Diyala University – collage of medicine
Hematology -5th stage

Hemolytic disorders

By:
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Definition:

- **Anemia** resulting from an **increased rate of red cell destruction**
- **Compensatory hemolytic** disorder mean hemolytic process not develop anemia, that the normal BM able to increase its RBC production to 6-8 folds before the destruction become more than the production and anemia will developed.

Classification

Inherited Hemolytic anemias (HEM)

1. Hemoglobinopathies
2. Enzymopathies
3. Membranopathies

Acquired Hemolytic anemias

1. Immune
2. Non immune

General laboratory evidence of hemolysis

1. **General hematological investigations:**
   - Low Hb.
   - High retic count.
   - Evidence by RBC morphology on Blood film as NRBC, Polychromasia, sickle cells, spherocytes, fragmented red cells.
   - Blood indices.

2. Biochemical findings:

- Increase S. Bilirubin.
- Increase S. Hb.
- Decrease S. Haptoglobin.
- Increase S.LDH.
- Increase U. HS.
- Increase U. Hb.
- Increase U. Urobilinogen.
- Increase fecal stercobilinogen.

3. **Shorting in RC life span (Cr 51).**
4. **Evidence of RC destruction by Ferrokinetic studies.**
5. **Nonspecific BM finding as marrow erythroid hyperplasia.**
6. **Investigation according to suspected cases** as Hb electrophoresis, osmotic fragility test, coomb's test

General clinical evidence

- Anemia.
- Jaundice.
- Crisis of disease.
- Leg ulceration.
- Splenomegaly.
- Skeletal changes.
- C/F of associated disease in acquired HA.

