ThalassemIA

PATHOPHYSIOLOGY & DIAGNOSIS

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Final year

DEFINITION:

- Heterogeneous group of disorders caused by inherited mutations that decrease/absence of the synthesis of adult hemoglobin, HbA ($\alpha_2\beta_2$).

- Depending upon whether genetic defect (mutations / deletions)lies in transmission of α or β globin genes

  (1) α-thalassemia
  (2)β-thalassemia

CLASSIFICATION

β-Thalassemias	α-Thalassemias	Misc.Thalassemic syndromes
Thalassemia major	Hydrops fetalis	HbS-Thalassemias
Thalassemia intermedia	Hb H disease	HbE-Thalassemias
Thalassemia trait	α-Thalassemia trait	HbD-Thalassemias
Thalassemia minima		δ-β-Thalassemias
		HPFH-Hereditary persistence of fetal hemoglobin

β-Thalassemias

- Caused by mutations that diminish the synthesis of β-globin chains.

Molecular Pathogenesis

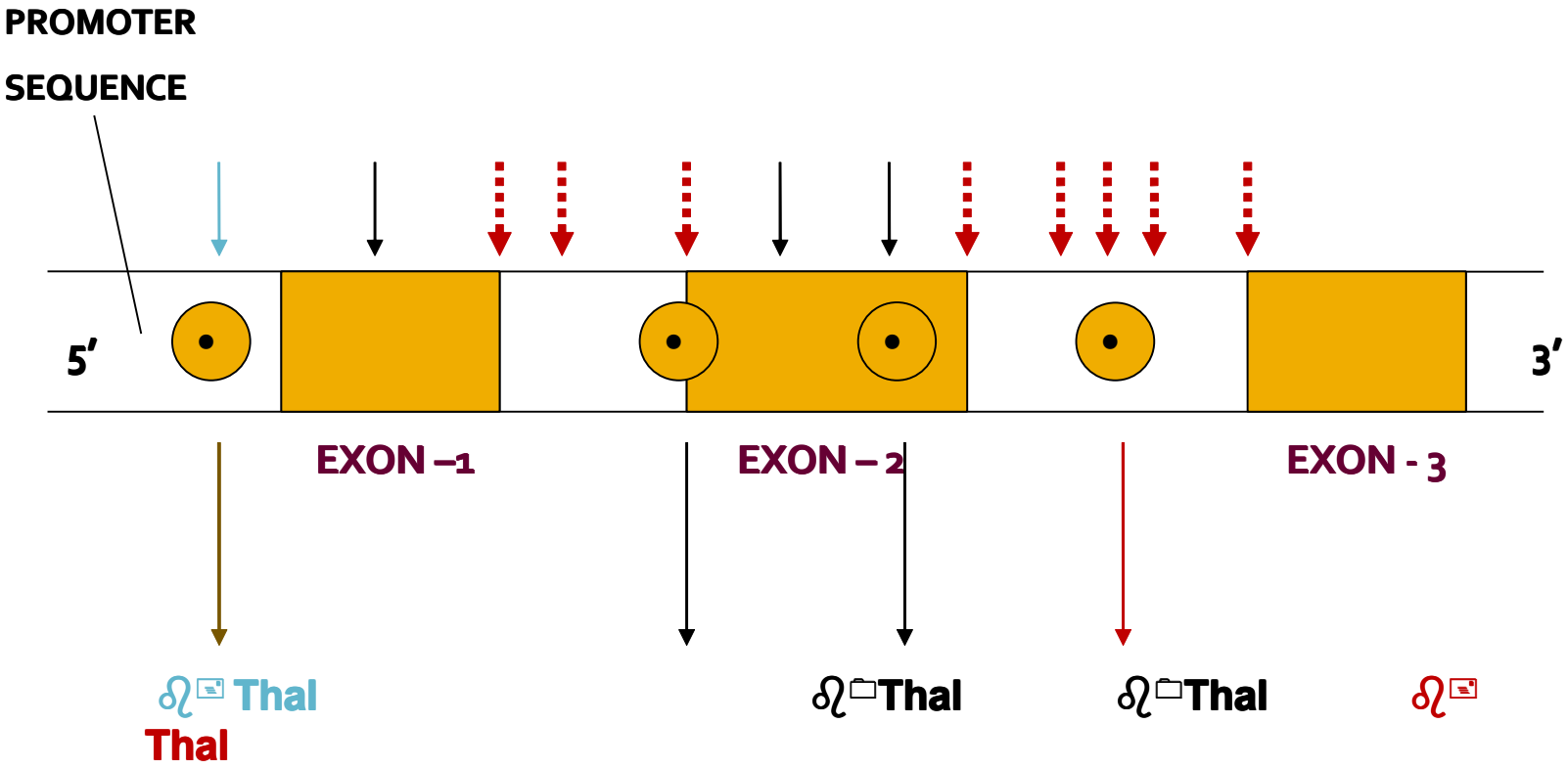
- Causative mutations (Point mutations)
  two categories:
  (1) β^0 *mutations* - absent β-globin synthesis
  (2) β^+ *mutations* - reduced (but detectable) β-globin synthesis.

- Splicing mutations: most common cause of β^+-thalassemia.

- Promoter region mutations: associated with β^+-thalassemia.

- Chain terminator mutations: most common cause of β^0-thalassemia.

GENETIC BACKGROUND



TRANSCRIPTION DEFECT	:	PROMOTER REGION MUTATIONS
RNA SPLICING DEFECT	:	SPLICING MUTATIONS
TRANSLATION DEFECT	:	CHAIN TERMINATOR MUTATIONS

Splicing mutations (m RNA splicing defect)

- Mutations leads to defective mRNA processing

abnormal mRNA

degraded in the nucleus.

- Depending upon whether part of splice site remains intact (or) is totally degraded

β^{+} or β^{o} thalassemia.

Promotor region mutations (Transcription defect)

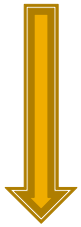
- Mutations affect transcriptional promotor sequence.

