

# HAEMOGLOBINOPATHIES

**DR. FATMA AL-QAHTANI**

**Consultant Haematologist**

**Head of Haematology Division**

**Department of Pathology**

# HAEMOGLOBINOPATHIES

**A - THALASSAEMIAS**

**B - ABNORMAL HAEMOGLOBINS**

# LEARNING OBJECTIVES

- To understand the normal structure and function of haemoglobin
- To understand how the globin components of haemoglobin change during development, and postnatally
- To understand the mechanisms by which the thalassaemias arise
- To appreciate the clinical presentations and complications of thalassaemia
- To appreciate the contribution of haemolysis and ineffective erythropoiesis to the pathophysiology of thalassaemia

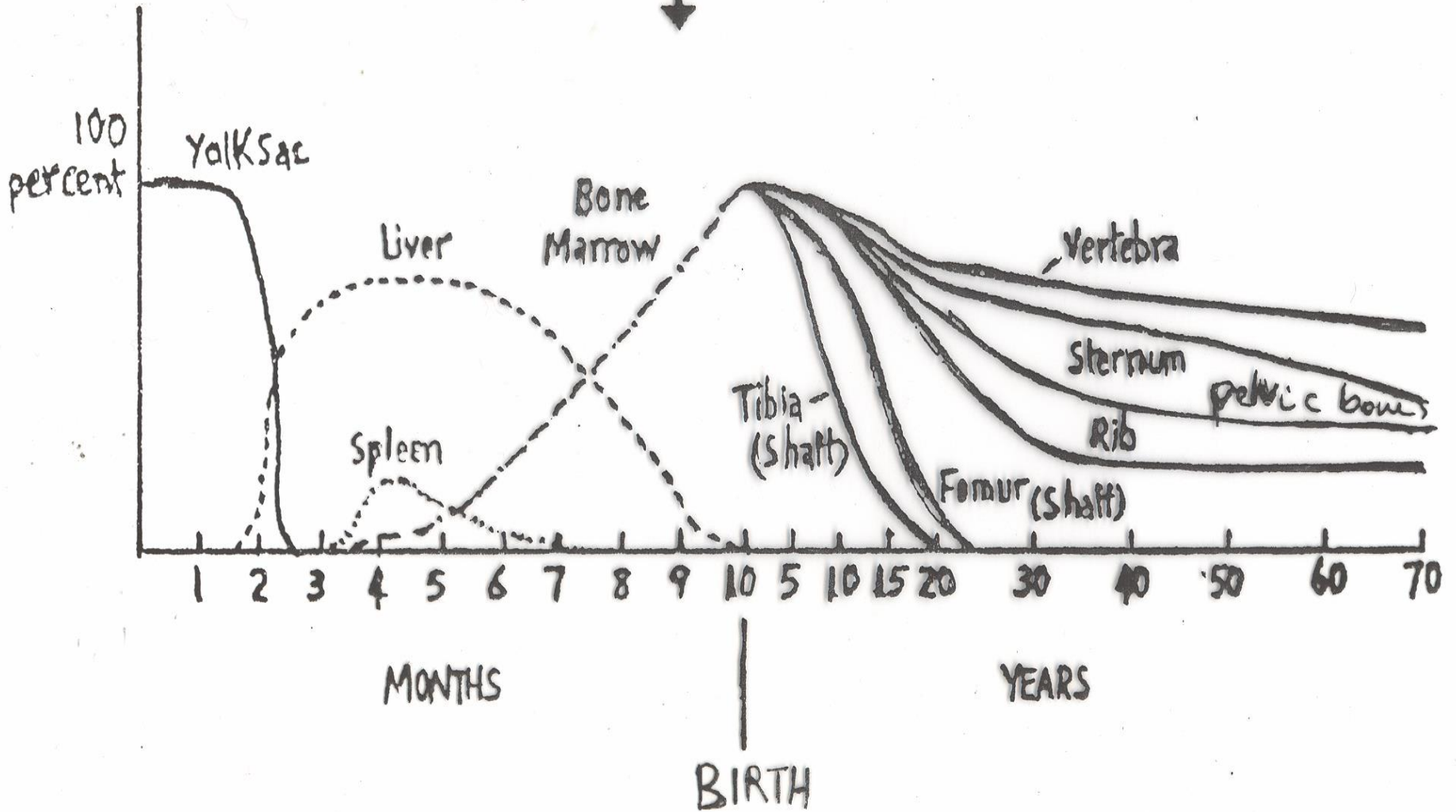
## *cont'd...*

- To understand the pathophysiology of sickle cell anaemia
- To be able to describe the clinical presentation and complications of sickle cell anaemia
- To understand the role of haemoglobin electrophoresis and high performance liquid chromatography in the investigation of globin disorders
- To appreciate the many other haemoglobin variants associated with disease

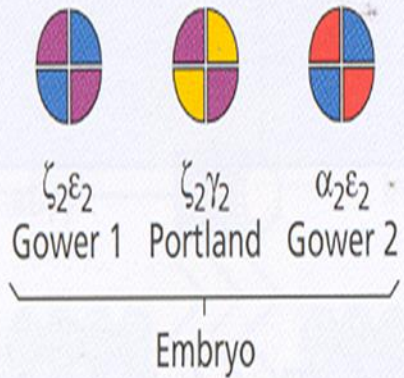
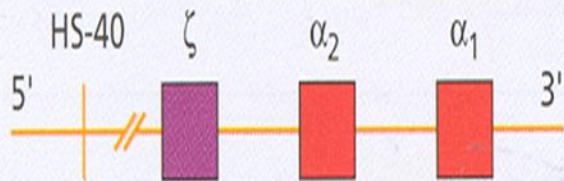
PRENATAL

POSTNATAL

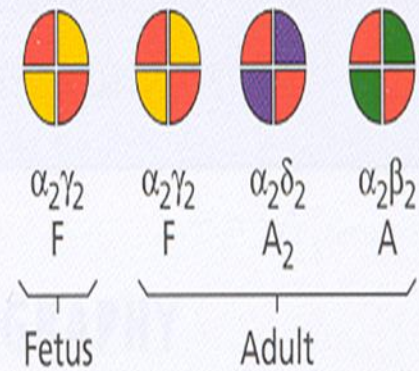
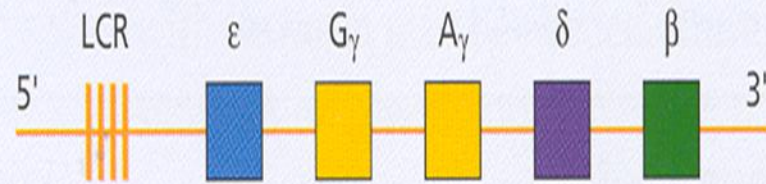
BIRTH

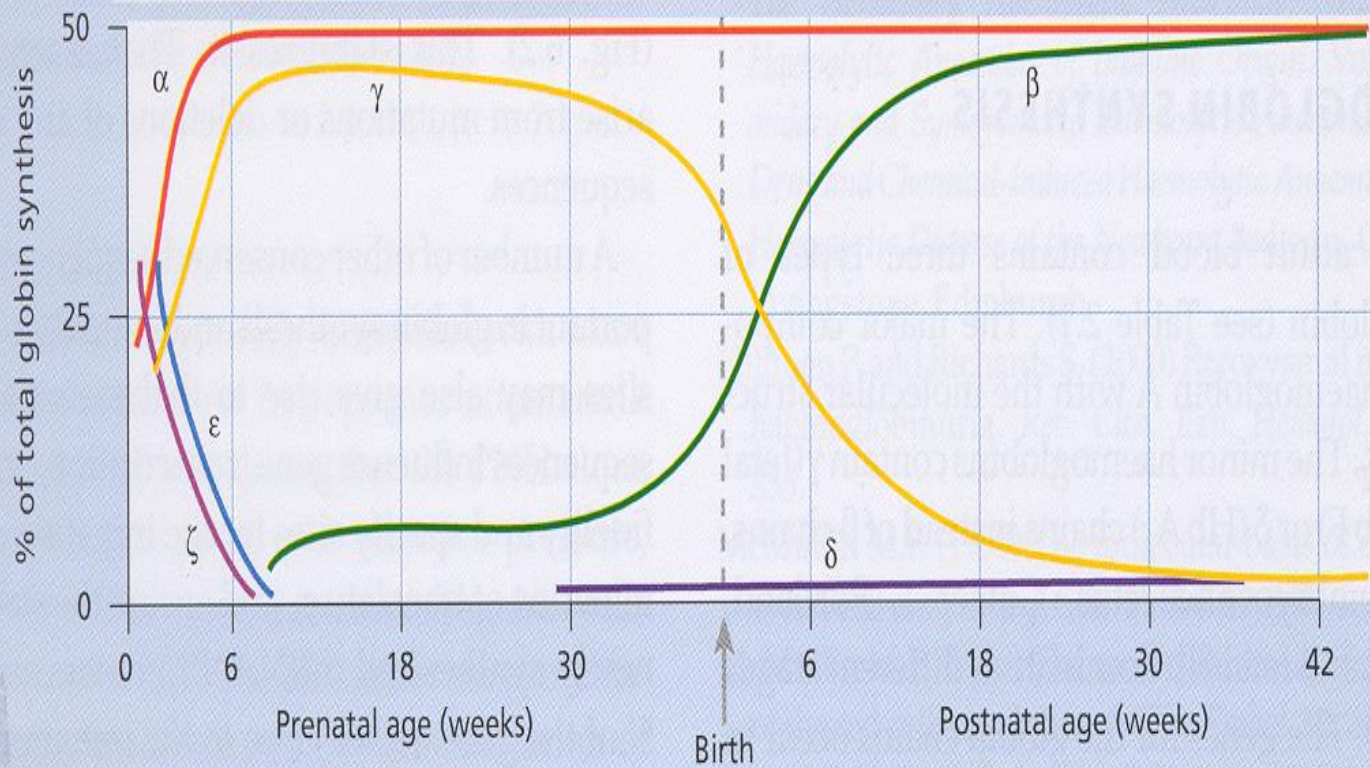
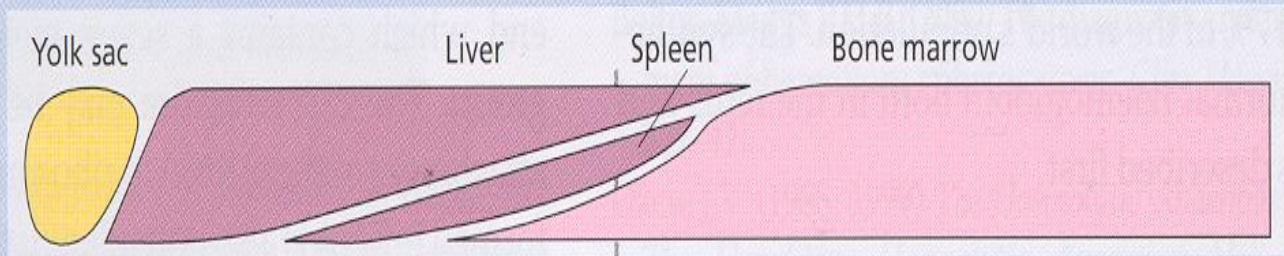


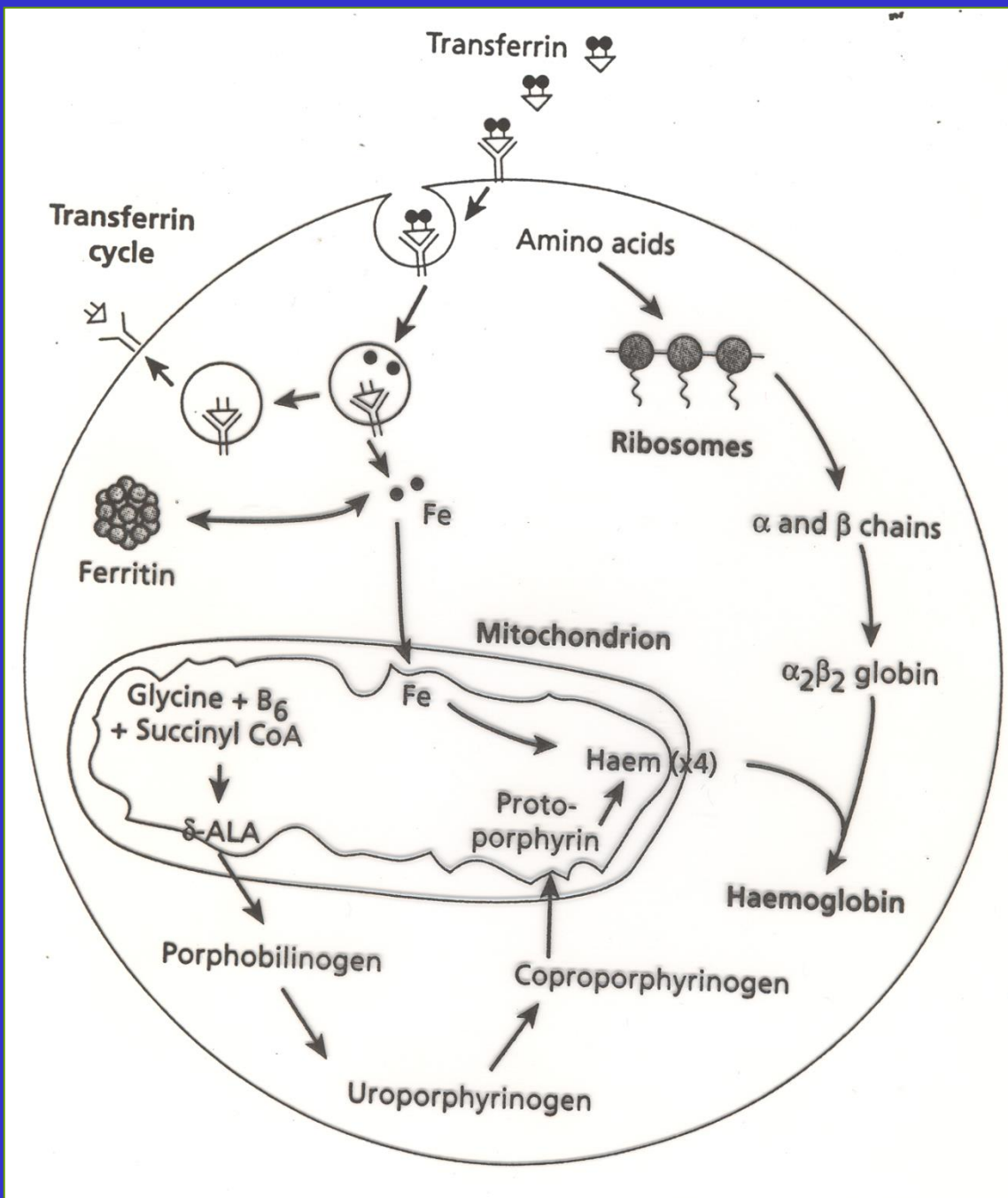
### Chromosome 16



### Chromosome 11









1 2 3 4 5 6 7 8 9 10 11 12 13 14 15  
VAL-LEU-SER-PRO-ALA-ASP-LYS-THR-ASN-VAL-LYS-ALA-ALA-TRY-GLY

16 17 18 19 20 21 22 23 24 25 26 27 28 29 30  
LYS-VAL-GLY-ALA-HIS-ALA-GLY-GLU-TYR-GLY-ALA-GLU-ALA-LEU-GLU

31 32 33 34 35 36 37 38 39 40 41 42 43 44 45  
ARG-MET-PHE-LEU-SER-PHE-PRO-THR-THR-LYS-THR-TYR-PHE-PRO-HIS

46 47 48 49 50 51 52 53 54 55 56 57 58 59 60  
PHE-ASP-LEU-SER-HIS-GLY-SER-ALA-GLN-VAL-LYS-GLY-HIS-GLY-LYS

61 62 63 64 65 66 67 68 69 70 71 72 73 74 75  
LYS-VAL-ALA-ASP-ALA-LEU-THR-ASN-ALA-VAL-ALA-HIS-VAL-ASP-ASP

76 77 78 79 80 81 82 83 84 85 86 87 88 89 90  
MET-PRO-ASN-ALA-LEU-SER-ALA-LEU-SER-ASP-LEU-HIS-ALA-HIS-LYS

91 92 93 94 95 96 97 98 99 100 101 102 103 104 105  
LEU-ARG-VAL-ASP-PRO-VAL-ASN-PHE-LYS-LEU-LEU-SER-HIS-CYS-LEU

106 107 108 109 110 111 112 113 114 115 116 117 118 119 120  
LEU-VAL-THR-LEU-ALA-ALA-HIS-LEU-PRO-ALA-GLU-PHE-THR-PRO-ALA