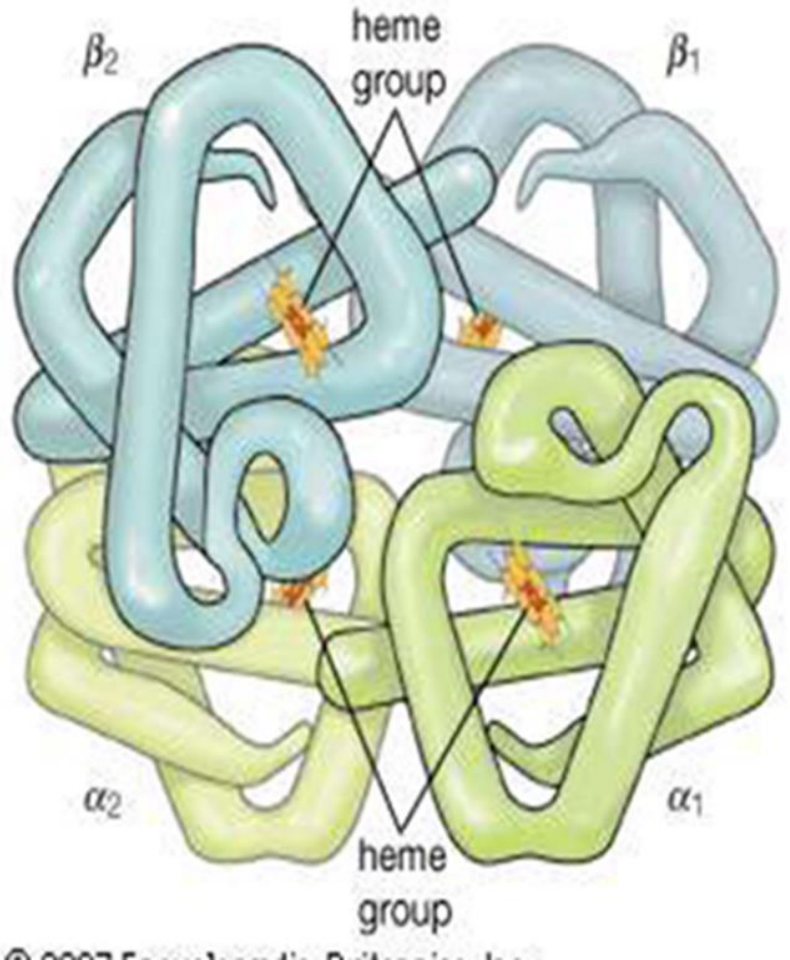
Structure and function of Hb

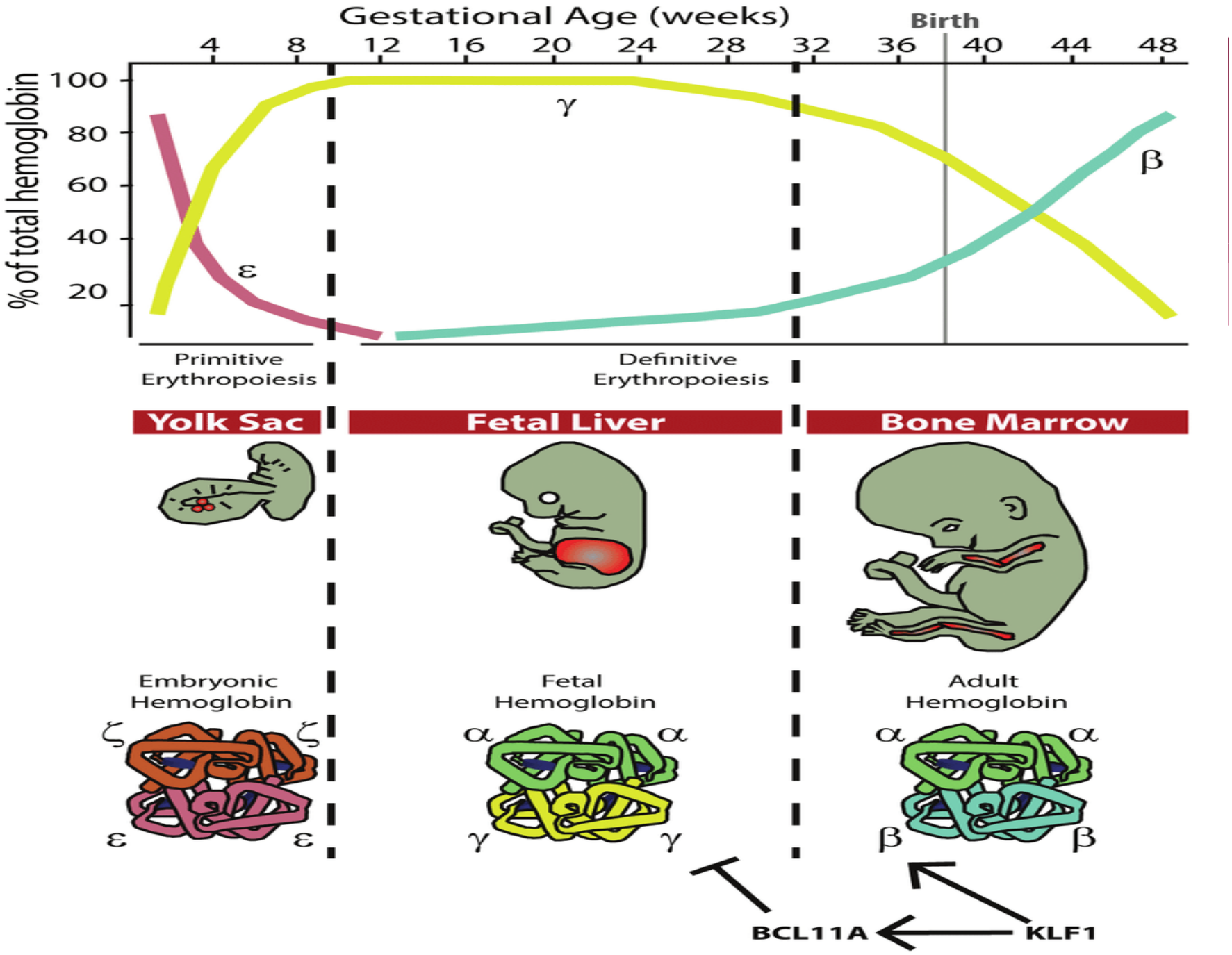
Types of human Hb

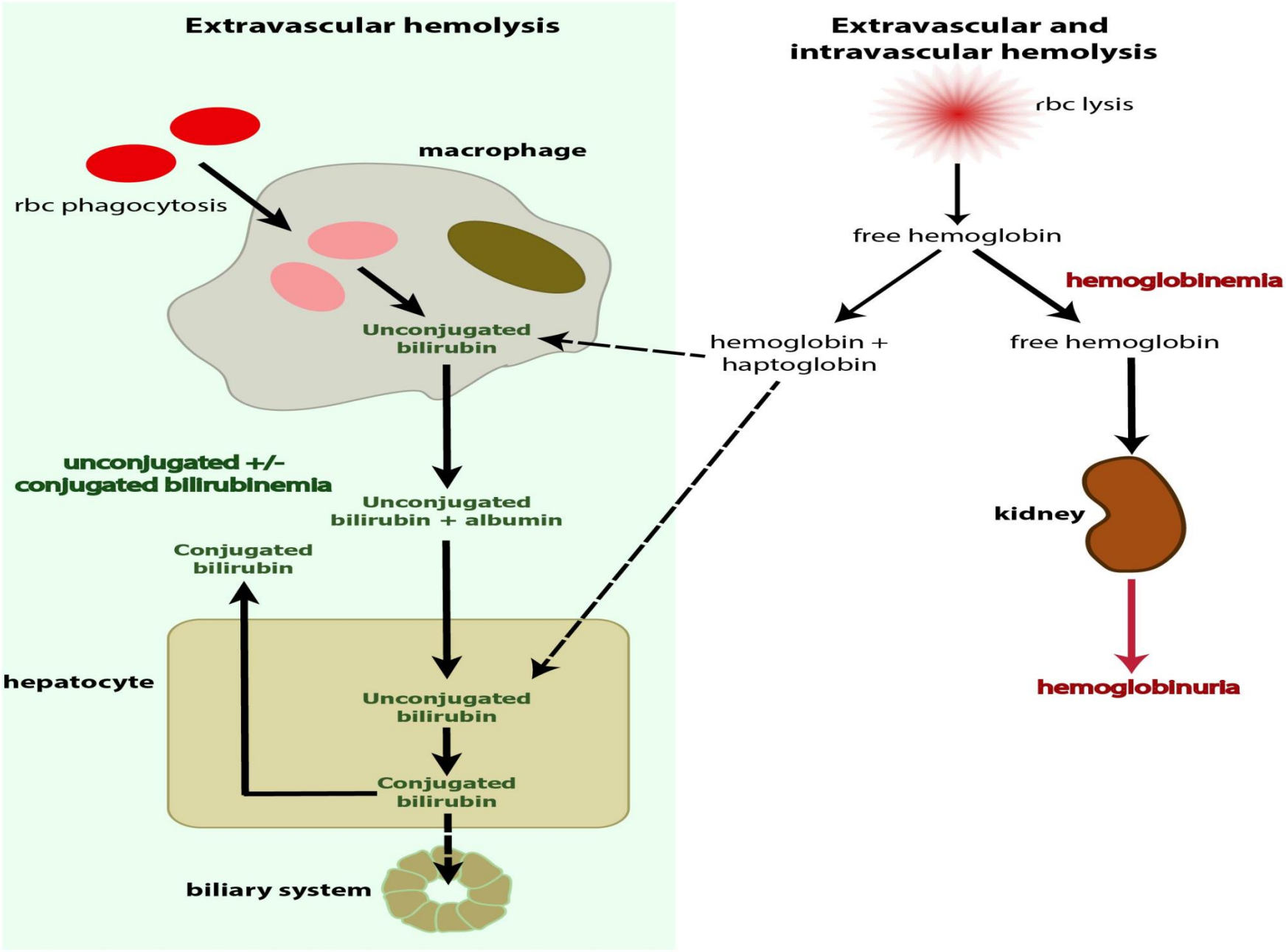
Haemoglobin proteins

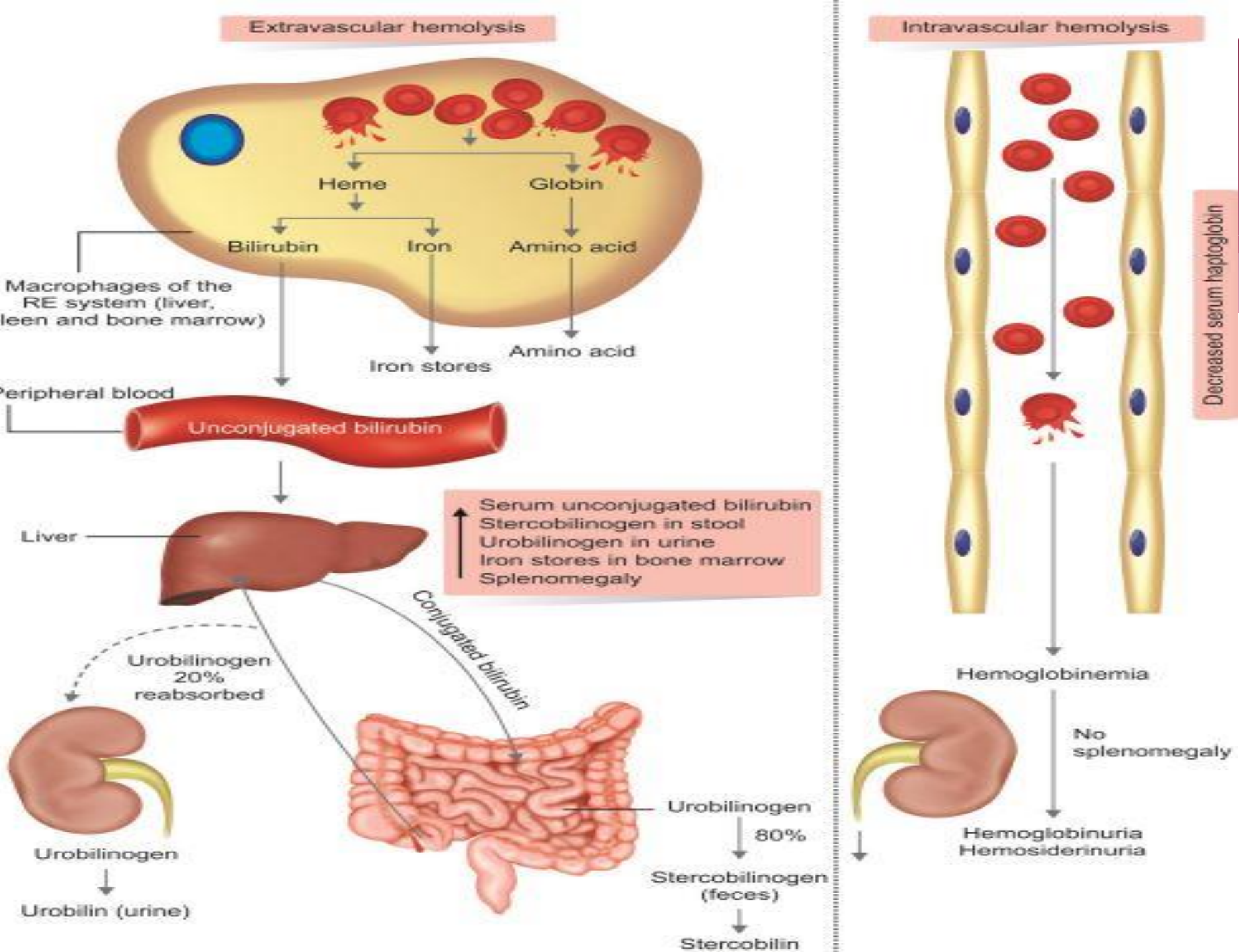
Embryonic	Fetal	Adult
Hb Gower 1	HbF	HbA
ζ ζ / ε ε	α α / Gγ Gγ	α α / β β
Hb Gower 2		HbA2
α α / ε ε	α α / Aγ Aγ	α α / δ δ
Hb Portland		
ζ ζ / γ γ		

Hb structure




Gestational Age (weeks)
Birth
4
8
12
16
20
24
28
32
36
40
44
48
% of total hemoglobin
100
80
60
40
20
γ
ε
β
Primitive Erythropoiesis
Definitive Erythropoiesis
Yolk Sac
Fetal Liver
Bone Marrow
Embryonic Hemoglobin
ζ
ζ
ε
ε
Fetal Hemoglobin
α
α
γ
γ
Adult Hemoglobin
α
α
β
β
BCL11A
KLF1


Extravascular hemolysis
rbc phagocytosis
macrophage
Unconjugated bilirubin
unconjugated +/- conjugated bilirubinemia
Unconjugated bilirubin + albumin
Conjugated bilirubin
hepatocyte
Unconjugated bilirubin
Conjugated bilirubin
biliary system
Extravascular and intravascular hemolysis
rbc lysis
free hemoglobin
hemoglobinemia
hemoglobin + haptoglobin
free hemoglobin
kidney
hemoglobinuria


Extravascular hemolysis
Heme
Globin
Bilirubin
Iron
Amino acid
Macrophages of the RE system (liver, leen and bone marrow)
Iron stores
Amino acid
eripheral blood
Unconjugated bilirubin
Liver
Serum unconjugated bilirubin
Stercobilinogen in stool
Urobilinogen in urine
Iron stores in bone marrow
Splenomegaly
Urobilinogen 20% reabsorbed
Conjugated bilirubin
Urobilinogen
Urobilin (urine)
Urobilinogen
80%
Stercobilinogen (feces)
Stercobilin
Intravascular hemolysis
Decreased serum haptoglobin
Hemoglobinemia
No splenomegaly
Hemoglobinuria
Hemosiderinuria

Structure and function of Hb

* Three types of Hb : (in adult)
  - Hb A (97%),
  - F or fetal Hb (1%),
  - And Hb A2 (2%)
* Hb F usually switches to adult Hb (Hb A) occurs after birth by decrease F and produce A.
* Hb A predominant Hb after one year of birth is composed of 2 beta globin chains and 2 alpha globin chains bonded to four iron containing heme groups

* The globin chains are protein made up of a precise sequence of amino acids that are coded by genes located on chromosome16 and chromosome 11 contained in the nucleus of the bone marrow stem cells.
* Hb production requires iron, the synthesis of the protoporphyrin ring and production of the globin chains so reductions in any of these leading to develop anemias.

* Lysis of red cell also produce free Hb this bind to Haptoglobin for transport to the RES
* A breakdown product of hemoglobin is bilirubin which is conjugated in the liver and excreted in the biliary system into the gut. Isolated increase in indirect bilirubin (unconjugated) is a very specific indicator of increased red cell breakdown

Hemoglobinopathies

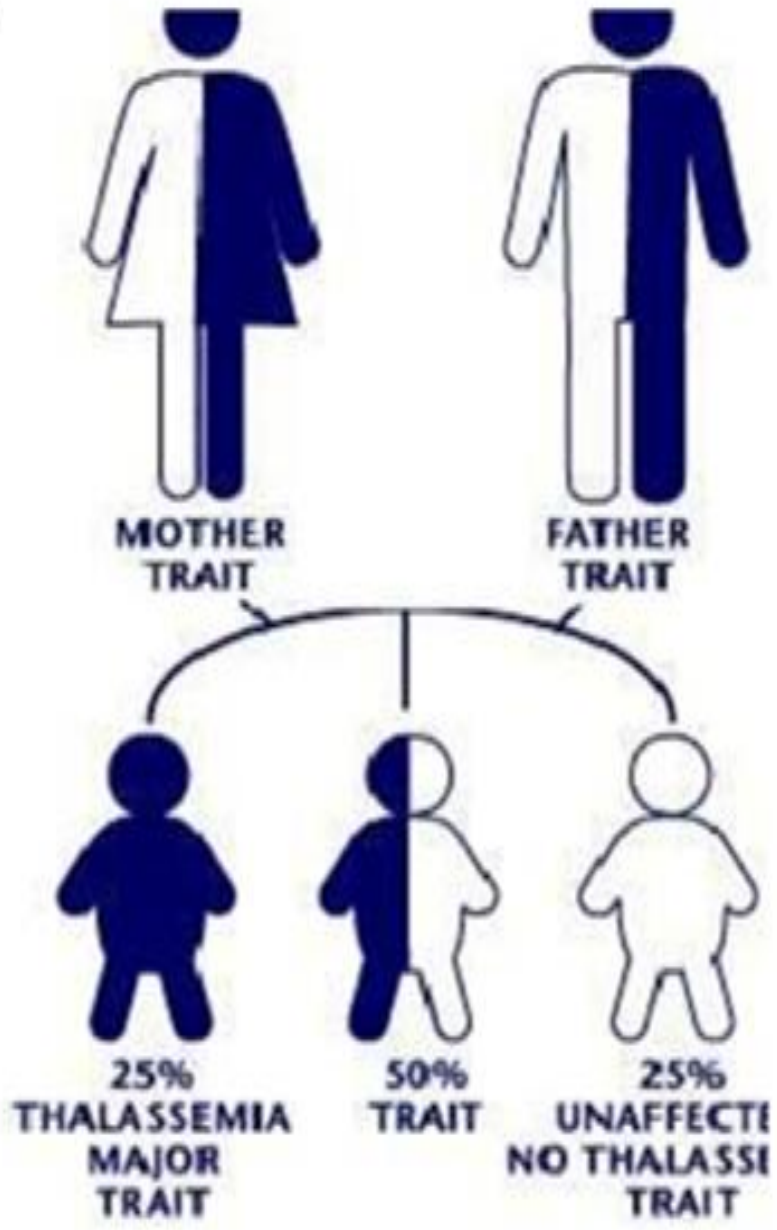
1. Quantitative like Thalassemia (α, β, etc).
2. Qualitative (structural abnormalities): Hb S, C, D, E.
3. Combination of both.

