HEMOGLOBIN:

- **Conjugated protein**
  Simple protein combined with a non-protein substance

Hemoglobin – HEME + GLOBIN

Nonprotein substance – HEME (prosthetic group)

- Red colour of blood is due to Hb in RBCs
- Normal level of Hb - male 14-16gm/dl
  female 13-15gm/dl
FUNCTIONS OF HEMOGLOBIN:

- Transport of Oxygen from lungs to tissues
- Transport of Carbon di oxide from tissues to lungs
- Transport of H from tissues to lungs and kidney
- Act as an intracellular buffer and is thus involved in acid base balance
STRUCTURE OF HEME:

- Heme is **iron protoporphyrin**

- Protoporphyrin – four pyrrole rings linked by methene bridge(=CH) to form porphyrin

- 4 Methyl (CH₃), 2 vinyl (CH₂-CH₂), 2 Propionate (CH₂-CH₂-COOH) side chain groups are attached to the porphyrin ring (protoporphyrinIX)
STRUCTURE OF HEME:
Iron of heme can form 6 coordinate bonds.

- 4 bonds are formed between the iron and nitrogen atoms of porphyrin.
- 5th bond is formed between nitrogen atom of histidine residue of the globin.
- 6th bond is formed with O₂.
STRUCTURE OF GLOBIN:

- Belongs to the class globulins
- 4 polypeptide chains
- 2 alpha(α) chains (141 amino acids each) is common for all types of hemoglobins
- 2 beta(β) or 2 gamma(γ) or 2 delta(δ) or 2 epsilon(ε) varies as per the type of hemoglobin, containing 146 amino acids each
- Normal HbA₁ - α₂ β₂ = 2 × 141 + 2 × 146 = 574 amino acids
## Normal Hemoglobin

<table>
<thead>
<tr>
<th>TYPE</th>
<th>COMPOSITION AND SYMBOL</th>
<th>% OF TOTAL HEMOGLOBIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1</td>
<td>$\alpha_2\beta_2$</td>
<td>97%</td>
</tr>
<tr>
<td>HbA2</td>
<td>$\alpha_2\delta_2$</td>
<td>2%</td>
</tr>
<tr>
<td>HbF</td>
<td>$\alpha_2\gamma_2$</td>
<td>&lt;1% (at birth 80%)</td>
</tr>
<tr>
<td>HbA1c</td>
<td>$\alpha_2\beta_2$-glucose</td>
<td>&lt;6%</td>
</tr>
</tbody>
</table>
What helps hemoglobin maintain its quaternary structure?

- 1) Hydrogen bonds
- 2) Salt bridges
- 3) Vander Waals forces
- 4) Ionic bonds
- 5) Hydrophobic Interactions
FUNCTION OF GLOBIN:

Forming a protective hydrophobic pocket for haem in order to protect the reduced form of iron (Fe^{2+})
2 FORMS OF HEMOGLOBIN

- **T FORM** (tense/taut/) – deoxyhemoglobin
- **R FORM** (relaxed) – oxyhemoglobin

**COOPERATIVE OXYGEN BINDING OF Hb:**
  i) Binding of the 1st O₂ to haem of the Hb enhances the binding of O₂ to the remaining haem of the same molecule of Hb
  ii) The shape of O₂ binding curve of Hb is Sigmoidal/S- shaped because O₂ binding is cooperative
The binding ability of Hb with O2 at different partial pressure of O2($pO_2$) is shown by ODC (i.e) it can load or unload O2 at different $pO_2$. 

OXYGEN DISSOCIATION CURVE

- Oxygen partial pressure ($pO_2$, mmHg)
- Percent saturation ($sO_2$, %)

Graph shows the relationship between oxygen partial pressure and percent saturation, indicating the ODC.
EFFECT OF pH ON O2 BINDING TO Hb

- TISSUES: CO2 IS LIBERATED
- CO₂ +H₂O→H₂CO₃
- H₂CO₃ →H⁺ + HCO₃⁻
- HbO₂ +H⁺ →HbH⁺ +O₂
The affinity of hemoglobin for oxygen is reduced when $H^+$ and $CO_2$ bind and oxygen is released into the tissues.