- Causes reduced synthesis of **β** globin chain

Partially preserved synthesis
(**β**$^{+}$ thalassemia).

Chain terminator mutations (Translation defect)

- Mutations in the coding sequence causing STOP CODON (chain termination) interrupting or blocking β-globin m RNA



No synthesis of β-globin chain
(β^o thalassemia)

When both you and your partner have traits there are three possible outcomes in every pregnancy

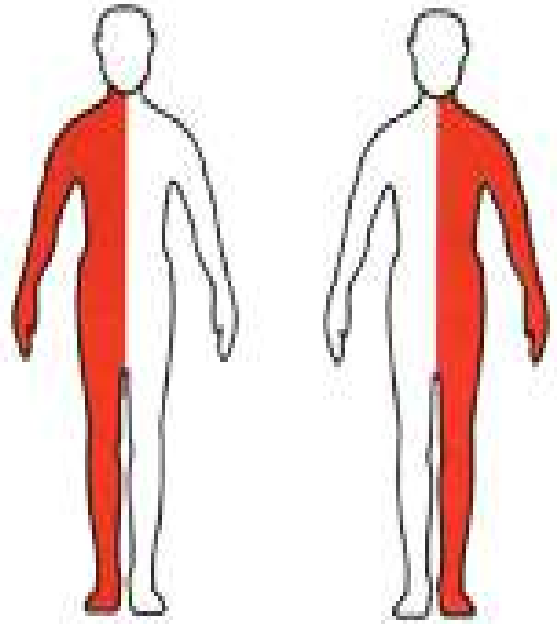
1 25% chance of a child without any trait

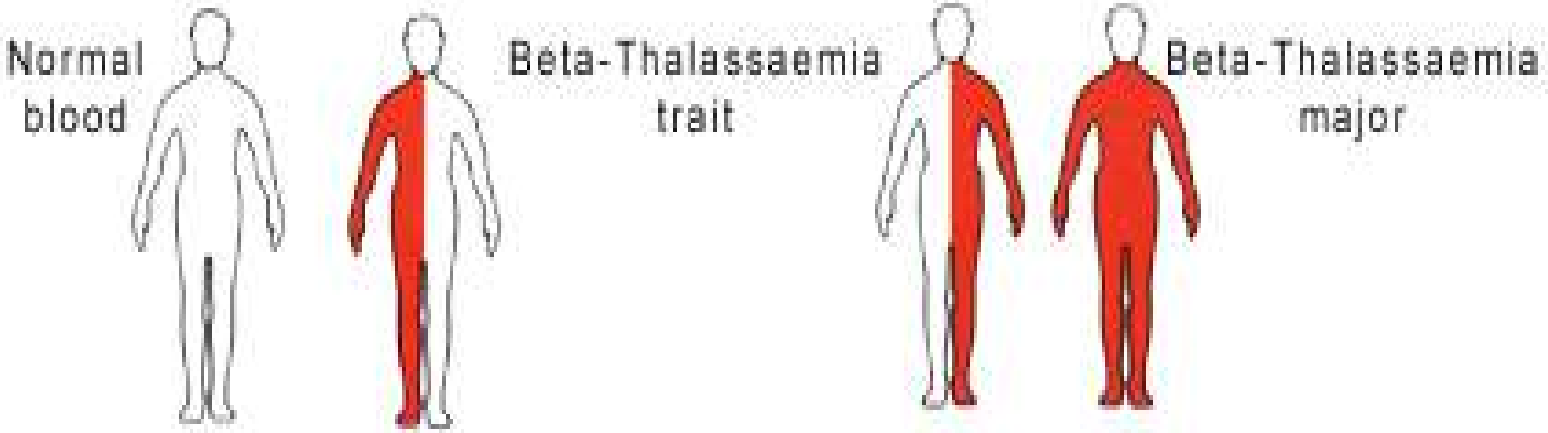
2 50% chance of a child with a trait (Minor)

