Haemoglobin

Protein formed of : 4 Haem

4 globin chains

MW: 64.500

It is a **tetramer:** composed of 2 α chains, & 2 non α chains (globins) & to each chain haem is attached.

Haem: Iron protoporphyrin

Globin:

It is the protein portion of Hb.

It is formed of 4 polypeptide chains: 2 α chains

& 2 non α chains

	α chains	**non α chains**
Formed of 141aa		Formed of 146 aa
Coded by a gene on chr. 16		Coded by a gene on chr. 11

Globin synthesis, starts at 3rd week of gestation

- **Embryonic**

  Haemoglobin Gower I ($\zeta_2\varepsilon_2$)

  Haemoglobin Portland ($\zeta_2\gamma_2$)

  Haemoglobin Gower II ($\alpha_2\varepsilon 2$)

- **Fetal :** 85% HbF ($\alpha_2\gamma_2$),

  5-10% HbA ($\alpha_2\beta_2$)

- **Adult :** 97% HbA,

  2.5% HbA2 ($\alpha_2\delta_2$),

  0.5% HbF.

Globin chain structure

1ry structure:

Sequence of amino acids in the polypeptide chain.

Each chain is formed of polar & non polar aa.

2ry structure:

- Each polypeptide chain is coiled on itself forming 8 helical structure from A⟹ H
- These helices r similar in all chains except for D-helix (7aa in βδg, and 2 aa in α chains).
- These helics r rigid & linear, seperated by non-helical 7 segments (flexible) w' allow binding of chains.
- **Haem** lies between E & F helices.

Tertiary structure:

- Every 3rd or 4th aa in the chain is non polar (non charged aa).
- So in tertiary structure:
- **Internal surface:**
- Formed of non polar aa (hydrophobic), important for haem function (prevent oxidation of iron).
- **External surface:**
- Polar aa (hydrophilic) w' allow interaction e' the external enviroment & to maintain Hb in a soluble form).

The internal non polar aa side chains bind e':

each other &

e' aa of adjacent helices &

e'haem group ⟶

Making 3ry structure stable, rigid & important for haem function.

So any aa replacement ⟶ Abnormal Hb.

External hydrophilic aa:

Interact e' environment of the cell⟶ keeping the chain soluble.

Their replacement rarely lead to abnormality**.**

Haem:

It is iron protoporphyrin

There r 4 haem groups, form the centre of Hb mol., each is attached to 1 globin chain in a deep pocket between E, F helices, e' its iron attached to proximal F8 histidine (hydrophobic).

So, abnormal substitution of histidine by tyrosine w' contains phenol gp → iron phenolate complex →iron in ferric state (irreversible) ⟶ M- Hb.

Tetramer:

A pair of α chains on top of a pair of non α chains.

Each chain contains a haem gp.

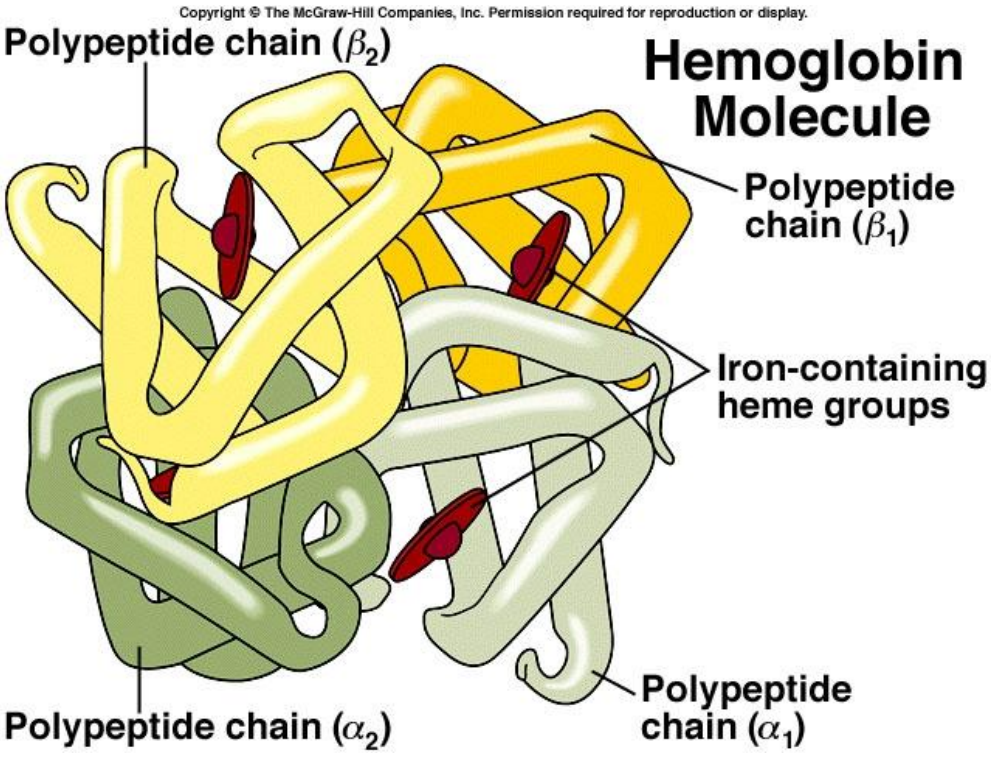
2 points of contact between α & β chains:

Structural contact:

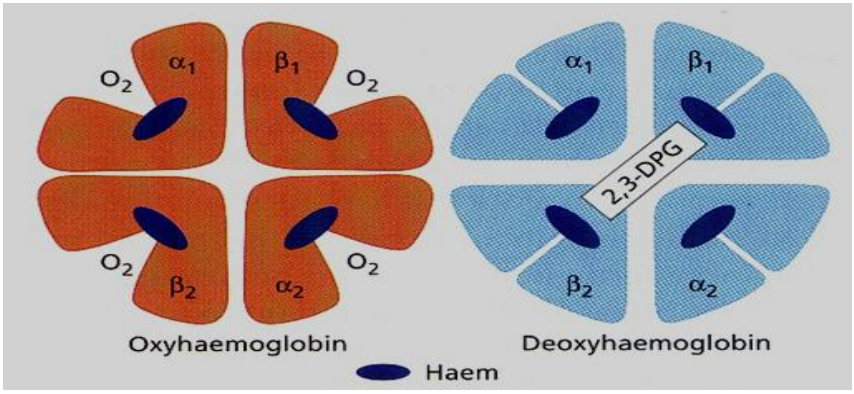
- $\alpha_1\beta_1$ and $\alpha_2\beta_2$ which confirms stability of the molecule.
- Any abnormality ⟶ unstable Hb.

Functional contact:

- $\alpha_1\beta_2$ and $\alpha_2\beta_1$ which confirms solubility of the molecule.
- Allows relation during oxygenation & deoxygenation.
- Any abnormality ⟶ Altered affinity Hb.



Oxy & deoxyhaemoglobin



Functions of Hb:

Transport of O2 into the tissues.

Factors affecting Hb affinity to O2:

1- Bohr effect:

↓ph→ shift of O2 dissociation curve to the right → ↓O2 affinity → giving O2 easily to tissues.

2- 2,3 DPG:

If ↑→ shift of curve to Rt,,,,,

3-aa structure:

Hb F→ high affinity to O2 due to inability to bind to 2,3 DPG.

\Hb S→ shift to the Rt.

