Molecular Basis Of Hemoglobin Disorders
الفريق الطبي الأكاديمي

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Today we are going to talk about hemoglobin disorders and we will concentrate mainly on the molecular mechanisms of the pathophysiology of these disorders and how we could use molecular diagnostic techniques to diagnose these disorders.

These are some of the specific objectives that you must understand by the end of this lecture:

1. Structural features of human hemoglobins
2. Human globin genes
3. Development expression of human globin genes
4. Clinically important hemoglobinopathies
5. Oxygen affinity of abnormal hemoglobin variants
6. Know what is Hb S and its clinical correlation
7. Know what is Hb C and its clinical correlation
8. Know molecular basis of beta thalassemia & types including Hb E
9. Know what is hemoglobin Lepore and its clinical correlation
10. Know the molecular basis of delta-beta thalassemia
11. Know the molecular basis of High Persistence of Fetal Hemoglobin
12. Know molecular basis of Alpha thalassemia and its types

So we will talk about different types of hemoglobin genes, different types of hemoglobin, different types of hemoglobin variants, thalassemia, sickle cell, etc...

لحد الآن لسنا ما يتائها كل هاي مقدمة ليست للدراسة فقط لمعرفة ما سدرس ...
الآن نبدأ, بلا طالعة...
This slide summarizes the different types of hemoglobin.

The first column represents the protein itself and the second column represents the quaternary structure of those proteins and the third represents the percentages of each of these proteins.

<table>
<thead>
<tr>
<th>protein</th>
<th>globin chain composition</th>
<th>distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A</td>
<td>α₂ β₂</td>
<td>major adult hemoglobin (90-95%)</td>
</tr>
<tr>
<td>Hb A₁₀</td>
<td>α₂ β₂</td>
<td>glycosylated β globin (3%; diagnostic of diabetes mellitus, up to 15%)</td>
</tr>
<tr>
<td>Hb A₂</td>
<td>α₂ δ₂</td>
<td>minor adult hemoglobin (1.5-3.5%)</td>
</tr>
<tr>
<td>Hb F</td>
<td>α₂ γ₂</td>
<td>major fetal hemoglobin (0.5-1% in adult)</td>
</tr>
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embryonic hemoglobins ζ₂ ε₂; α₂ ε₂; ζ₂ γ₂
chromosome 11: ε γ δ β
chromosome 16: ζ α₂ α₁

modified from Bunn (2001)
- In adult we mainly have Hb A which is composed of 2 α subunits and 2 β subunits (it is a tetramer).

- Hb A1c: it is called the glycosylated hemoglobin in which glucose will be covalently attached to hemoglobin and it is an important marker for diabetes in the past three months for every diabetic patient. So in order to know the situation of diabetic patient in the previous three months, you just need to determine the amount of Hb A1c, and nowadays it is determined routinely in medical laboratories or in the hospitals. It has specific physiological concentration up to 5.8% if it exceeds this value, this means that your sugar was not well controlled in the previous 3 months.

- In adult form we have also another type of hemoglobin which is hemoglobin A2 which is composed of 2 α subunits and two δ subunits. Its concentration is low in the adult stage, it does not exceed 2% that stage.

- Hb F which is composed of 2 α subunits and 2 γ subunits; in the adult stage it does not exceed 1%; it is found mainly in the fetus stage.

- Embryonic hemoglobins: they are 3 types ζ2 ε2, α2ε2, ζ2 γ2.

  ζ (zeta) is α like globin gene.

  ε and γ are β like globin gene.

In a moment we are going to see the location of these genes on chromosomes and they are arranged according to the developmental expression.

- The β like globin genes are located on chromosome 11.
- The α like globin genes are located on chromosome 16.

Embryonic hemoglobins: they are expressed in the embryonic stage and they are not expressed in the adult and once that stage is finished they are switched off and the other hemoglobins will be switched on.
Adult hemoglobins: they expressed in the adult stage.