Thalassemia

* **Definition**: A group of inherited disorders of Hb synthesis, characterized by reduced or absent in synthesis of one or more of the globins chains of Hb.
* **Thalassa** (Greek word mean the sea)1st description in 1925 by Cooley (Cooley's anemia)

INHERITED AS

- Autosomal recessive
- Beta thal - point mutations on chromosome 11
- Alpha thal - gene deletions on chromosome 16



B-Thalassemia

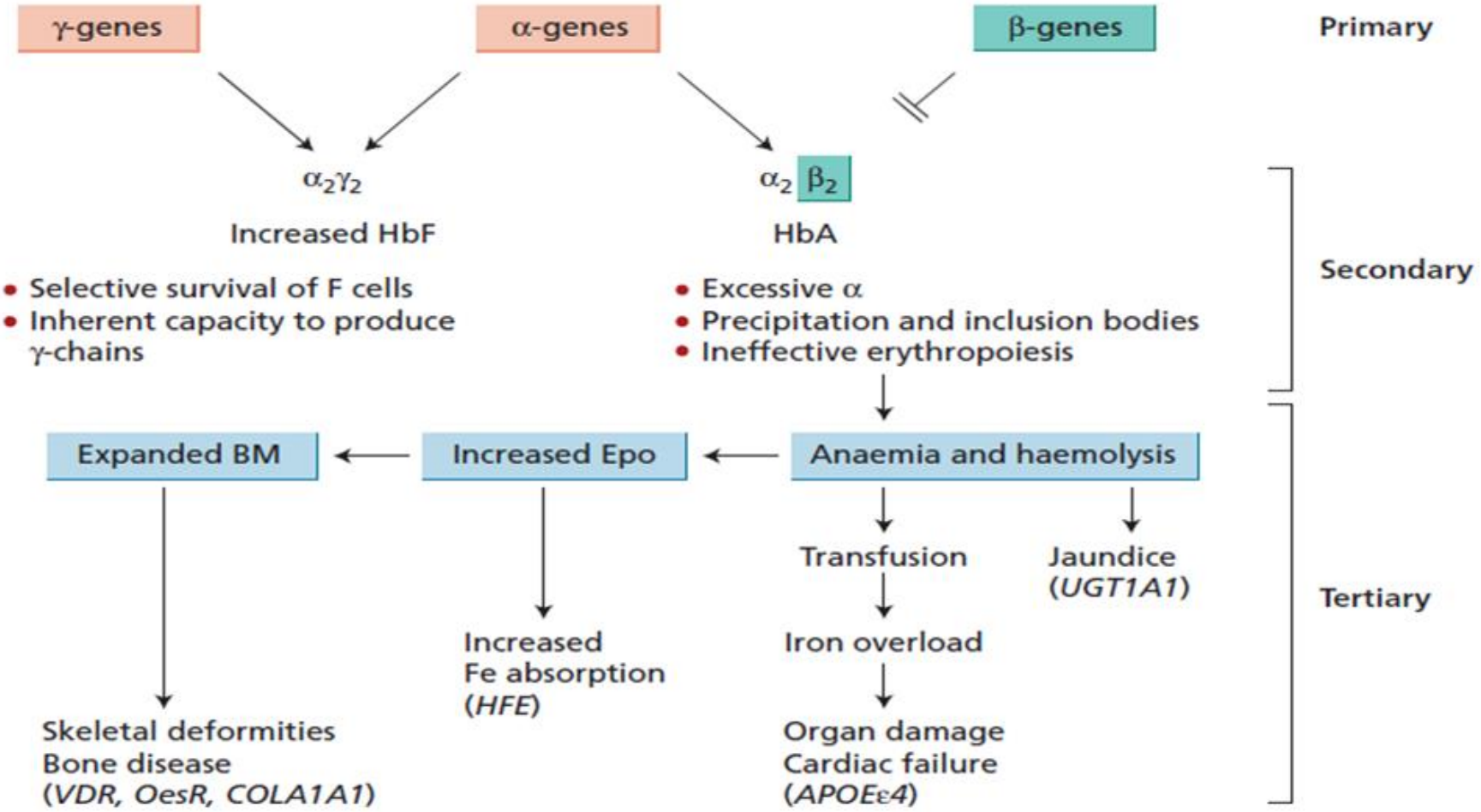
* This is one of the most common inherited hematological disorders **in Iraq.**
* It was estimated that around 4-5% of the population of the **country carry thalassemia genes**.
* Beta thalassemia more common in **Mediterranean** region.
* The inheritance of this disorder is autosomal recessive, so that heterozygous are usually symptomless, while homozygous are severely or moderately affected.

Clinically β thalassemia could be classified into :

Clinical syndromes	*Genotype*	*Clinical features*
β-thalassemia major	Homozygous (β^0/β^0,β^+/β^+) or double heterozygous (β^0/β^+)	Severe form, severe anemia and transfusion dependent High level of HbF in the blood
β-thalassemia intermedia	Variable (β^0/β^+, β^+/β^+, β^0/β, β^+/β)	Moderately severe and not transfusion dependent
β-thalassemia minor/ β-thalassemia trait	Heterozygous (β^0/β, β^+/β)	Mild anemia and asymptomatic

β^0 = Total absence of β-globin synthesis; β^+ = Markedly reduced or diminished β-globin synthesis; β-normal β-globin synthesis

Pathogenesis of β- Thalassemia major



.5 The pathophysiology of β-thalassaemia. BM, bone marrow; Epo, erythropoietin. (Source: Thein, 2004 [*Br J Hae*
Reproduced with permission of Wiley.)

Clinical features β- Thalassemia major:

* First diagnosis between age of **6 months and 2 years**
* **Presentation usually with** Pallor, poor feeding, failure to thrive, abdominal swelling (due to HSM) and sometimes Jaundice.
* **Later clinical picture** depends on management, and falls into two categories
* **At end of 1st decade** feature of **iron over load** will appear mainly growth retardation, endocrine disorders, liver and heart failure, and delay in sexual development.

