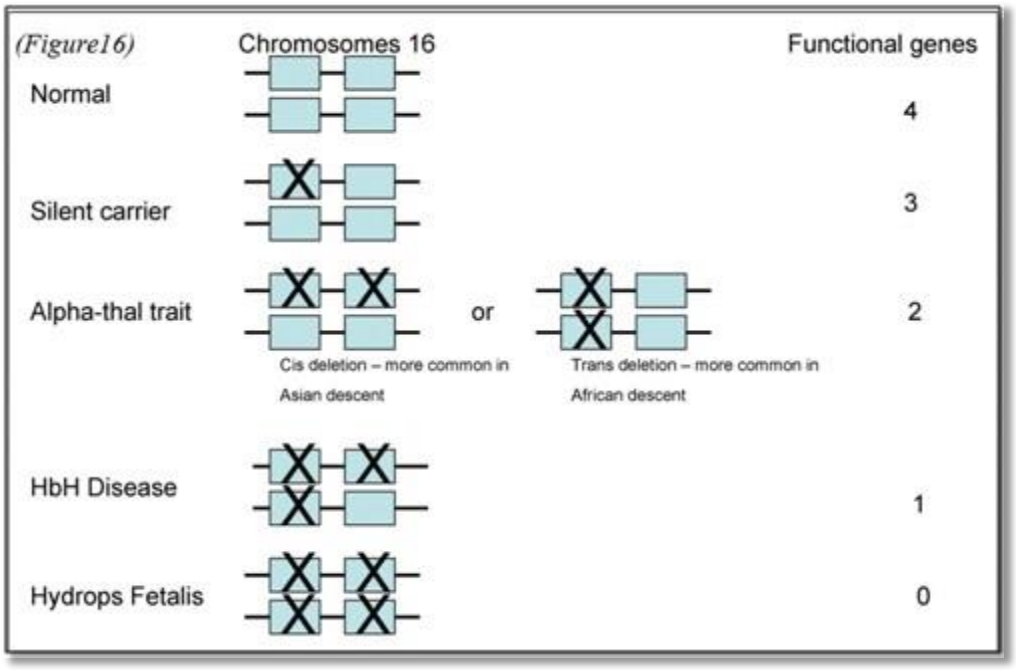
What's the importance in β thalassemia minor? <u>Differentiate it from iron deficiency anemia</u>.

High RDW → iron deficiency anemia
Low RBCs count →iron deficiency anemia.
In β thalassemia minor the RBCs count is high or normal.

β thalassemia minor has a role in <u>Genetic counseling.</u>

- ✓ **Alpha thalassemia**

We have 4 genes when the four genes are normal that's normal, if **one gene** is affected that's a silent carrier (no symptoms & no finding for the peripheral blood).
If **2 genes** are affected we call it alpha thalassemia trait, it has 2 types: **cis** and **Trans**; cis when the 2 genes are in the same chromosome (common in Asian), in Trans they are on two different chromosomes (common in African).
In **HbH** we have **3 affected genes**, and if we have **4 affected genes** (that is incompatible with life) so they die of **hydrops fetalis** in utero.
Remember that there is deference between alpha and beta; in **beta** thalassemia the mutations are usually **small point mutations**, while in **alpha** thalassemia they are **large deletions**.

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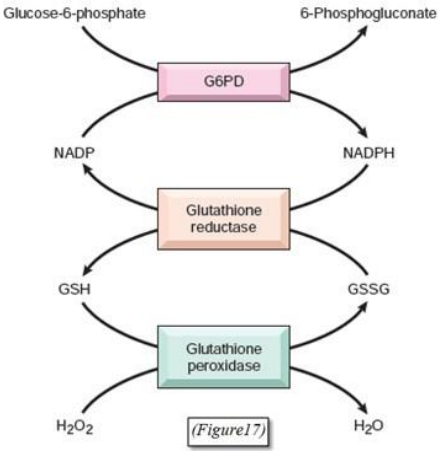
## ❖ G6PD deficiency




*(Figure17*) G6PD is enzyme produces NADPH; NADPH on the other hand reduces glutathione which will go ahead and find reactive oxygen species ($H_2O_2$).