What makes the embryonic hemoglobin genes to be switched off and stop synthesizing embryonic hemoglobin in the adult stage? what is the mechanism of hemoglobin switching?

(8:54-13:00min)

In the slide, we can see alpha like and beta like...

- In the alpha like we have sequence that resembles enhancers and it is called LCR (locus control region) this sequence on the DNA will control the regulation of the expression of these genes, we can see the zeta alpha like, alpha 2 and alpha 1, they are located according to the developmental expression, this (zeta) is the first to be expressed in the embryonic stage as alpha like, then when it is switched off one or both of these will be expressed in the adult stage (α1 and α2).

- These are the beta like globin located on chromosome 11, they are also under the control of LCR and they are arranged.
according to the time of expression during development, so we can see the globin gene which is called **epsilon that is going to be expressed first in the embryonic stage** the rest are switched off, that expression (zeta) will coordinate with this expression and both will make the embryonic hemoglobin as zeta 2 epsilon 2, then the gama globin gene after epsilon is switched on those genes will be expressed in the fetal stage and once the fetal stage is off these genes will be switched off and delta and beta will start to be expressed, so there will be coordination between the components of the beta like as well as the components between alpha like and there is an intercoordination between alpha like and beta like to produce a balanced production of globin and that to produce that specific type of hemoglobin.

*Now remember that in the fetal stage if you measure the hemoglobin for the newly born baby, you will see it about 20 g/dL, it is rich in fetal hemoglobin.

*Fetal hemoglobin has high oxygen affinity than maternal adult hemoglobin.*
These are some types of disorders

Here we have hemoglobins with decreased of oxygen affinity, you remember the oxygen affinity for hemoglobin and how it follows the sigmoidal, cooperative binding and dissociation... (We are not going to repeat that ذكرنا من أجل التذكير بهم لا أكثر).

Some types of hemoglobin, they have decreased oxygen binding affinity and the clinical symptom on those patients who have hemoglobin variants that have decreased oxygen affinity, they will show cyanosis, and in the other side, there are some hemoglobins with increased oxygen affinity and as a result polycythemia symptoms will be produced.

Met hemoglobin in which iron is not in ferrous state but in the ferric state, cyanosis also will be produced, why?!

Because it will not bind oxygen (very low), because of the ferric versus ferrous.
We are going to concentrate on thalassemia in which there is a decreased synthesis of alpha or beta globin proteins due to defects in the alpha or beta globin genes, alpha thalassemia due to defects in the synthesis of the alpha globin proteins due to defects in the alpha globin genes and the same applied for beta thalassemia.

The purpose of this slide is to show you the oxygen affinity for different types of hemoglobin variants, some of them have high affinity, some of them have low affinity; and these are due to variations in the structure of hemoglobin due to some mutations or structural changes in that hemoglobin or in the tertiary structure of the hemoglobins that prevent or decrease oxygen affinity to that type of hemoglobin.
Genetic disorders of hemoglobin

- structural variants in the proteins (hemoglobinopathies)
  - sickle cell disease
    - affects codon 6 of β-globin
    - GAG (glutamic acid) to GTG (valine)
    - results in a severe sickling disease
  - hemoglobin C disease
    - affects codon 6 of β-globin
    - GAG (glutamic acid) to AAG (lysine)
    - results in a mild hemolytic anemia
- thalassemias (decreased α- or β-chains)
- hereditary persistence of fetal hemoglobin (HPFH)

(16:14-20:00min)

This is to show you what is the biochemical lesion in sickle cell disease, it is in **codon 6** (What do we mean by codon? It is a 3 nucleotides that will indicate an amino acid), so codon 6 in beta globin gene, there is a point of mutation in GAG (What is GAG? It is a codon for glutamic acid)), this GAG has been changed to GTG, only one nucleotide in the middle has been changed by mutations and GTG is the codon that indicates another amino acid which is called **valin**, so you have beta globin gene which is about 146 amino acids, only one amino acid has been changed from **glutamic acid to valin** and that on single change of amino acid due to a single nucleotide mutation in codon 6 and that produces a hemoglobin which is insoluble inside the cell called **hemoglobin S** that will cause destruction of the membrane of the RBCs causing the sickling structure and causing hemolysis, why?! It is due to defect in primary structure of beta globin gene and converts
the normal beta into beta S globin gene which is defected, not functional and cause diseases.