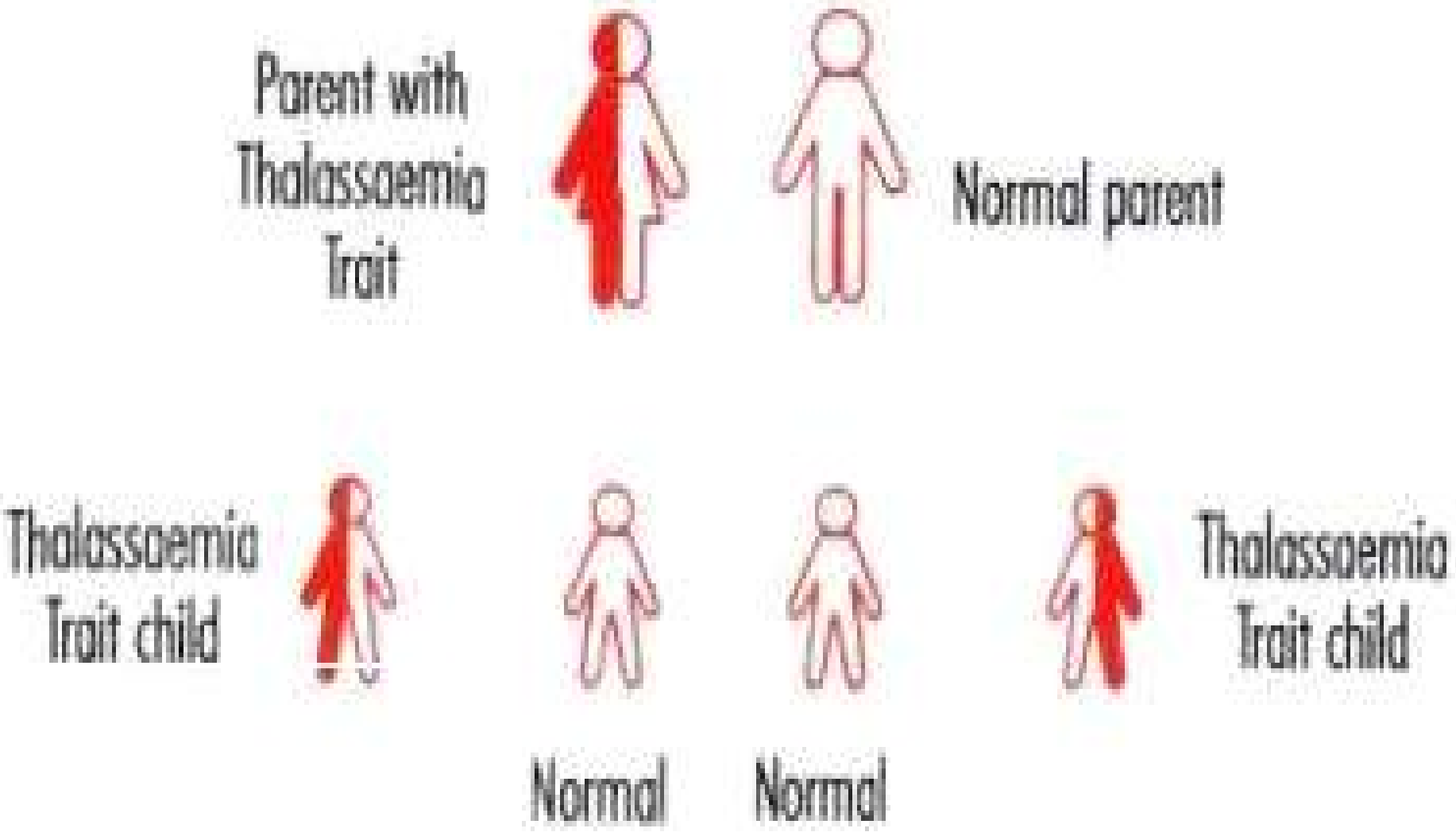
3 25% chance of a child with Beta-Thalassaemia Major

A parent with Beta-Thalassaemia trait

A parent with Beta-Thalassaemia trait






Parent with Thalassaemia Trait
Normal parent
Thalassaemia Trait child
Normal
Normal
Thalassaemia Trait child

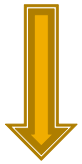
- Impaired **β** globin syntheis results in anemia by 2 mechanisms

  * Defect in Hb A synthesis
    - produces "underhemoglobinized" hypochromic microcytic RBCs.

  * Diminished survival of RBC and their precursors
    - imbalance in α- and **β** globin synthesis.
    - unpaired α-chains precipitate within RBC precursors forming insoluble inclusions

Membrane damage
(ineffective eRYTHROpoiesis)

- RBCs released from marrow also bear inclusions and prone to splenic sequestration.



EXTRAVASCULAR HAEMOLYSIS

- In severe **β** thalassemia

Ineffective erythropoiesis

Erythropoietic drive
in response to severe uncompensated anemia

Massive erythroid hyperplasia in marrow
(erodes bony cortex, impairs bone growth, produces skeletal abnormalities).

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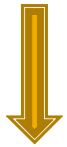
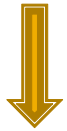
Extensive extramedullary hematopoiesis
(liver, spleen, and lymph nodes)

- Ineffective erythropoiesis

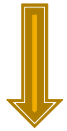


Suppresses circulating levels of
hepcidin
(negative regulator of iron absorption)