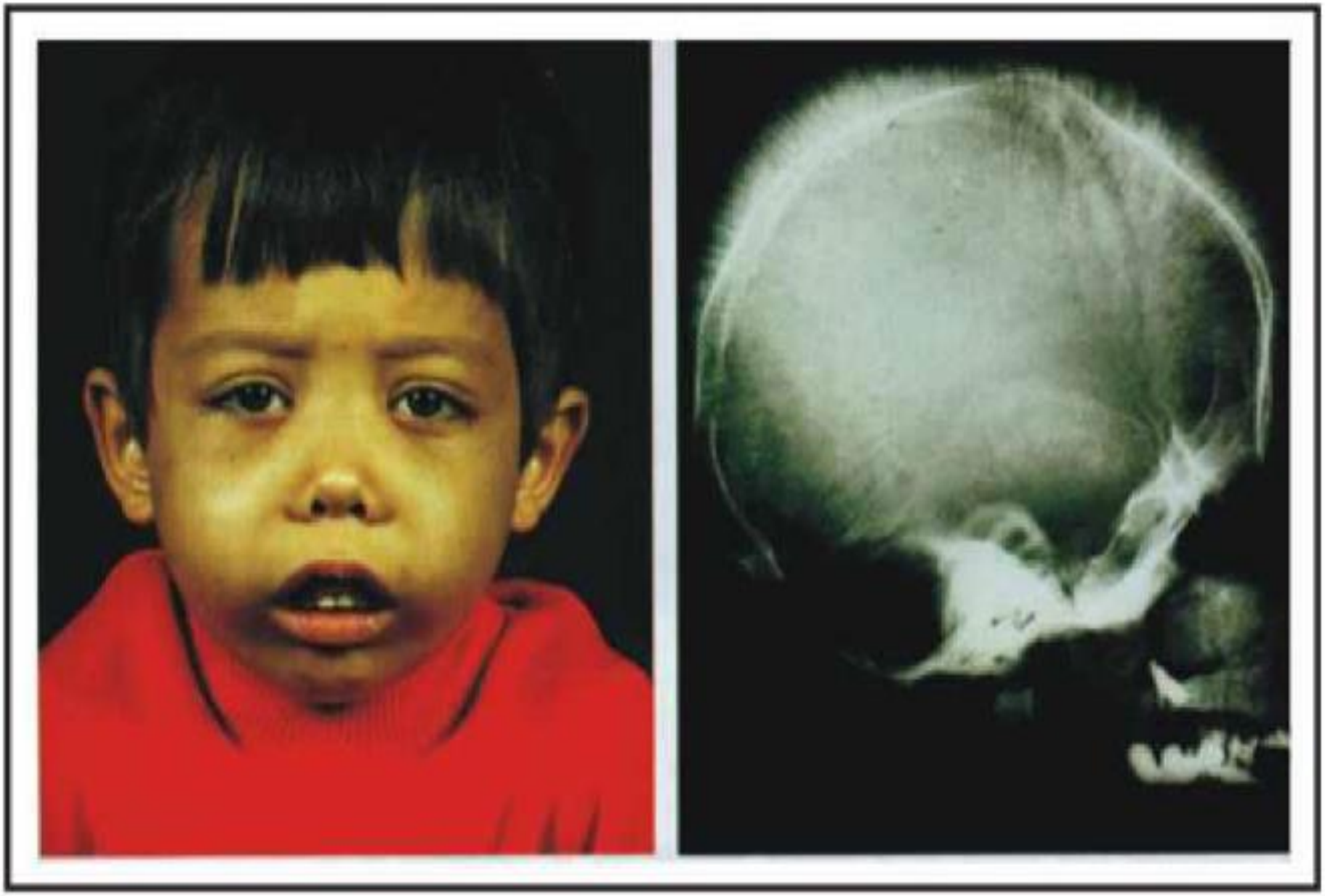
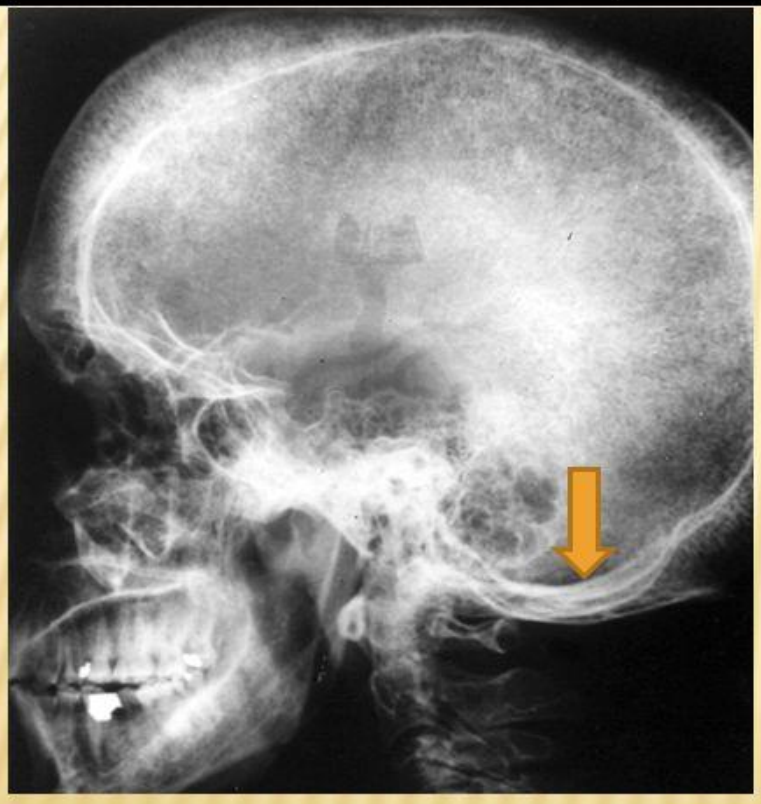
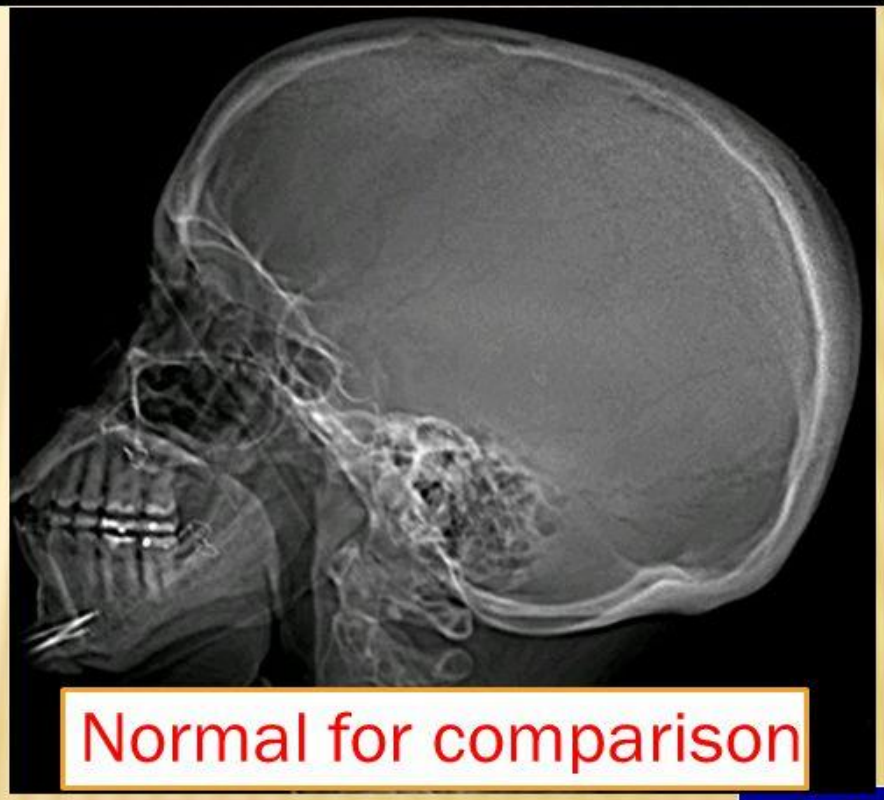


Fig. 2 Beta thalassemia major – bone changes

25-year-old man with β-thalassemia.
Lateral skull radiograph shows
expansion of diploic space with hair-on-end appearance
widened groove for middle meningeal artery
Spared occipital bone (arrow)





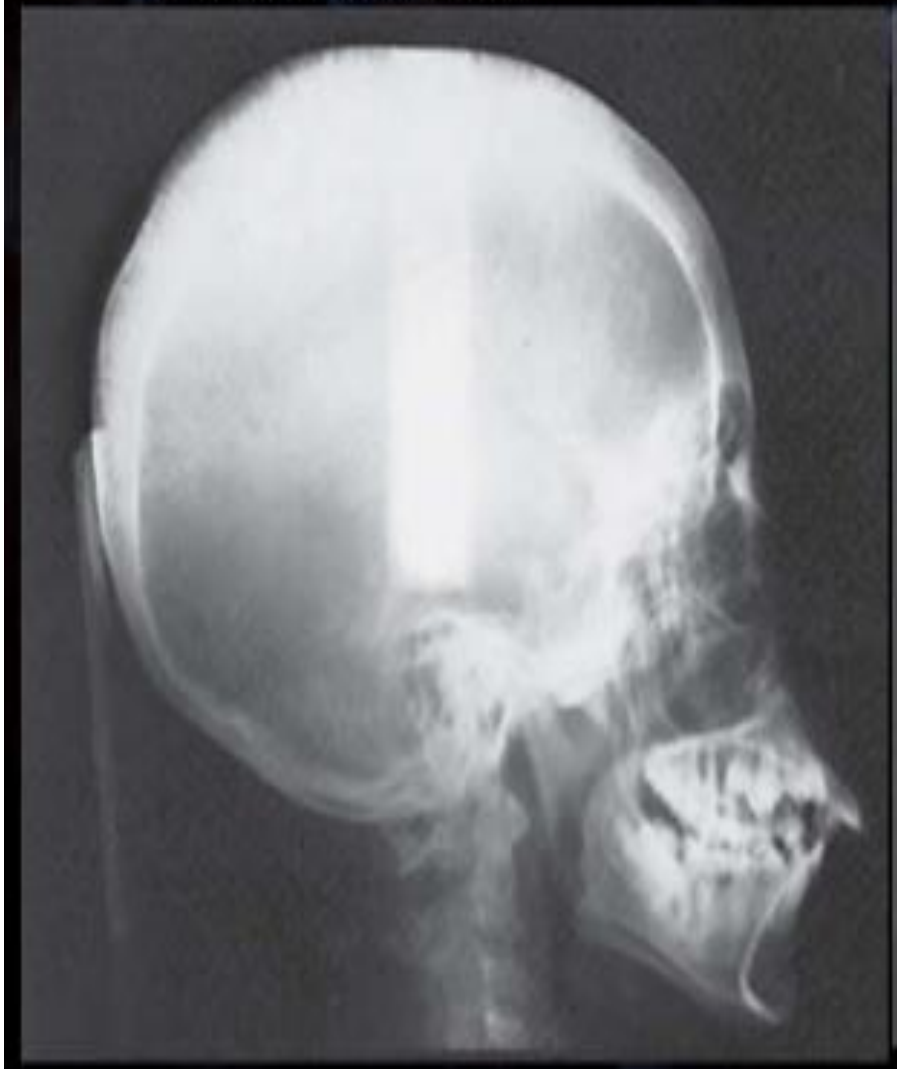
Normal for comparison

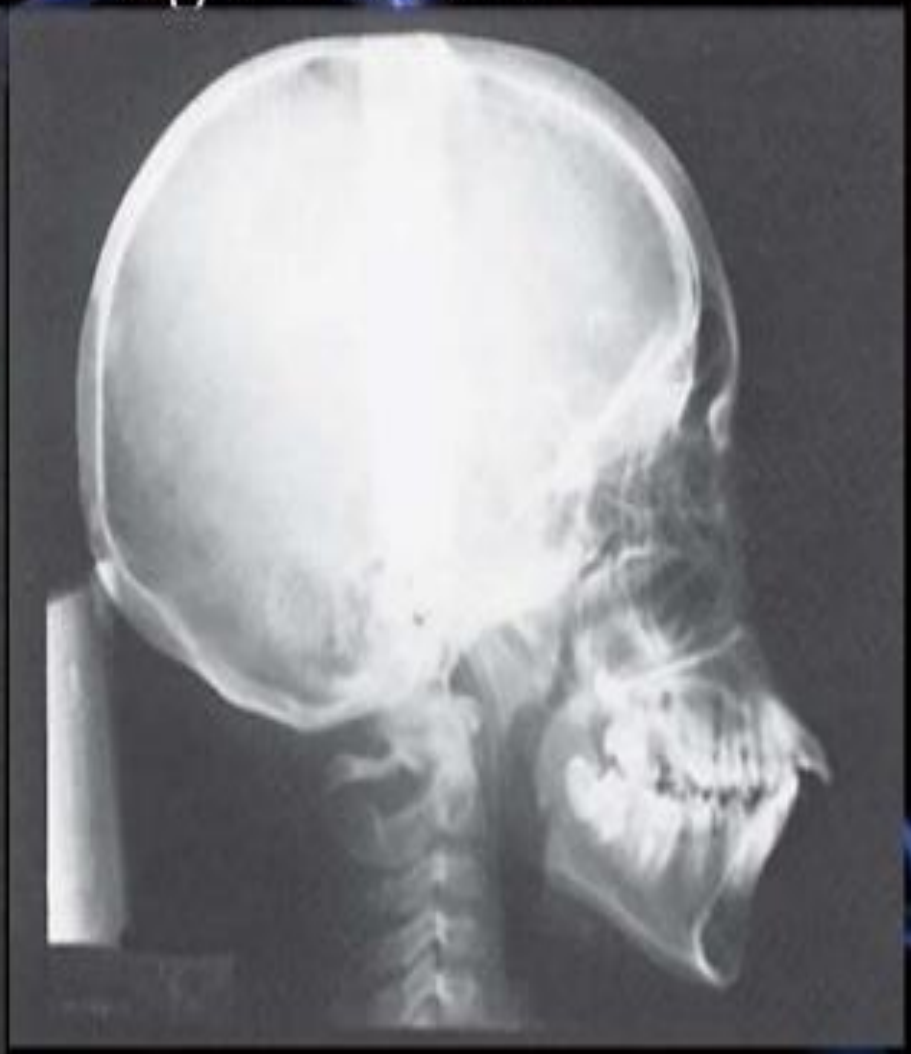
Georgiades C S et al. AJR 2002;179:1239-1243