The same thing for another disease called hemoglobin C disease, it also in codon 6, GAG (glutamic acid) has been changed to AAG to produce lysine, but the severity of sickle cell is more than the severity of the hemoglobin C.

**Sickle Cell Disease**

Sickle cell disease is an inheritable, multisystem disorder, with physiological and psychosocial manifestations

- **cardinal features:** hemolytic anemia and recurrent pain
- **biochemical basis:** \( \beta \)-globin gene point mutation - Glu6\( \beta \) → Val

This amino acid change reduces solubility and promotes polymerization of deoxygenated hemoglobin S (Hb S) molecules.

**spectrum of genotypes:**

- sickle cell anemia (Hb SS) - severe clinical problems
- sickle cell trait (Hb AS) - few clinical problems
- sickle cell - \( \beta^- \)-thalassemia - more benign clinical problems; co-inheritance of two hemoglobin variants

The hematologic severity of sickle cell disease is inversely proportional to the amounts of Hb A (3-25%) and Hb F (up to 15%).

(20:04-25:00min)

Now remember that every one of us have 2 beta globin genes and 4 alpha globin genes, so in case of sickle cell we have two beta globin genes, if both beta globin genes that we inherited from our parents are beta S then the progeny will have sickle cell disease (homozygous for beta S) if one of the parents have the beta S and the other has the normal beta then progeny or some of progeny will have beta beta S that is sickle cell trait , it is not sever , it is mild, may be it is asymptomatic
comparable to sickle cell disease homozygous beta S beta S, the same thing applies for hemoglobin C disease.

**Thalassemia Syndromes**

As a consequence of abnormal or non-functional globin genes, the insufficient synthesis of either hemoglobin subunit leads to a hemolytic anemia. Severity is a function of the number of mutated genes.

**β-thalassemia** - decreased level (β’) or complete absence (β°) of β-globin protein

There may be some compensation by continued production of Hb F (α2γ2).

**α-thalassemia** - decreased level of α-globin protein

This is more complicated, since there are two α-globin genes; the clinical picture extends from mild anemia to stillbirth.

There is some compensation by formation of Hb H (βδ) and Hb Bart’s (γδ). These proteins exhibit neither allosteric transitions nor a Bohr effect; both remain in the R state.

Now remember in case of thalassemias (alpha or beta) the defect here is the decrease in the synthesis of alpha globin or beta globin, it is not structural dysfunction, you will have no enough amount of alpha or beta (no enough synthesis of alpha or beta) and under that condition you will have alpha or beta thalassemia.

In sickle cell, you have complete synthesis (no decrease synthesis of hemoglobin) but they are defected in structure and they are not functional.

Sometimes, sickle cell could appear in some people as double or compound heterozygote (you can see people with sickle cell disease have hemoglobin with beta S beta S, beta S beta A (sickle cell trait), beta S beta thal (combination between sickle trait and thalassemia
trait), beta S beta C, these are called compound heterozygote of the disease.

For example, sickle cell- β⁺-thalassemia, it is double heterozygote

What is the difference between β⁺ and β₀

In β⁺ we have some synthesis of beta globin and beta node there is no synthesis of beta globin.

How you explain that sometimes there is something synthesis of beta globin and sometimes there is no synthesis at all at beta globin according to the molecular genetics and gene expression?

A compensatory mechanism for sickle cell anemia is the production of Hb F because there is something wrong in β-gene so the γ-gene will be switched on to produce γ-globin and with abundant α-globing so Hb F will be produced (α₂ γ₂), in this case you will see that the percentage of Hb F is 10% instead of 2% in normal condition.

