HEMATOLOGY
&LYMPH SYSTEM

Biochemistry sheet

Number	4
Done BY	Fahed Al Karmi
Correction	Sufian Alhafez
Doctor	Dr nayef karadsheh

Genetic variants of hemoglobin

Hemoglobinopathies (abnormal variants of hemoglobin) are divided into:

1. Structural abnormalities: Any change in the genes responsible for the globin proteins that leads to changes in the structure.

2. Quantitative abnormalities: In which the amount of alpha or beta chains produced is affected.

Structural changes may lead to a change in any of the following:

1. Solubility (Hb becoming less soluble).

2. Hb with higher tendency to oxidize Fe^{2+} (ferrous) to Fe^{3+} (ferric), leading to methemoglobinemia.

3. Unstable Hb

4. Hb with increased or decreased O_2 affinity.

By studying the various hemoglobin variants we sometimes can deduce the relationship between the physical changes and the location of the structural change.

Regional changes in amino acids and their corresponding physical change:

Surface amino acids (altered exterior): Despite the fact that nearly all changes in surface amino acids are harmless (not associated with any symptoms), some are in fact associated with symptoms due to the fact that these changes in the structure of Hb made the whole protein less soluble in the cytosol, then it forms polymers such as: HbS and HbC (common in black people), HbE is rare (common in Ceylon and Malaysia) - Hb Punjab (common in India and Pakistan).

Active site alteration: Certain amino acid substitutions in the active site allow ferrous to become ferric, producing methemoglobin.

- Hydrophobic amino acids are substituted with hydrophilic ones. Hydrophilic residues allow water to get in and aid in the oxidation of ferrous iron to ferric iron.

-Proximal histidine might be substituted with tyrosine (tyrosine can't prevent the oxidation of ferrous to ferric , so it is oxidized and it binds H2O not O2), thus producing methemoglobin.

Examples: Hb.M $_{Iwate}$ $^{87\alpha\ his>>tyr}$ Hb.M $_{Hide\ park}$ $^{92\beta\ his>>tyr}$

-Distal histidine might be substituted, examples: Hb.M $_{Boston}$ $^{58\alpha\ his>>tyr}$

Hb.M $_{Saskatoon}$ $^{63\beta\ his>>tyr}$

Unstable Hb: gene mutations affect the **tertiary structure** leading conformational changes which disrupt the 3D shape.

- The denatured protein will precipitate leading to formation of Heinz bodies (not in the record but in the slides).

- Some of these changes involve breaking certain α helices by proline. Remember that α helices never contain proline. Therefore, proline disrupts the helix.

-Substitution with large amino acids that are normally not found at contact sites. Subunits are linked together by interactions between small amino acids. If these were substituted by other large amino acids, the interaction would be weakened. Thus, cooperativity will be affected and oxygen affinity as well. Also charged or polar amino acids could disrupt the protein.

Examples: Hemoglobin Hammer-Smith is an unstable hemoglobin resulting When phenylalanine is replaced by serine, that substitution will disrupt heme binding. Hemoglobin Riverdale-Bronx is an unstable hemoglobin resulting from the substitution of arginine for glycine at helical residue B6 of the B beta polypeptide chain, this substitution will disrupt the interaction between B6 and E8 (8^{th} residue in helix E).

Altered affinity:

-Oxygen affinity is affected by the quaternary structure of hemoglobin (the association of the subunits together), so a change in a point of contact between the subunits might change the affinity.

- Substitution in the sites of allosteric effectors binding (BPG binding site, proton binding site) affects oxygen affinity. Any increase or decrease in oxygen affinity is considered pathological.

- Substitution of His146 (responsible for the Bohr effect) to leucine (Hbcowtown) will result in more hemoglobin in the R state (increase affinity).