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135  
VAL-HIS-ALA-SER-LEU-ASP-LYS-PHE-LEU-ALA-SER-VAL-SER-THR-VAL

136 137 138 139 140 141  
LEU-THR-SER-LYS-TYR-ARG

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15  
VAL-HIS-LEU-THR-PRO-GLU-GLU-LYS-SER-ALA-VAL-THR-ALA-LEU-TRY

16 17 18 19 20 21 22 23 24 25 26 27 28 29 30  
GLY-LYS-VAL-ASN-VAL-ASP-GLU-VAL-GLY-GLY-GLU-ALA-LEU-GLY-ARG

31 32 33 34 35 36 37 38 39 40 41 42 43 44 45  
LEU-LEU-VAL-VAL-TYR-PRO-TRY-THR-GLN-ARG-PHE-PHE-GLU-SER-PHE

46 47 48 49 50 51 52 53 54 55 56 57 58 59 60  
GLY-ASP-LEU-SER-THR-PRO-ASP-ALA-VAL-MET-GLY-ASN-PRO-LYS-VAL

61 62 63 64 65 66 67 68 69 70 71 72 73 74 75  
LYS-ALA-HIS-GLY-LYS-LYS-VAL-LEU-GLY-ALA-PHE-SER-ASP-GLY-LEU

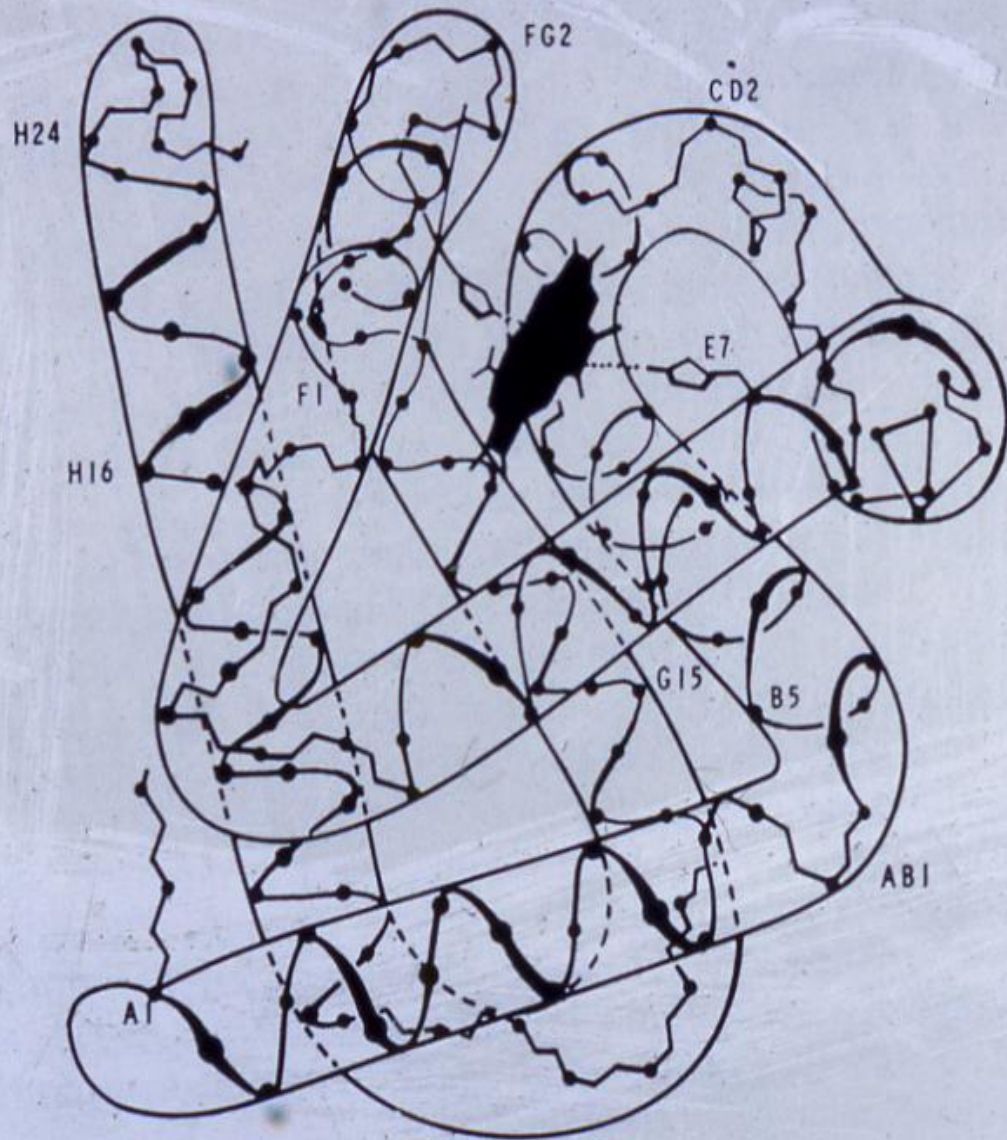
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90  
ALA-HIS-LEU-ASP-ASN-LEU-LYS-GLY-THR-PHE-ALA-THR-LEU-SER-GLU

91 92 93 94 95 96 97 98 99 100 101 102 103 104 105  
LEU-HIS-CYS-ASP-LYS-LEU-HIS-VAL-ASP-PRO-GLU-ASN-PHE-ARG-LEU

106 107 108 109 110 111 112 113 114 115 116 117 118 119 120  
LEU-GLY-ASN-VAL-LEU-VAL-CYS-VAL-LEU-ALA-HIS-HIS-PHE-GLY-LYS

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135  
GLU-PHE-THR-PRO-PRO-VAL-GLN-ALA-ALA-TYR-GLN-LYS-VAL-VAL-ALA

136 137 138 139 140 141 142 143 144 145 146  
GLY-VAL-ALA-ASN-ALA-LEU-ALA-HIS-LYS-TYR-HIS



| NAME                 | Chains     |                   |
|----------------------|------------|-------------------|
| Haemoglobin A        | $\alpha_2$ | $\beta_2$         |
| Haemoglobin A2       | $\alpha_2$ | $\delta_2$        |
| Haemoglobin F        | $\alpha_2$ | $\gamma_2$        |
| Haemoglobin H        | -          | $\beta_4$         |
| Haemoglobin Bart's   | -          | $\gamma_4$        |
| Haemoglobin Gower I  | $\zeta_2$  | $\epsilon_2$      |
| Haemoglobin Gower II | $\alpha_2$ | $\epsilon_2$      |
| Haemoglobin portland | $\zeta_2$  | $\gamma_2$        |
| Haemoglobin Lepore   | $\alpha_2$ | $(\delta\beta)_2$ |

# THE HAEMOGLOBINS PRESENT AT BIRTH IN NORMAL NEWBORN

| <u>NAME</u>      | <u>%</u> |
|------------------|----------|
| HbA              | 15 – 40  |
| HbA <sub>2</sub> | < 0.3    |
| HbF              | 60 – 85  |
| Hb Bart's        | < 0.5    |

# THE NORMAL HUMAN HAEMOGLOBINS

## EMBRYONIC

(Upto 8 Weeks gestation)

$\zeta_2 \epsilon_2$  Hb Gower I

$\zeta_2 \gamma_2$  Hb Portland

$\alpha_2 \epsilon_2$  Hb Gower II

## FETAL

$\alpha_2 \gamma_2$  HbF 60 - 85%

$\alpha_2 \beta_2$  HbA 15 - 40 %

## ADULT

### Caucasian

$\alpha_2 \beta_2$  HbA 97.0%

$\alpha_2 \delta_2$  HbA<sub>2</sub> 2.5%

$\alpha_2 \gamma_2$  HbF 0.5%

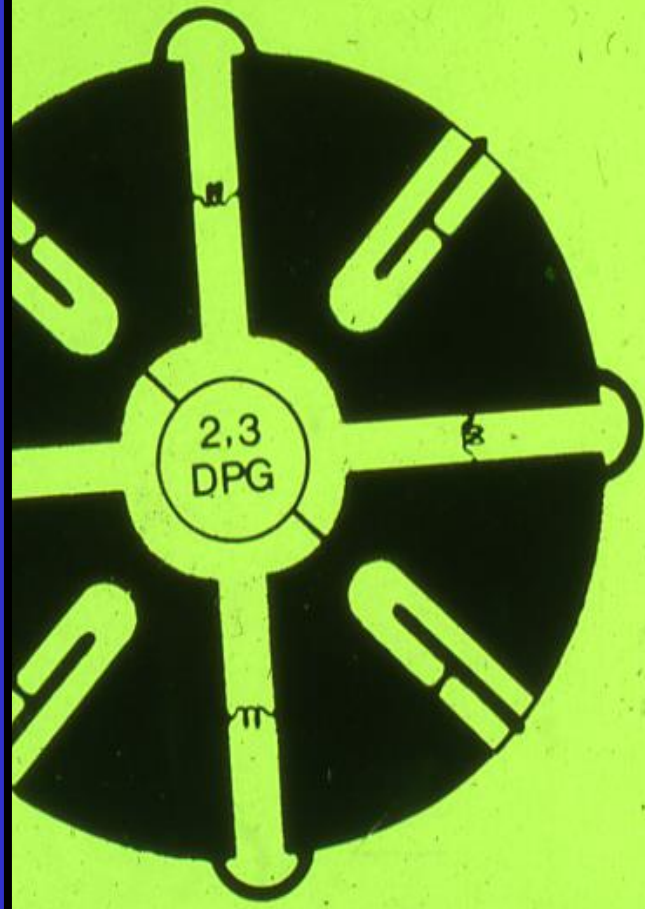
### Saudi

95.0%

3.5%

1.5%

Deoxy



Oxy



modified from Pe

# $\alpha$ and $\beta$ THALASSAEMIA

- The thalassaemias are divided into two main groups, the  $\alpha$ -thalassaemias and the  $\beta$ -thalassaemias, depending on whether the defect lies in the synthesis of  $\alpha$ - or  $\beta$ -globin chains respectively.
- The pathophysiology reflects the impact of an imbalance in the expression of  $\alpha$  and  $\beta$  globin chains.

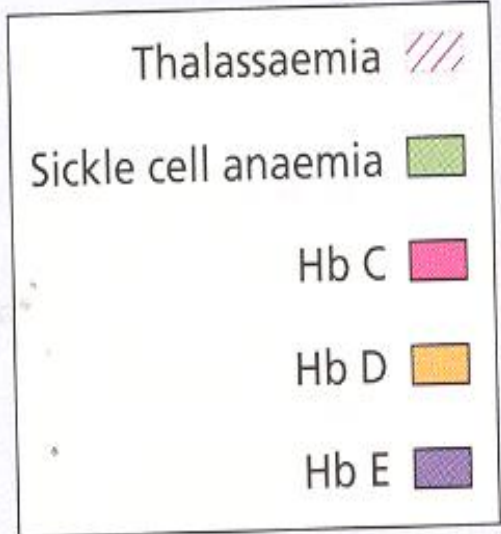
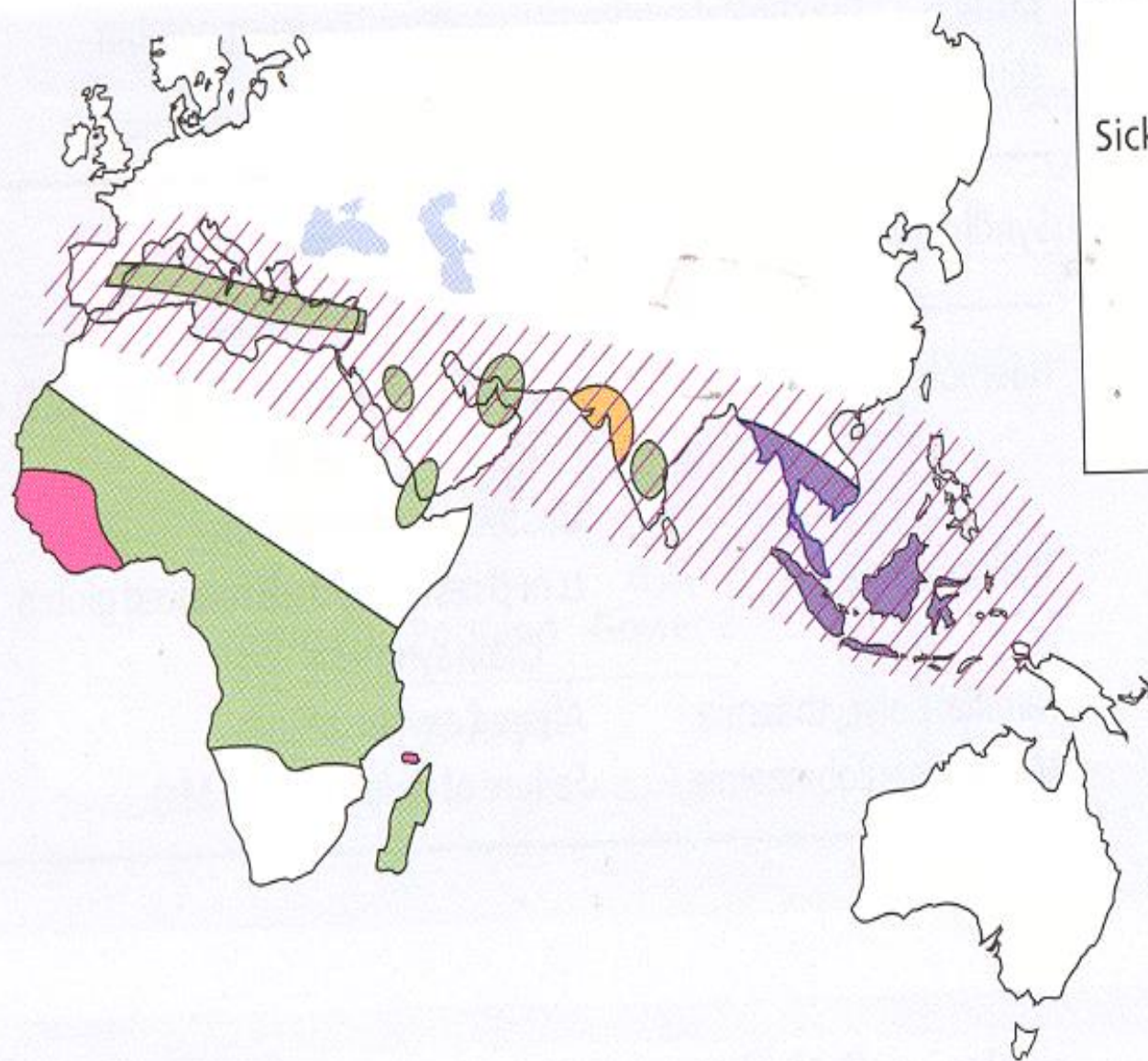


*cont'd...*

- The chains which are present in excess will precipitate in the precursor red cells, leading to their premature death prior to release from the bone marrow (ineffective erythropoiesis).
- The resulting anaemia leads to an increased erythroid drive.

*cont'd...*

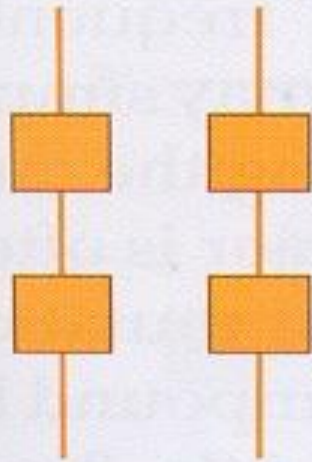
- There is further expansion of the marrow into bones not typically used for haemopoiesis, and into the spleen.
- The long-term consequences of thalassaemia therefore include splenomegaly, bony deformities and iron excess as well as chronic anaemia.