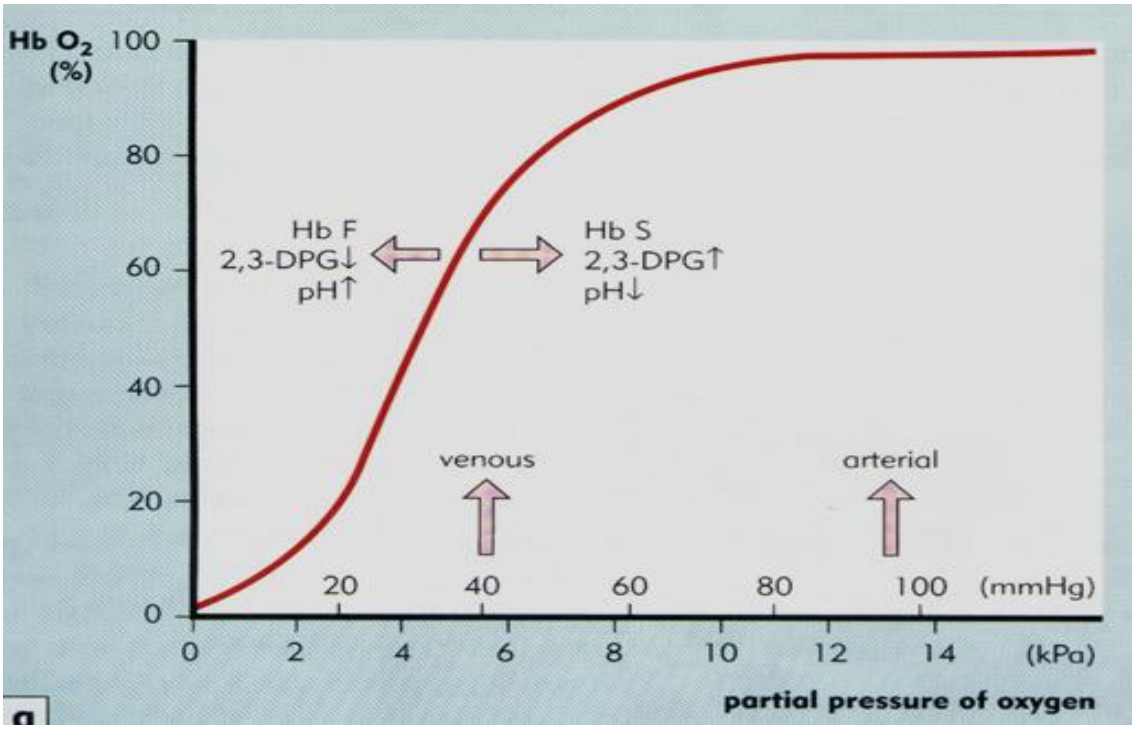


Fig: O2 dissociation curve

Haem-Haem interaction:

O2 dissociation curve of Hb is sigmoid→ giving advantage of delivery of more O2 to tissues at any given O2 tension.

While O2 dissociation curve of myoglobin is hyperbolic.

Genetic control of globin synthesis:

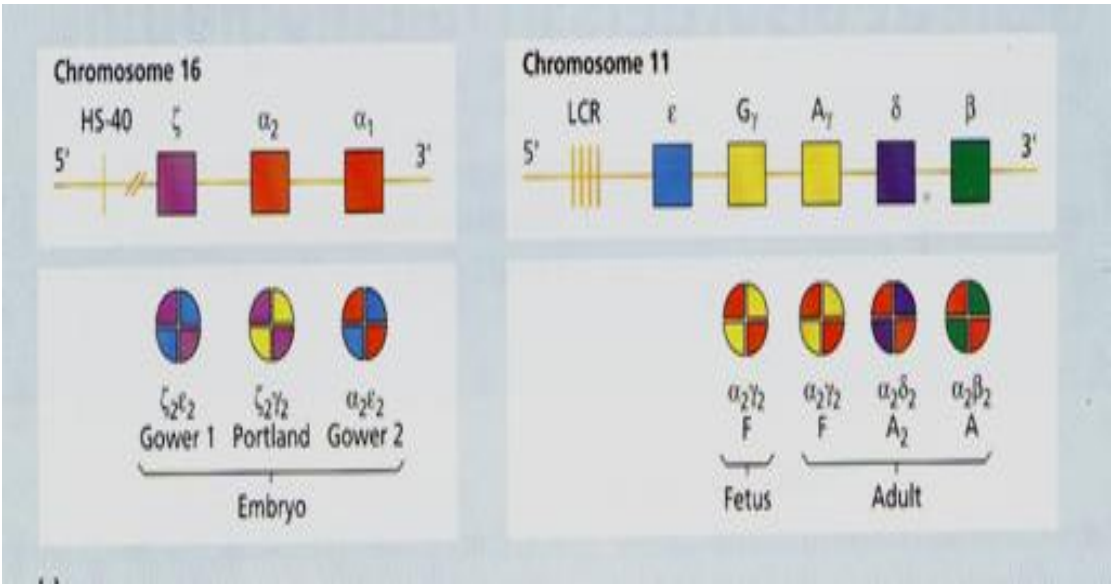
α genes:

A pair of non allelic genes (2 for α1, 2 for α 2) placed on chromosome no.16.

β genes:

A pair of allelic genes on chromosome no. 11, except for γ chain w' is under the control of non allelic genes (2 for γA (alanine) & 2 for γG (glycine).

Globin gene clusters



Gene structure:

Each globin gene is formed of:

3 exons separated by 2 introns (intervening sequences).

Promotor:

Located 5' upstream to coding sequences & control gene expression.

Steps of globin chains synthesis:

1-Transcription:

Genetic information is transmitted from DNA (gene) to m RNA.

2-Translation:

Genetic information of mRNA is translated into protein.

Transcription

Extension of mRNA from 5' → 3'

Each base in mRNA is complementary to a corresponding base in DNA i.e: C-G , T-A, A-U.

Post transcription modification (Processing):

1-splicing:

Excision of introns & splicing of exons.

2-5' capping:

Addition of methylated Guanine to 5' end → more stable

3-Poly adenylation:

Adding polyadenine at 3' end → make m-RNA more resistant to digestion by nucleases.

Translation

Occur in the cytoplasm on ribosomal surface.

t-RNA recognizes triple codons of m-RNA by anti codon region & carry the specific aa into ribosomes, where aa will be arranged to form polypeptide chain.