So if you break this cycle we will have G6PD deficiency; the RBC will be more prone for oxidative stress. The cells that are more prone to oxidative stress are the **old RBCs**, the young RBCs -even in G6PD deficient patients- are not affected as severely as the old RBCs.

G6PD deficiency is X-linked disorder; more common in males.

Numerous mutations, more than 100 mutations, those required are G6PD A- (common in African Americans) and Mediterranean (common in Middle East).the Mediterranean is more severe than the A-.

These patients will present with acute episodic hemolysis not chronic hemolysis, usually induced by one of those 3:

- Infections ; the most common
- Fava beans; the most famous
- Drugs: antimalarials, nitofurantoin, etc…

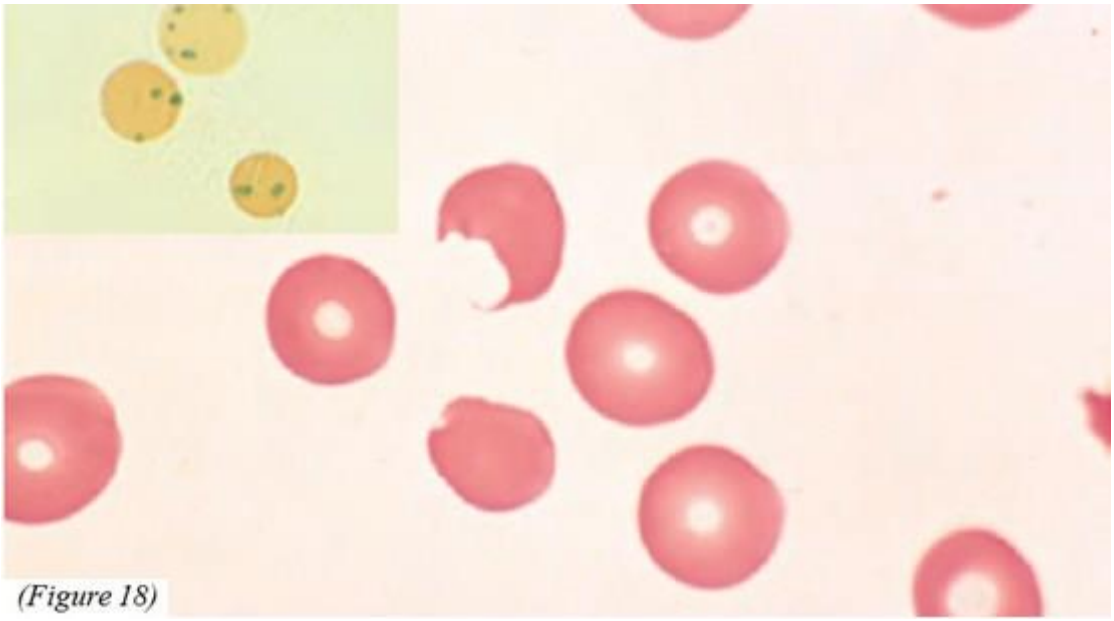
*(Figure 18)* Why the picture above is yellow (upper left)? Why can't we see it in the peripheral blood? **Reticulocytes and Heinz bodies** are not seen by Wright Giemsa stain which we use normally (our routine stain that we use in every blood test; peripheral blood bone marrow ), that's why we do a special stain known collectively as **supravital stains**, specifically **crystal violet**, those are the special stain that we need to use.

Heinz bodies are denaturing hemoglobin; when there is oxidative stress on the hemoglobin, the sulfhydryl groups within the amino acids will combine to each other causing precipitation (Heinz bodies). The RBCs that contain Heinz bodies will go to the spleen, which will pluck out these Heinz bodies resulting in a "bite cell" that you see in the center of the figure.

Precipitation by itself causes damage to the membrane, if it was **severe** enough it will cause **intravascular hemolysis**, if it was **moderate** then it will be damaged by the **spleen**, so hemolysis is either intra- or extra vascular.

Old RBCs are affected more. If the patient have G6PD hemolysis; old RBCs will die, young RBCs will survive. So even if the patient was taking the drug that causes the problem, hemolysis will stop because young RBCs are able to fight this oxidative stress.