In the lungs, $CO_2$ is released from $Hb$ and the affinity of $Hb$ for $O_2$ increases.
This effect of pH and CO2 on binding and release of O2 by hemoglobin is called Bohr effect.
The binding ability of Hb with O2 at different partial pressure of O2 (pO2) is shown by ODC (i.e.) it can load or unload O2 at different pO2.
EFFECT OF 2,3-BPG

- Intermediary Metabolite formed mainly in RBCs during glycolysis
- Found in RBC nearly the same concentration as Hb.
- Reduces the affinity of Hb for O2
- Reduced affinity allows Hb to release O2 efficiently and shifts ODC to right

**SIGNIFICANCE:** Without BPG, Hb is inefficient O2 transporter
CLINICAL SIGNIFICANCE:

- HYPOXIA & ANEMIA:
  2,3- BPG is elevated, it helps in unloading O2 to the tissues

- COPD

- BLOOD TRANSFUSION:
  Storage of blood results in decreased concentration of 2,3BPG, such blood when transfused fails to supply O2 to the tissues immediately
HbF HAS HIGH AFFINITY FOR O₂

- HbF (α₂γ₂) – The fetal Hb continues to be in the newborn babies up to 6 months.
- In γ chain, Basic amino acid His-143 of β chain replaced by neutral serine amino acid.
- Reduces the affinity of fetal Hb for 2,3-BPG.
- O₂ binding affinity of fetal Hb increases.
- This helps in transporting O₂ from maternal blood to fetus.
### EMBRYONIC HEMOGLOBINS

<table>
<thead>
<tr>
<th>TYPE</th>
<th>COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb Gover-1</td>
<td>$z_2\varepsilon_2$</td>
</tr>
<tr>
<td>Hb Gover-2</td>
<td>$\alpha_2\varepsilon_2$</td>
</tr>
<tr>
<td>Hb Portland</td>
<td></td>
</tr>
</tbody>
</table>

- These hemoglobins are produced from 3rd to 8th week of gestation
ABNORMAL HEMOGLOBIN

- Mutations in the genes that code for globin chains can affect their formation and biological function of hemoglobin called as abnormal hemoglobin

- HEMOGLOBINOPATHY:
  Biological function is altered due to mutation in hemoglobin
  - **QUANTITATIVE:**
    - ↓ synthesis of globin chain
    - Chains are structurally normal
• α Thalassemia: Reduction in α chain synthesis
• β Thalassemia: Reduction in β chain synthesis

□ QUALITATIVE:
  altered sequence of amino acids usually in any one of the globin chain
  1. Sickle cell disease
  2. Hb C disease
  3. Hb M disease
THALASSAEMIA

- Genetically transmitted disorders of hemoglobin synthesis
  - ↓ synthesis of α or β chain
  - ↓ production of normal hemoglobin
- The synthesis of 1 globin chain reduced, there is a relative excess synthesis of the other globin chains
- Hemolysis of cell resulting in Hypochromic anaemia
α THALASSAEMIA

- Due to deletion of alpha genes in the chromosomes production of α chain is impaired.
- α- globin chain is structurally normal
- Results in the excess of β and γ globin chains
  - β-globin tetramer(β₄) – HbH
  - γ-globin tetramer(γ₄) – Hb Bart
- globin chains precipitate causing hemolysis
The binding ability of Hb with O2 at different partial pressure of O2(pO2) is shown by ODC (i.e) it can load or unload O2 at different pO2.
HYDROPS FETALIS

- $\alpha$-Thalassaemia – total absence of $\alpha$-chain synthesis
- Most clinically severe form
β THALASSAEMIA

- β-globin chain is structurally normal
- ↓production of β-globin chain
- Compensated by excess synthesis of alpha, gamma and delta chains
  - α-globin tetramer(α4) – Heinz bodies
  - hemolysis occurs
INHERITANCE PATTERN

- Thalassaemia major – Homozygous – symptomatic
  - Anemia due to destruction of premature red cells and shortened red cell life span – Hypochromic, microcytic anemia

- Thalassaemia minor – Heterozygous – βThalassemia asymptomatic
  - Thalassaemia trait
  - Completely normal or mild anemia
SICKLE CELL DISEASE/SICKLE CELL ANEMIA

- HbS
- Due to mutation in globin genes
- Sickle cell disease: Symptomatic-Anemia
  - Homozygous
  - Due to defective genes from each parent

Sickle cell trait: Asymptomatic
- Heterozygous
- Abnormal globin gene from only one parent
GENETIC DEFECT: β chain

HbA:

1  2  3  4  5  6  7  8
Val – His – leu – Thr – Pro – **Glu** – Glu – lys

HbS

Val – His – leu – Thr – Pro – **Val** – Glu – lys
REPLACEMENT OF Glu BY Val

- Loss of –ve charge in each of 2 β chains
- Polar Glu replaced by non-polar Val produces sticky patch at 6th position of β chain
- Reduces the solubility of deoxygenated HbS
- Bind to another deoxygenated HbS
- This binding causes polymerization of deoxy-HbS forming insoluble long tubular fibrous precipitates
- Deforming the RBCs to Sickle shape
CHARACTERISTIC FEATURES