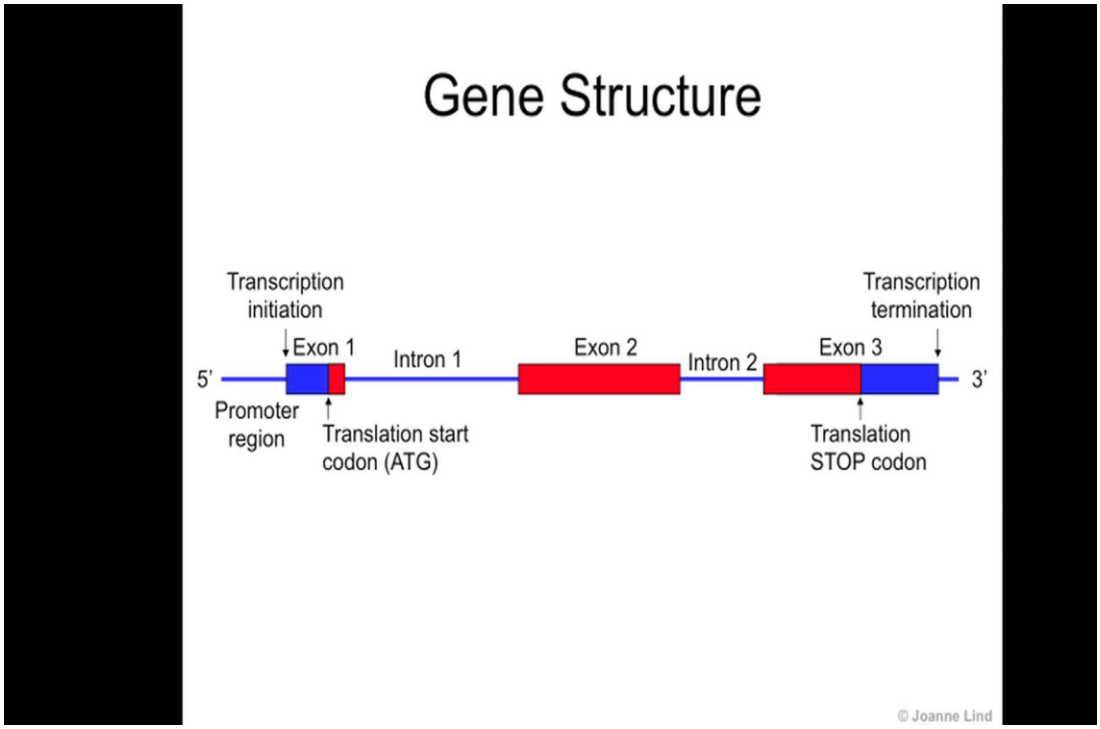
Codon:

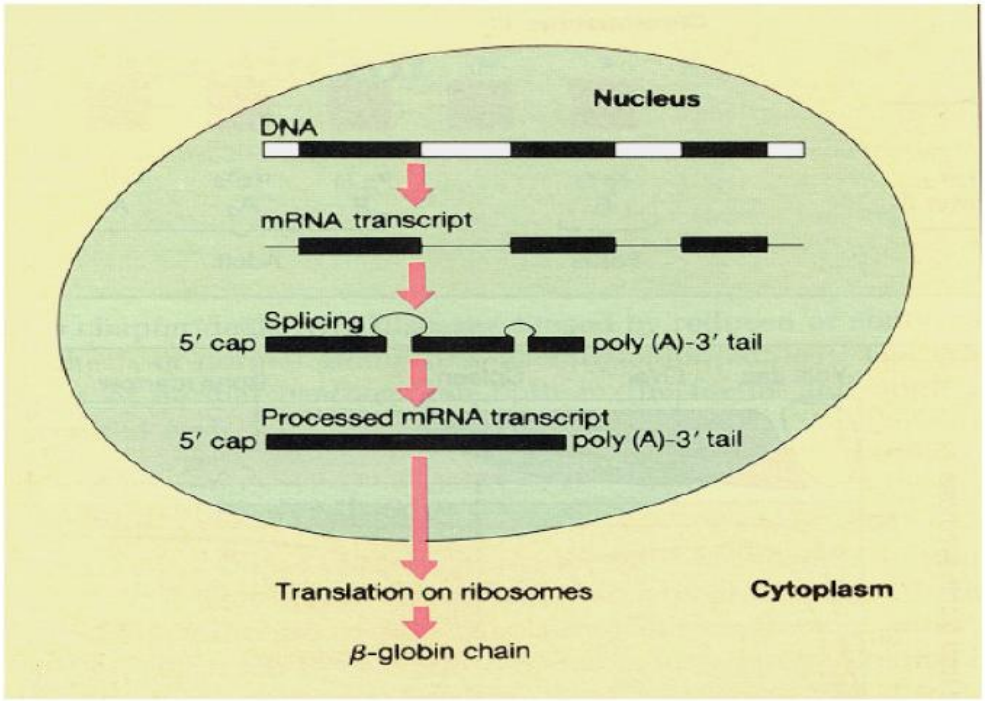
Consists of 3 nitrogenous bases i.e: GAG or AGG genetic code for each aa.