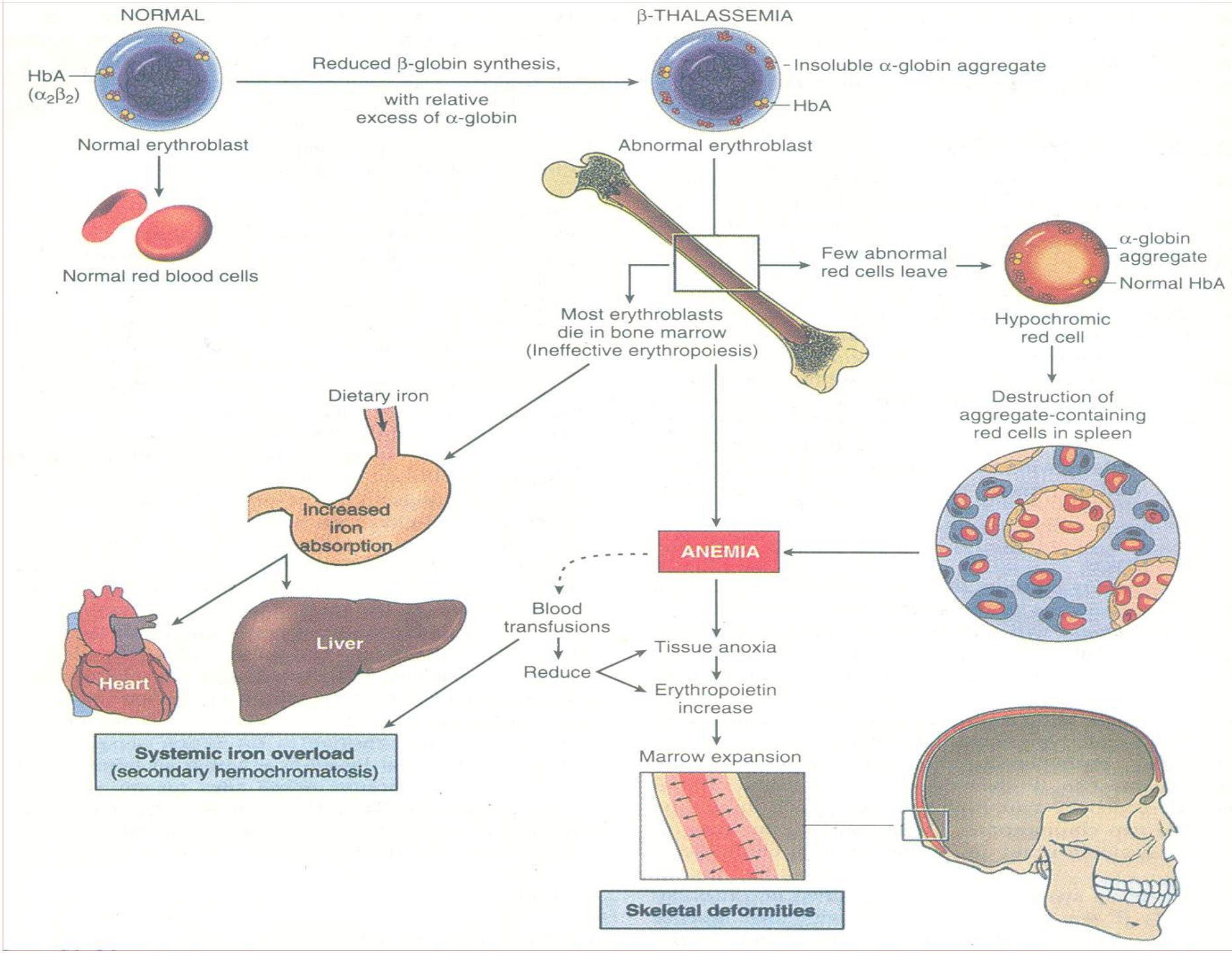
- Repeated blood transfusions



severe iron overload.



liver, HEART, PANCREAS
(secondary hemochromatosis).


NORMAL
β-THALASSEMIA
HbA
($\alpha_2\beta_2$)
Reduced β-globin synthesis,
with relative
excess of α-globin
Insoluble α-globin aggregate
HbA
Normal erythroblast
Abnormal erythroblast
Normal red blood cells
Few abnormal
red cells leave
α-globin
aggregate
Normal HbA
Hypochromic
red cell
Most erythroblasts
die in bone marrow
(Ineffective erythropoiesis)
Dietary iron
Destruction of
aggregate-containing
red cells in spleen
Increased
iron
absorption
ANEMIA
Heart
Liver
Blood
transfusions
Reduce
Tissue anoxia
Erythropoietin
increase
Marrow expansion
Systemic iron overload
(secondary hemochromatosis)
Skeletal deformities

Clinical Syndromes

- Clinical classification:
  - Based on the severity of the anemia,
  - Depends on the genetic defect (β^+ or β^0) and gene dosage (homozygous or heterozygous).

β-Thalassemia Major

- M.C in Mediterranean countries, parts of Africa, and Southeast Asia.
- Individuals have 2 β thalassemia alleles (β^+/β^+ or β^0/β^+ or β^0/β^0)
- Anemia manifests 6 to 9 months after birth
- In untransfused patients, Hb levels are 3 to 6 gm/dL.

- Red cells may completely lack HbA (β^o/β^o genotype) or contain small amounts (β^+/β^+ or β^o/β^+ genotypes).
- Major red cell hemoglobin is HbF, markedly elevated. HbA_2 levels-normal or low.
- Transfusion depent anaemia.

LABORATORY TESTS:

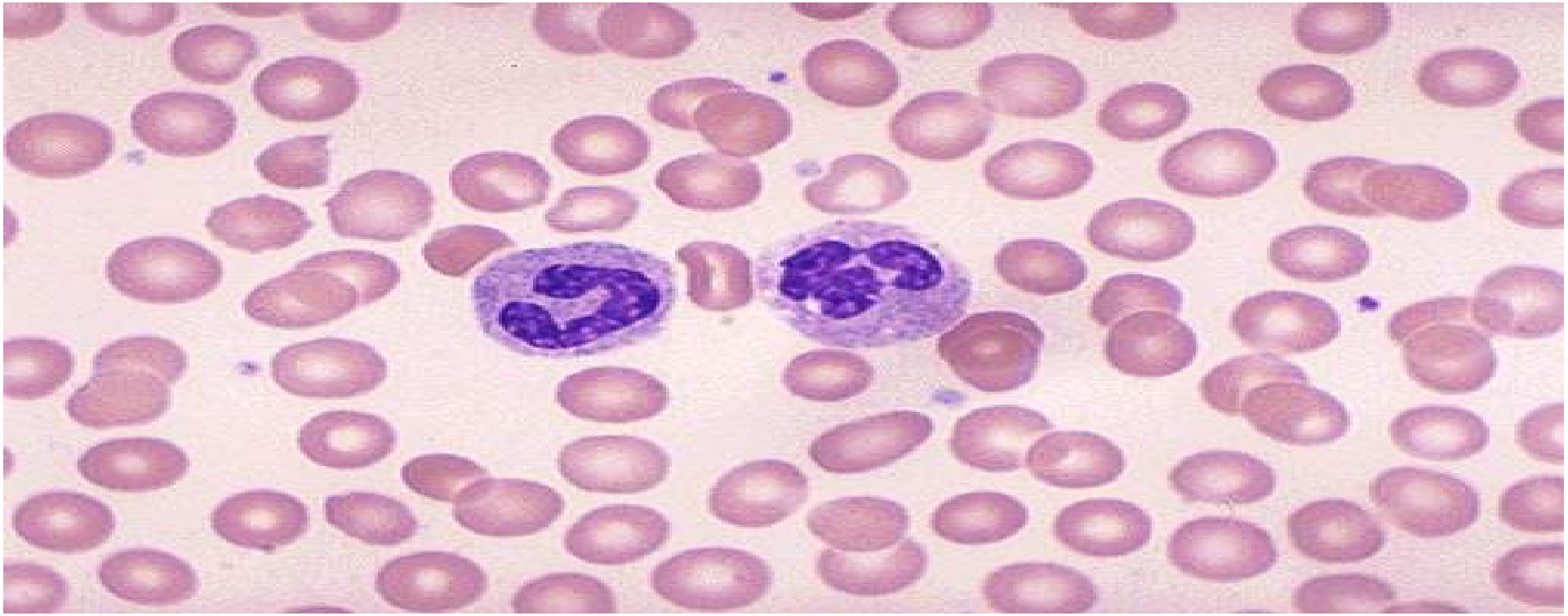
- Blood smears :