Now, the next table mentions some examples of mutant hemoglobins and we will focus on few ones (the required details from the table are mentioned separately):

Table 4.1
Amino acid substitutions in mutant hemoglobins

Mutant Hemoglobin[a]	Position Number[b]	Normal Residue	Substitution
α Chain			
$G_{Honolulu}$	30	Glu	Gln
$G_{Philadelphia}$	68	Asn	Lys
I	16	Lys	Glu
M_{Boston}	58	His	Tyr
Norfolk	57	Gly	Asp
$O_{Indonesia}$	116	Glu	Lys
β Chain			
C	6	Glu	Lys
D_{Punjab}	121	Glu	Gln
$G_{San\ Jose}$	7	Glu	Gly
E	26	Glu	Lys
S	6	Glu	Val
Zurich	63	His	Arg

[a] The hemoglobins are often named for the cities where they were first discovered.
[b] The numbering for an amino acid position begins at the N-terminus.

HbS & HbC:

-HbS is the result of substitution of the negatively charged glutamic acid on the beta chain with the hydrophobic valine residue. This is found in sickle cell disease. ($\alpha_2^A \beta_2^{6Glu>>Val}$, that means normal alpha and altered beta).

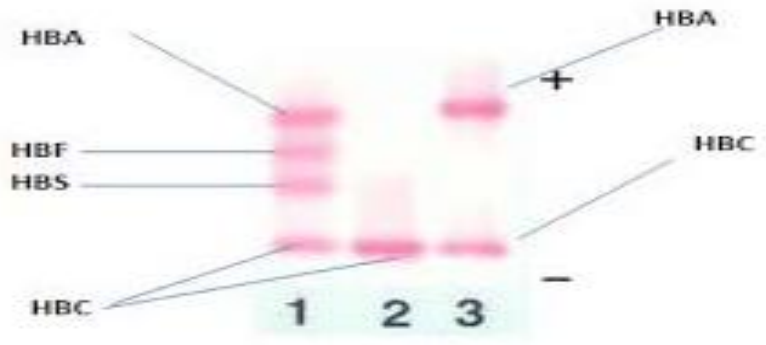
-HbC is the result of substitution of the negatively charged glutamic acid on the beta chain with a positively charged lysine. ($\alpha_2^A \beta_2^{6Glu>>Lys}$).

Sickle Cell Disease

-It is the most common hemoglobinopathy. Sickle cell disease is an autosomal recessive trait, patients could be heterozygous (carriers) or homozygous (2 copies of the affected gene).

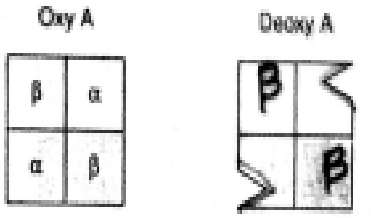
-The disease was studied among African Americans (because they make up a significant portion of the American population).1 out of every 500 people were homozygous and 1 of 10 people were carriers.

-The disease can be detected easily by electrophoresis in an alkaline pH, HbA (contains negatively charged glutamic acid) will migrate from the cathode (negative charge) to the anode (positive charge) at a faster rate than HbS (contains hydrophobic valine instead of glutamic acid) and HbC (contains positively charged lysine instead of glutamic acid).