AJR

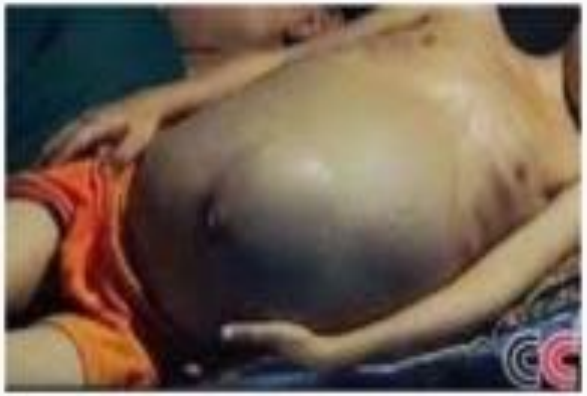
thickened diploic space and thinning of the skull cortex

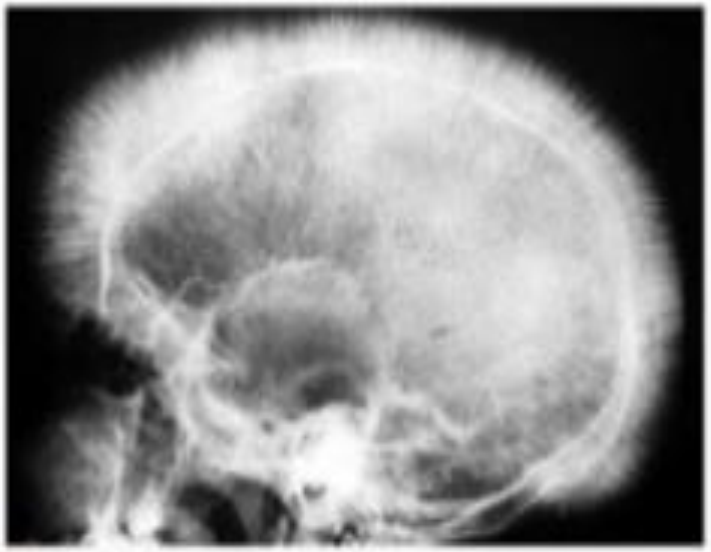
right=normal





CLINICAL PRESENTATION

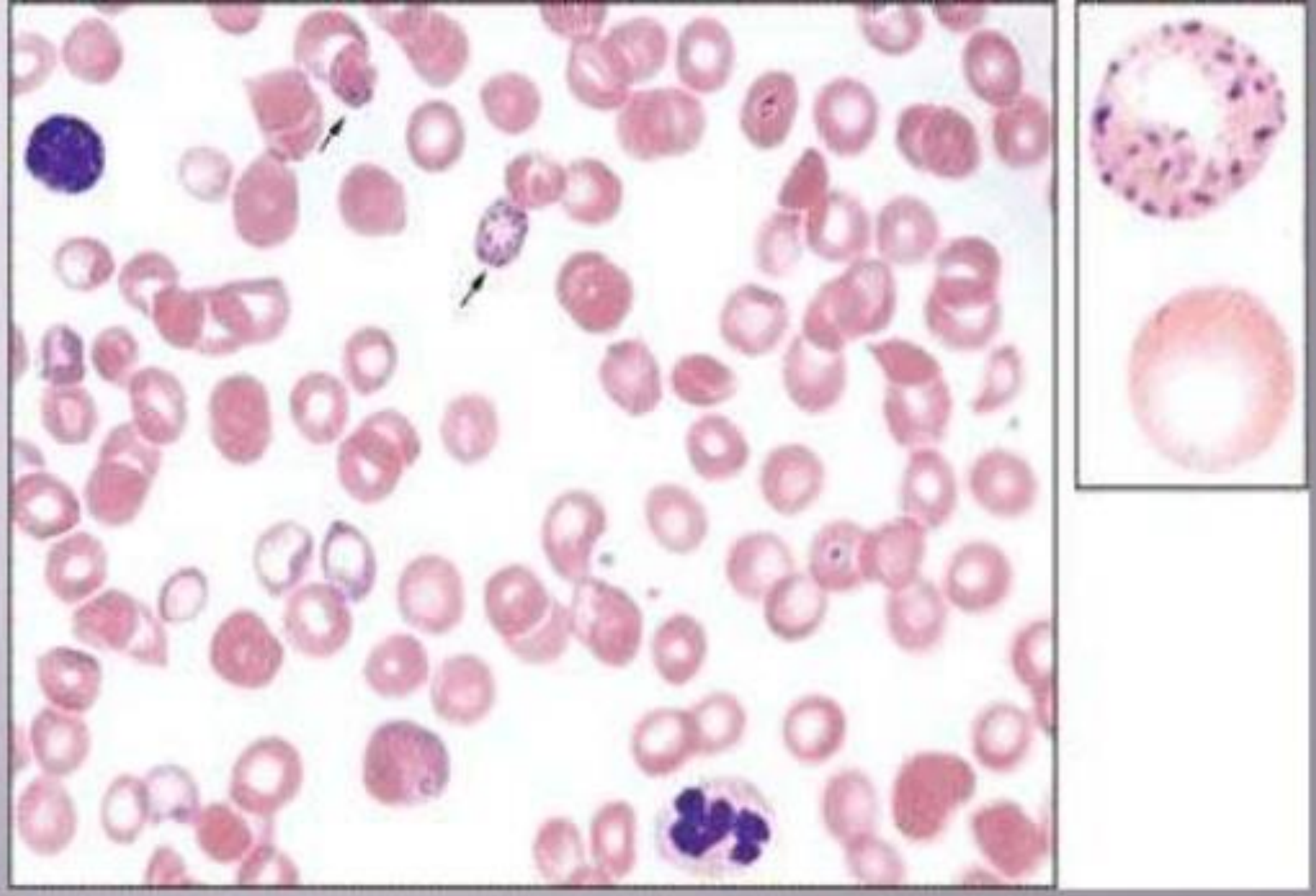




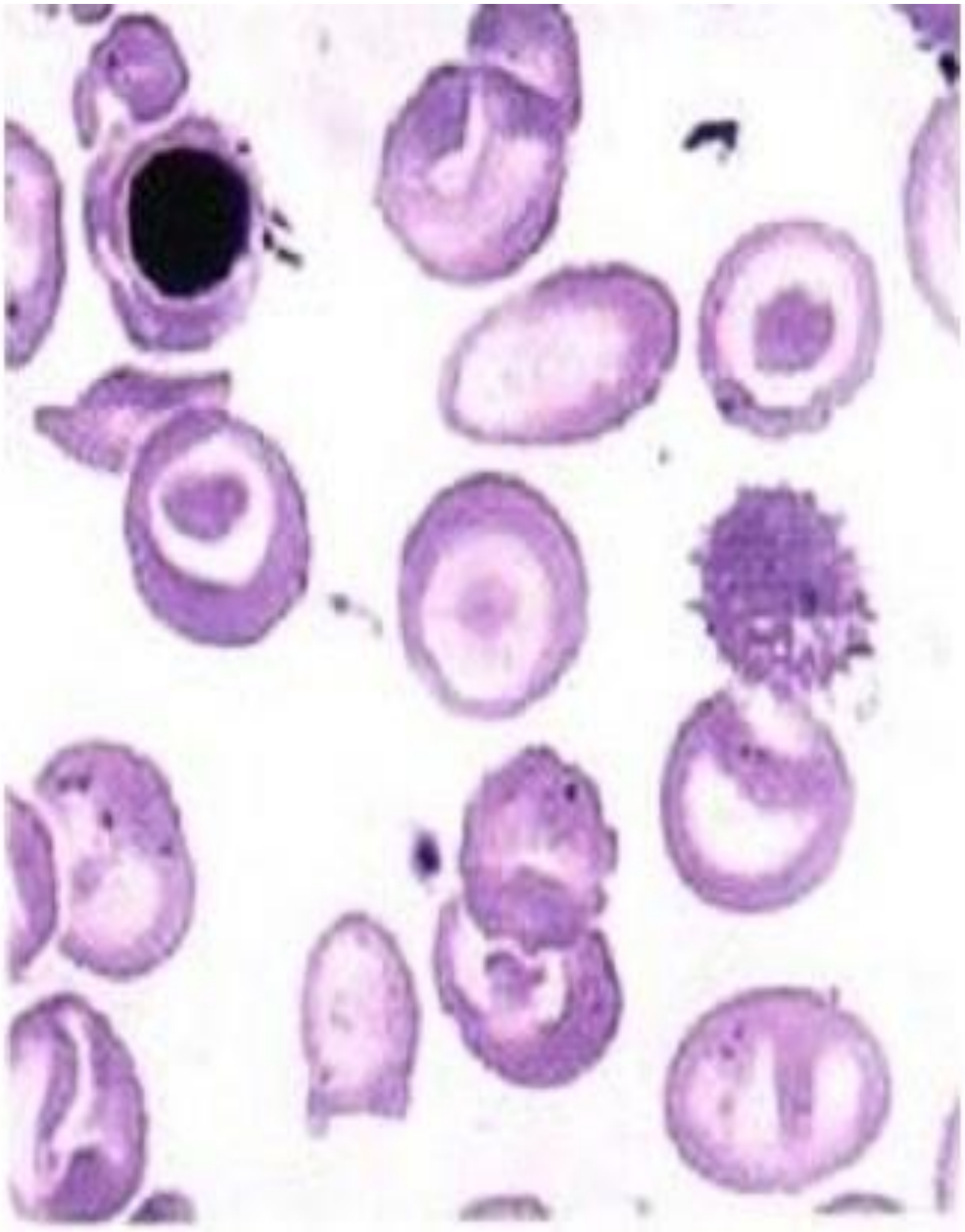
Laboratory findings:

- **CBC**: Usually severe anemia, variable WBC and platelets counts. MCV and MCH are both reduced.
- **Blood film**: Sever hypochromic microcytic anemia, NRBC, sever anisopoikilocytosis, target, tear drop cells and basophilic stipling.
- **Reticulocytes**: usually range 2-8%.
- **BM showing** eryth. hyperplasia, with many RC precursors of ragged inclusions due to alpha chains ppt.