β-Thalassemia (28:00)

due to mutations in β gene we have decrease or no synthesis of β-globin and we’ll see here as a compensation mechanism Hb A₂ (α₂ δ₂) as δ gene will be switched on to produce δ-globin to be combined with α-globin and produce Hb A₂. This is a marker for β-thalassemia. (If Hb A₂ is normally below 1% , you will see 5%)

☐ About 200 mutations worldwide but only about 30 of these reach a frequency of 1% or more in the at-risk groups.

☐ the common mutations are all point mutations because of single base substitutions, insertion or deletion of a few bases
For α-thalassemia the molecular lesion is deletion of (1 or 2 or 3 or 4) genes (the severity of the disease depends on the dose of deletion).

In case of α-thalassemia because there is a decrease in α-globin and excess of β-globin, you will see different types of hemoglobin that are composed of tetramer of β-globin or γ-globin.

Because there is a decrease of α-globin we’ll see hemoglobin with tetramers like Hb H and Hb Bart’s which are not functional.

- Point mutations in the promoter.
- Mutations in the translational initiation codon.
- Point mutation in the polyadenylation signal.
- Array of mutations leading to splicing abnormalities.

In β-thalassemia, where the β-globins are deficient, the α-globins are in excess and will form α-globin homotetramers.

- The α-globin homotetramers are extremely insoluble which leads to premature red cell destruction in the bone marrow and spleen

(33:40)

- β 0-thalassemia : A complete lack of HbA
- β +-thalassemia : Production of a small amount of functional β- globin

- Individuals homozygous for β-thalassemia have what is termed thalassemia major. genotype is (β-thal, β-thal) → require multiple blood transfusion,

- Individuals heterozygous for β-thalassemia have what is termed thalassemia minor. → doesn’t require blood transfusion.

- Mutations that disrupt splicing
• \( \beta^o \)-thalassemia - no \( \beta \)-chain synthesis

• \( \beta^+ \)-thalassemia - some \( \beta \)-chain synthesis

(35:40)

• Normal splice pattern:

Intron 2 acceptor site \( \beta^o \) mutation: no use of mutant site; use of cryptic splice site in intron 2

what happens here is point mutation in acceptor site (AG) in intron 2 that become (GG) so it will no longer be used as the acceptor site, the cell here will start to search in the intron 2 for a sequence that resembles the acceptor site which is called cryptic acceptor site.

Unfortunately the sequence here has a stop codon therefore a functional protein cannot be made.

Intron 1 \( \beta^+ \) mutation creates a new acceptor splice site: use of both sites
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CCUAUUAAG/U: β+ mutant site (used 90% of the time)
CCUAUUGG U: Normal intron sequence (never used because it does not conform to a splice site)

Translation of the retained portion of intron 1 results in termination at a stop codon in intron 1

Exon 1 b+ (Hb E) mutation creates a new donor splice site: use of both sites

GGUG/GUAAGGCC: b+ mutant site (used 40% of the time)
GGUG GUGAGGCC: Normal sequence (never used because it does not conform to a splice site)

The GAG glutamate codon is mutated to an AAG lysine codon in Hb E

The incorrect splicing results in a frameshift and translation terminates at a stop codon in exon 2

الدكتور ما حكى اشي عن اخر رسمتين وقال انهم تقربهما نفس فكرة اول رسمة ف اللي بحب يقرأ اشي عنهم الكلام التالي كان موجود تحت السلايده عنهم 😊
This figure shows two examples in which splicing mutations have given rise to beta(plus)-thalassemia. In these cases there is some remaining beta-globin synthesis because the normal splice sites were not mutated - they are still functional. Instead, new splice sites were created from sequences that closely resembled splice sites but did not normally function as such. (For an understanding of these mutations, it would be instructive to view the slide showing the frequency of bases in each position of the splice sites).