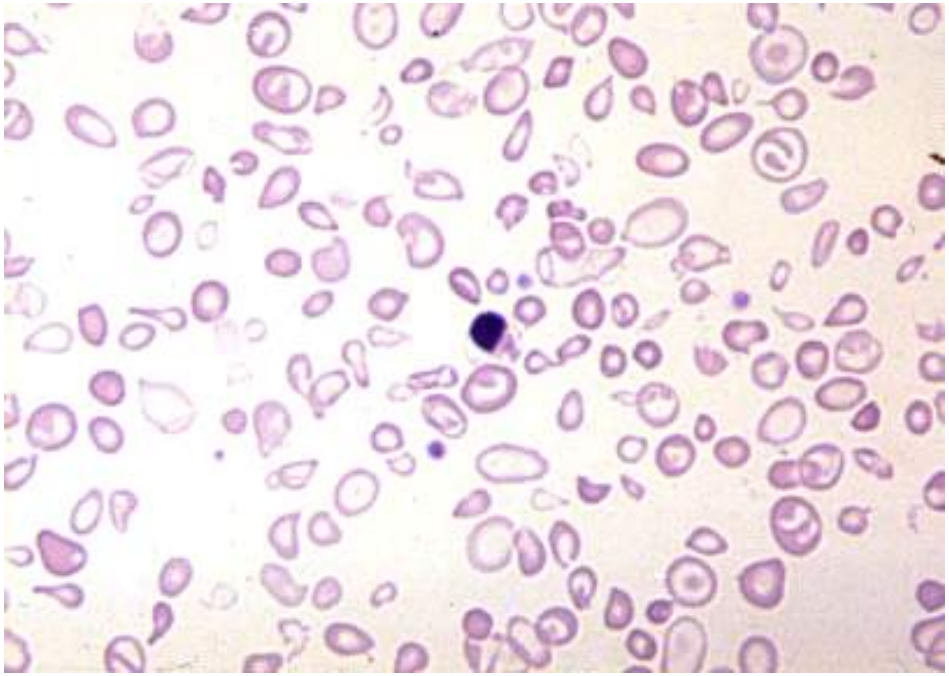
  - Severe anisocytosis, poikilocytosis, microcytosis, and hypochromia.

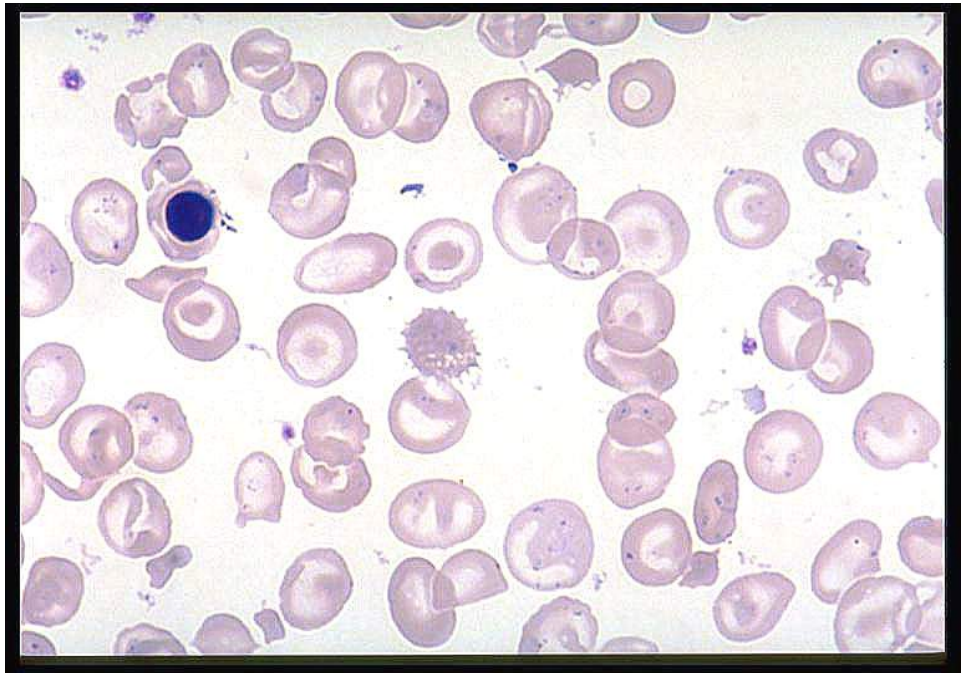
  - Target cells, basophilic stippling, and fragmented red cells.

  - Variable numbers of nucleated red cell precursors (late normoblasts) 5-40/100 WBCs

