Lecture 1

Haemoglobin

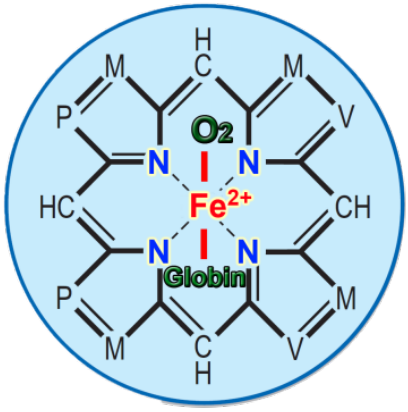
It is a haem containing globular protein. Its major role is to take up oxygen from areas of high oxygen tension (lungs) , release it in areas of low oxygen tension (tissues) and transporting CO_2 back to the lungs.

The haemoglobin molecule consists of 2 parts **Haem** and **Globin**

Haem: consists of a porphyrin ring with iron held in the center. The haem Fe^{2+} is bound to the four nitrogens of the porphyrin ring.

Iron forms two additional bonds:

1- One is to bind to the side chain of a histidine residue of globin molecule.
2- Whereas the other is available to bind oxygen, so the iron atom is the site of reversible oxygen attachment.



Globin: which is the protein portion, Hb consists of two pairs of globin chains that are twisted together .

So the complete haemoglobin molecule contains four haem groups attached to each of four globin chains and may carry up to four molecules of oxygen.

Normal Hb types in adults:

The main type of Hb is **HbA_1** (97%) consisting of 2 pairs of globin chains α (141 AA residues) and β (146 AA residues) ($\alpha_2\beta_2$). The other type of Hb which is also found in adult but in small amount is **HbA_2** ($\alpha_2\delta_2$) (2-3%).

Other normal Hbs:

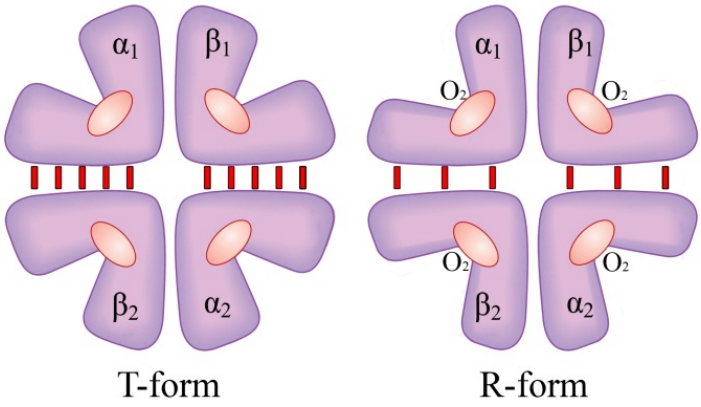
- **HbF:** ($\alpha_2\gamma_2$) : This type of Hb is present primarily in embryonic life, and usually disappears from the circulation by the age of 6 months. HbF represents less than 1% of the Hb in adults.
- **Gower 1 and 2:** are synthesized by the embryonic yolk sac during the first month of embryonic life and ceased by the 4^{th} month of embryonic life to be replaced by HbF .

Forms of Haemoglobin:

The Hb molecule exists in two forms, **T** and **R**.

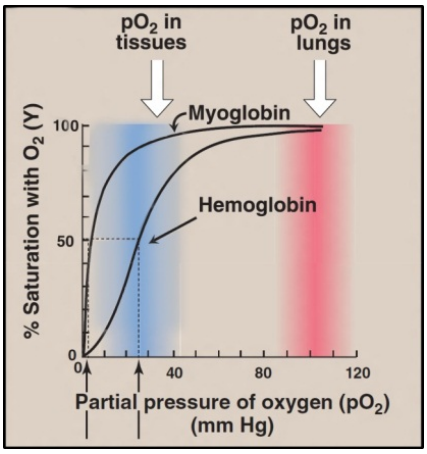
1. The T (tense or taut) form: of deoxyhaemoglobin is characterized by the globin units being held tightly together by electrostatic bonds.