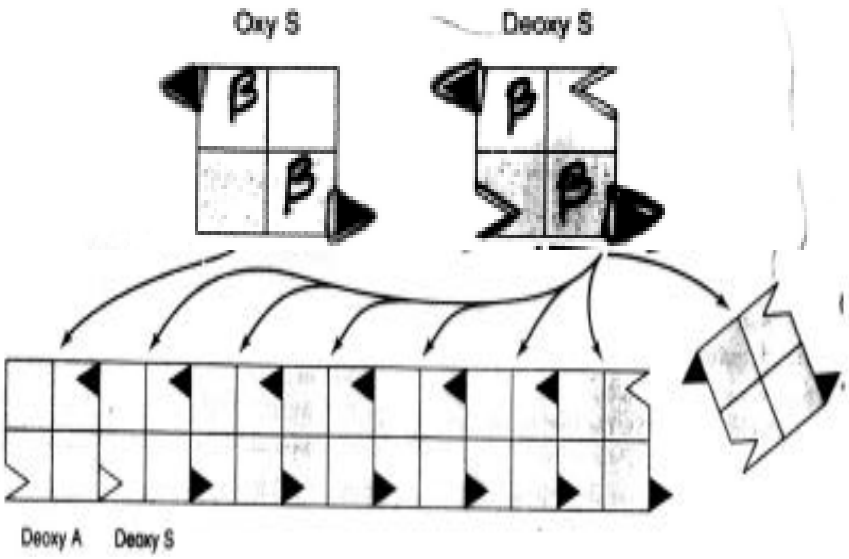


-HbS in its deoxy form polymerizes and forms long aggregates in the form of insoluble fibers, hence distorts the shape of the erythrocytes converting them from flexible biconcave cells to rigid crescent or sickle shape cells. These new erythrocytes will hardly pass through small capillaries; this will occlude small vessels and cause anoxia, infarction (which causes severe pain sometimes

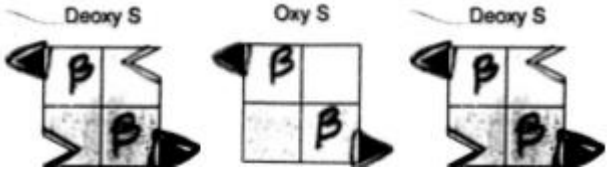
manifested in the form of severe headaches due to the small size of capillaries in the head).



HbA (normal Hb): Even if there is a condition favoring an increase in the deoxy state (for example: decreased O2 tension by high altitude or flying in a non-pressurized plane, increased 2,3-BPG, increased CO_2 and decreased pH), normal Hb will not polymerize because the ends are not complementary to each other



HbS (sickle cell): Due to the high amount of hydrophobic amino acids, protrusions on the Hb are present. These protrusions are complementary to neighboring pockets, which leads to the formation of insoluble aggregations and fibers.

	
Conditions favoring the deoxy state, aggregation will be high and symptoms are more severe.	Conditions favoring the oxy state will disrupt the aggregation and make symptoms of anoxia milder.

-The aggregate formation shortens the erythrocytes' life span from 120 days to 17 days because erythrocytes lose water and become fragile, after which hemolytic anemia occurs.

-Heterozygous individuals (carriers) have mild symptoms, they have 50% HbA and 50% HbS , these patients can live normally as long as they avoid hypoxic conditions (high alititudes, strenuous exercise, anesthesia, etc..). There are no symptoms unless the patient is hypoxic.

-The presence of the HbF, HbA and the oxy HbS will disrupt the polymer.

-HbS trait (heterozygous) prevails in areas where malaria is endemic. Sickle cell gene mutations provide natural protection against deadly Plasmodium falciparum (malaria). There is an overlap between the distribution of sickle cell anemia and malaria in Africa. Protection is due to the decrease in the life span of erythrocytes. It will not be 17 days as in sickle cell anemia (homozygous) but it is definitely going to be less than 120 days. This will prevent the malaria parasite from completing its life cycle. Other abnormalities that provide protection against malaria include: G6PD deficiency, Thalassemia and PK deficiency.

-Management for sickle cell disease: hydration, antibiotics, intermittent blood transfusions, and analgesics.

-Investigational studies showed that the presence of higher extent of HbF will help prevent sickle cell crisis. A drug called hydroxyurea (anti-cancerous drug) was found to increase HbF, reducing symptoms of sickle cell crisis.

HbC and HbSC

In patients with HbC, erythrocytes are not sickle shaped. HbC patients have mild chronic anemia without infarctive crisis and no special therapy is required.

Double heterozygous (HbSC) patients have some sickling (but less than that in HbS), anemia (which is milder than HbS) and less frequent painful crises.