Hemoglobinopathies

Prof. Mamoun Ahram Hematopoietic-lymphatic system
Resources

- This lecture
- Mark’s Basic Medical Biochemistry, Ch. 44
What are hemoglobinopathies?

- Hemoglobinopathies: Disorders of human hemoglobin (deficiency in the oxygen transport system).
- The most common genetic disease group in the world—so they are really serious diseases—(5% of people are carriers) with substantial morbidity (about 300,000 born each year).
- Hemoglobin disorders account for 3.4% of deaths in children < 5 years.

Notice the distribution of hemoglobinopathies around the world that concentrated in the old world—including Jordan—
Types of abnormalities:

- Qualitative abnormalities: mutations resulting in structural variants in hemoglobin that makes it defective in O2 transport, (each amino acid has a function in a hemoglobin molecule, and there is a good chance that one of them would be mutated, and this results in a defective hemoglobin)
  - Over 800 variants have been identified.

- Quantitative abnormalities are abnormalities in the relative amounts of α and β subunits (thalassemias) [the hemoglobin is fine in structure]. Jordan has high prevalence of thalassemias.

- Hereditary persistence of fetal hemoglobin (HPFH): impairment of the perinatal switch from γ to β globin.
  - NO switching from gamma to beta globin-
Qualitative abnormalities
Classification of molecular mutations

- Mutations in surface residues
  - Usually asymptomatic (e.g. HbE); an exception is HbS >>sickle cell anemia[ it is a single point mutation that really causes a dramatic effect on the function of hemoglobin molecule.]

- Mutations in internal residues (remember that Hb is a globular protein with a hydrophilic AAs outside and hydrophobic AAs inside)
  - Often producing unstable hemoglobin, Heinz bodies and causing hemolytic anemia (e.g. Hb Hammersmith)

- Mutations stabilizing methemoglobin – a hemoglobin with iron in the ferric state-
  - Stabilizing heme-Fe $^{+3}$; resulting in cyanosis – cannot bind to oxygen-

- Mutations at $\alpha$1-$\beta$2 contacts
  - Altered oxygen affinity (mainly higher; a condition known as polycythemia)
Sickle cell hemoglobin (HbS)

- It is caused by a change of amino acids in the 6th position of \( \beta \) globin (negatively charged Glu to non-polar Val).
- The hemoglobin is designated \( \alpha_2\beta_s2 \) - there are 2 beta chains that sickled - or HbS.
- The hemoglobin tetramers aggregate into arrays upon deoxygenation in the tissues.
- This aggregation leads to deformation of the red blood cell because we have millions of these hemoglobin molecules and they all aggregate together as chains.
- It can also cause hemolytic anemia (life span of RBCs is reduced from 120 days to <20 days).

It is caused by a single point mutation in one nuclide in DNA.

We have the hemoglobin proteins as aggregates, forming a chain of proteins instead of having hemoglobin proteins as individual molecules.
Cellular effect on system

- Repeated cycles of oxygenation and deoxygenation (that would cause clumping and clustering of the hemoglobin molecules, so blood flow is restricted) lead to irreversible sickling.
- Cells cannot squeeze through capillaries in a single file and therefore block blood flow causing local hypoxia (low oxygen levels in tissue).
- Long-term recurrent clogging of the capillary beds leads to damage to the internal organs, in particular the kidneys, heart and lungs.
How does the fiber form?

- Fiber formation only occurs in the deoxy or T-state.
- The mutated valine of $\beta 2$ chain is protruded and inserts itself into a hydrophobic pocket on the surface of $\beta 1$ chain.

### Variables that increase sickling
- Decreased oxygen pressure (high altitudes)
- Increased $pCO_2$
- Decreased pH
- Increased 2,3-BPG
- Dehydration (why?)

Deoxyhemoglobin S polymerizes into filaments.
we have here in (1) a hemoglobin molecule that's oxygenated, if it's deoxygenated as (2) it will cause a formation of a hydrophobic pocket within the beta 1 chain.

In a sickled hemoglobin we have the formation of a Valine protrusion (hydrophobic) that includes a Valine molecule - the sickled hemoglobin here is (3) -

So, when it becomes deoxygenated, the protrusion still exists + we have the hydrophobic pocket = so what happens is this protrusion of one molecule would fit the pocket of another molecule and that would cause clumping of the hemoglobin molecules

- remember that sickling increases with repeated oxygenation-deoxygenation cycles

So, WHAT does increase the sickling?