2. The R (relaxed) form: in which the bonds are broken when oxygen binds to haemoglobin, resulting in the oxygen binding sites being more exposed and have a much higher affinity for oxygen than in the T form.



Oxygen Dissociation Curve

- The oxygen dissociation curve for haemoglobin is sigmoidal in shape.
- The binding of one oxygen molecule to deoxyhaemoglobin increases the oxygen affinity of the remaining binding sites.
- This property is known as **'cooperativity'** and is the reason for the sigmoid shape of the oxygen dissociation curve.
- The oxygen dissociation curve for myoglobin has a hyperbolic shape.
- Myoglobin consists of a single polypeptide chain that is structurally similar to the individual globin chain of the Hb molecule. Therefore it can bind only to one molecule of oxygen with high affinity.



Factors that affect Oxygen Dissociation Curve of Hb:

1. Increase in temperature (shifts the curve to the right).

2. pH of environment and PCO_2 (Bohr effect): The release of oxygen from haemoglobin is enhanced when the pH is lowered or when there is an increase in PCO_2. Both decrease oxygen affinity of Hb and, therefore, a shift to the right in the oxygen dissociation curve.

In the tissues, CO_2 is converted by carbonic anhydrase to carbonic acid:

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow HCO_3^- + H^+$$

The Bohr effect can be represented schematically as:

$$HbO_2 + H^+ \longleftrightarrow HbH^+ + O_2$$

3. The availability of 2,3-BPG (2,3-DPG): RBC metabolism produces 2,3-BPG from glycolysis. 2,3-BPG decreases the oxygen affinity of Hb by binding to deoxyhaemoglobin but not to oxyhaemoglobin. This binding stabilizes the taut form of deoxyHb. The effect of binding 2,3-BPG can be represented schematically as:

$$HbO_2 + \text{2,3-BPG} \longleftrightarrow \text{Hb-2,3-BPG} + O_2$$

* Why do patients with severe anaemia may be compromised if transfused with large quantities of long stored blood?

4. Carbon monoxide (CO): binds tightly but reversably, to Hb iron, forming carboxyHb.

When CO binds to one or more of the haem sites, Hb shifts to the relaxed form causing the remaining haem sites to bind oxygen with high affinity. This shifts the oxygen dissociation curve to the left.

* Why does patient with CO poisoning have cherry red or pink cheek appearance?

- **So in summary:**
- 1- Factors that shift the oxygen dissociation curve to the right are:
- A- ↑ temperature.
- B- ↑ PCO_2 or ↓pH
- C- ↑ 2,3 DPG
- 2- Factors that shift the oxygen dissociation curve to the left are:
- A- ↓ temperature.
- B- ↓ PCO_2 or ↑pH
- C- ↓ 2,3 DPG
- D- CO poisoning.

Lecture 2

Haemoglobin Synthesis

Globin Synthesis:

Hb consists of 2 pairs of globin chains: **α** and **non-α** chains

The genes coding for the α globin are found as 2 genes on chromosome 16. While the genes coding for non-α globin are found on chromosome 11: One gene for β-globin, one gene for δ-globin and 2 genes for γ-globin.

Haem Synthesis:

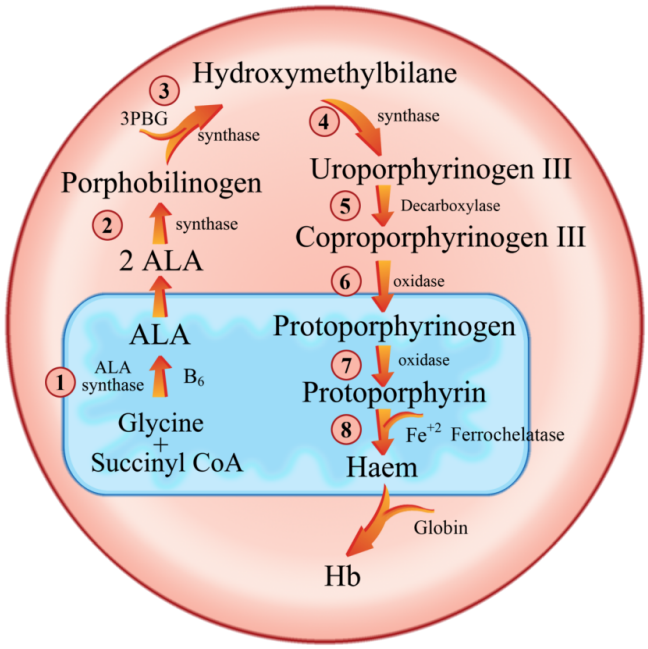
The major sites of haem biosynthesis are:

1. Erythrocyte-producing cells of the bone marrow where over 85% of all haem synthesis occurs there.

2. The liver, which synthesizes a number of haem proteins (particularly cytochrome P450 proteins) (microsomal drug oxidation system).

Haem synthesis in erythroid cells is relatively constant, and is matched to the rate of globin synthesis. While in the liver, the rate of haem synthesis is highly variable, responding to alterations in the demands for haem proteins.

Haem synthesis occurs in the mitochondria and cytoplasm of the developing RBC. It is an eight-step process in which the first step and the last three steps take place in the mitochondria while the middle four steps take place in the cytoplasm:



The Rate limiting step in haem synthesis:

The reaction glycine with succinyl CoA to form δ-aminolaevulinic acid (ALA) (catalyzed by ALA synthase) is the major rate-limiting step in Hb synthesis. It is inhibited by **haem** and stimulated by **erythropoietin**.

PORPHYRIAS

These are rare, mostly autosomal dominant, inherited defects in haem metabolism caused by abnormalities of enzymes involved in the biosynthesis of haem, resulting in the accumulation and increased excretion of porphyrins or porphyrin precursors. The word porphyria is derived from Greek word meaning " **Purple pigment** ".

Clinical Features: Are categorized into 2 main types :

1- Neurovisceral manifestations.
2- Skin manifestations (photosensitivity)

1. Neurovisceral manifistations:

- Individuals with an enzyme defect prior to the synthesis of the tetrapyrrole ring show abdominal and neuropsychiatric signs.
- Patients present with acute abdominal pain, constipation and vomiting with features of autonomic dysfunction such as tachycardia, hypertension and neurological symptoms such as anxiety and delirium.

2. Photosensitive skin manifestations:

- Those with enzyme defects leading to the accumulation of tetrapyrrole intermediates show photosensitivity causing pruritus when exposed to visible light.
- Photosenstivity is a result of the oxidation of colorless porphyrinogens to colored porphyrins, which are photosensitizing molecules that are thought to participate in the formation of superoxide radicals from oxygen which can oxidatively damage membranes, and cause the release of destructive enzymes from lysosomes.
- The excess of porphyrins accumulate in the skin, causing pain, erythema, bullae, erosions, hirsutism and hyperpigmentation, mainly on areas of the skin that are exposed to sunlight.

Types of porphyrias

1- X-linked sideroblastic anaemia
2- ALA dehydrase porphyria
3- Acute intermittent porphyria
4- Congenital erythropoietic porphyria
5- Porphyria cutanea tarda
6- Hereditary coproporphyria
7- Variegate porphyria
8- Erythropoietic protoporphyria

The most common types of porphyrias are:

1. Acute intermittent porphyria **2. Porphyria Cutanea Tarda**

1.Acute Intermittent Porphyria:

- It is due to partial deficiency of Hydroxymethylbilane synthase leading to accummulation of ALA and Porphobilinogen.
- The attack is usually precipitated by alcohol and drugs such as barbiturates and oral contraceptives.
- The acute attacks present with abdominal pain and neuropsychiatric symptoms.
- ALA and Porphobilinogen levels are greatly increased in plasma and urine, especially during acute attacks.