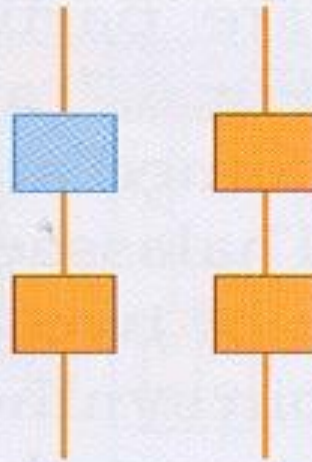
# $\alpha$ - THALASSAEMIA

- **HETEROZYGOUS**
- **HOMOZYGOUS**

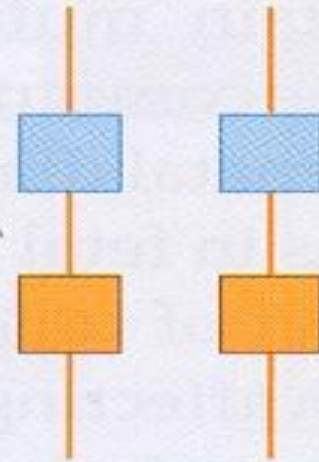
Normal



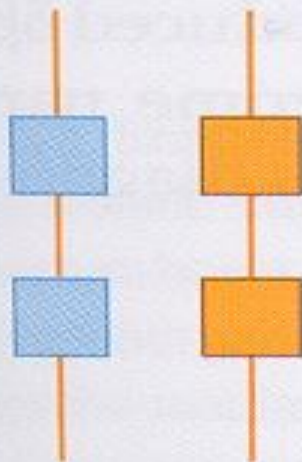
$\alpha^+$  trait



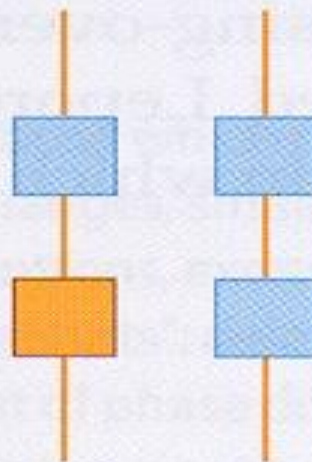
Homozygous  $\alpha^+$  trait



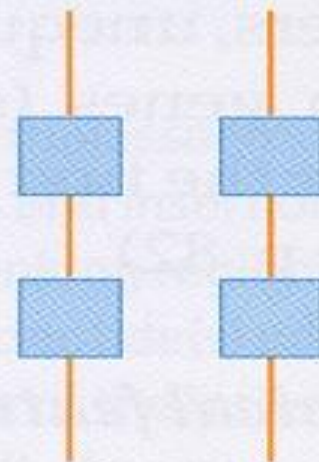
$\alpha^0$  trait



Hb H disease



Hydrops fetalis



## $\alpha^+$ -Thalassaemia trait (deletion of one or two $\alpha$ globin genes)

This is seen when an individual inherits the  $\alpha^+$ -thalassaemia allele from one parent or two parents and a normal chromosome 16 from one or two parents (i.e. heterozygotes for the  $\alpha^+$  determinant or homozygous  $\alpha^+$  trait). Affected individuals are asymptomatic, although they have minor haematological changes such as slight reductions in mean cell volume (MCV) and mean cell haemoglobin (MCH).

**$\alpha^0$ -Thalassaemia trait (deletion of both  $\alpha$ -globin genes on one chromosome 16)**

The Hb is either normal or slightly reduced and the MCV and MCH are low.

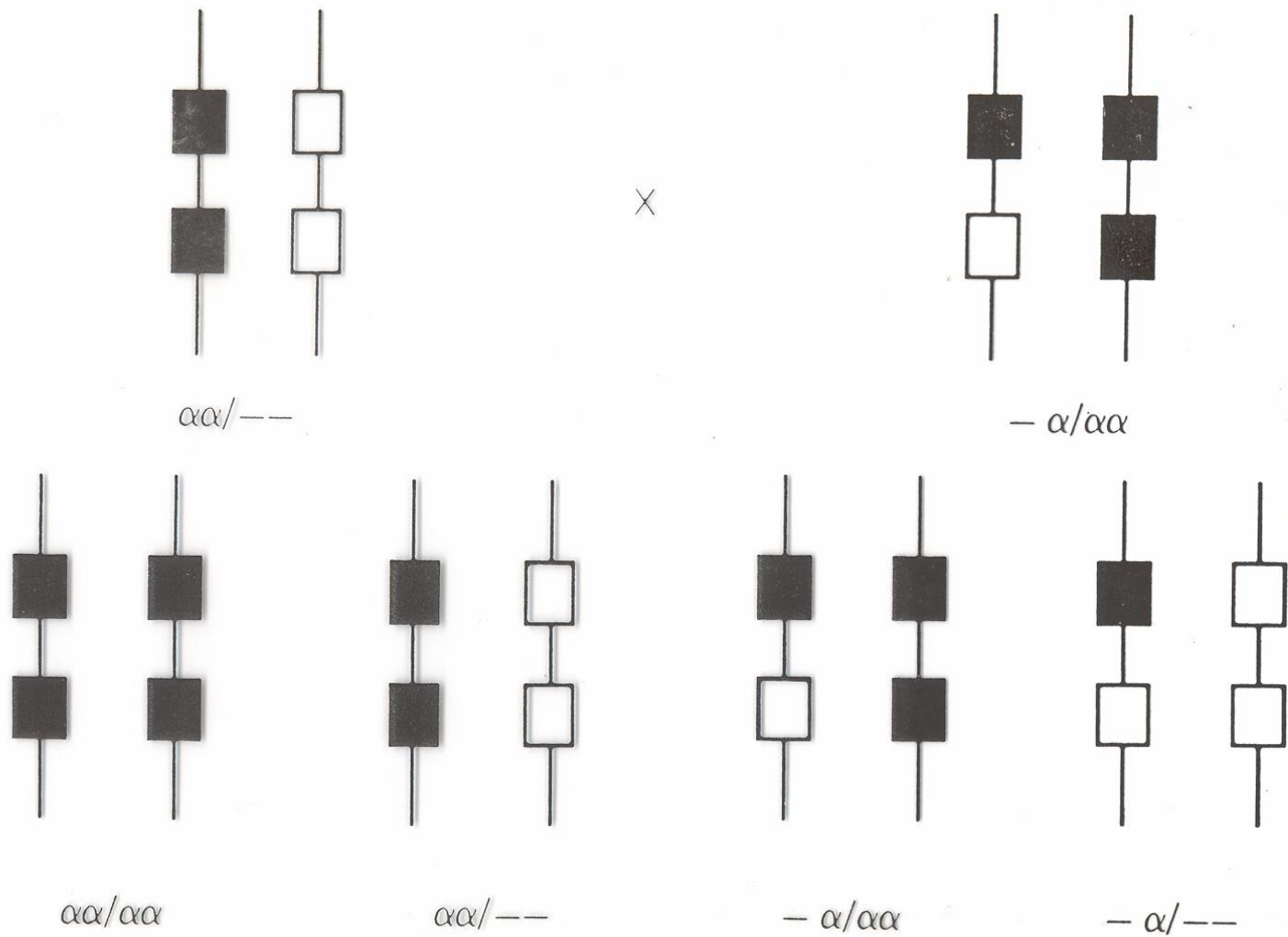
## Haemoglobin H disease (deletion of three $\alpha$ -globin genes)

- This chronic haemolytic anaemia results from the inheritance of both the  $\alpha^+$ - and  $\alpha^0$ -thalassaemia alleles, leaving one functioning  $\alpha$ -globin gene per cell.  $\alpha$ -globin chains are produced at very low rates, leaving a considerable excess of  $\beta$ -chains, which combine to form tetramers ( $\beta_4$ ). This tetramer is known as HbH.
- HbH is unstable and precipitates as the erythrocytes age, forming rigid membrane-bound inclusions that are removed during the passage of affected red cells through the spleen. The damage to the membrane brought about by this removal results in a shortened red cell lifespan.



## *cont'd...*

- Most patients are moderately affected, with anaemia of 7-11g/dl and markedly hypochromic, microcytic indices.
- Supravital staining of the blood film demonstrates cells with many HbH inclusions, giving a characteristic 'golf-ball' appearance.
- Most patients will be transfusion independent.
- Splenomegaly is seen in most patients.

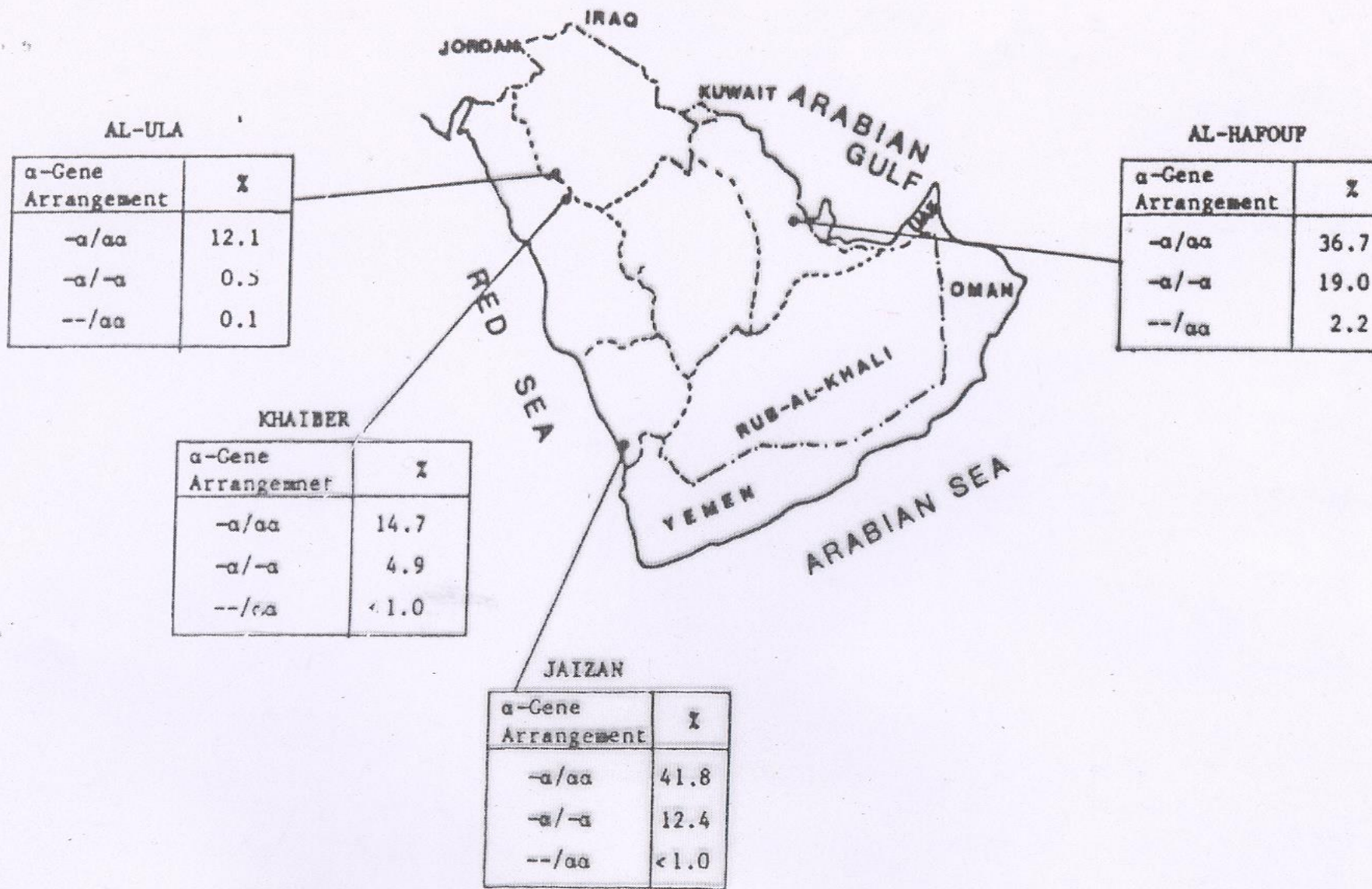


*Inheritance of HbH (–α/–) disease. Normal α-globin genes are shown by closed boxes, and deleted or otherwise inactivated α-globin genes by open boxes.*

## **Hb Bart's hydrops fetalis syndrome (deletion of all four $\alpha$ -globin genes)**

No  $\alpha$ -chains can be formed, and the fetal  $\beta$ -like chain  $\gamma$ -globin forms tetramers known as Hb Bart's. This haemoglobin is not useful for oxygen transport and, despite the persistence of the embryonic haemoglobin Hb Portland ( $\zeta_2\gamma_2$ ), there is intrauterine or neonatal death due to hydrops.