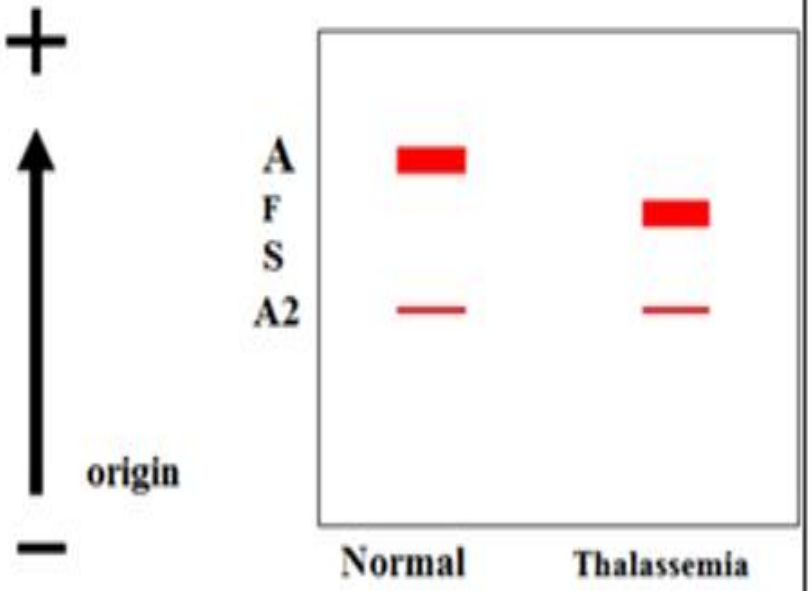
- Hb electrophoresis:
  - HbF 10-98%
  - HbA may or may not present
  - HbA2 variable
- DNA analysis to predict the severity or in prenatal diagnosis.
- Biochemical findings: increase in S.Bilirubin, S.Iron, S.Ferritin, with normal or low TIBC.
- Assessment of iron status usually by S.Ferritin



Basophilic stippling in thalassemia. Peripheral blood film demonstrating microcytic hypochromic RBCs and basophilic stippling (*arrows*). *Basophilic stippling occurs in*
thalassemia as well as in other hematologic disorders.

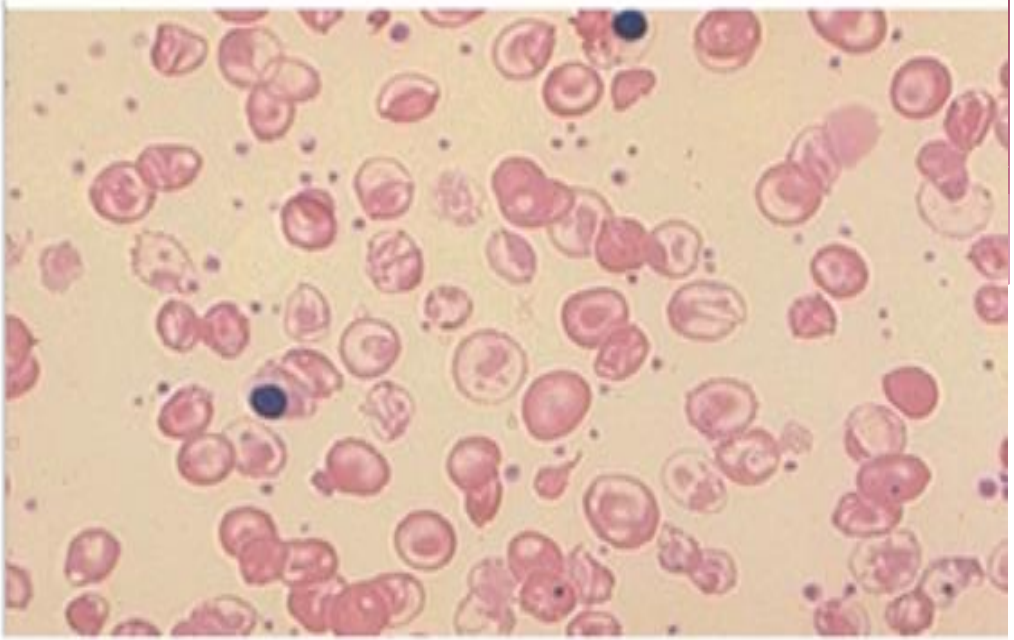


Hb- electrophoresis



- Depends on the molecular defect responsible for the thalassaemia
- Hb F is increased from 10-98%, Hb A may or may not be present, Hb A2 variable.
- Remember that on birth Hb F is the major Hb , and it goes down to <1% at the age of 6 months, therefore a definitive diagnosis is best achieved > 6 months.

Blood film in βThalassemia major



Hypochromic, microcytic, target cells, and NRC

Management of Thalassemia major

1. **Prevention**
2. **BM transplant** with successful rate up to 90% when done in 1st few years before iron loading with appropriate donors.
3. **Gene therapy** with gene replacement.
4. **Symptomatic treatment**:
   * Adequate Transfusion with Iron chelation. Maintain Hb 10-12 g/dl.
   * Transfusion depends on general condition rather than Hb level.
   * usually required 2-3 unit/4-6wk
   * Use of Iron chelation e.g.Desferoxamine (Desferral) with vitamin C, indicated when S. Ferritin level reach 1000 µg/L or patient receive 12-15 transfusion.
   * Folate supplements (5mg/day).
   * Splenectomy in frequent transfused patient or symptomatic SM.
   * Management of complications

Prognosis:

* If no Transfusions, death usually occurs in the first few years of life.
* If iron overload is allowed to occur then death in 2nd or early third decade.
* However if measures to prevent Iron overload by Iron Chelation are instituted early on, with the transfusion, Iron overload consequences maybe limited, although delayed puberty and stunted growth may still be encountered, but otherwise patients may develop normally.

β- Thalassemia Minor

* **Heterozygous** to ..... (β β+) or (β βo).
* **Clinically** usually symptom free and discovered by chance
* **Laboratory findings:**
  - ✓ Hemoglobin is usually reduced 1-2 g/dl less than normal.
  - ✓ MCH and MCV are reduced.
  - ✓ RBC count is > 5 x 1012/L in 85% of cases.
  - ✓ Reticulocyte count is slightly increased or normal.
  - ✓ Blood film: slight hypochromic, mild anisocytosis, target cells.
  - ✓ Increase in Hb A2: (Normal range of Hb A2 is 1.8-3.5%)
* **Management**: No need for specific management. Except in periods of stress like pregnancy

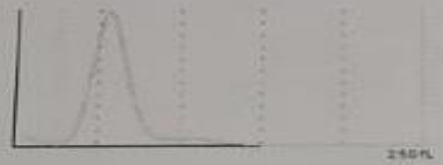
Patient ID:
Doctor: wesam waled

Birth:
Sex:

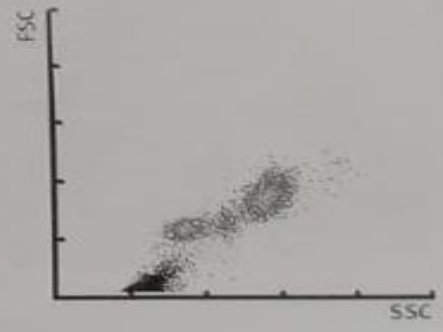
Parameters				Normal Ranges
WBC	6.35	[10^3/uL]		(3.00 - 15.00)
NEUT	4.11 *	[10^3/uL]	64.8 * [%]	(1.50 - 7.00
LYMPH	1.62 *	[10^3/uL]	25.5 * [%]	(1.00 - 3.70
MONO	0.53 *	[10^3/uL]	8.3 * [%]	(0.00 - 0.70
EO	0.06	[10^3/uL]	0.9 [%]	(0.00 - 0.4
BASO	0.03	[10^3/uL]	0.5 [%]	(0.00 - 0.
RBC	6.42 +	[10^6/uL]		(2.50 - 5
HGB	11.5	[g/dL]		(8.0 - 1
HCT	36.8	[%]		(26.0 -
PLT	186 *	[10^3/uL]		(50 -
MCV	57.3 -	[fL]		(86.0 - 1
MCH	17.9 -	[pg]		(26.0 -
MCHC	31.3	[g/dL]		(31.0 -
RDW-SD	30.9 -	[fL]		(37.0 -
RDW-CV	16.2 +	[%]		(11.0 -
PDW	----	[fL]		(9.0 -
MPV	----	[fL]		(9.0 -
P-LCR	----	[%]		(13.0 -
PCT	----	[%]		(0.17
IG	0.10 *	[10^3/uL]	1.6 * [%]	(0.00