2. Porphyria Cutanea Tarda:

- It is a chronic disease of the liver. The disease is associated with a deficiency in uroporphyrinogen decarboxylase, but clinical expression of the enzyme deficiency is influenced by various factors, such as hepatic iron overload, alcohol ingestion, and the presence of hepatitis B or C, or HIV infections. Porphyrin accumulation leads to cutaneous symptoms.
- Urine is red to brown in natural light due to presence of uroporphyrinogen.
- Urinary ALA may be slightly elevated but urinary porphobilinogen is normal.

Lecture 3

Abnormalities in globin synthesis (Haemoglobinopathies):

Haemoglobinopathies are defined as a family of genetic disorders caused by production of a structurally abnormal hemoglobin molecule, synthesis of insufficient quantities of normal hemoglobin or both.

So, abnormalities occur in:

- Structure of the globin chain (ex: sickle cell disease).
- Globin chain production (ex: thalassaemia).

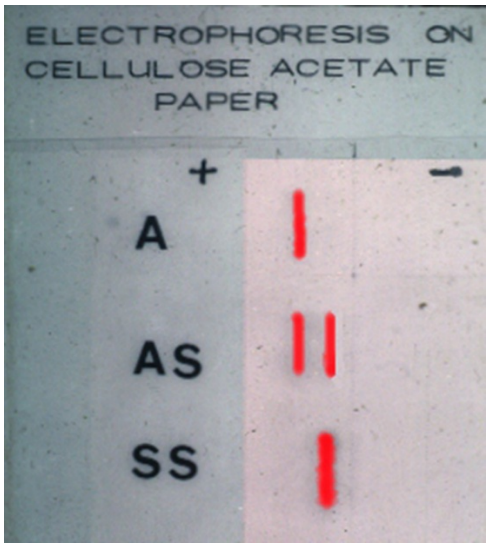
Sickle Cell Anaemia:

- The sickle cell disease is a type of haemolytic anaemia caused by a single base mutation (point mutation) in the β-globin gene that changes the sixth amino acid from glutamic acid (polar AA) to valine (nonpolar AA).
- Homozygotes only produce abnormal β-chains that make HbS and this results in the clinical syndrome of sickle-cell disease.
- Heterozygotes produce a mixture of normal and abnormal β-chains that make normal HbA and HbS (HbAS), and this results in the clinically asymptomatic sickle-cell trait.
- When HbS is deoxygenated, the molecules of haemoglobin polymerise to form pseudocrystalline structures known as 'tactoids'. These distort the red cell membrane and produce characteristic sickle-shaped cells.

Clinical features: The disease usually does not manifest itself until the 6 months of age.**(why?)**
Sickling is precipitated by hypoxia, acidosis, dehydration, fever and infection.
Vaso-occlusive crisis: Plugging of small vessels in the bone produces acute severe bone pain. This affects areas of active marrow: the hands and feet in children or the femur, humerus, ribs, pelvis and vertebrae in adults.

Investigations:

- Hb (6-8 g/dl)
- Blood film (sickle cells and increase in reticulocyte count to 10-20%).
- Liver Function tests: Bilirubin (D+I), ALT, AST, Alkaline phosphatase, GGT.
- Sickle solubility test. A mixture of HbS in a reducing solution such as sodium dithionite gives a turbid appearance because of precipitation of HbS, whereas normal HbA gives a clear solution.
- Hb electrophoresis (the definitive diagnosis):
  * No HbA band and predominance of HbS (homozygote).
  * HbA and HbS bands (sickle cell trait) (heterozygote).



- Why do the attacks of sickle cell crisis increase in high altitude ?

Thalassaemia:

They are hereditary haemolytic diseases in which there is a reduced rate of production of one or more of the globin chains of haemoglobin. This basic defect results in imbalanced globin chain synthesis.

The pathology behind thalassemias results from mutations in the globin gene resulting in the generation of a stop codon in the coding region of the mRNA. This causes premature termination of globin chain synthesis and leads to the production of a shortened or absent globin chain.