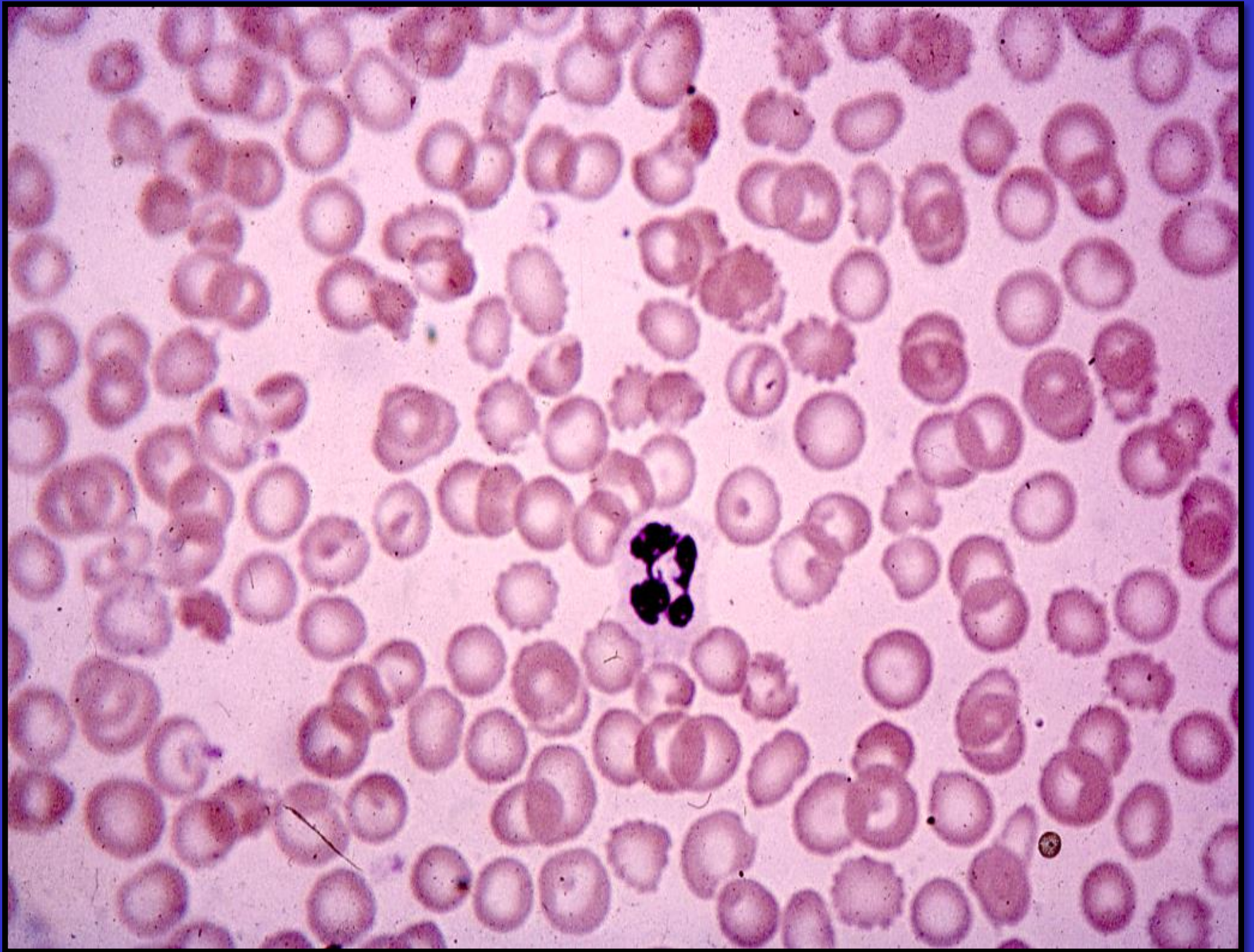


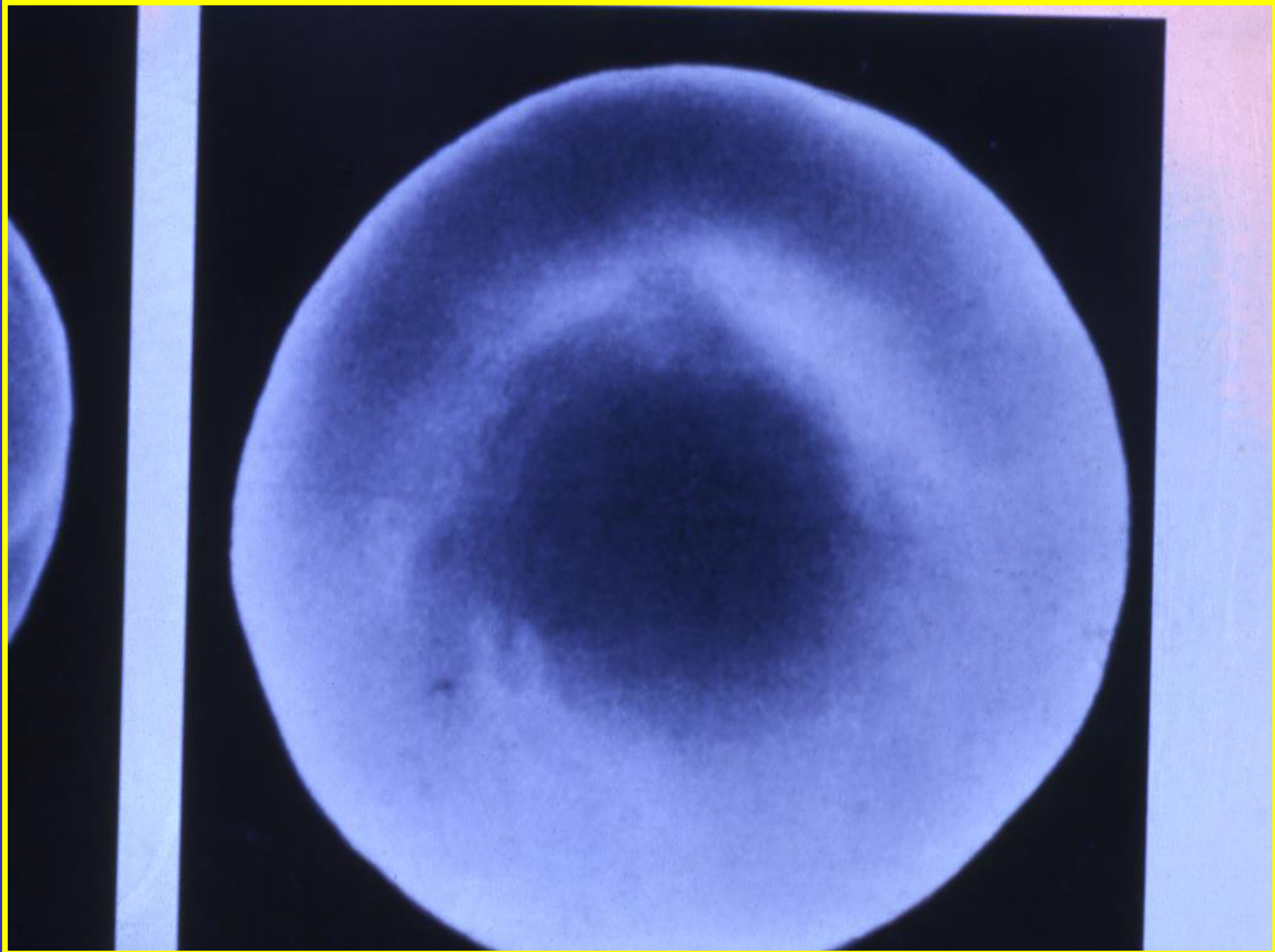


Frequency of  $\alpha$ -thalassaemia due to  $\alpha$ -gene deletion in different regions of Saudi Arabia (diagnosed using restriction endonuclease Bam HI).

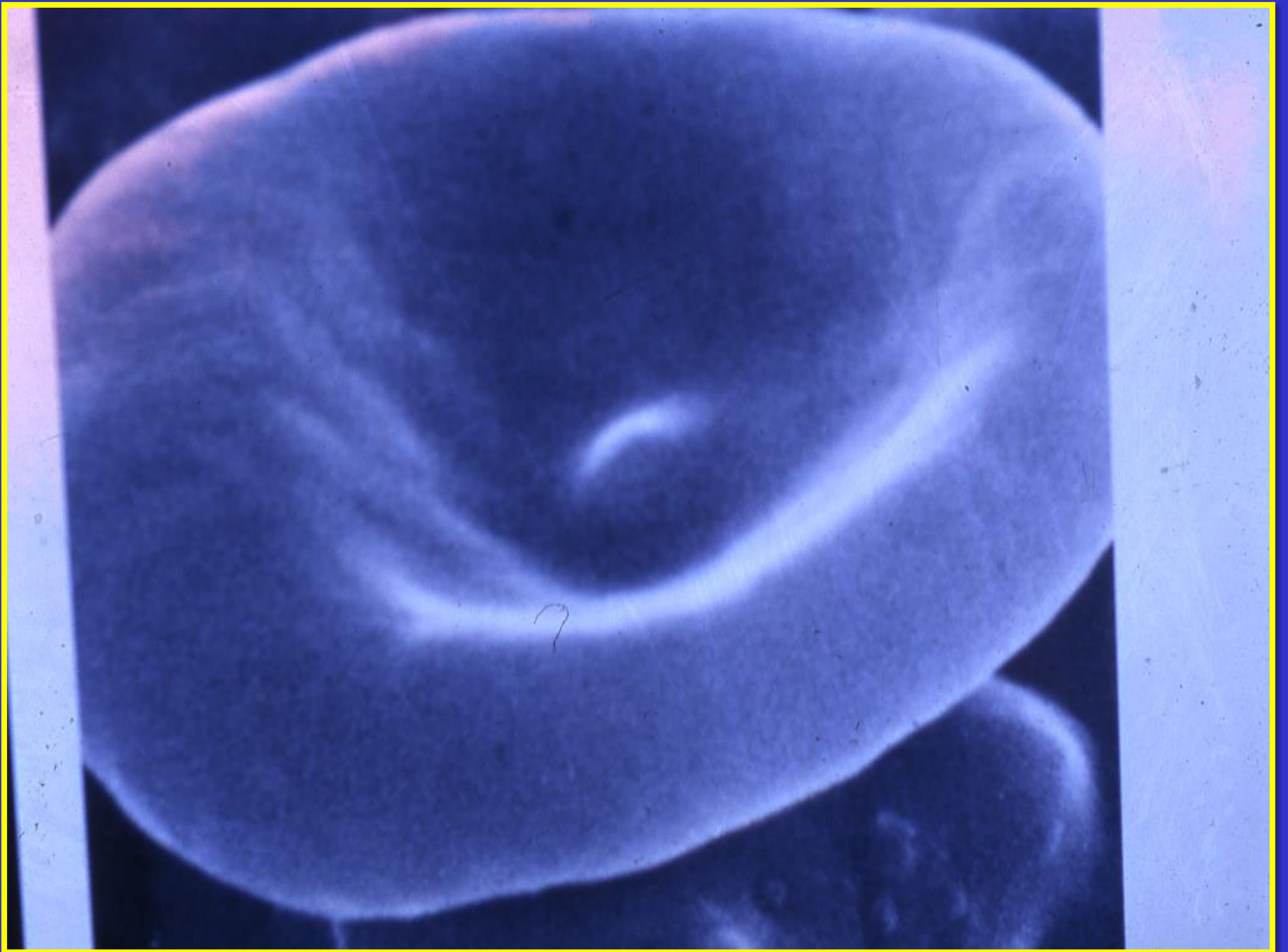
# LABORATORY DIAGNOSIS OF ALPHA THALASSEMIA SYNDROME

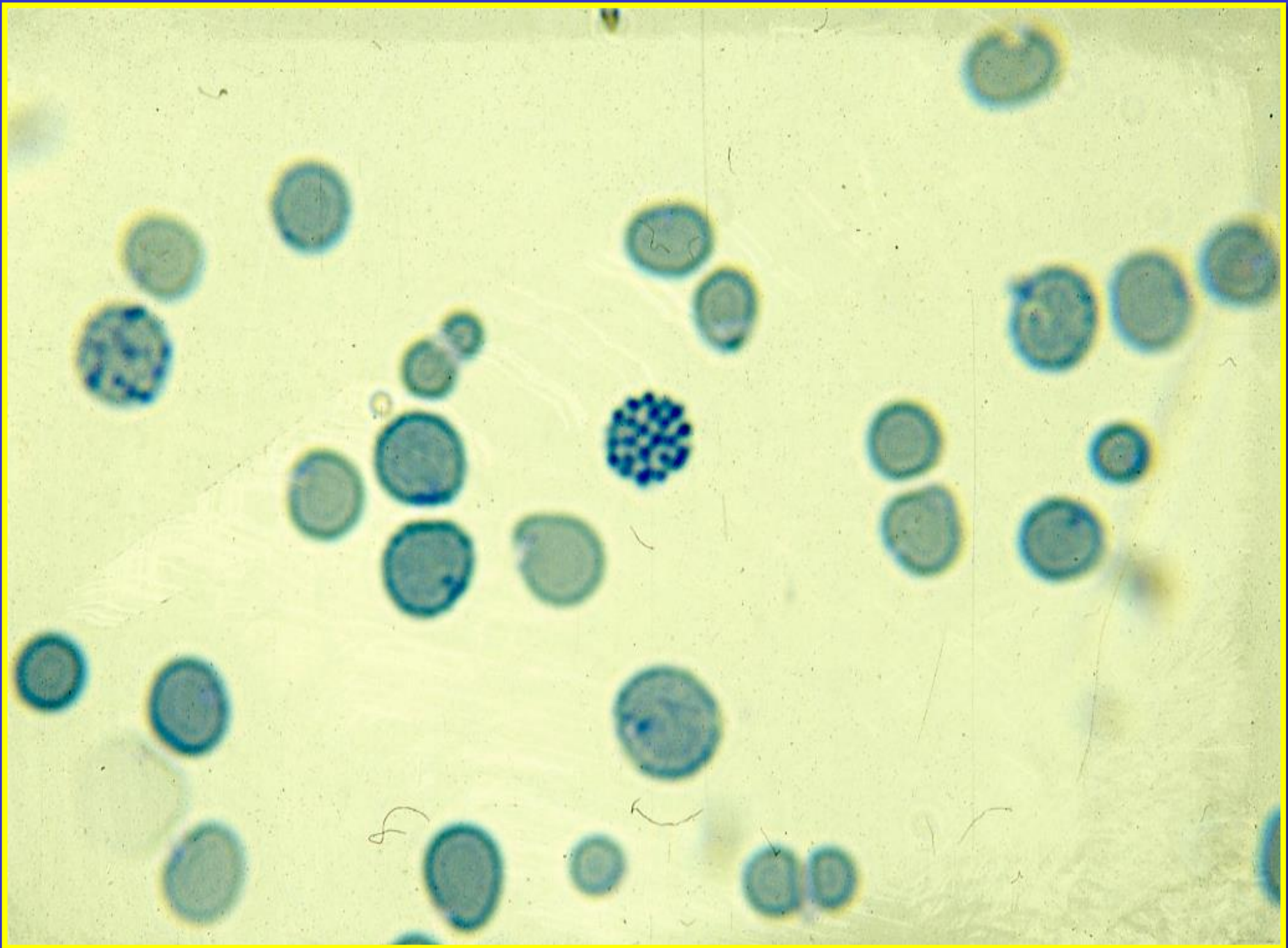
- **High red cell count in the trait**
- **Hypochromic microcytic red cells & target cells**
- **Normal serum iron or low in children**
- **Normal total iron binding capacity or high in children**
- **Positive Hb H inclusion bodies in the blood film preparation & positive Heinz bodies with vital stains**
- **Hemoglobin electrophoresis show presence of hemoglobin H (Hb H disease)**
- **Hemoglobin electrophoresis show low Hb A2 level**
- **Genetic study to confirm the diagnosis**