WDF

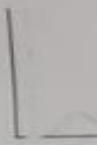
RBC



WDF - CBC



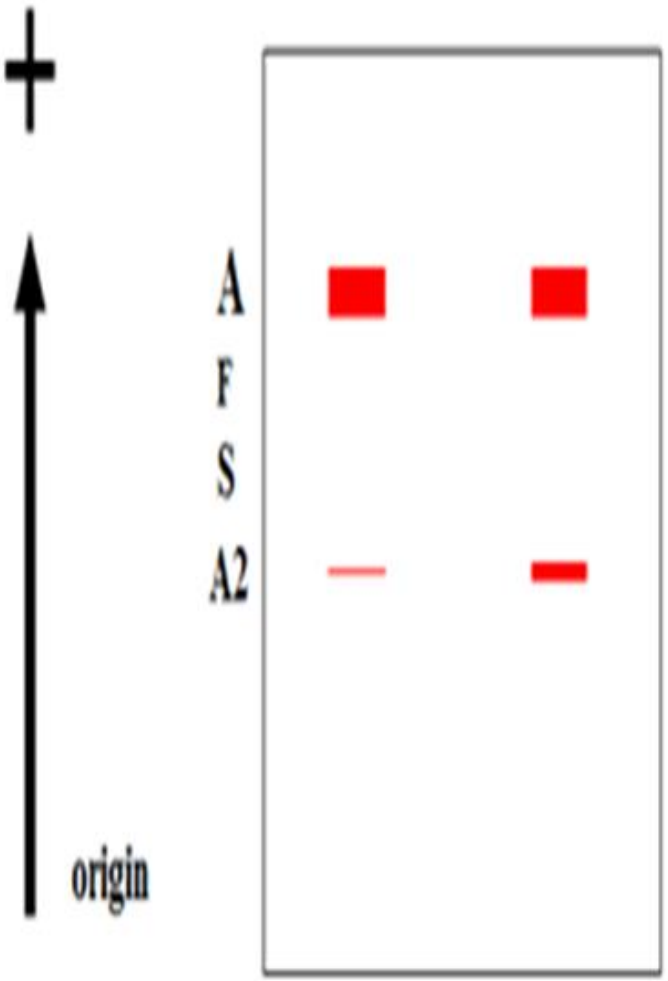
PLT

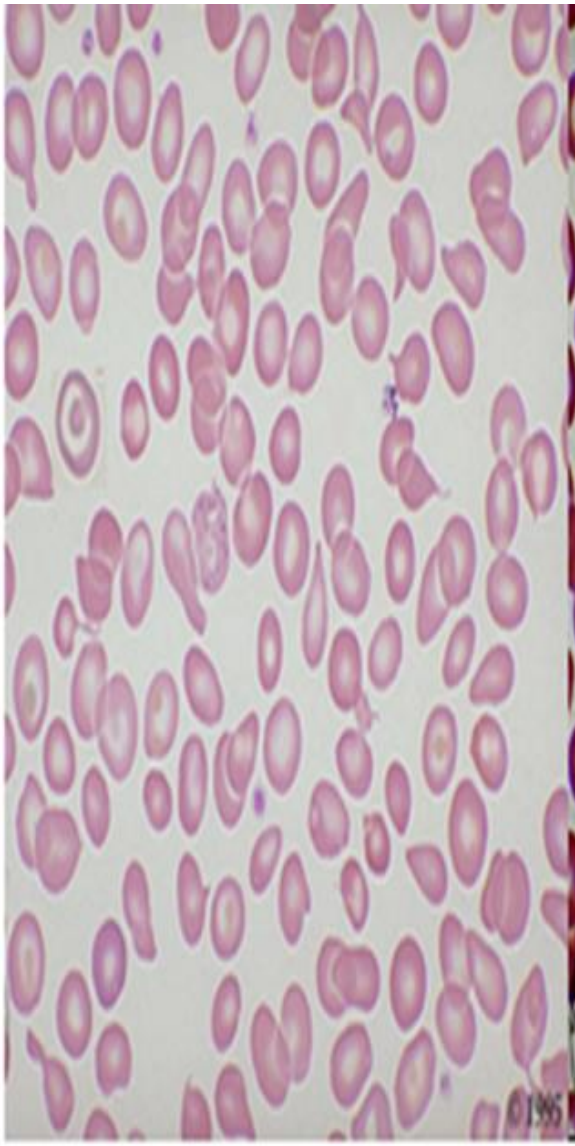


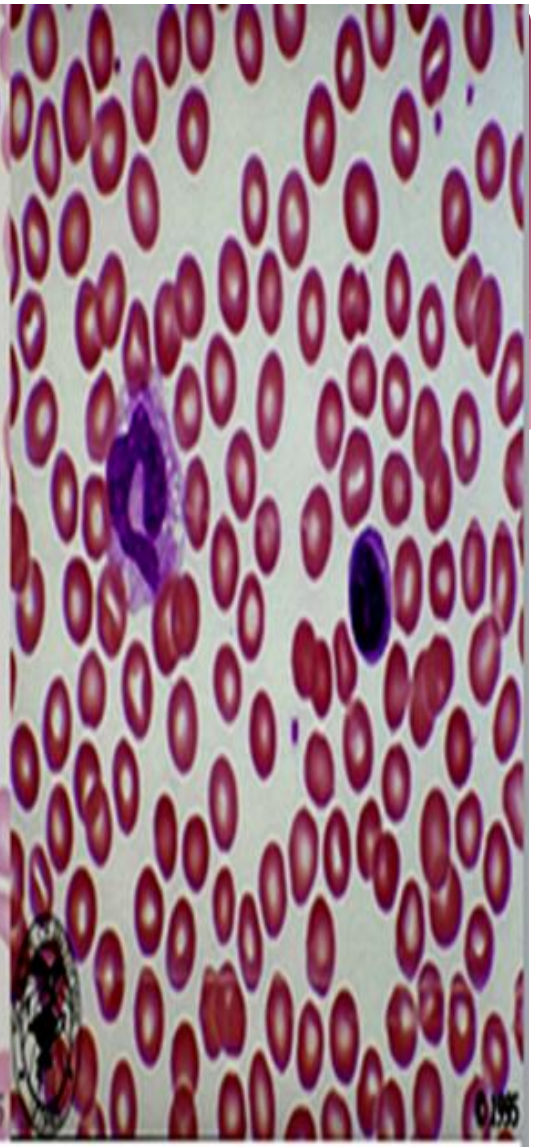
Hematology

RBC (Erythrocytes)	* H	6.26	4.06	-	5.30	10^6/uL
HCT(Haematocrit)		38.9	38	-	52	%
HGB (Hemoglobin)		12.3	12.0	-	16.0	g/dL
MCV	* L	62.1	76	-	96	fL
MCH	* L	19.6	26	-	32	pg
MCHC	* L	31.6	32	-	36	g/dL
RDW-SD	* L	31.6	37	-	54	fL
RDW-CV	* H	15.6	11.5	-	14.5	%
WBC (Leukocyte)		7.50	3.70	-	11.00	10^3/uL
NEU%		66.4	39.3	-	73.7	%
NEU#		4.98	1.63	-	6.96	10^3/uL
LYM%		22.3	18	-	45.3	%
LYM#		1.67	1.09	-	2.99	10^3/uL
MON%	* H	9.3	2	-	8	%
MON#		0.70	0.240	-	0.79	10^3/uL
EOS%		1.7	.7	-	6	%
EOS#		0.13	.03	-	.44	10^3/uL
BASO%		0.3	0.0	-	1.70	%
BASO#		0.02	0.0	-	1	10^3/uL
PLT (Platelet Count)		325	155	-	450	10^3/uL
MPV		10.4	6.9	-	10.6	fL
PDW		12.5	9	-	17	fL
PCT		0.34	0.17	-	0.35	%
P-LCR		29.6	13	-	43	%

Hb- electrophoresis





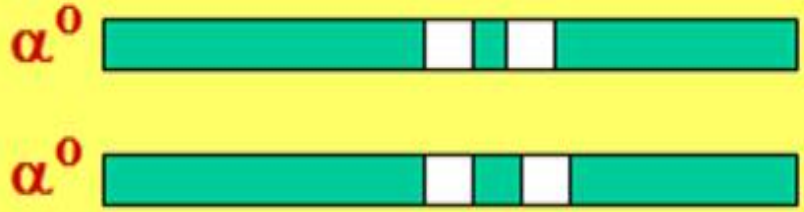


Normal Thalassemia minor **Thalassemia minor** **Normal blood film**

α - Thalassemia

* Much Less common in our country than Beta thalassemia, and of much less clinical significance.
* It due to reduced or absent synthesis of alpha (α) globin chains of hemoglobin (α chains are constituents of all three normal Hb A, A2 and F).
* Clinical Phenotypes of Alpha thalassemia (relevant to number of alpha genes remaining):