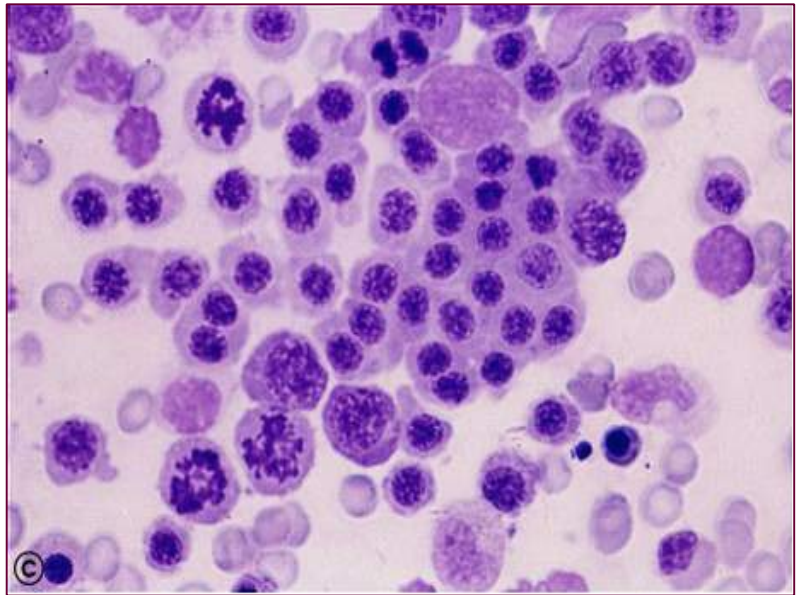
  - Reticulocyte count - elevated



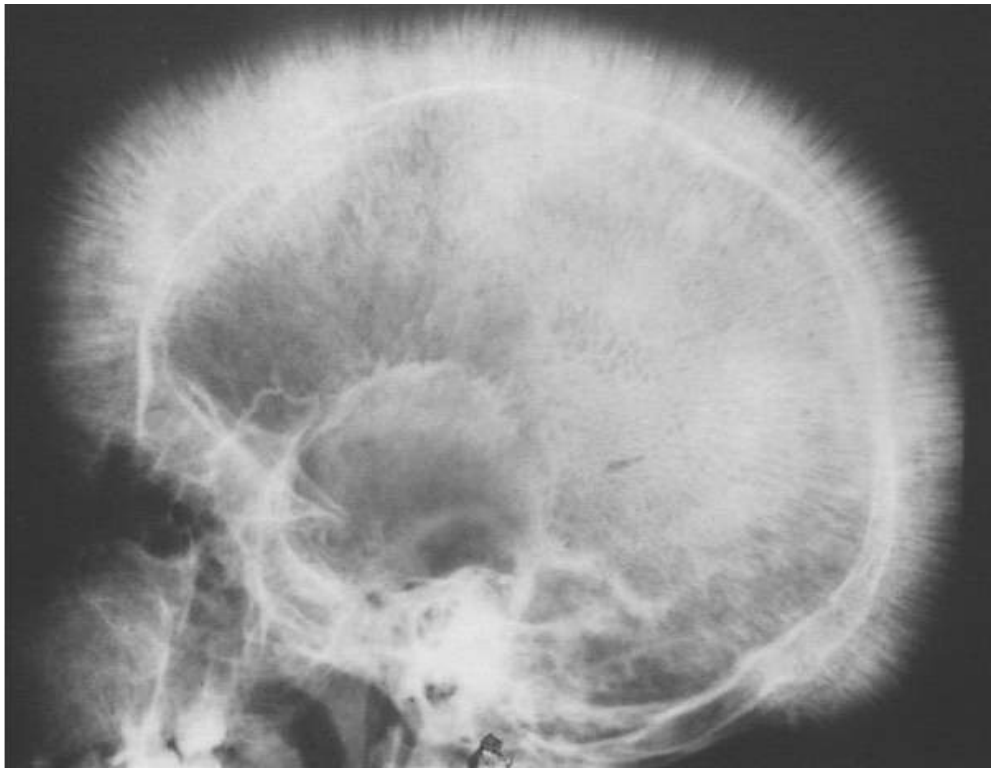




- BONE MARROW:
  - Hypercellular
  - Erythroid hyperplasia is marked (M:E ratio-reversed)
  - Normoblasts with pink inclusions and basophilic stippling
  - Myelopoiesis and megakaryopoiesis : Normal



- Striking expansion of active bone marrow.
  - Bones of face and skull, bone marrow erodes cortical bone and induces new bone formation ("crew-cut" appearance on x-ray).



- IRON STATUS:

  - s. Iron : increased

  - s. Ferritin : usually >1000 µg /litre

  - Transferrin saturation: markedly elevated to 55-90%

  - TIBC : reduced to 250-300µg%

IRON STUDY		IRON DEFICIENCY	THALASSEMIA
SERUM IRON	:	DECREASED	N / INCREASED
SERUM FERRITIN	:	DECREASED	N / INCREASED
TIBC	:	INCREASED	N / DECREASED
PERCENT SATURATION	:	DECREASED	INCREASED
STORAGE IRON	:	ABSENT	INCREASED