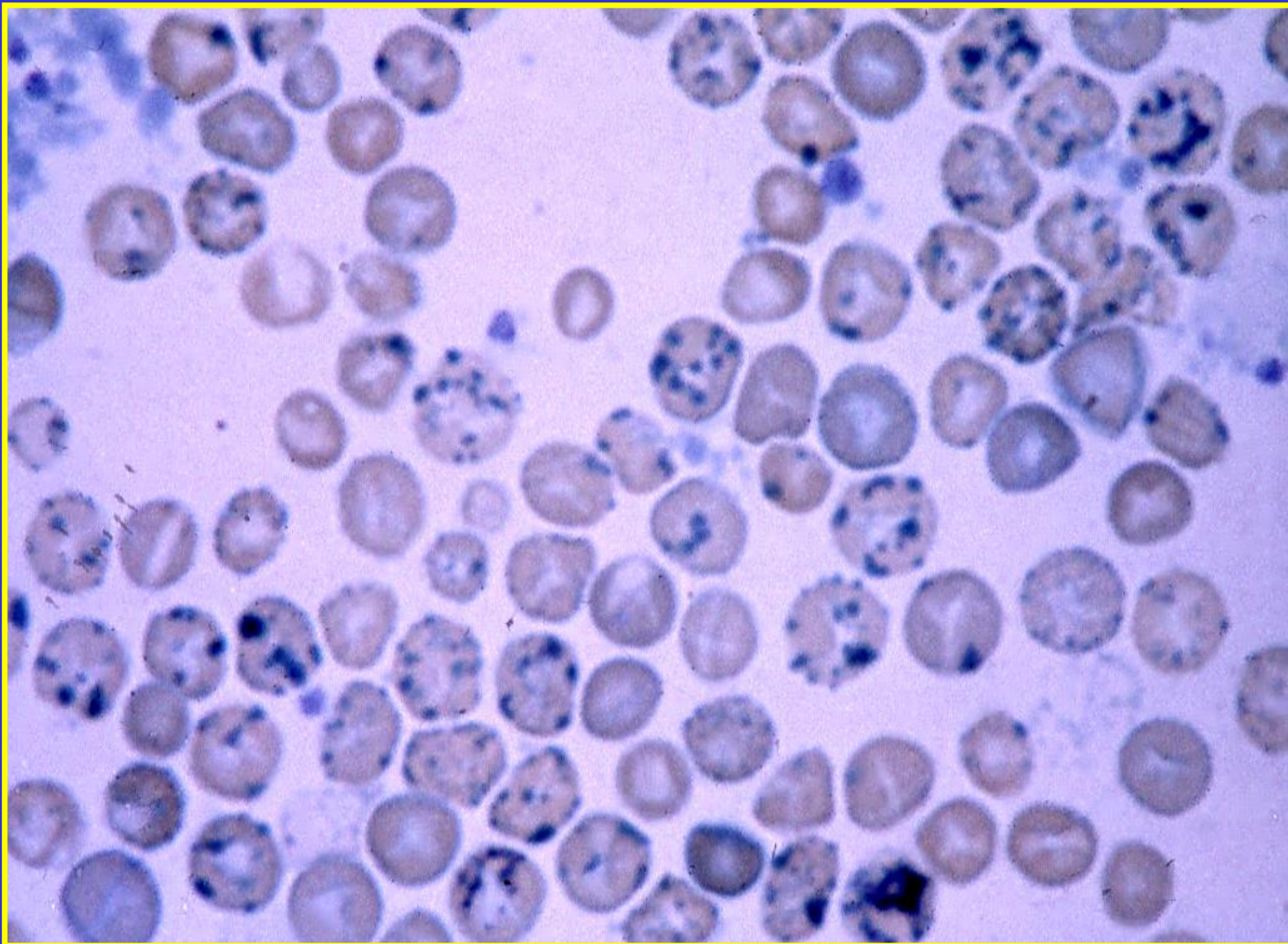


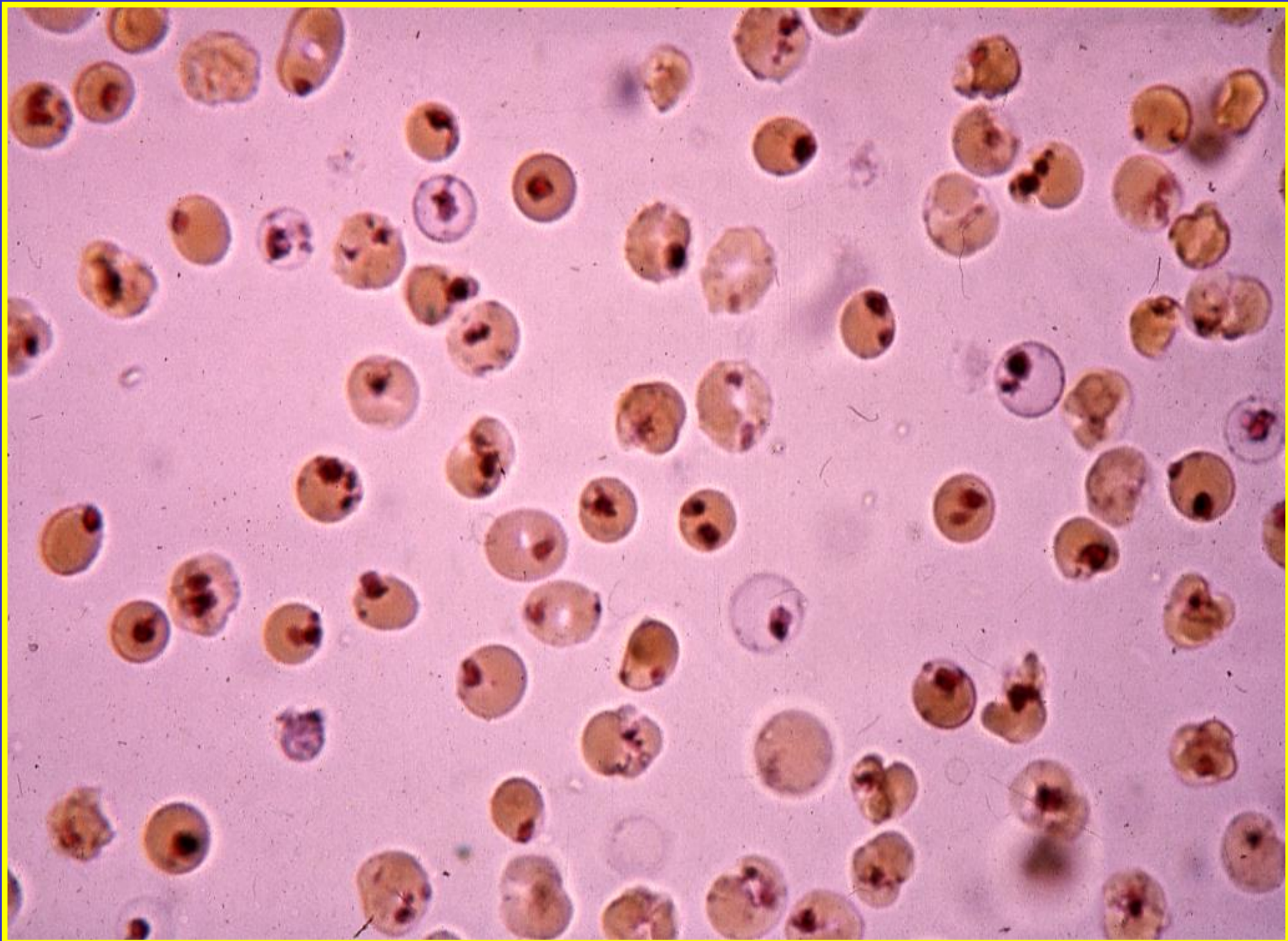




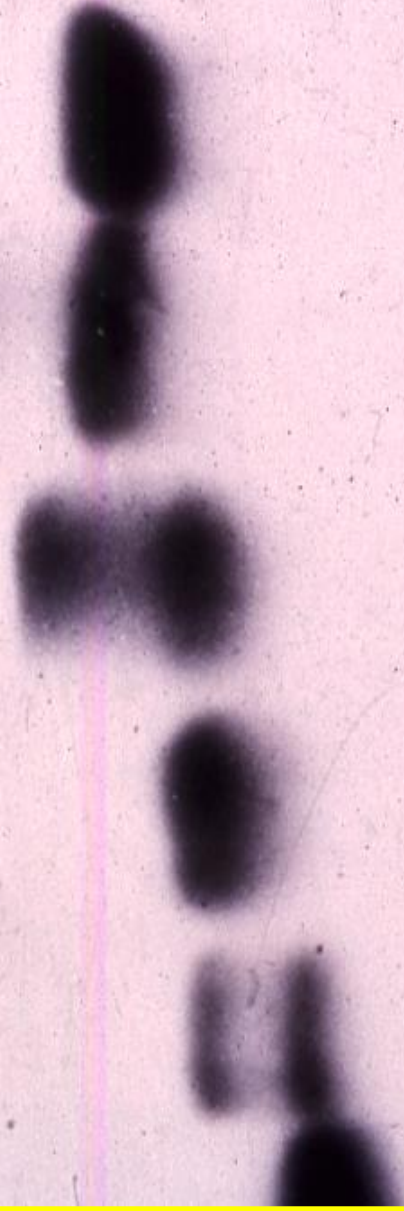






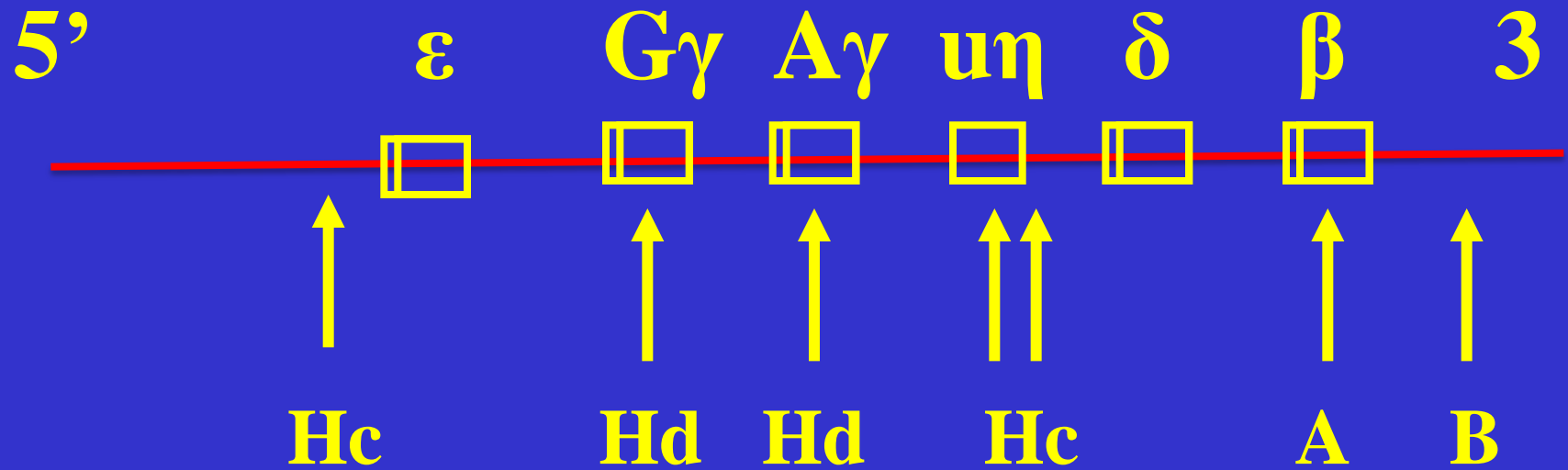


HTA S C



**$\beta$ -THALASSAEMIA**

**DR. FATMA AL-QAHTANI**



**The  $\beta$ -globin gene cluster showing the position of various common restriction endonuclease polymorphic sites. (Hc, Hinc II; Hd, Hind III; A, Ava II; B, Bam H1).**

# $\beta$ -Thalassaemia

- The World Health Organization estimates that 1.5% of the world's population are carriers of  $\beta$ -thalassaemia. The prevalence of the  $\beta$ -thalassaemia trait is particularly high in southern Europe (10-30%) and south-east Asia (5%), common in Africa, the Middle East, India, Pakistan and southern China.
- $\alpha$ -thalassaemia typically arises from gene deletions.
- $\beta$ -thalassaemia usually results from a multiplicity of different single nucleotide substitutions, insertions or small deletions affecting the  $\beta$ -gene itself.



# Heterozygous $\beta$ -thalassaemia (Beta-thalassaemia trait)

- Most affected subjects with beta thalassaemia trait are asymptomatic.
- The Hb concentration is either normal or slightly reduced, hypochromic and microcytic red cell indices are seen.
- Examination of peripheral blood film may show red cell abnormalities such as target cells and poikilocytes.
- HbA<sub>2</sub> levels will be raised above the normal range to 3.5-7.0%.
- Slightly increased HbF levels, in the range of 1-5%.

# Homozygous $\beta$ -Thalassaemia

- Defects of  $\beta$ -globin on both copies of chromosome 11
- Marked anaemia
- Transfusion dependent

# Clinical classification of the thalassaemias

- 1) *Thalassaemia minima* describes the presence of a thalassaemia mutation that is without clinical consequences.
- 2) *Thalassaemia minor* describes patients with microcytosis and hypochromic red cells secondary to thalassaemia mutations, but with only mild anaemia or a normal haemoglobin. Patients who inherit a single affected allele are usually in this category.

*cont'd...*

3) *Thalassaemia intermedia* patients will also have a microcytic hypochromic anaemia, increased erythroid drive to maintain their haemoglobin, packed bone marrow with a decreased myeloid:erythroid ratio, and extramedullary haematopoiesis, giving splenomegaly. Transfusion may be required to maintain the haemoglobin at times of additional physiological stress.

*cont'd...*

4) *Thalassaemia major* have severe anaemia and are transfusion dependent. Their increased erythroid drive leads to a packed erythroid marrow and splenomegaly, development of bony abnormalities secondary to unchecked marrow expansion. Patients in this category are those with complete loss of  $\beta$ -globin expression from both copies of chromosome 11.

# Molecular Defects in the $\beta$ -Thalassaemia Syndrome

|  | $\beta$ -Globin synthesis | $\beta$ -mRNA       | $\beta$ -Globin Gene         | $\delta$ -Globin Synthesis | $\gamma$ -Globin Synthesis |
|--|---------------------------|---------------------|------------------------------|----------------------------|----------------------------|
| 1. $\beta^+$ -Thalassaemia<br>2. $\beta^0$ -Thalassaemia | Decreased<br>Absent       | Decreased<br>Absent | Present<br>Present           | Present<br>Present         | Present<br>Present         |
| Ferrara Variant<br>Indian Variant                        | Absent<br>Absent          | Inactive<br>Absent  | Present<br>Partially Deleted | Present<br>Present         | Present<br>Present         |
| 3. $\delta\beta$ -Thalassaemia<br>4. HPFH                | Absent<br>Absent          | Absent<br>Absent    | Deleted<br>Deleted           | Absent<br>Absent           | Increased<br>increased     |

## Hemoglobin Fractions in the Genotypic Variants of the $\beta$ -Thalassaemia Syndromes

| Genotype  | HbA     | HbA <sub>2</sub> | HbF (%)             | Other Hemoglobins     |
|---|---------|------------------|---------------------|-----------------------|
| <b>Normal</b><br>$\beta/\beta$                                  | 97      | 2.5 – 3.2        | <1                  | None                  |
| <b>Thalassaemia major</b><br>$\beta^0/\beta^0$                  | 0       | 1.0 – 5.9        | >94                 | Free $\alpha$ -chains |
| $\beta^+/\beta^+$ Mediterranean                                 | Present | 2.4 – 8.7        | 20 – 90             | Free $\alpha$ -chains |
| $\beta^0/\beta^+$   | Present | 0.6 – 3.4        | >75                 | None                  |
| ( $\delta\beta$ ) Lepore/ ( $\delta\beta$ ) Lepore              | 0       | 0                | 70 – 92             | Hb Lepore (8-30%)     |
| <b>Thalassaemia intermedia</b><br>$\beta^+/\beta^+$ , black     | Present | 5.4 – 10.0       | 30 – 73             | None                  |
| $\beta^0/(\delta\beta)^0$                                       | 0       | 0.3 – 2.4        | 60 – 99             | None                  |
| $\beta^+/(\delta\beta)^0$                                       | 20 – 30 | Decreased        | Increased           | None                  |
| $\beta^0/(\delta\beta)^0$ Lepore                                | 0       | Decreased        | Increased           | Hb Lepore (10%)       |
| $\beta^+/(\delta\beta)^0$ Lepore                                | Present | Decreased        | Increased           | Hb Lepore (10%)       |
| $\beta^0/\beta$   | Present | >3.2             | 1.5 – 12            | None                  |
| ( $\delta\beta$ ) <sup>0</sup> / ( $\delta\beta$ ) <sup>0</sup> | 0       | 0                | 100                 | None                  |
| ( $\delta\beta$ ) <sup>0</sup> / ( $\delta\beta$ ) Lepore       | 0       | 0                | 92                  | Hb Lepore (8%)        |
| $\alpha/\beta$  | Present | Increased        | Normal or increased | ± Hb H                |

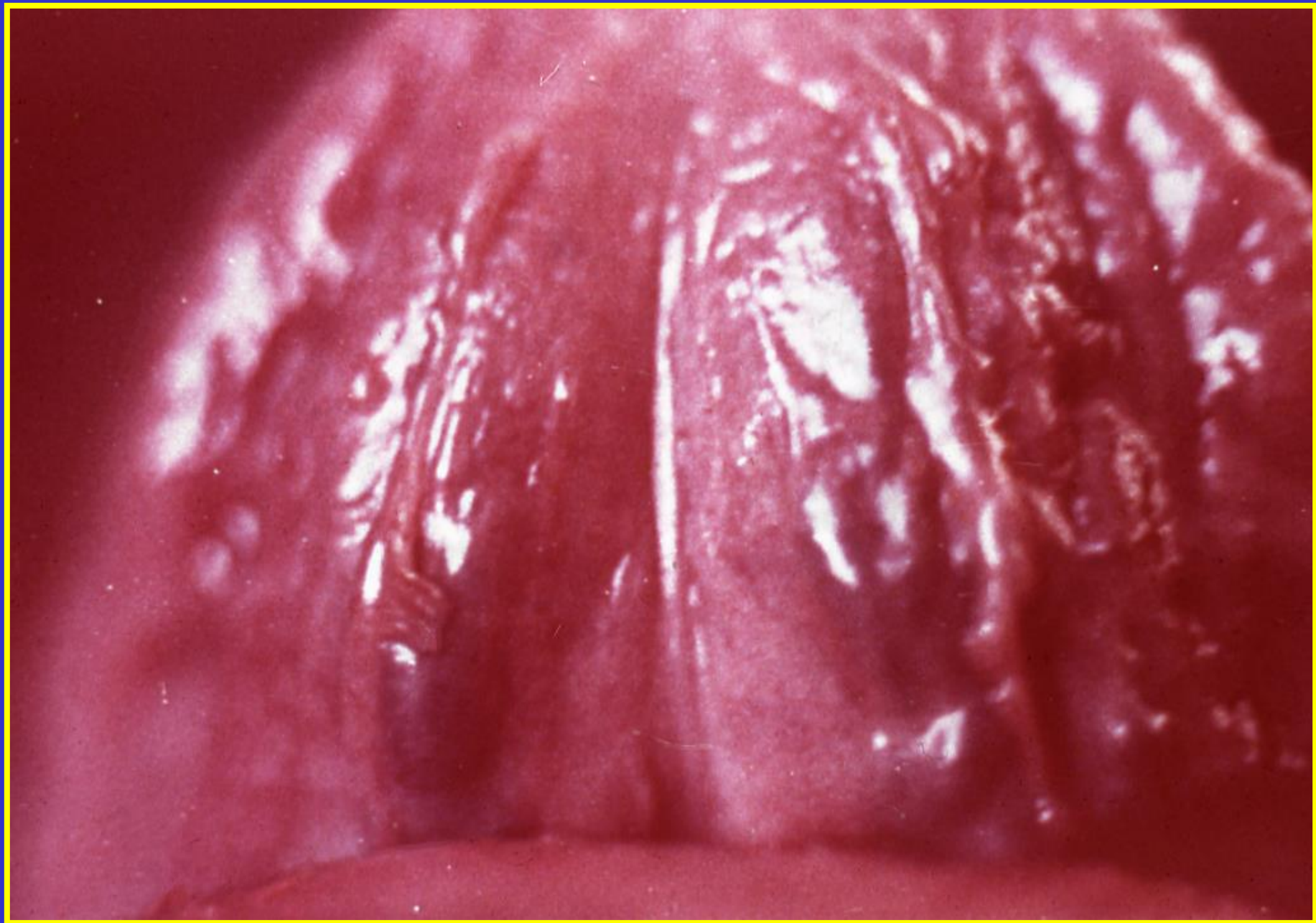
## Hemoglobin Fractions in the Genotypic Variants of the $\beta$ -Thalassaemia Syndromes (Continued)

| Genotype                        | HbA     | HbA <sub>2</sub> | HbF (%) | Other Hemoglobins    |
|---------------------------------|---------|------------------|---------|----------------------|
| <b>Thalassaemia minor</b>       |         |                  |         |                      |
| $\beta^{+}/\beta$               | >90     | 3.5 – 8.0        | 1 – 2   | None                 |
| $\beta^0/\beta$                 | >90     | 3.5 – 8.0        | 1 – 2   | None                 |
| $(\delta\beta)^0/\beta$         | >90     | 2.5 – 8.0        | 5 – 20  | None                 |
| $(\delta\beta)$ Lepore/ $\beta$ | Present | 1.2 – 2.6        | 1 – 3   | Hb Lepore ( 5 – 15%) |
| $(\gamma\delta\beta)^0/\beta$   | Present | 2.5 – 3.2        | < 1 – 2 | None                 |
| <b>Thalassaemia minima</b>      |         |                  |         |                      |
| $\beta^{\text{silent}}/\beta$   | 97      | <3.2             | <1      | None                 |