Since its acute episodic not chronic, complications of chronic hemolysis (gallbladder stones and splenomegaly**) are not seen in G6PD.**



*(Figure 18)*

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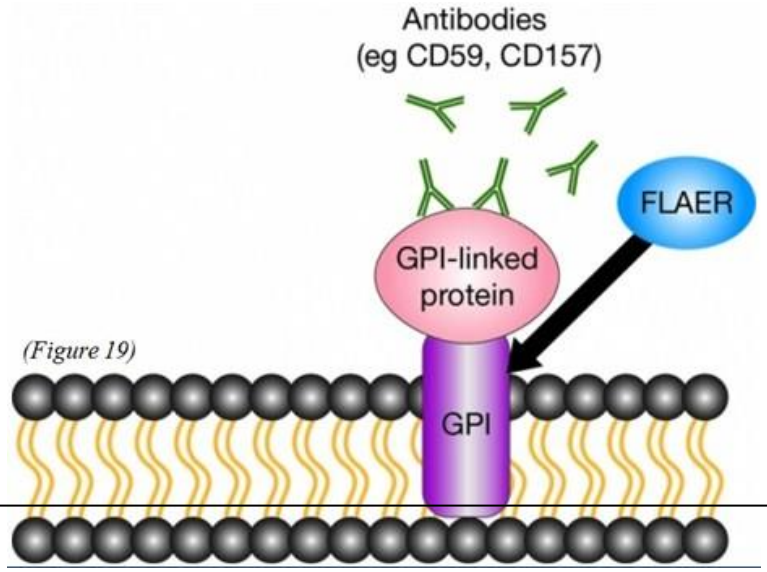
### ❖ Paroxysmal nocturnal hemoglobinuria (PNH)

- The probability to see it in your life practice is exceptionally rare - the probability to see it in the exam is 100%.
- RBC membranes (all membranes basically) have proteins to protect them from complement.
- Paroxysmal nocturnal hemoglobinuria is kind of a weird disease; there is **acquired mutation** not inherited. Not all cells are affected; its **one clone** in the body that has this mutation, it's kind of similar to leukemia or any cancer; one clone of the disease is affected not the whole cells in the body but here it's not neoplastic.
- *(Figure19)*What happens is mutation in **PIGA** gene will result in decreased production of PIGA protein on the membrane → less CD55 and 59 which fight complement → the cell will be more prone to complement fixation and hemolysis.
- It is the only hemolytic anemia resulting from an acquired genetic mutation.

- **PIGA** gene, present on the **X** chromosome.
- Usually Low level of chronic hemolytic anemia
- It's NOCTURNAL, why? Because PH will **decrease** a little bit making cells more prone to hemolysis.
- It has some association with aplastic anemia and thrombosis that's why the most clinical scenario when you suspect PNH is a young patient with anemia and thrombosis.
- The **treatment of those patients will increase the risk** of Niesseria infection, why? Because the treatment will fight complement, and complements -last 5 proteins specifically, which are called membrane attack complex (MAC) are really important to fight Niesseria, if you oppose these proteins you will risk that the patient might be affected by Niesseria.



*(Figure 19)*

*Questions*

1- What is the mode of inheritance in the vast majority of spherocytosis cases?
A. Autosomal dominant
B. Autosomal recessive
C. X-linked dominant
D. X linked recessive

2- The amino acid present at the sixth position of the normal alpha-globin chain is replaced by which one of the following amino acids in sickle cell disease?
A. Lysine
B. Valine
C. Serine
D. Alanine
E. None of the above

3- In thalassemia disorders, when only one alpha gene is affected, what do we call that?

A. Normal
B. Silent carrier
C. Thalassemia trait-cis
D. Thalassemia trait-trans
E. HbH disease

4- Gallbladder stones are a frequent complication of G6PD deficiency?

TRUE FALSE

5- Paroxysmal nocturnal hemoglobinuria results from an acquired mutation in which of the following genes:

A. Alpha hemoglobin
B. Beta hemoglobin
C. Erythropoietin
D. PIGA
E. G6PD

*Answers*

1. A 2.E 3.B 4.false 5.D

- *This sheet has been written according to the record of section 3 –mainly- and the record of section 2.*
- *I apologize for any mistake I may have made,*
  *Best regards :)*