No alpha genes

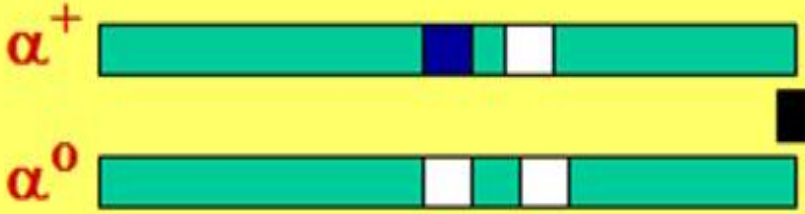


No alpha chains

1. Hb Barts Hydropes Fetalis

Incompatible with life

One Alpha gene

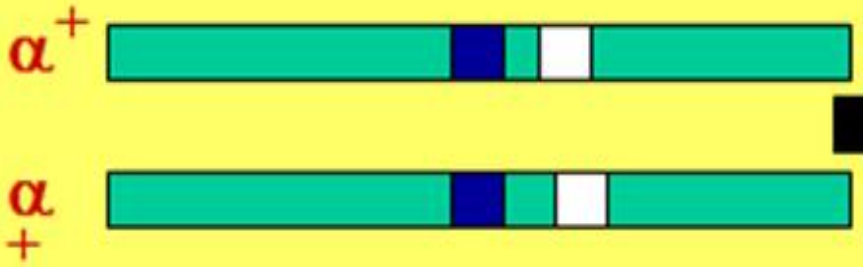


Markedly reduced alpha chain production

2. Hb H disease

Thalassaemia intermedia phenotype

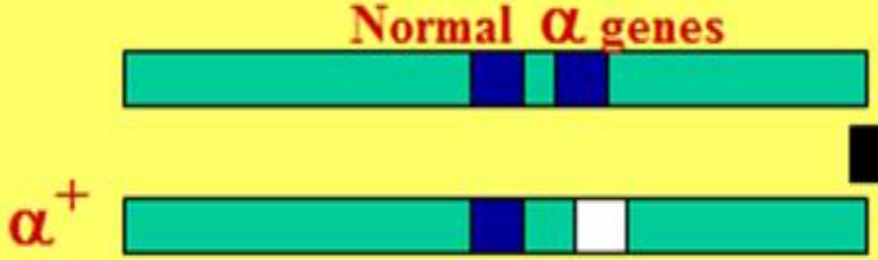
Two alpha genes



Moderately reduced Alpha chains

3. α Thalassemia minor

Three Alpha genes



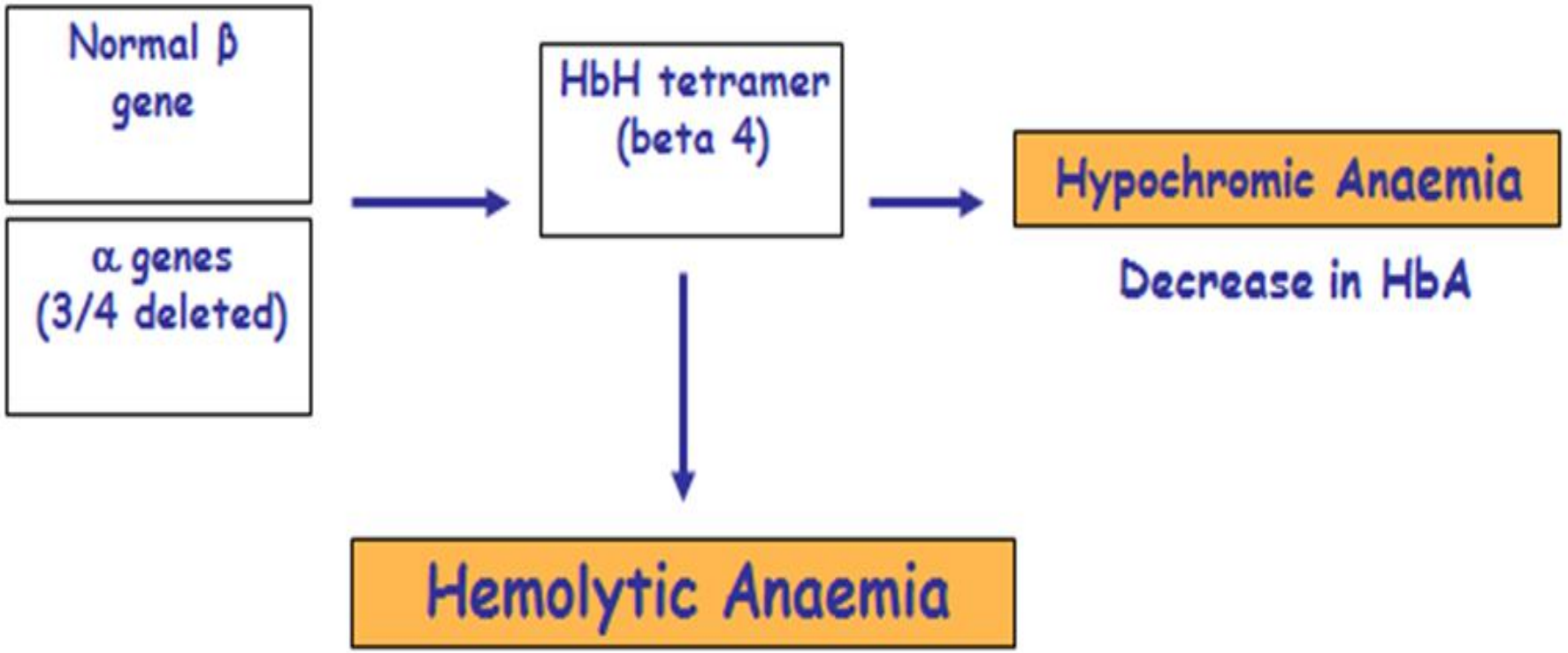
Minimally reduced Alpha chains

4. Silent α thal. carrier state

Hemoglobin H disease(Hb H) (β4 tetramer)

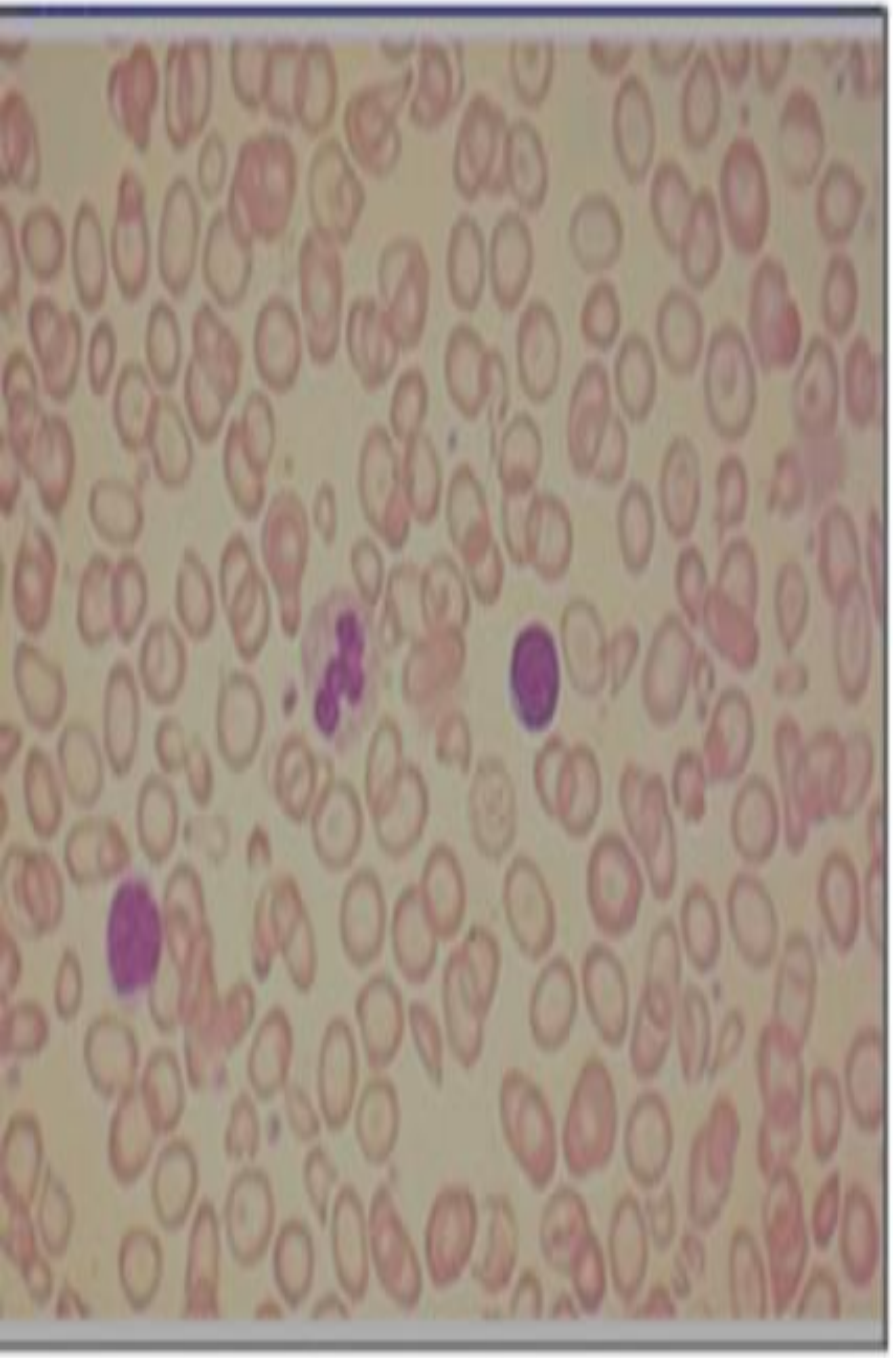
* Common in South East Asia, less so in Mediterranean countries, Sporadic in Iraq
* The only clinical phenotype of alpha thalassemia of **clinical significance.**
* **Clinically**: Variable anemia, Hb 7-10, variable degrees of splenomegaly, sometimes Jaundice
* **unusual to see severe** thalassaemic skeletal changes or growth retardation, usually survive to adult life
* **Management**: Depends on severity, unlikely to require regular transfusions. Folate supplements

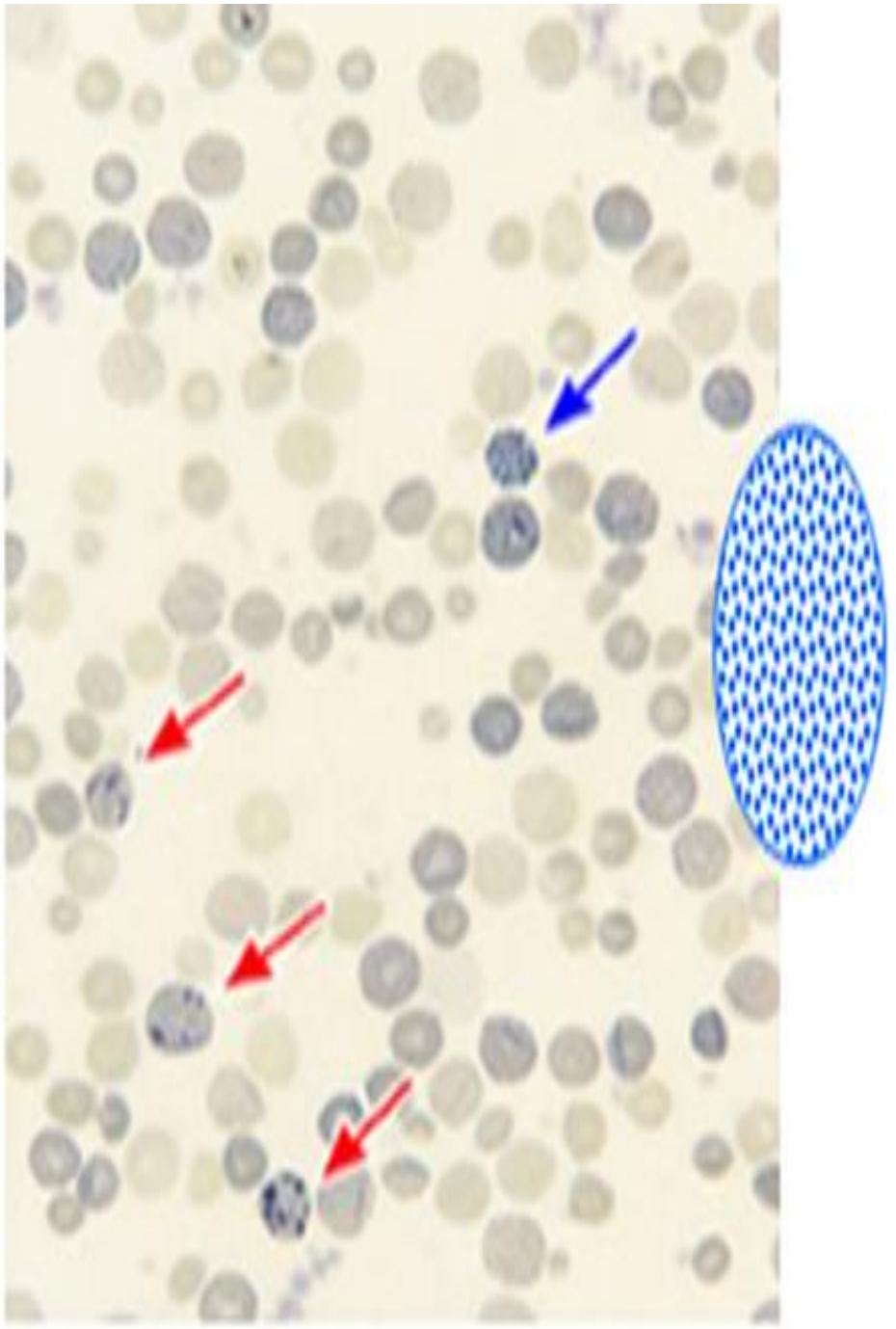
Pathophysiology of Hb-H disease



- Hemolysis of RBC due to inclusions of unstable HbH or membrane damage.
- tetramer Hb is useless and of high O2 affinity.

* **Lab.findings:**
  - ✓ Hb is usually 7-10 g/dl, MCV and MCH reduced, Reticulocyte count is moderately increased.
  - ✓ Blood film shows picture of hypochromia, moderate aniso-pokilocytosis, and target and tear drop cells.
  - ✓ On modification of the retics stain: characteristic Hb H inclusions could be seen in RBCs (Golf ball appearance).
  - ✓ Hb electrophoresis: Hb H 5-40% and Hb A.






Thank You!