# Clinical Manifestations in Thalassaemias

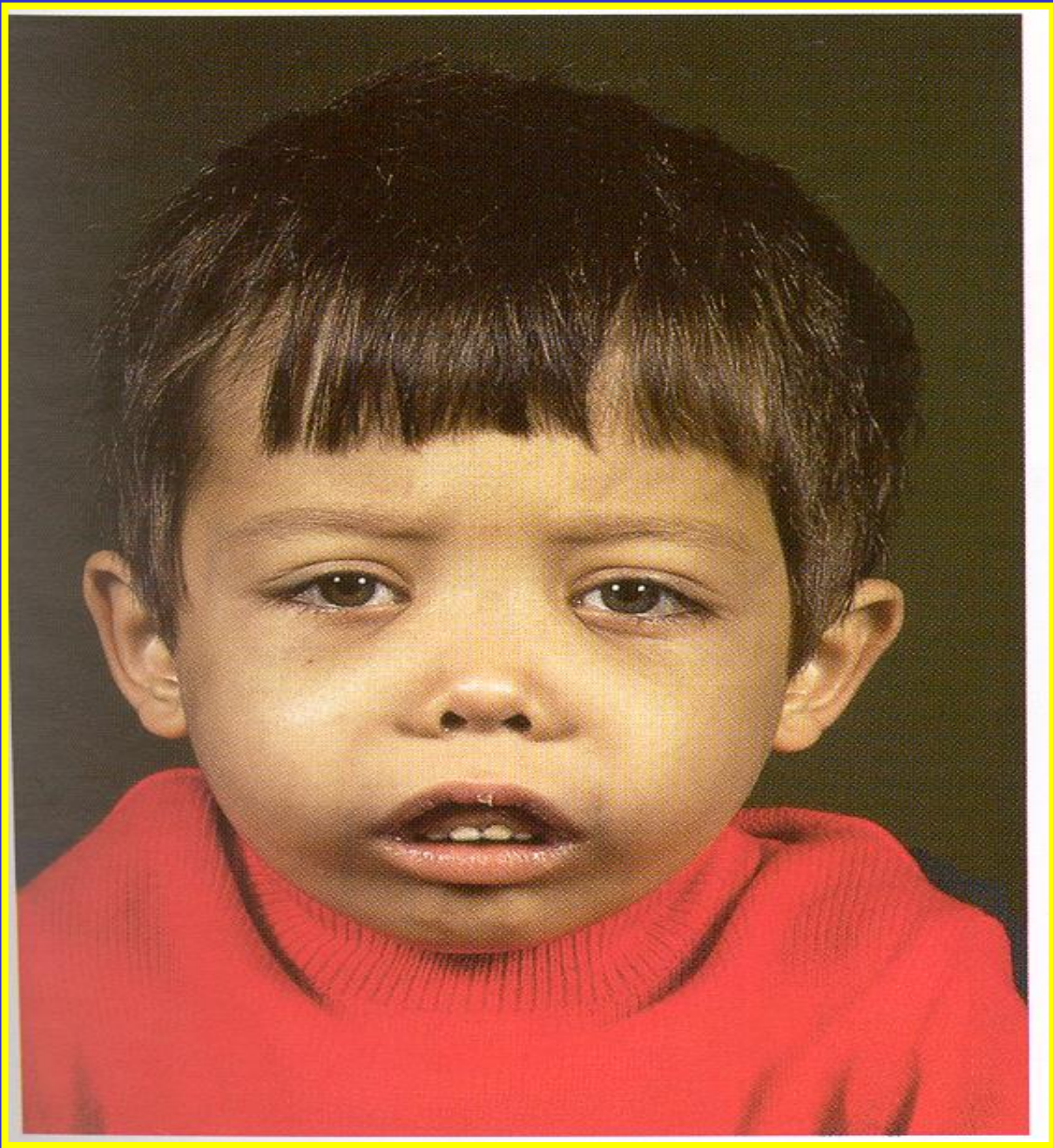
- **Pallor**
- **Jaundice**
- **Apathy and Anorexia**
- **Failure to Thrive**
- **Hepato-splenomegaly**
- **Skeletal Deformity**
- **Iron Overload manifestations**