Producing more T form, like:
1. Decreased O2 pressure
2. Increased CO2 pressure
3. Decreased PH
4. Increased 2,3 BPG
5. Dehydration
Sickle cell trait

- It occurs in heterozygotes (individuals with both HbA and HbS), who are clinically normal, but their cells sickle when subjected to low oxygen.

Advantage: selective advantage from plasmodium falciparum that causes malaria – they become resistant to infection by malaria -.

Why?

Check the next slide
About the previous slide

Why??
One reason is that sickled RBCs have low half life (40 days or less) and the reproductive duration of malaria is 60 days and malaria infects RBCs so this prevents malaria from completing its growth inside cells.

About the map
If you look at the map (on the left) which shows the distribution of sickle cell allele in Africa, you can see that it actually matches the distribution of Malaria in these countries. (On the right) > so there is an advantage for theses individuals.
Hemoglobin C (HbC)

- (HbC) is also due to a change at the 6th position of β globin replacing the glutamate with lysine - positively charged - (designated as βc).
- This hemoglobin is less soluble than HbA so it crystallizes in RBCs reducing their deformability in capillaries – decrease the ability of RBCs to change their shape as the squeeze in capillaries –
- HbC also leads to water loss from cells leading to higher hemoglobin concentration.
- This problem causes only a minor hemolytic disorder.

(Insignificant effect on function contrary to HbS)

If you look at the blood sample you will see the abnormality in the morphology of RBCs in individuals having HbC

You have finished 1/3 of the slides, GO ON :)
Individuals with both $\beta_c$ and $\beta_s$ mutations have HbSC disease, a mild hemolytic disorder which may have no clinical consequences, but it is clinically variable.

They produce defective hemoglobin molecule just like normal people or patients with sickle cell trait – mild clinical abnormalities.

1st allele : Glu to Val
2nd allele : Glu to Lys
Hemoglobin E

- It is common in Southeast Asia
- It has both quantitative and qualitative characteristics.
- It is caused by a point mutation in beta chain in codon 26 that changes glutamic acid (GAG) to lysine (AAG) creating an alternative RNA splice site and a defective protein (it's not stable as the normal protein, so it would tend to be degraded)

- Individuals with this mutation make only around 60% of the normal - low - amount of β-globin protein. and Hb has deficient ability to carry O2.
Hb Hammersmith

- Hb Hammersmith results from a point mutation that leads to formation of unstable hemoglobin and denaturation of the globin protein.
- The most common point mutation of Hb Hammersmith substitutes an internal phenylalanine (which exists in the heme pocket) with a serine (polar) within the β globin, reducing the hydrophobicity of the heme-binding pocket, heme positioning, and oxygen binding affinity causing cyanosis. That will cause destabilization of the interaction between heme and hemoglobin molecule.
Mutations at α1-β2 contacts

- Hb Cowtown: Substitution of His146 (responsible for the Bohr Effect) to Leucine produces more hemoglobin in the R state (increased affinity).
- Elimination of hydrogen bonds between the chains can also alter the quaternary structure:
  - Hb Kansas: stabilization of the T state (Asn G4 (102) to thr); Decreased cooperativity.
  - Hb Yakima: stabilization of the R state (Asp G1 (99) to His or Asn).

There are different mutations of the same amino acid

You would have in this case destabilization of the T state.
We talked about non-covalent interactions that take place between the polypeptide chains and how they stabilize the T or R states, one of them is **His 146 – beta chain- with Lys 40 – alpha chain**.

Also, **His 146** can also interact non covalently **with aspartate 94 – in the same chain (beta)** which called **intra-molecular interaction** and this stabilizes the T state.

**SO WHAT?**

If His 146 is mutated to leucine for example that would actually stabilize the R state – high affinity - because there is no more interaction and the T state is destabilized and this variant is known as Hb CowTown.

There are many single mutations of hemoglobin and these are given the names of the towns where these mutations were identified such as Hb Kansas and Hb Yakima.
Stabilization of T/R state

- Hb Cowtown: Substitution of His146 (responsible for the Bohr Effect) to Leucine produces more hemoglobin in the R state (increase affinity).
- Elimination of hydrogen bonds between the chains can also alter the quaternary structure:
  - Hb Kansas: stabilization of the T state (Asn to thr).
  - Hb Yakima: stabilization of the R state (Asp to His).