There r 64 genetic codes but there r only 20 aa.

So 1 aa is coded by more than 1 code.

64 genetic codes: because there r 4 bases (A,G,T,C) in 3 kinds of arrangement I;e: $4^3 = 64$.





Haemoglobinopathies

It is either:

Quantitative:

↓ synthesis of normal Hb e.g: thalasamia

Qualitative:

synthesis of structurally abnormal Hb e.g Hb S

Causes:

Molecular basis of haemoglobinopathies

1-Mutations:

replacement of 1 aa by another aa → abnormal Hb e.g **Hb S.**

2-Mutations:

Leading to premature stop codon → short chain.

3-Mutations:

Affecting terminal codons → elongated chain → **Hb Constant Spring**

4-Deletion:

Missing some of aa e.g α thalasamia.

5- Deletion of closely linked genes:

e.g: HPFH where both ↓ β , δ ↑ γ

6-Transcription mutations:

defect in m-RNA processing → ↓ m-RNA e.g: β thalasamia.

Clinical presentation of Haemoglobinopathies:

1- Silent: most of external aa substitutions

2- H.A : eg: Hb S

3-Cyanosis: eg: Hb M

4-Polycythemia.

5-Erythroblastosis fetalis:

eg: homozygous α thalasamia.

6-Thalasamia: mild, moderate, severe.

Qualitative Haemoglobinopathies

Structural variants: either:

Substitution of external (polar) aa:

- Hb S,C,D,E

c/p in homozygous state.

Substitution of internal (non-polar) aa:

- Unstable Hb
- Altered affinity Hb
- Hb M

c/p in heterozygous state.

I- Variants due to substitution of internal (non-polar) aa:

Manifest in heterozygous state

1- Unstable Hb

Congenital Heinz body anaemia.

Molecular defect & pathogenesis:

- Replacement of aa at structural contact, w' is responsible for stability of tetrameres.
- Replacement of aa w' surround haem group → H_2O passes in oxidative damage to haem → transform to oxidized met Hb → ppt as Heinz bodies w' interact e' RBCs membrane → ↓ deformability of RBCs pitting in spleen or its destruction (EVH).
- Substitution of non polar aa by polar aa.

C/P:

H.A varies in severity:

1- compensated H.A: no haemolysis.

2- Chronic H.A: MHA; moderate or severe.

3-Special test: Dipyroloria:

Dark urine due to excretion of dipyrol rings into urine resulted from metabolism of free haem or heinz bodies.

4-Drug induced haemolysis:

Hb-Zurich: it is harmless untill the patient is exposed to oxidizing drugs.

D.D:

Heinz bodies in :

- Unstable Hb
- G6PD def.

<u>Lab findings:</u>

1- evidence of H.A

2-CBC:

MHA, ↓MCH, aniso, pokilio, basophilic stippling, ↑retics.

3-Special tests:

A- Heat stability test:

- Heat haemolysate $\xrightarrow{\text{1-2 hs 50°C}}$ turbidity (denaturated Hb).
- Heating 1/25 diluted Hb in phosphate buffer (7.4) at 50-60°C → heat ppt Hb (turbidity).

B-Heinz bodies:

R seen by supravital stains as: MB, briliant cresyl blue, seen only after splenectomy.

C-Isopropanol stability test:

Isopropanol → denaturation of haem → turbidity

D- Electrophoresis:

Abnormal Hb

E- DNA study

2-Altered affinity Hb

Pathogenesis:

Shape & structure of Hb mol. Alters between oxygenated & deoxygenated states:

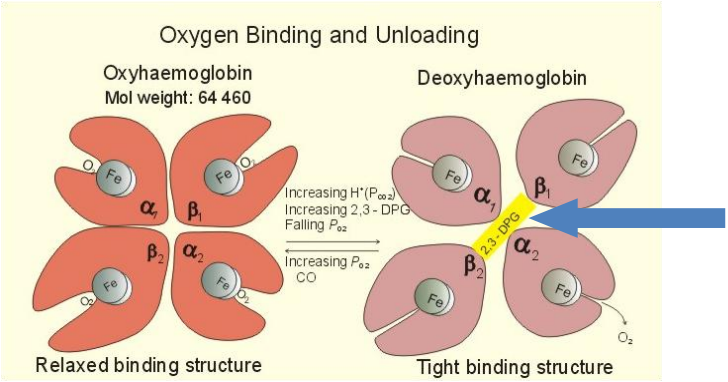
On oxygenation:

- The sliding movement contracts the central cavity → 2,3 DPG is dislodged ↓ Hb→ relaxed form (w' has 100 times O2 affinity as deoxy Hb).