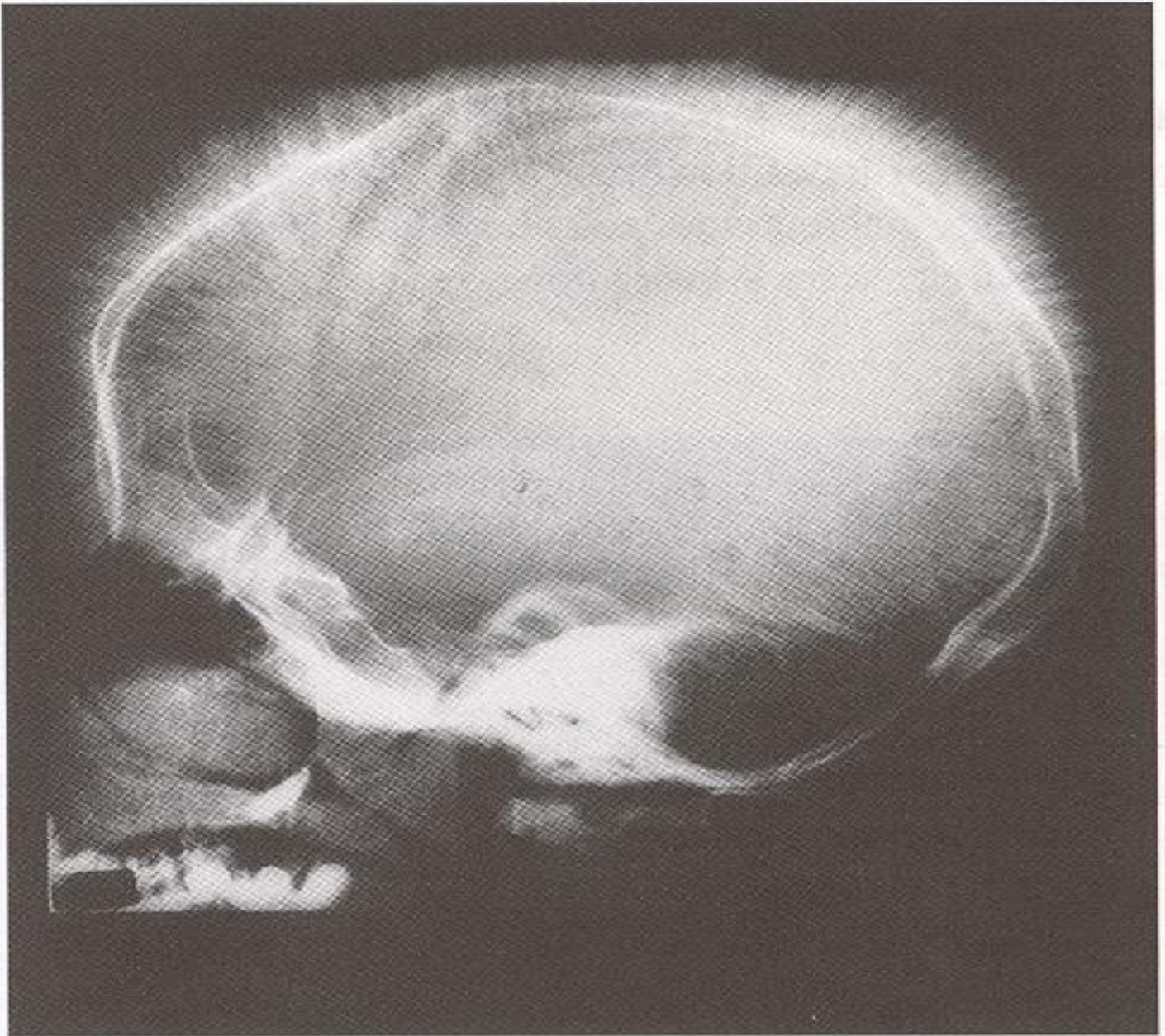


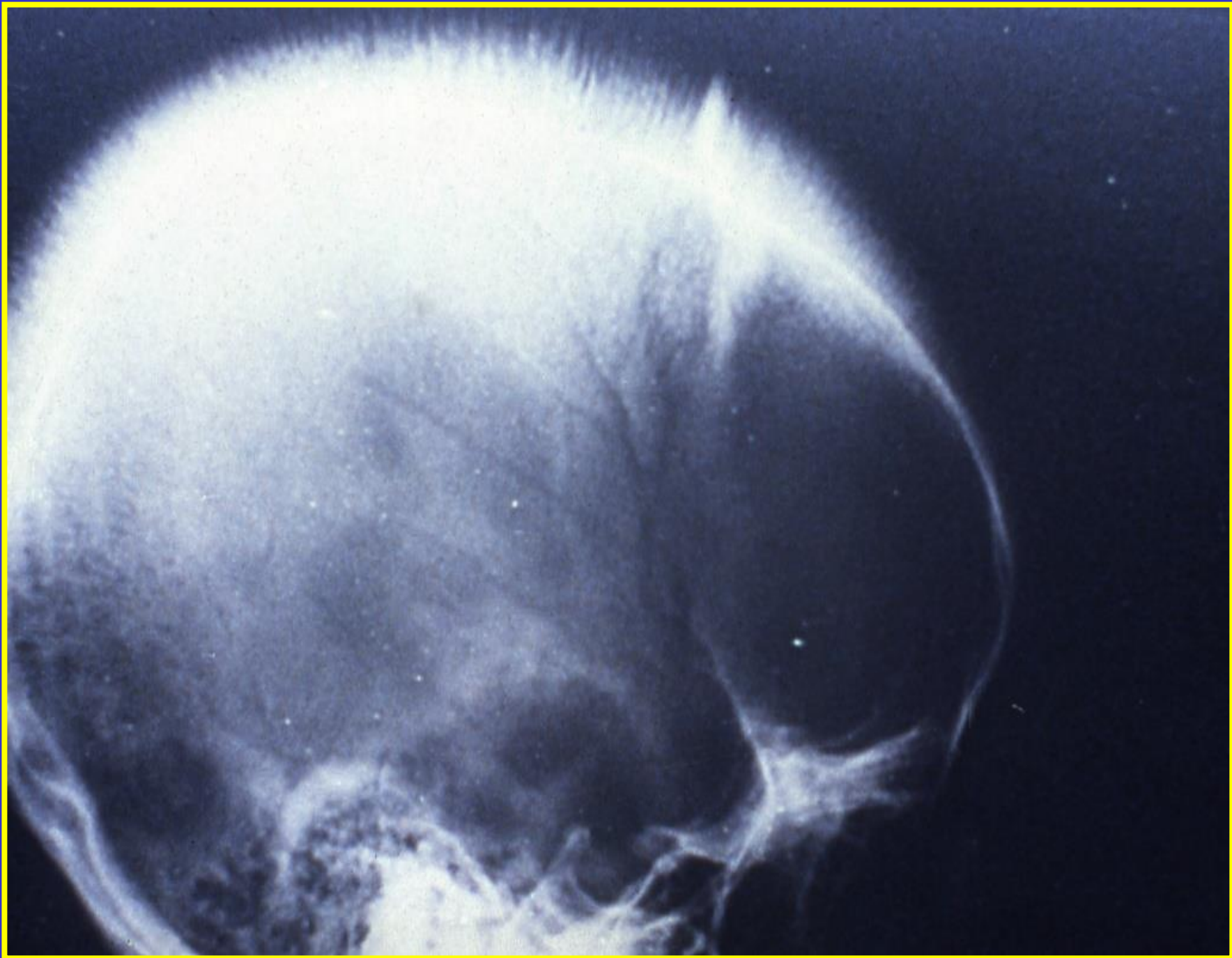




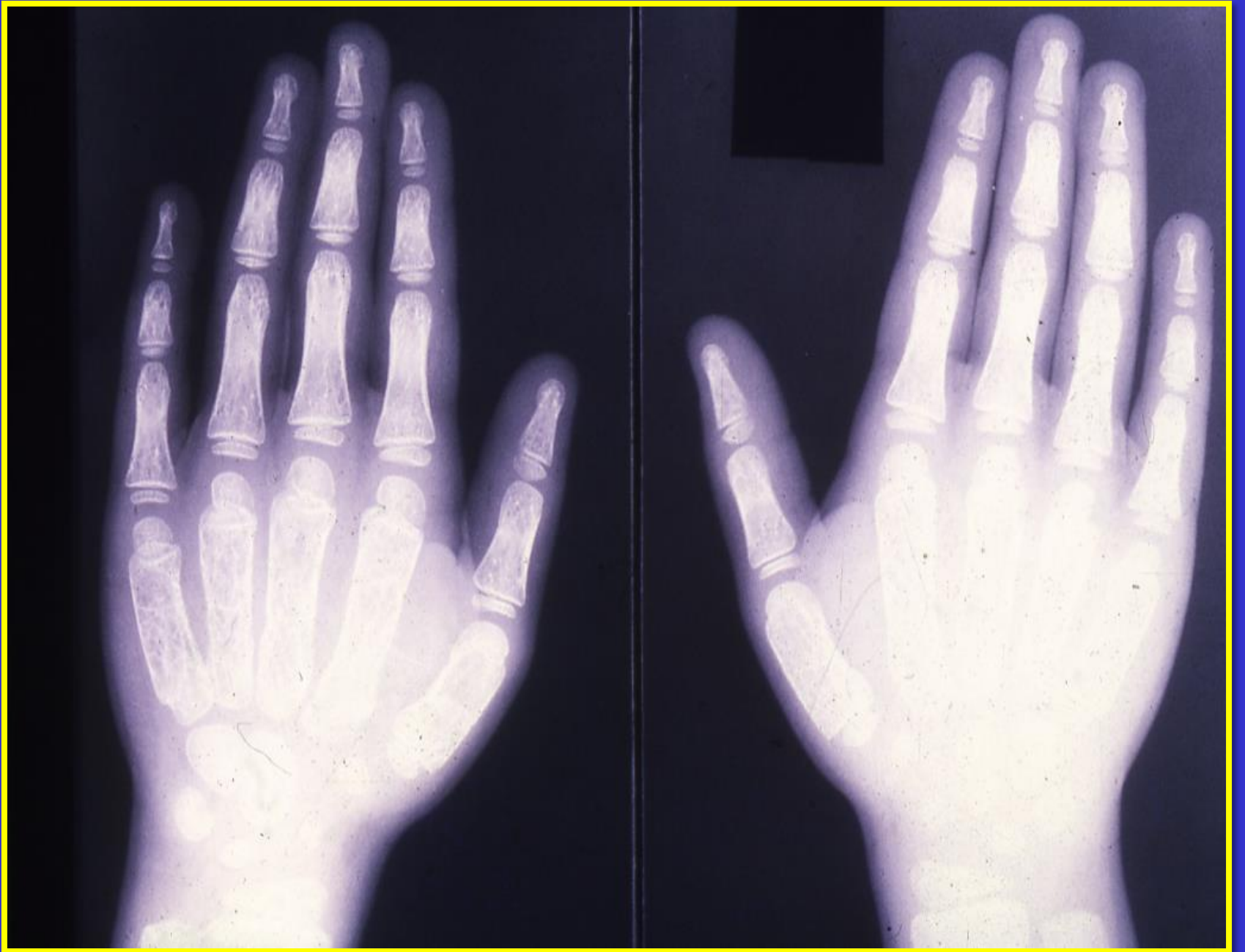












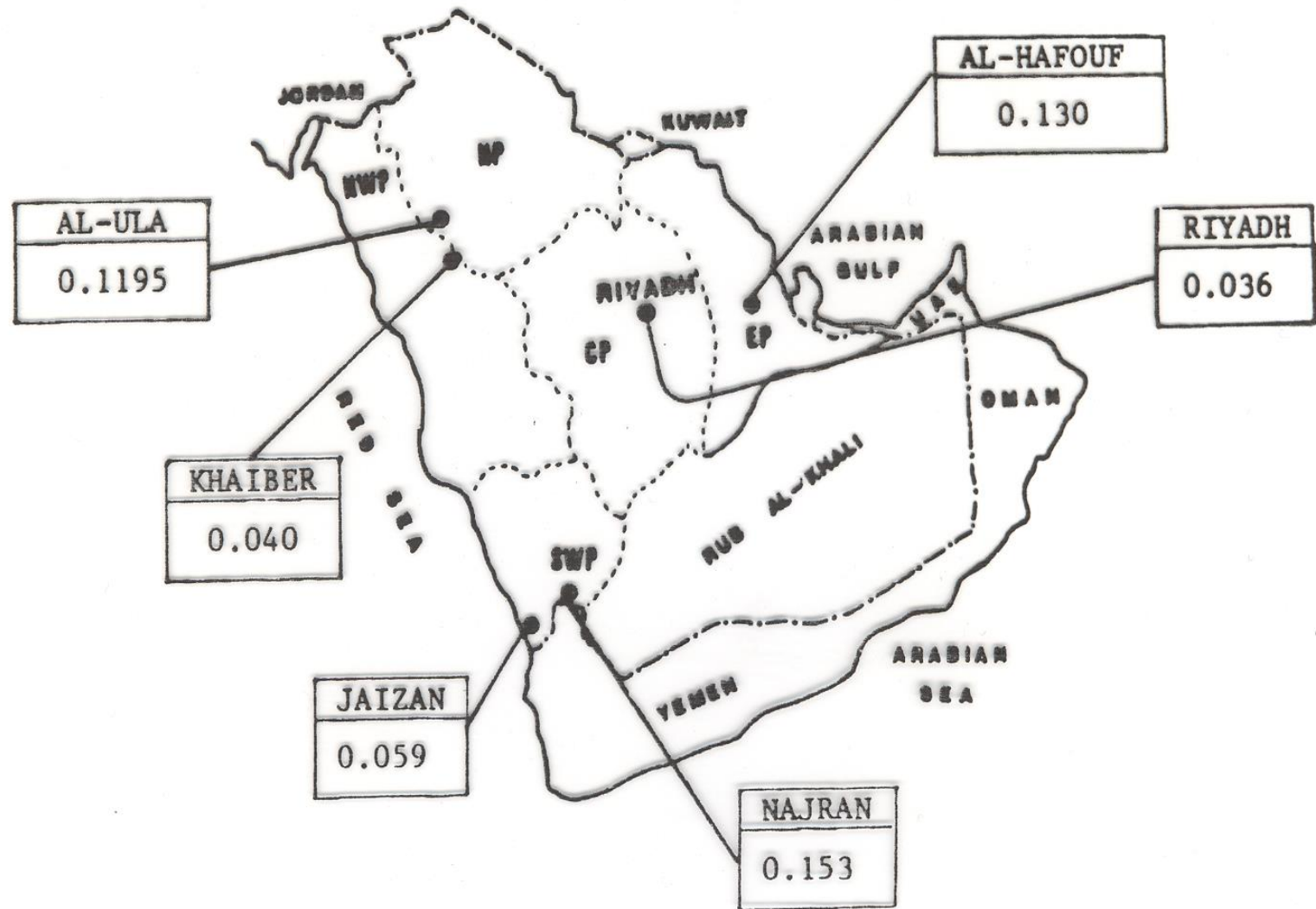


## Clinical and Hematological Features of the $\beta$ -Thalassemia Syndrome

|                                   | <b>Major</b>                             | <b>Intermedia</b>  | <b>Minor</b>         | <b>Minima</b>        |
|-----------------------------------|--|--|----------------------|----------------------|
| <b>Severity of manifestations</b> | ++++                                     | ++   | +, $\pm$             | $\pm$ , 0            |
| <b>Genetics</b>                   | <b>Homozygotes, double heterozygotes</b> | <b>Homozygotes, double heterozygotes, rarely heterozygotes</b> | <b>Heterozygotes</b> | <b>Heterozygotes</b> |
| <b>Splenomegaly</b>               | ++++                                     | ++, +++  | +, 0                 | 0                    |
| <b>Jaundice</b>                   | +++                                      | ++, +  | 0                    | 0                    |
| <b>Skeletal changes</b>           | ++++, ++                                 | +, 0   | +, 0                 | 0                    |
| <b>Anemia (Hb, g/dl)</b>          | <7                                       | 7 – 10   | >10                  | <b>Normal</b>        |

## Clinical and Hematologic Features of the $\beta$ -Thalassemia Syndrome (Continued)

|   | <b>Major</b>    | <b>Intermedia</b> | <b>Minor</b> | <b>Minima</b> |
|---|-----------------|-------------------|--------------|---------------|
| <b>Hypochromia</b>  | +++++           | +++               | ++           | +             |
| <b>Microcytosis</b>   | +++             | ++                | +            | 0             |
| <b>Target cells</b>   | <b>10 – 35%</b> | ++                | +            | ±             |
| <b>Basophilic stippling</b>   | ++              | +                 | +            | 0, +          |
| <b>Reticulocytes (%)</b>  | <b>5 – 15</b>   | <b>3 – 10</b>     | <b>2 – 5</b> | <b>1 – 2</b>  |
| <b>Nucleated red cells</b>  | +++             | +, 0              | 0            | 0             |
| <b>±, little or no abnormality; +, mild abnormality; +++++, prominent abnormality</b> |                 |                   |              |               |



Frequency of  $\beta$ -thalassaemia in different regions of Saudi Arabia. (From Ref. No. 20.) (No. investigated: Al-Hafouf 300; Riyadh 250; Al-Ula 427; Khaiber 500; Jizan 1271; Najran 301.)  $f=8.8353$ ;  $df=10$ ;  $p<0.01$

# **Diagnosis of Haemoglobinopathies including Thalassaemias**

## **A. Personal & Family History**

## **B. Physical Examination**

## **C. Laboratory Investigation**

**1. Haematological Tests – CBC, Red cell indices, blood film Morphology, reticulocyte count.**

**2. Sickling Tests – Sickle cell test, Sickle cell solubility test.**

**3. Hb Electrophoresis at alkaline/acidic pH and quantitation.**

**4. Quantitation of HbA<sub>2</sub> and HbF**

**5. Serum iron total iron binding capacity and ferritin level**

**6. Biochemical tests:**

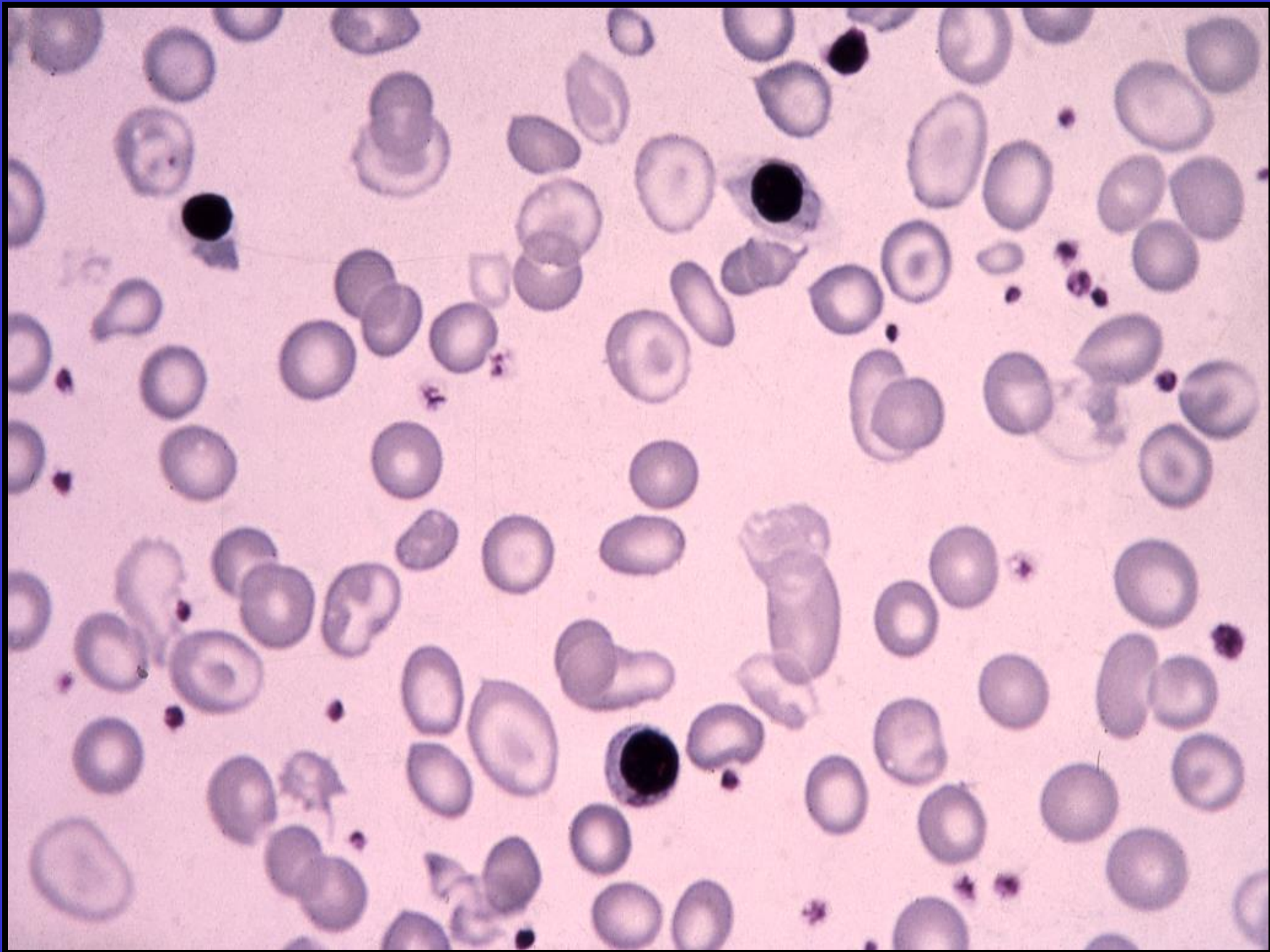
**Liver functions tests, renal function tests, blood gases and acid-base status, bone profile and urine analysis.**

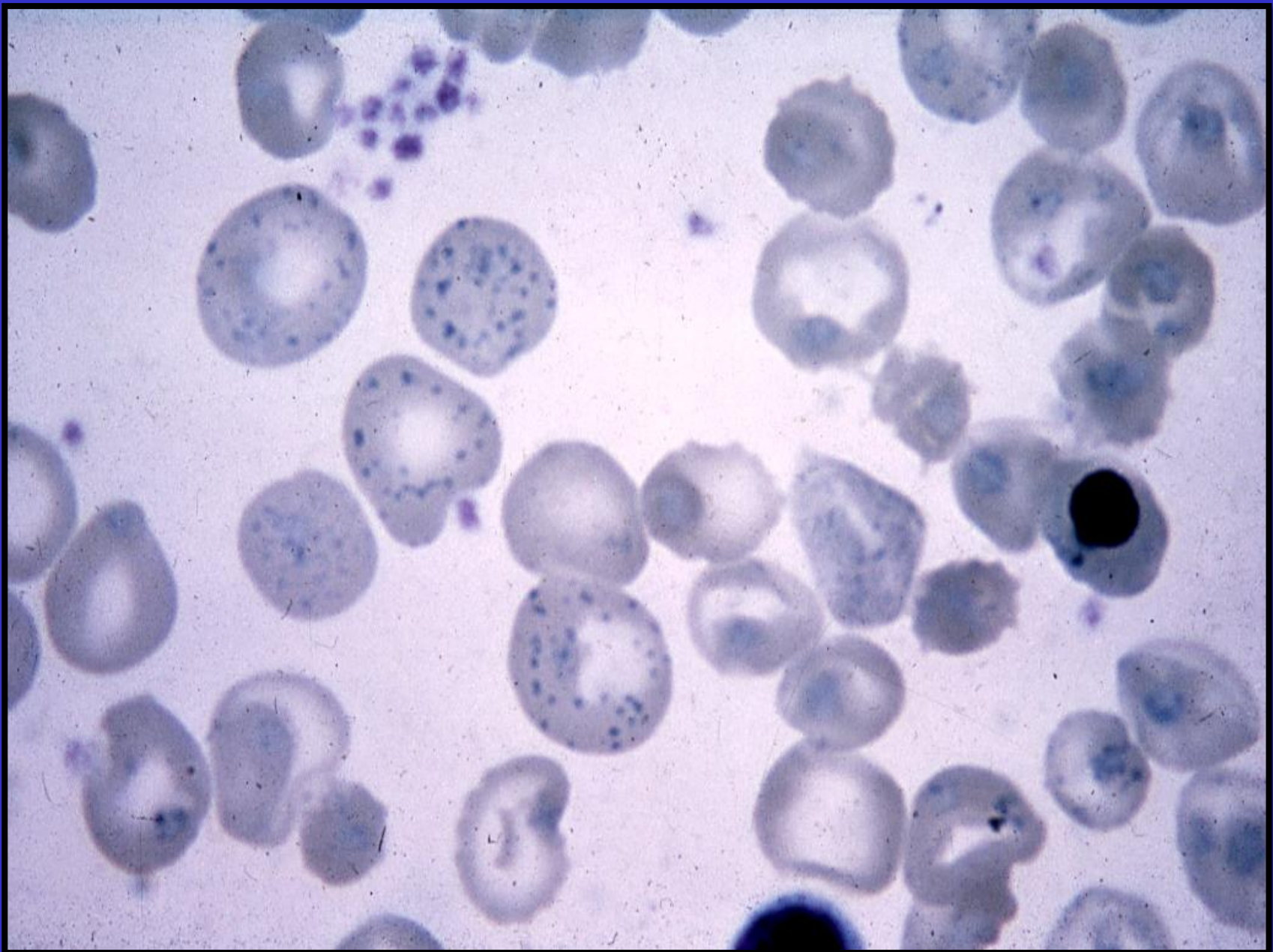
**7. Special Tests**

**A. Family studies (Laboratory Investigations)**

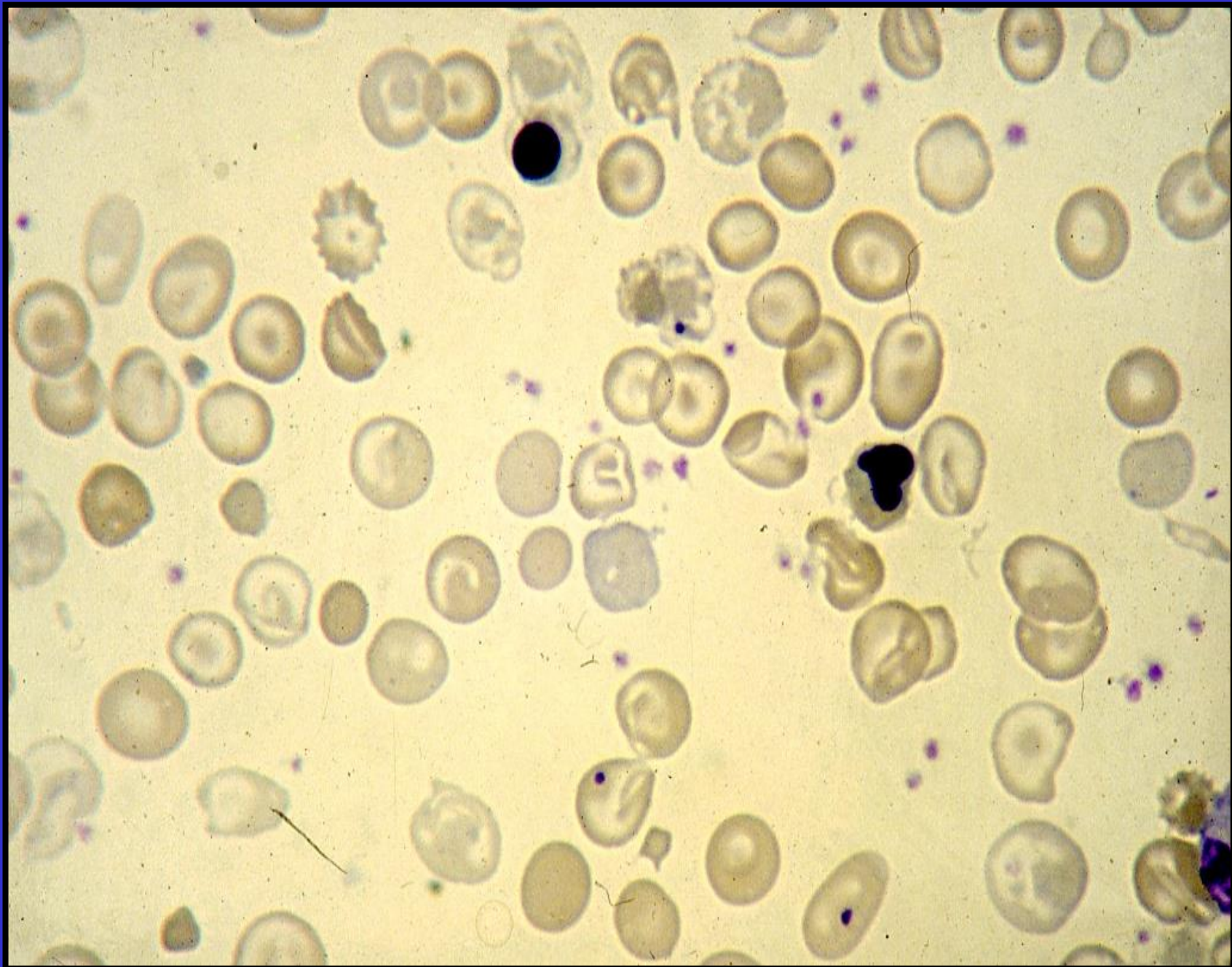
**B. Measurement of Alpha/Non-Alpha chain ratio**