Classification:

Thalassaemia is classified according to which globin chain is produced in reduced amounts into:

1. α-thalassaemia:

The α-thalassaemia results from a defect in the synthesis of α globin chain, and because both HbA and HbF have α-chains, genetic disorders of α-chain synthesis result in defective fetal and adult haemoglobin production.

α-thalassemia can be classified, genetically, into 4 types:

- **Single-gene mutation** : Clinically silent.
- **Two-gene mutation** : presents as a microcytic anaemia that can be mistaken for iron-deficiency anaemia.
- **Three-gene mutation HbH disease**: Moderately severe, microcytic anaemia. The excess β chains precipitate as β4 tetramers (HbH).
- **Four-gene mutation** (**hydrops fetalis**) (Incompatible with life): Most pregnancies spontaneously terminate prematurely. Free γ-globin chains accumulate, forming γ4 tetramers (Hb Bart's)

2. β-thalassaemia:

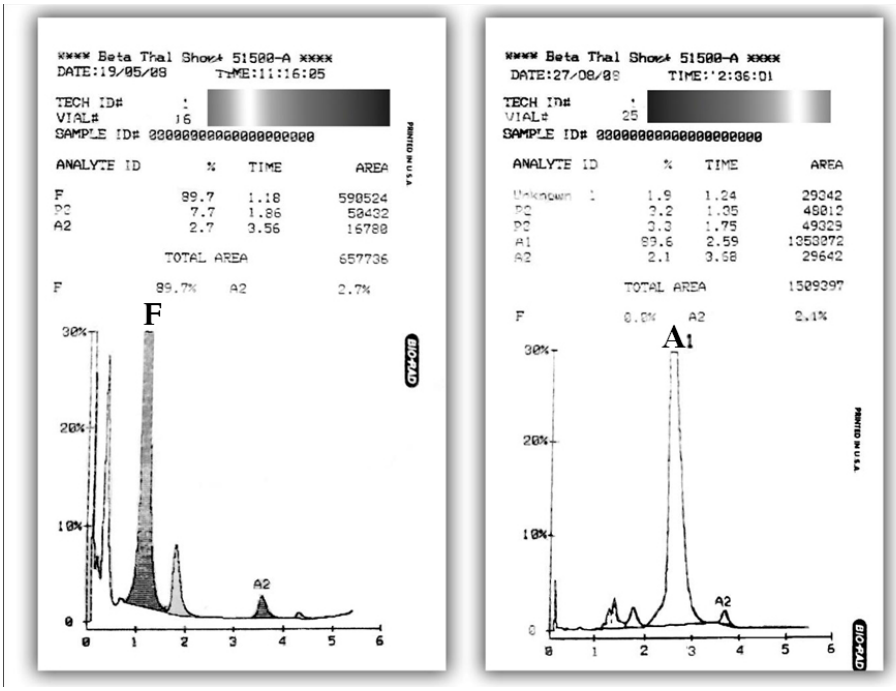
The β-thalassemias can be classified ,clinically, into three groups:
The severe transfusion-dependent **(thalassaemia major)** (Cooley's Anemia).
Intermediate in severity called **thalassaemia intermedia).**
The symptomless carrier state **(thalassaemia minor)** .

Clinical features of β-thalassaemia major:

Patient presents within the first year of life with failure to grow, poor feeding, recurrent attacks of infection. **On examination:** the patient is pale (anaemic), jaundiced, hepatosplenomegally.

Investigations:

- Hb 2-8gm/dl
- Blood film : microcytic hypochromic anaemia with reticulocytosis.
- Liver Function tests: Bilirubin (D+I), ALT, AST, Alkaline phosphatase, GGT.
- Hb Electrophoresis: The HbF level is always elevated. In thalassaemia major : only HbF and HbA2 (no HbA1).
- HPLC (High Performance Liquid Chromatography) is used to separate and quantify various normal and abnormal hemoglobin.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