In the top case, a G to A mutation has occurred in intron 1 creating an AG dinucleotide, making that site more dominant over the normal acceptor site. In the bottom case, a G to A mutation has occurred in exon 1 creating a somewhat better overall donor splice site than was already there (and normally unused). It is important to see that there is a region in exon 1 that already has a GU dinucleotide, but that the G in the third position (while tolerable in a splice site) was not good enough in the overall context of that sequence to render it functional under normal circumstances. However, mutating the G (which is found in only 29% of donor sites) to an A (which is found in 62% of donor sites) was able to "tip to balance" and make it functional (unfortunately for patients with disease). The Hb E mutation is very common – it is estimated that there are 30 million heterozygotes for the Hb E amino acid variant in Southeast Asia.

**Thalassemia Intermedia** (40:12)

- Both β-globin genes express reduced amounts of protein or where one gene makes none and the other makes a mildly reduced amount.

Or sometimes double heterozygotes (β+ and α-thalassemia) will cause thalassemia intermedia.

- A person who is a compound heterozygote with α-thalassemia and β+-thalassemia will also manifest as thalassemia intermedia.
Anemia
Do not require transfusions.

Hemoglobin lepore

- unequal crossing over
  - hemoglobin Lepore (β-thalassemia)

- unequal crossing over occurred due to the close homology of the δ- and β-genes: only 10 out of 146 residues differ (the genes are ~90% homologous to each other)
- the consequence can be severe β-thalassemia due to decreased synthesis of the δβ-fusion (due to the weak δ-globin promoter)

Hb Lepore happens due to fusion of β and δ genes.

This happens because of the homology of the sequence of δ and β-globin genes and this could lead to misalignment and unequal crossing over to produce chromosome with part of δ and β genes

The severity of Hb lepore is because the δβ-fusion gene is under the regulation of δ-globin promoter which is very week so there will be very low synthesis of δβ-globin.
Delta-Beta thalassemia, or Hereditary Persistence of Fetal Hemoglobin (HPFH)

- Large deletions of the delta-beta region of chromosome 11 can give rise to delta-beta thalassemia, or to hereditary persistence of fetal hemoglobin (HPFH), which is a benign disorder with no apparent disease symptoms.

- In delta-beta thalassemia there is some compensation by continued (or persistent) expression of the gamma genes.

- In HPFH there is complete compensation by this persistent gamma chain expression.

- It has been hypothesized that these large HPFH mutations delete regulatory sequences that control the decreased expression of the gamma chain genes that occurs during hemoglobin switching shortly after birth.

- Large deletions (examples of large deletions with little or no phenotype)
  - δ/β-thalassemia -- some compensation by γ-chain synthesis
  - HPFH -- entirely compensated by γ-chain synthesis

(deleted regions are indicated by the boxes below the chromosome)
Thalassemia as we said is due to point mutation but in these two cases it’s deletion of large area and in these cases the γ-gene will be switched on even in the adults as a compensation mechanism this will cause in Hb F production because the δ and β genes are deleted (part of them or all as shown in the figure above)

α-THALASSEMIA

- Unequal crossing over between the alpha1 and alpha2 genes.
- Crossing over results in the fusion of the two genes and therefore in essence the deletion of one of the genes.
- This event by itself would not give rise to alpha thalassemia because in diploid cells there would still be three alpha genes, which is sufficient.
- However, if two individuals who are heterozygous for the deleted gene have children, there is a 25% chance that a child will inherit two of these deletion chromosomes.
· normally there are four α-globin genes in heterozygotic somatic cells
· loss of α-globin genes results in different severities of α-thalassemia depending on the number of genes lost in combination with deletion chromosomes

α2  α1  normal

α2-thalassemia (silent carrier state)

α1-thalassemia (no significant anemia)

Hb H disease (mild to severe anemia)

hydrops fetalis (fetal or early neonatal death)

α2-thalassemia usually used when there is only 1 α-gene deleted

Hb H is produced when there is 3 α-genes deleted because there is no α-globin so the β-globin for tetramers (β₄)