- Sickled RBCs lose water and become fragile
  - Shorter life span – 17 days – lysis of RBC accounting for low Hb level causing anaemia
  - Small blood capillaries blocked by abnormal RBCs which interrupts oxygen supply and leads to anoxia
  - People with sickle cell trait show resistant for Plasmodium falciparum
  - The parasite cannot complete the stage of its development
Any factors increasing the formation of deoxy-Hb will increase sickling:

i) ↓O2 tension in high altitude
ii) ↑CO2 concentration
iii) ↓pH
iv) ↑ Concentration of 2,3-BPG in RBCs

Separation of HbS from HbA:

- Electrophoresis
- Absence of 2 –vely charged glutamate residues results in slow movement of HbS than HbA
HbC OR COOLEY’S:
  i) Glu in 6\textsuperscript{th} position replaced by lysine
  ii) HbC crystals form within the cell
  iii) Mild hemolytic anaemia

HbM DISEASES:
  • Mutation in either proximal or distal histidine residue of either $\alpha$ or $\beta$ chains, which bound with the iron in the haem molecule.
  • Histidine replaced by tyrosine
  • Iron is stabilized in the Fe$^{3+}$ form leads to cyanosis
Normal blood contains the following derivatives:

1) **OXY-Hb** – reversible, Iron remains in Fe$^{2+}$ state

   Reduced Hb + 4O$_2 \leftrightarrow$ HbO$_2$(Oxy-Hb)

2) **Reduced Hb** – When O$_2$ is released from Oxy-Hb, it is called as reduced Hb/Ferro Hb

3) **Carbamino Hb** – Hb+CO$_2$

   Affinity of Hb to CO$_2$ is 20 times more than O$_2$
ABNORMAL Hb:

1) Methemoglobin
2) Sulphhemoglobin
3) Carboxyhemoglobin

1) METHEMOGLOBIN:
   - Oxidized hemoglobin
   - Fe2+ oxidized to Fe3+
**METHEMOGLOBIN**

- Oxidized hemoglobin
- Ferrous iron(Fe$^{2+}$) oxidized to ferric state(Fe$^{3+}$)
- Normally, MetHb formed in the RBCs reduced back to Fe$^{2+}$ by MetHb reductase enzyme system
  - i) NADH cyt b5 – 75%
  - ii) NADPH dependent system – 25%
  - iii) Glutathione dependent MetHb reductase accounts for the rest 5% activity
METHEMOGLOBINEMIA

- Normal blood has < 1% as MetHb
- Decreased capacity for O2 binding & transport
- ↑ in MetHb – manifested as Cyanosis (>5%)
- Classified as i) Inherited
  - ii) Acquired
INHERITED METHAEMOGLOBINAEMIA:
1) Deficient activity of MetHb reductase
2) Hemoglobinopathies (HbM):
   - Due to mutation in the gene

ACQUIRED METHAEMOGLOBINAEMIA:
: Certain drugs and chemicals
TREATMENT

- Oral administration of
  - i) Methylene blue 100-300mg/day
  - ii) Ascorbic acid (vit-C) 200-500mg/day

  decreases Met-Hb and reverses Cyanosis
Laboratory Diagnosis:
- Ferricyanide can oxidize oxy-Hb or deoxy-Hb to MetHb.
- Colour changes to dark brown. Absorption spectra by Spectroscopic analysis show a band in red region with oxy-Hb bands.
- 540 – oxyHb
- 576 – oxyHb
- 630 – MetHb
- Sodium hydrosulphite or dithionate reconverts MetHb to oxyHb
SULPHHEMOGLOBINEMIA

- When drugs like Sulphonamide, Phenacetin, Dapzone are taken, SulpHb formed in the presence of Sulphur containing compounds such as Hydrogen sulphide that may arise from bacterial action in the intestine.
- It cannot be converted back to Oxy-Hb.
- Patients are cyanosed.
- To differentiate from MetHb.
- Seen as basophilic stippling of RBC, throughout its life span.
CARBOXYHEMOGLOBIN

- $\text{CO} + \text{Hb} \rightarrow \text{COHb}$
- Carbon Monoxide
- Hemoglobin
- CarboxyHb

- Affinity is 210 times greater than O2

- CAUSES:
  - Acute poisoning due to smoke inhalation or car exhaust fumes and heavy smoking

- SYMPTOMS:
  - Lethargy, Headache, nausea leading to confusion, agitation and deep coma
  - >40% is fatal
GLYCATED HEMOGLOBIN

- Measurement of glycated Hb is to know the long term control of blood glucose level – HbA1C
- Non Enzymatic addition – Glycation
- In Hyperglycemia, protein in the body undergo glycation
- Glucose forms a schiff base with N-terminal amino group of proteins
Interpretation of GlycoHb values

- HbA1c – Indicator for response to the treatment
- Reveals the mean glucose level over the previous 10-12 weeks
- Normal value: <6%
- Elevated GlycoHb indicates poor control of Diabetes Mellitus