SPECIAL LAB TESTS

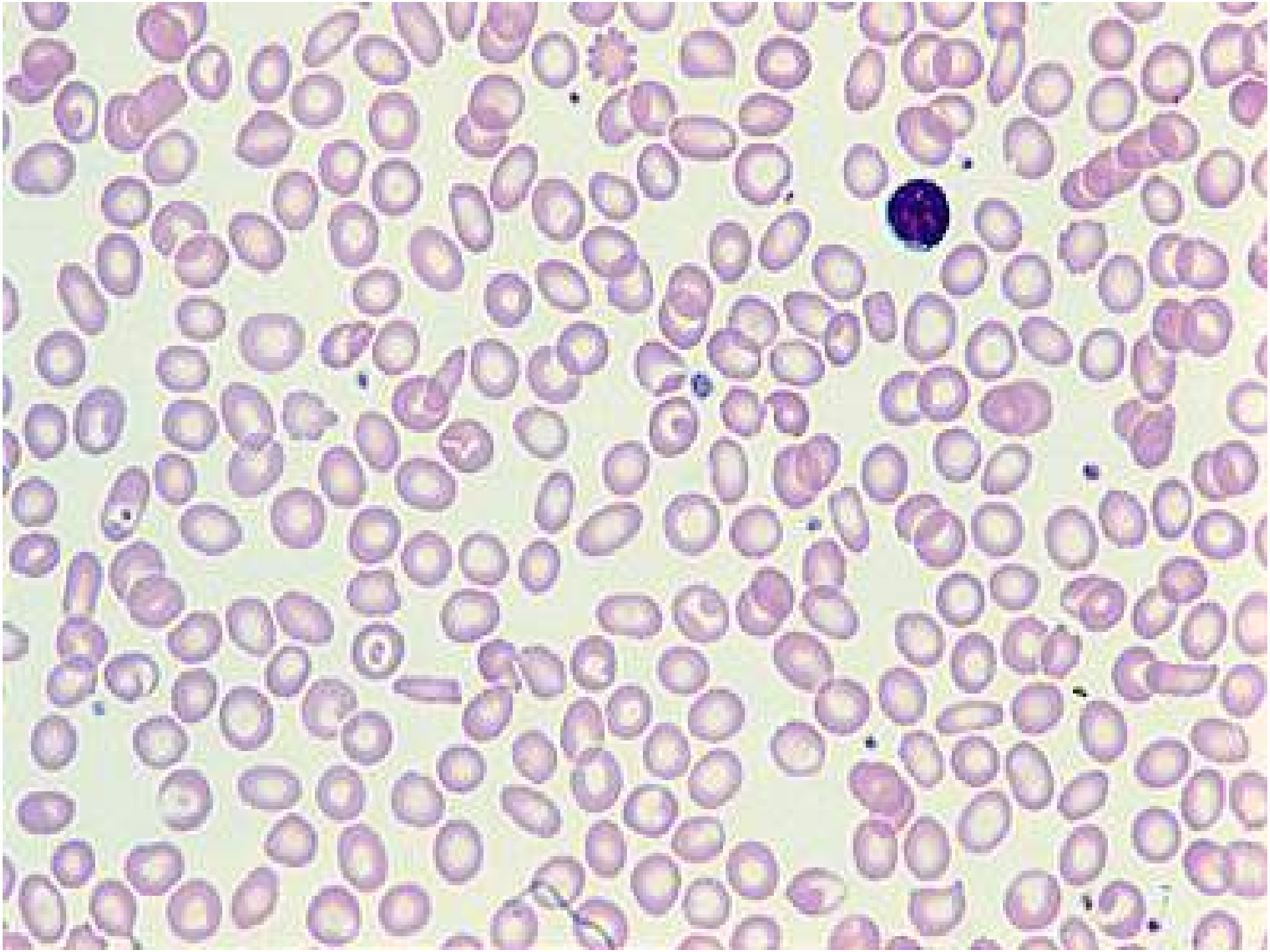
- ACID ELUTION TEST:
  - HbF levels are high in thalassemia(β^o > β^+)
  - Cells containing HbA appear as ghost cells while HbF cells are well stained

- Hb ELECTROPHORESIS:
  - Demonstrates bands of both HbA and HbF in β^{+}
  - In β^{0} thalassemia, HbF is >90%

- GLOBIN CHAIN SYNTHESIS:
  - α:β globin chain synthesis is altered to 2-30:1 (N=1:1)

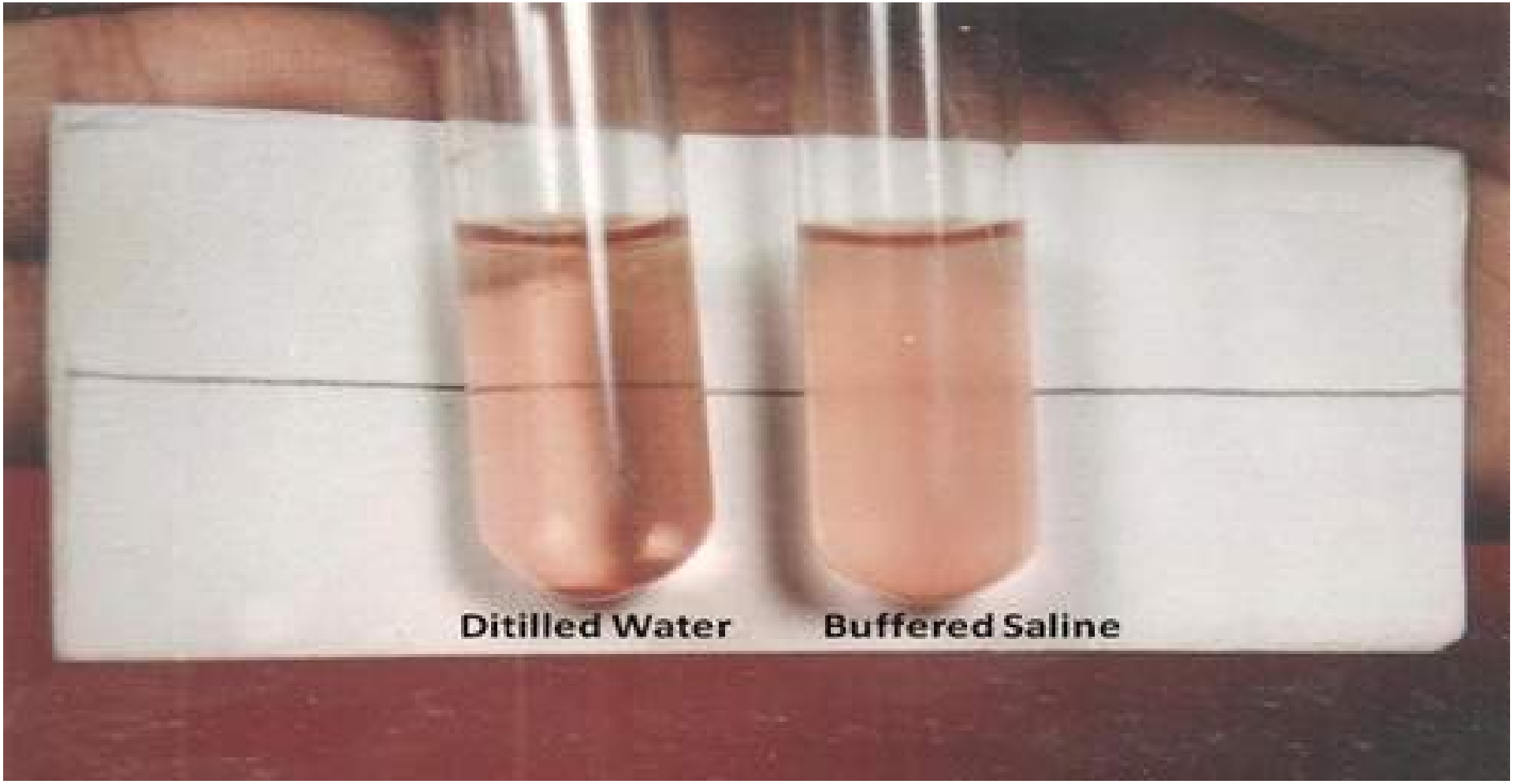
β-Thalassemia Minor

- Much more common than β-thalassemia major.
- Patients are heterozygous carriers of a β^{+} or β^{o} allele.
- Usually asymptomatic.
- Anemia, if present, is mild.
- Peripheral blood smear-Hypochromia, Microcytosis, Basophilic stippling, and target cells.