**NOTE:** this slide is not in the original file of slides on e learning or in live lecture but I found it in the 2021 recorded lecture and it is almost a review so check the next slide for the explanation :)
The mutations change the equilibrium between the 2 states of the hemoglobin molecule.

Remember,
In the T state we have interactions between His - in beta- with Asp - in beta-, and His - in beta-with Lys - in alpha-, that stabilize the T state.

Once we have oxygen formation hemoglobin changes to the R state and what happens is sliding of the alpha chain and loss of interaction between His with Asp and His with Lys and Instead of that we have formation of interaction between Asn-in beta- with Asp - in alpha-

So, when there is a mutation and Asn changes to Thr, so the reaction between Asn and Asp that stabilizes R state will not happen which would stabilize and fix the hemoglobin molecule in the T state (Hb Kanses)

And when Asp -beta chain- changes to His we won't have the interaction that stabilizes the T state, so the equilibrium changes the formation of R state and the molecule will stay in the R state (Hb Yakima)

The doctor said that he may ask a question about this idea but with different amino acids mentioned in the lectures so check the modified slides and make sure that you memorized them.
Altered Oxygen Transport

These are examples about qualitative hemoglobinopathies and remember that there are 700 variants, so we can talk about every single amino acid and there are different names for different hemoglobin variants.
Methemoglobin can undergo reversible oxygenation because its heme iron is in the reduced (ferrous, Fe\(^{+2}\)) state.
- During oxygen release from heme, Fe\(^{+2}\) is oxidized to Fe\(^{+3}\), forming methemoglobin (HbM), except that the enzyme methemoglobin reductase reduces iron back.
- If not, a condition known as methemoglobinemia develops.

Methemoglobin is a Hemoglobin molecule that is bound to iron in the ferric state rather than the ferrous state. So it is not able to bind oxygen.

Methemoglobin reductase AKA NADH-Cytochrome b5 reductase

Chocolate lips because of cyanosis
Normal people have HbM but in very low quantities because of:

1. The hydrophobic pocket surrounding the heme molecule prevents the iron oxidation from the ferrous to ferric state.

2. Individuals have methemoglobin reductase so it immediately reduces HbM into Hb with iron in the ferrous state, and this requires cytochrome molecule within the enzyme itself and NADH.

So, we oxidize NADH and transfer the electrons to iron.

- this process takes place in RBCs
- The source of NADH is glycolysis
Some mutant globins ($\alpha$ and $\beta$) bond heme in such a way as to resist the reductase.

- Hb Boston: distal histidine – that stabilizes the oxygen binding to a heme molecule - is mutated into a tyrosine resulting in oxidation of ferrous iron by tyrosine’s oxygen. It also attracts H2O into the pocket.
- HbM Iwate: it was discovered in Japan- proximal histidine (that binds to iron in the 5th coordinate) is replaced by a tyrosine.

A deficiency of the reductase enzyme.

Certain drugs or drinking water containing nitrates.

All of these causes increase the probability of oxidizing Iron from Fe+2 to Fe+3
NADH from glycolysis is the main source of electrons.

Methylene blue can be used in treating patients with methemoglobinemia.

NADPH is the source of electrons that would be used to reduce methylene blue into leukomethylene blue.

These electrons would be donated ultimately to Fe+3, converting it to Fe+2.

The second source of electrons for methemoglobin resulting in the production of the normal globin molecule.

In pentose phosphate pathway, we have production of NADPH.
Quantitative abnormalities (thalassemias)
• Thalassemias: the most common human single-gene disorder specifically in the old world including the middle east.
• They are caused by a reduced amount of either the α or β protein, which alters the ratio of the α:β ratio—in normal RBCs we have an equal amount of alpha and beta chains.
The Alpha-Thalassemias

- Alpha-thalassemia: underproduction of the $\alpha$-globin chains
  - we have more beta than alpha.
- HbA ($\alpha2\beta2$), HbF ($\alpha2\gamma2$), and HbA2 ($\alpha2\delta2$) are all affected in $\alpha$-thalassemia.

Remember that,
- Alpha chain is produced at the end of embryonic stage and continues throughout the life.
- Beta chain: starts to be produced slowly in the early fetal stage but there is a big jump right before birth and it continues throughout the life having equal quantities of alpha and beta chains.
With reduction of α chain production, and β-chain production is established, homotetramers of β (β4 or HbH) are formed.

The HbH tetramers have a markedly reduced oxygen carrying capacity.