Upon deoxygenation:

- Mol.opens
- 2 β chains slide over 2 α chain at α1β2 contact
- 2,3 DPG enters ↑ the centre of mol. + β chain, Hb become low affinity & O2 passes to tissues.

Hemoglobin Structure Changes



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So substitution of any aa at any of these imp. States→ altered affinity to O2.

1- substitution at α1β2 contact w' allows rotational movement during oxy & deoxy states → high affinity Hb.

2-aa subststitution at 2,3 DPG binding site on β chain → high affinity Hb.

21 variants r known:

18 high affinity → polycythemia

3 low ,, → cyanosis

C/P:

Polycythaemia (isolated erythrocytosis) in high affinity types, but unlike polycythaemia rubra vera: condition is mild & stable.

Low affinity Hb: anaemia & cyanosis (↑deoxy Hb)

Diagnosis:

Hb electrophoresis : abnormal Hb.

D.D:

Polycythaemia ,Cyanosis.

D.D of polycythemia

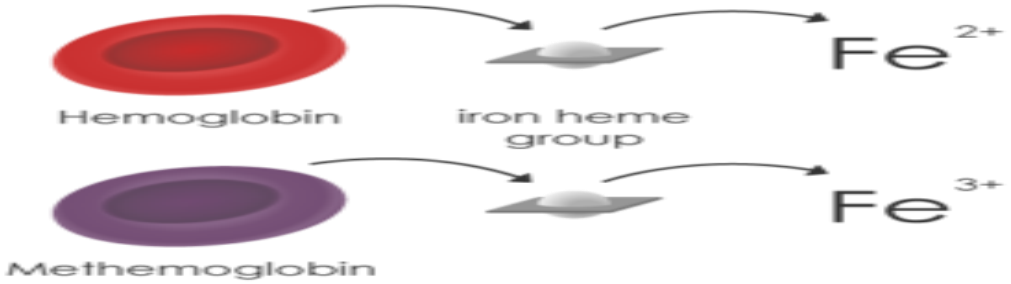
	Abnormal Hb (altered affinity)	Renal disease	Pulmonary disease	Polycythemia vera
Arterial PO2	N	N	↓	N
Lung function	N	N	Abnormal	N
IVP	N	Abnormal	N	N
O2 dissociation curve	Abnormal	N	N	N
Abnormal Hb	+ve	-	-	-
Blood picture	↑RBCs	↑RBCs	↑RBCs	↑ RBCs, WBCs, platelets

3- Hb M

Pathogenesis:

- Substitution of proximal or distal Histidine where haem is inserted (E7,F8) by Tyrosine.
- Phenol gp of tyrosine forms iron-phenolate complex w' stabilizes iron in ferric form & makes it resistant to reduction (met Hb).

- 7 types where described, all → congenital methaemoglobinaemia.



C/P:

- Cyanosis due to ↑met Hb
- Presented at birth: if α chain is affected.
- ,, ,, 6 months: if β chain is affected.
- No H.A, but there is compensated H.A.

D.D: cyanosis or met haemoglobinaemia

D.D of chronic methaemoglobinaemia:

	Drug toxcicity (G6PD def.)	**↓NADH reductase enzyme**	**Hb M**
Heinz bodies:	+	-	-
History:	Recent drug exposure	Long life	At birth or at 6 ms
Met Hb:	variable	variable	25-40%
Optical spectrum:	Like met Hb A	Like met Hb A	Specific for Hb M
Electrophoresis	Like Hb A	Like Hb A	Hb A+ Hb M
Enzyme assay:	↓G6PD	↓Met Hb reductase	normal
Incubation of blood e' MB:		Reduction of met Hb	Resist reduction

II- Variants due to substitution of external (polar) aa:

Manifest in Homozygous state.

1-Hb S (Sickle cell anaemia)

Pathogenesis:

Substitution of the 6th aa (Glutamic) in the β chain by Valine w' is non polar aa.

Under oxy Hb state: no difference between Hb S & Hb A

Under deoxy state: ↓solubility of Hb S helical structure transforms into helical firm gel⟶ distorted sickle cells.