- Bone marrow-Mild erythroid hyperplasia.
- Hemoglobin electrophoresis- reveals increase in HbA_2 to 4 - 8% (normal, 2.5% ± 0.3%),
  Elevated ratio of δ-chain to β-chain synthesis.
- HbF levels -Normal or slightly increased.

NESTROF test:



β-Thalassemia Intermedia

- Intermediate degree of severity.
- Does not require regular blood transfusions.
- These are genetically hetrogenous(β^+/β or or β^+/β).
- Moderate degree of anisopoikilocytosis with microcytic hypochromic red cells, target cells and basophilic stippling.
- HbF is 10-30%, HbA2 is <3.5%

Comparison of β Thalassemias

Parameter	Minor	Intermedia	Major
Hb	10-13	6-10	2-8
MCV (fl)	60-78	50-70	50-60
MCH (pg)	28-32	22-28	16-22
RDW	Normal	S. increased	Increased
Micro/hypo Film	Mild	Moderate	Severe
Polychromasia	V. Little	Moderate	Marked
Anisocytosis	None	Moderate	Marked
Poikilocytosis	None	Moderate	Marked
Targetting	Present	Present	Present

Comparison of β Thalassemias

GENOTYPE	Hb A	Hb A_2	Hb F
NORMAL	Normal	Normal	Normal
SILENT CARRIER	Normal	Normal	Normal
β THAL MINOR	Dec	Increased	N to Inc
β THAL INTERMEDIA	Dec	N to Inc	Increased
β THAL MAJOR	Dec	Usually Inc	Increased

α-ThalassemiaS

- Caused by inherited deletions
  result in reduced or absent synthesis of α-globin chains.

- Normally, there are 4 α-globin genes.
  Severity of α-thalassemia depends on number of
  α-globin genes affected.

- Anemia results from
  - Lack of adequate hemoglobin
  - Effects of excess unpaired non-α chains (β, γ, and δ)

- Free β and γ chains are more soluble than free α chains and form stable homotetramers

- Hemolysis and ineffective erythropoiesis are less severe.

Clinical Syndromes:

- Clinical syndromes are determined and classified by number of α-globin genes that are deleted.

Silent Carrier State:

- Caused by deletion of a single α-globin gene
- Causes barely detectable reduction in α-globin chain synthesis.
- Individuals are completely asymptomatic, but they have slight microcytosis.

α-Thalassemia Trait

- Caused by deletion of two α-globin genes from a single chromosome (α/α -/-). [M.C in Asia]

  or

  deletion of one α-globin gene from each of two chromosomes (α/- α/-). [M.C in Africa].

- Both genotypes produce similar quantitative deficiencies of α-globin.

- Children are at risk of clinically significant α-thalassemia (HbH disease or hydrops fetalis) only when at least one parent has the (-/-)haplotype.
  So, symptomatic α-thalassemia is common in Asian populations.

- Clinical picture-Similar to β-thalassemia minor.

- HbA_2 levels are normal or low.

Hemoglobin H Disease

- Caused by deletion of three α-globin genes.
- Most common in Asian populations.
- Synthesis of α chains is markedly reduced, and tetramers of β-globin, called HbH, form.

- HbH- has an extremely high affinity for oxygen
  - not useful for oxygen delivery
  - leads to tissue hypoxia
  - prone to oxidation, precipitates and forms intracellular inclusions

R.B.C sequestration

Phagocytosis in spleen.

- Produces moderately severe anemia resembling β-thalassemia intermedia.

Hydrops Fetalis

- Most severe form of α-thalassemia.
- Caused by deletion of all four α-globin genes.
- In the fetus, excess γ-globin chains form tetramers (Hb Barts)
  - Have high affinity for oxygen
  - Deliver little oxygen to tissues.
- Survival in early development
  - Due to the expression of ζ chains, an embryonic globin that pairs with γ chains to form a functional $\zeta_2\gamma_2$ Hb tetramer.

- Signs of fetal distress evident by third trimester .
- Fetus shows severe pallor, generalized edema, and massive hepatosplenomegaly
- Lifelong dependence on blood transfusions.
- Bone marrow transplantation -curative.

Comparison of α-Thalassemias

State	Genotype	Genes	Features
Normal	αα/αα	4	normal
Hetero α⁺ α-thal-2	αα/ – α	3	Essentially normal
Hetero α° α-thal-1	αα/ – –	2	Micro / Hypo Mild Anemia Bart's 2-8% (at birth) Hb H <2%
Homo α⁺ α-thal-1	– α/ – α	2	
α⁺ + α° Hb-H Disease	– α/ – –	1	Moderate Micro/Hypo anemia: Barts <10%, Hb H <40%
homo α° Hydrops	– – / – –	0	Hb A 0%, Bart's 70-80% Portland 10-20%

Comparison of α-Thalassemias

Phenotype	Hb A	Hb Barts	Hb H
Normal	97-98%	0	0
Silent Carrier	96-98%	0-2% (At birth)	0
α Thalassemia Trait	85-95%	2-8% (At birth)	<2%
Hb H Disease	Dec	<10% (At birth)	5-40%
Hydrops Fetalis	0	70-80% (with 20% Hb Portland)	0-20%

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THANK YOU