Main type of mutation is deletion (rarely point mutations)

Remember that we have 4 alpha genes – 2 on each chromosome.
Variable severity

• With the $\alpha$-thalassemias, the level of $\alpha$-globin production can range from none to very nearly normal levels.
• This is due in part to the fact that each individual has 4 genes.
Hydrops fetalis

- 4 of 4 genes are deleted.
- The predominant fetal hemoglobin is a tetramer of $\gamma$-chains.
- $\gamma 4$ or Hb Bart: a homotetramer of $\gamma$.
- Hb Bart has no oxygen carrying capacity resulting in oxygen starvation in the fetal tissues.
- This situation is called hydrops fetalis.
- Stillbirth or death shortly after birth occurs.
Hemoglobin H disease

- 3 of 4 genes deleted one only is active.
- Mild to moderate hemolytic anemia in adults.
- A high level of β4 tetramer is present (high HbH).
- Clinically, it is known as hemoglobin H disease.
- The disease is not fatal but it is symptomatic.
Minor $\alpha$-thalassemia and silent carrier

- $\alpha$-Thalassemia trait -minor-: If 2 of the 4 genes are inactivated.
  - The individuals are generally asymptomatic.

- Silent carrier: 1 of 4 genes deleted.
  - Individuals are completely asymptomatic.
## Summary of α-thalassemias

<table>
<thead>
<tr>
<th>Genotype</th>
<th>α-globin gene number</th>
<th>Name</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>αα / αα</td>
<td>4</td>
<td>Normal state</td>
<td>None</td>
</tr>
<tr>
<td>αα / α−</td>
<td>3</td>
<td>Silent carrier</td>
<td>None (values for Hb and MCV may be near the lower limits of normal)</td>
</tr>
<tr>
<td>α− / αα</td>
<td>2</td>
<td>Thalassemia trait</td>
<td>Thalassemia minor: asymptomatic, mild microcytic anemia</td>
</tr>
<tr>
<td>-- / αα</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>or</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α− / α−</td>
<td>1</td>
<td>Hb H disease</td>
<td>Thalassemia intermedia: mild to moderate microcytic anemia</td>
</tr>
<tr>
<td>-- / --</td>
<td>0</td>
<td>Alpha thalassemia major</td>
<td>Thalassemia major: hydrops fetalis</td>
</tr>
</tbody>
</table>

Deletion of alpha alleles on one chromosome or on each chromosome.

*Number of normal alpha globin genes*
The beta-thalassemias

Normal people have 2 alleles one on each chromosome

- β-globins are deficient and the α-globins are in excess and will form α-globin homotetramers.
- Main type of mutation is point mutations, mutations within the coding region or the promoter, translation initiation codon, splicing positions, or poly-adenylation termination signal.
- The α-globin homotetramers are extremely insoluble, which leads to premature red cell destruction in the bone marrow and spleen.
• A complete lack of HbA is denoted as β0-thalassemia or β-thalassemia major. (no production of beta globin at all)

• Afflicted individuals suffer from severe anemia beginning in the first year of life and need blood transfusions.
  – Long-term transfusions lead to the accumulation of iron in the organs, particularly the heart, liver and pancreas and, finally, death in the teens to early twenties.
There is some production of beta globin

- Individuals heterozygous for \( \beta \)-thalassemia is termed \( \beta \)-thalassemia minor.
- Afflicted individuals carry one normal \( \beta \)-globin gene and a mutated gene or two that produce low amount of beta globin.
- Thalassemia minor individuals are generally asymptomatic.
### Classification and types of β-thalassemia