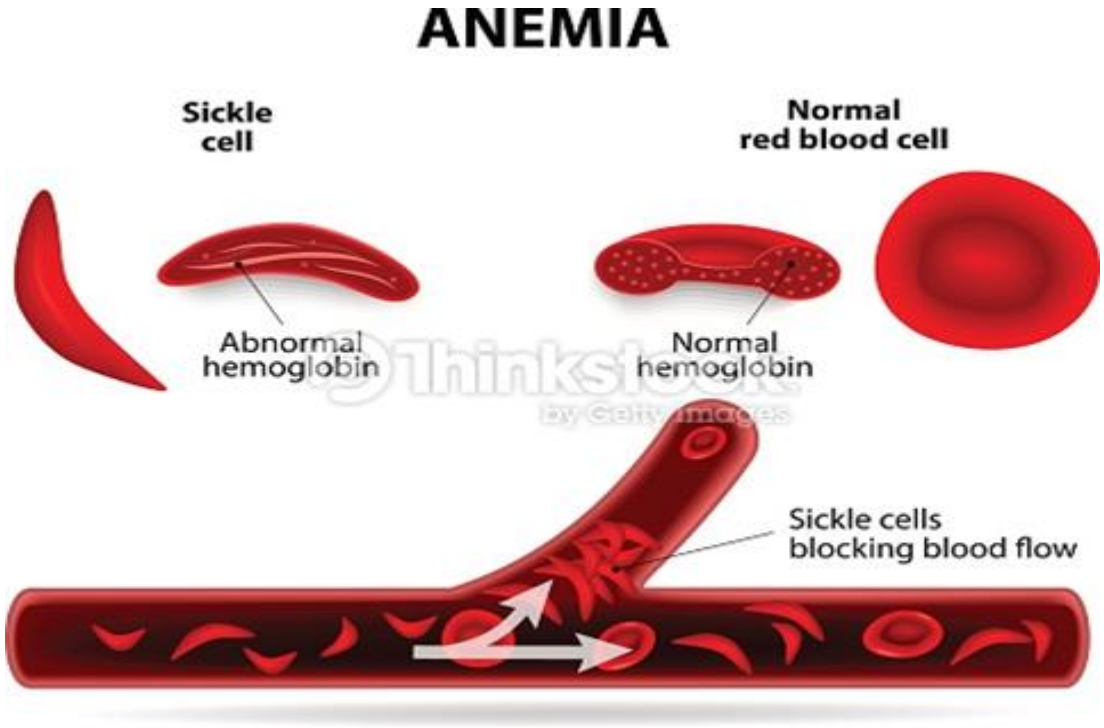
The condition is 1st reversible upon deoxygenation, but repetition of this condition ⟶ 2ry membrane changes ⟶ irreversible sickling.

2ry membrane changes:

- ↑ intra cellular Ca+
- ↓ „ K+
- ↓ ATP
- Acquired Ig on cell surface
- Phospholipid asymmetry

These changes lead to:

Ingestion by MQ more rapidly than normal cells.



Factors affecting sickling:

1- Hb A:

Sickling occurs at PO2 20mm Hg → mild haemolysis → Hb S/A trait.

2- Hb D/ Hb E:

Gives protection: mildest form, S/D, or S/E trait.

3- Hb C:

No protection → sickling occurs at PO2: 40 mm Hg → haemolysis in S/C trait.

4-Stasis:

As in spleen & BM: ↓O2 → sickling → further stasis & sickling vascular occlusion & infarctions → painful sickle cell crises.

5-In vitro: ↑ ph → sickling

urea → ,,

cyanide → ,, (but carcinogenic)

C/P:

A- S/S:

Presented after 6 months when β chain replaces g chain through N switching mechanism.

1- chronic H.A

2- Complications:

Sickle cell crisis:

Stasis → sickling → vascular occlusion → infarctions:

In small children : in ends of fingers → dactylitis

In older children & adolescence: in spleen, head of long bones as femur or humerous.

In adults: pulmonary infarctions, cerebral infarctions or optic nerve infarction

C/P of sickle cell crisis:

- Fever of acute onset
- Pain
- Hb level remains constant
- Leucocytosis
- ↑No. of sickle cells
- ↑CRP
- Autosplenectomy: due to repeated splenic infarctions

Lab diagnosis:

1- 3 evidence

2-CBC:

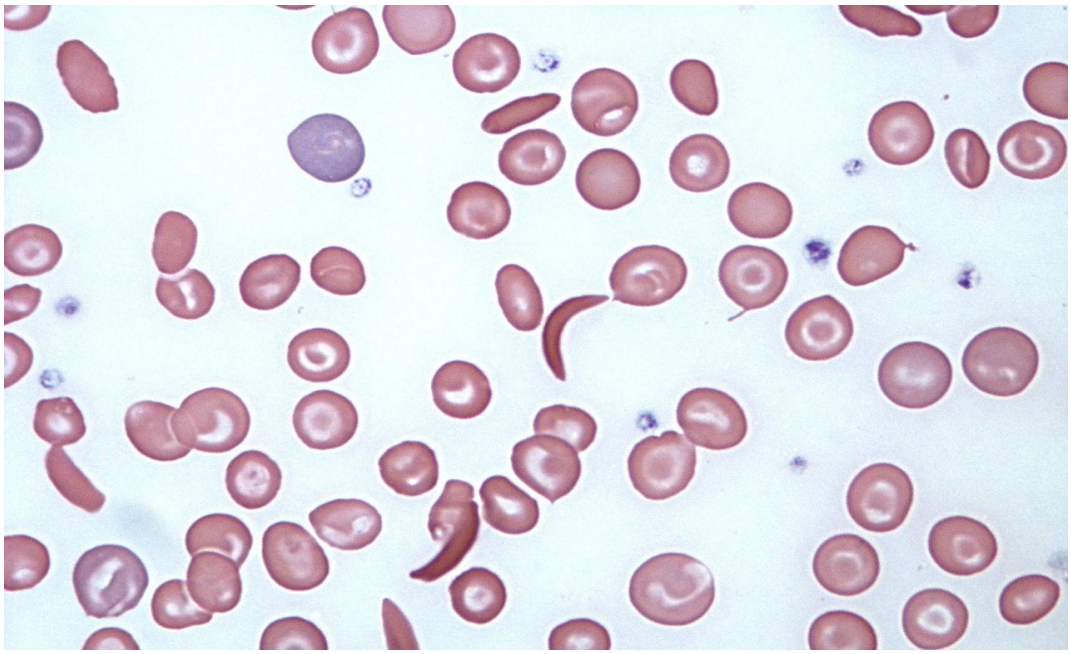
NNA+ sickle cells

↑WBCs & platelets: due to functional asplenia

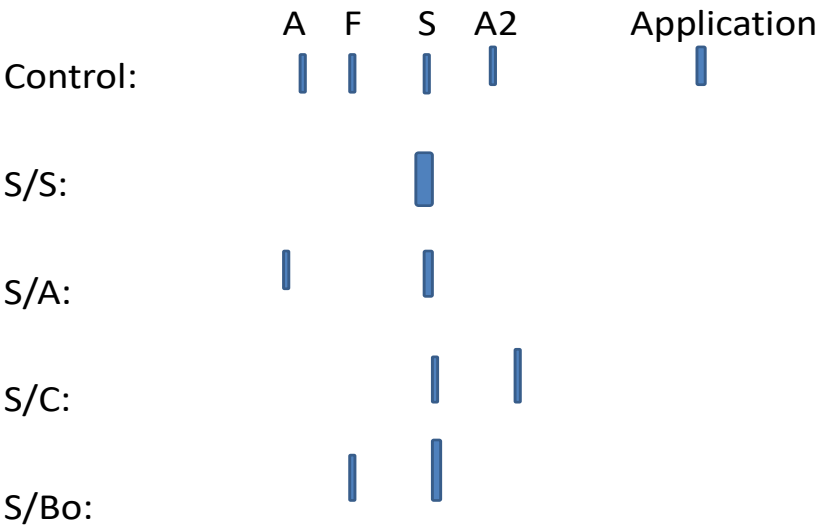
3- Special tests:

A- Sickling test:

Inducing sickling by reducing agent (drop blood+ drop Na meta bisulphite under sealed cover)⟶ hypoxia ⟶ sickling



B- Hb electrophoresis:



Management

- Prevention of infection
- Supplementing factors:
  - Folic acid, iron.
- Regular examination of heart, liver, respiratory tract.
- **Ttt of sickle cell crisis:**

-rest , rehydration & analgesics

-if not ttt:

- dextran, I.V Na bicarbonate, I.V urea

- **Ttt of aplastic crisis:**

-Blood transfusion, folic acid , ttt of infection

B- Hb S-trait (sickle cell trait) Hb S/A:

Carrier state, benign form

Asymptomatic or only mild ↓ in Hb

Symptoms appear under certain conditions:

Flying , anaesthesia, high altitude ⟶ hypoxia

Diagnosis:

Hb electrophoresis: Hb A,S, A2

Hb A> S

C-Hb S/C:

Severe as homozygous S/S

Sickling occurs at PO2: 40 mm Hg

Infarcts episodes r common

Diagnosis:

Hb electroph.:

S band & C band (at A2)

CBC:

Sickle cells, target cells

D-S/D or S/E:

Difficult to distinguish it from homozygous S/S by Hb electroph. As D band migrates as S band on cellulose- acetate paper (ph 8.6)

So repeat electrophoresis on Na- acetate agar gel acidic ph (6.2) → D band separates from S band.

2- Hb C ↖
3- Hb D | mild haemolysis
4- Hb E ↙