<table>
<thead>
<tr>
<th>Common genotypes</th>
<th>Name</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>β/β</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>β/β&lt;sup&gt;0&lt;/sup&gt;</td>
<td>Beta thalassemia trait</td>
<td>Thalassemia minor: asymptomatic, mild microcytic hypochromic anemia</td>
</tr>
<tr>
<td>β/β&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Beta thalassemia intermedia</td>
<td>Variable severity</td>
</tr>
<tr>
<td>β&lt;sup&gt;+&lt;/sup&gt;/β&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Beta thalassemia intermedia</td>
<td>Mild to moderate anemia</td>
</tr>
<tr>
<td>β&lt;sup&gt;+&lt;/sup&gt;/β&lt;sup&gt;0&lt;/sup&gt;</td>
<td>Beta thalassemia intermedia</td>
<td>Possible extramedullary hematopoiesis</td>
</tr>
<tr>
<td>β&lt;sup&gt;+&lt;/sup&gt;/β&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Beta thalassemia intermedia</td>
<td>Iron overload</td>
</tr>
<tr>
<td>β&lt;sup&gt;+&lt;/sup&gt;/β&lt;sup&gt;0&lt;/sup&gt;</td>
<td>Beta thalassemia major (Cooley’s Anemia)</td>
<td></td>
</tr>
<tr>
<td>β&lt;sup&gt;+&lt;/sup&gt;/β&lt;sup&gt;0&lt;/sup&gt;</td>
<td>No producing of beta globin at all</td>
<td>Severe anemia</td>
</tr>
<tr>
<td>β&lt;sup&gt;+&lt;/sup&gt;/β&lt;sup&gt;0&lt;/sup&gt;</td>
<td>Beta thalassemia major (Cooley’s Anemia)</td>
<td>Transfusion dependence</td>
</tr>
<tr>
<td>β&lt;sup&gt;+&lt;/sup&gt;/β&lt;sup&gt;0&lt;/sup&gt;</td>
<td>Beta thalassemia major (Cooley’s Anemia)</td>
<td>Extramedullary hematopoiesis</td>
</tr>
<tr>
<td>β&lt;sup&gt;+&lt;/sup&gt;/β&lt;sup&gt;0&lt;/sup&gt;</td>
<td>Beta thalassemia major (Cooley’s Anemia)</td>
<td>Iron overload</td>
</tr>
</tbody>
</table>

β<sub>0</sub>: complete lack of β chain  
β+: some expression of β chain  
β: normal expression of β chain  
β<sub>E</sub>: HbE
Hereditary persistence of fetal hemoglobin (HPFH)

These individuals have an abnormality in not transitioning from gamma to beta during development so they produce HbF throughout their life and they do not know that they have abnormality, so everything is functional and okay.

- Persons with HPFH continue to make HbF as adults.
- Because the syndrome is benign most individuals do not even know they carry a hemoglobin abnormality.
- Many HPFH individuals harbor large deletions of the δ- and β-coding region of the cluster.
- There is no deletion of the fetal globin genes.

HbF has higher affinity to oxygen but this doesn’t give an advantage.

- Think: treatment for β-thalassemia!!!!

There is an idea of switching on the fetal globin gene (gamma globin) in case of beta globin gene is not functional.
Hemoglobin Electrophoresis

For diagnosing hemoglobinopathies

Molecules such as proteins can be separated according to its charge or size.
Amino acid substitution in abnormal Hbs results in an overall change in the charge of the molecule.

Therefore, Hb migration in a voltage gradient is altered.

Electrophoresis of hemoglobin proteins from individuals is an effective diagnostic tool in determining if an individual has a defective hemoglobin and the relative ratios of the patient’s hemoglobin pattern.
Examples

• In Sickle Cell hemoglobin, replacement of a negatively-charged glu in the standard HbA by a neutral val in HbS results in a protein with a slightly reduced negative charge.
• In homozygous individuals, the HbA tetramer electrophoreses as a single band, and the HbS tetramer as another single band.
• Hemoglobin from a heterozygous individual (with both alleles) appears as two bands.
• Since HbC contains a lysine instead of the normal glutamate, HbC will travel even faster to the cathode.
Results

- Lanes 1 and 5 are hemoglobin Standards (a sample that contains all forms of hemoglobin)
- Lane 2 is a normal adult
- Lane 3 is a normal neonate
- Lane 4 is a homozygous HbS individual
- Lanes 6 and 8 are heterozygous sickle individuals
- Lane 7 is a SC disease individual
Band 1 and 5: we can see the migration pattern is different among the different hemoglobin molecules, for example HbC is traveling close to the cathode (negative charge end), HbS is migrating a little bit slower than HbC and so on.

Why we see this difference in migration patterns?
Because in HbC we have a mutation of Glu to Lys so we have a loss of one negative charge and an addition of one positive charge, that's why it travels closer to the cathode.

In HbS the we have a loss of a negative charge( Glu to Val which is with no charge) so it will travel away from the anode and closer to the cathode.

HbF as a whole molecule is more positively charged than HbA.

Band 2: This individual has only HbA and this is an adult individual. And that’s good, there is no problem.
Band 3: This individual has both HbA and HbF with more HbF than HbA, so this is a neonate most probably.
Band 4: This individual has only HbS so this is someone with sickled cell anemia
Band 6: This individual has both HbA and HbS so this someone with sickled cell trait
Band 7: This is someone with HbSC
Band 8: This is someone with sickled cell trait