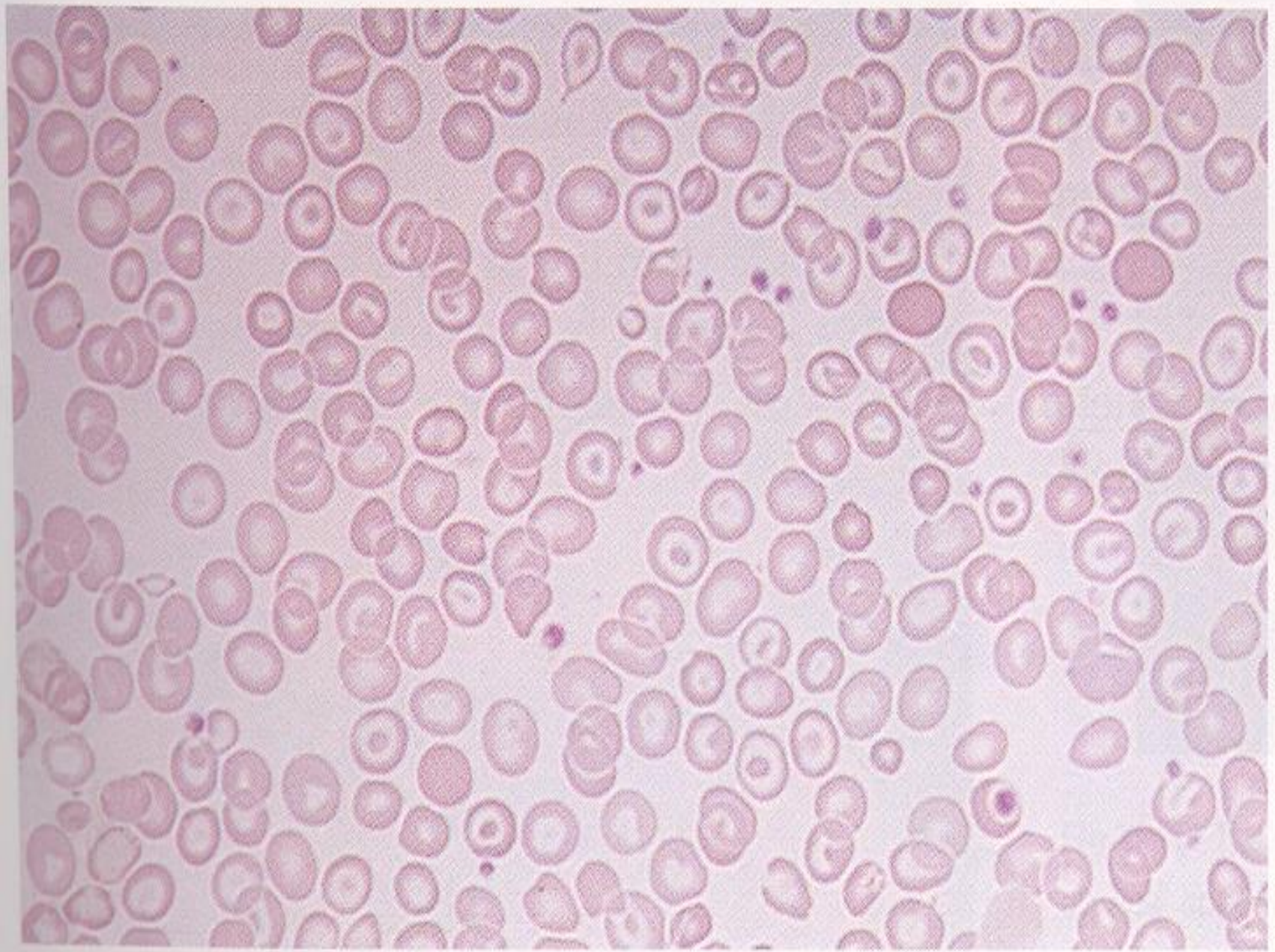
**C. Gene Studies**

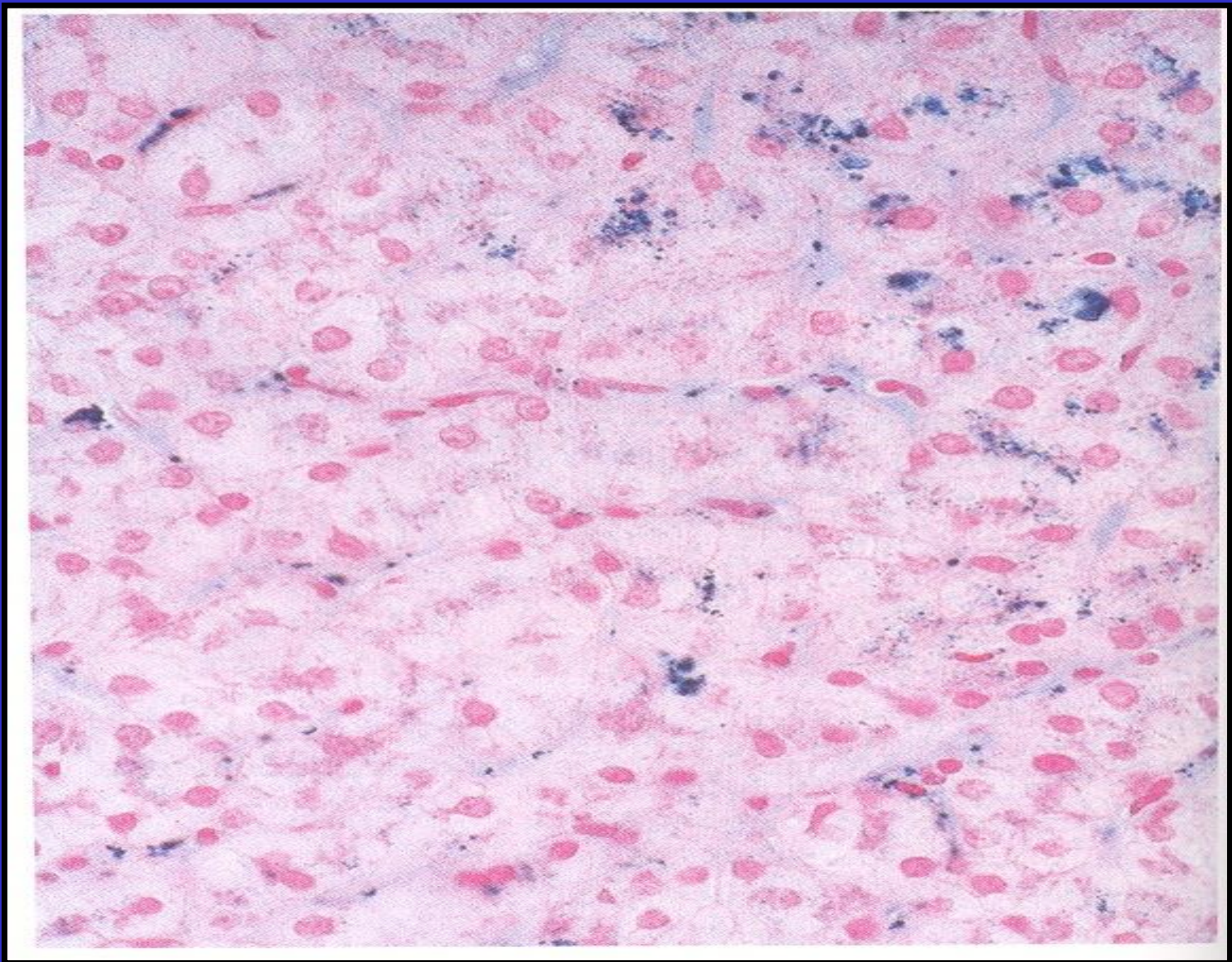


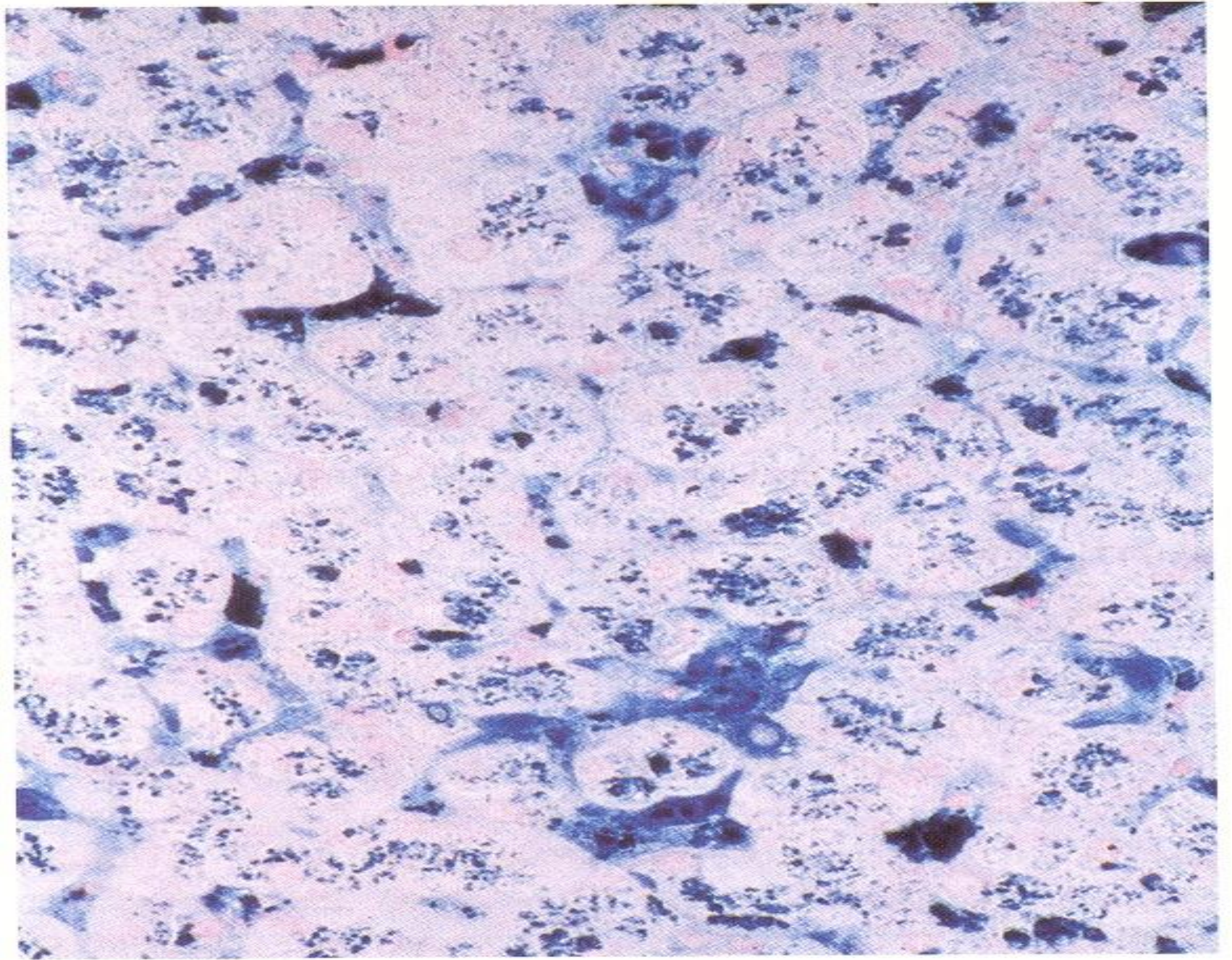












ING KHALID HOSP.  
O BOX 7805 RIYADH

HEMATOLOGY UNIT

Pat.No  
Name:

Page No.:1

Hospital:KING KHALID UNIVERSITY HOSPITA DOB: 14 Jun 61  
Location: (PCF01) PCC (Female)  
Doctor:UNKNOWN \*

ref:

Req No.:H02022419 Date Coll.:04/01/23(18/03/02) Date Recd.:04/01/23(18/03/02)  
Printed:09/01/1423(23/03/02)08:32 Time Recd.:10:30

MDTA Whole Blood

Full Blood Count

|       |       |       |   |              |           |
|-------|-------|-------|---|--------------|-----------|
| [ * ] | WBC   | 5.60  |   | 4 - 11       | x10.e9/L  |
| [ ]   | > RBC | 5.67  | H | 4.2 - 5.5    | x10.e12/L |
| < [ ] | HGB   | 98    | L | 120 - 160    | g/L       |
| < [ ] | HCT   | 31.0  | L | 37 - 47      | %         |
| < [ ] | MCV   | 54.6  | L | 80 - 94      | fl        |
| < [ ] | MCH   | 17.3  | L | 27 - 32      | pg        |
| < [ ] | MCHC  | 315   | L | 320 - 360    | g/L       |
| [ ]   | > RDW | 15.6  | H | 11.5 - 14.5  | %         |
| [ * ] | PLT   | 426   |   | 140 - 450    | x10.e9/L  |
| [ * ] | MPV   | 7.9   |   | 7.2 - 11.1   | fl        |
| < [ ] | PDW   | 15.6  | L | 20 - 70      | %         |
| [ ]   | > PCT | 0.339 | H | 0.150 - 0.32 | %         |

Differential

|       |       |      |   |           |          |
|-------|-------|------|---|-----------|----------|
| [ * ] | %NEUT | 74   |   | 40 - 75   | %        |
| < [ ] | %LYMP | 19   | L | 20 - 45   | %        |
| < [ ] | %MONO | 2    | L | 3 - 9     | %        |
| [ * ] | %EOS  | 5    |   | 0 - 6     | %        |
| [ * ] | #NEUT | 4.14 |   | 2 - 7.5   | x10.e9/L |
| [ * ] | #LYMP | 1.06 |   | 1 - 5     | x10.e9/L |
| < [ ] | #MONO | 0.11 | L | 0.2 - 0.8 | x10.e9/L |
| [ * ] | #EOS  | 0.28 |   | 0.0 - 0.8 | x10.e9/L |

Morphology

Flag Comments 3+ ,3+

Flag Comment 1

ANISO  
MICRO MK  
MACRO  
POIKILO  
HYPO MK  
Polychromasia  
LSHIFT

TARGET CELLS SL

Ovalocytes SL

[ \* ] Retic Count 1.4 0.2 - 2.0 %

[ ]> ESR 35 H 3 - 9 mm/hr

KING KHALID HOSPITAL

DEPARTMENT OF SPECIAL BIOCHEMISTRY

BOX 7805 RIYADH

Hosp No. 12258

Page No.: 1

Patient: AL HANAN, TRAJAA

Hosp Srce: KING KHALID UNIVERSITY HOSPIT DOB: 14 Jun 61

Location: (EHC) Employee Health Clinic

Doctor: UNKNOWN \*

Ref:

Req No.: S0202265 Date Coll.: 04/01/23(18/03/02) Date Recd.: 04/01/23(18/03/02)

Printed: 09/01/1423(23/03/02)08:34 Time Recd.: 10:51

Arterial Blood

Hemoglobin Electrophoresis

|           |   |      |               |      |   |
|-----------|---|------|---------------|------|---|
| 95 - 99   | % | <[ ] | Hemoglobin A  | 93.5 | L |
| 0 - 2.0   | % | [ *] | Hemoglobin F  | 2.0  |   |
| 2.0 - 3.5 | % | [ ]> | Hemoglobin A2 | 4.5  | H |
|           |   |      | Hemoglobin S  | 0.0  |   |
|           |   |      | Hemoglobin E  | 0.0  |   |
|           |   |      | Hemoglobin C  | 0.0  |   |
|           | % |      | Hemoglobin O  | 0.0  |   |



### نموذج فحص ما قبل الزواج

تاريخ سحب العينة : / / ١٤هـ رقم الملف الطبي: ..... رقم المختبر .....

اسم الطبيب المعالج: ..... رقم التحويل/النداء ..... العيادة .....

#### **البيانات الشخصية:**

الاسم ..... الجنسية: ..... العمر: ( ) الجنس :  ذكر  أنثى.

رقم السجل المدني/الإقامة: ..... العنوان: ..... الهاتف: .....

الفحوصات المطلوبة:

١- تعداد الدم الكامل (CBC).  
٢- اختبار الخلايا المنجلية (Sickling).

٣- الرحلان الكهربائي لخضاب الدم (Hb Electrophoresis).  
٤- اختبارات أخرى (Other Tests).

#### LABORATORY RESULT

| TEST                    | NORMAL RANGE              | RESULT      | REMARKS        |
|-------------------------|---------------------------|-------------|----------------|
| RBCX10 <sup>12</sup> /L | M:4.7 - 6.1 ....F:4.2-5.5 |             |                |
| HBg/dL                  | M:13 -18 ....F:12-16      |             |                |
| Het%                    | M:42 - 52 ....F:37- 47%   |             |                |
| MCV fL                  | 80 - 94                   |             |                |
| MCH pg                  | 27 - 32                   |             |                |
| MCHCg/dL                | 32 - 36                   |             |                |
| RDW                     | 11.5 - 14.5%              |             |                |
| Retic                   | 0.5 - 2%                  |             |                |
| Sickling Test           | Positive or Negative      |             |                |
| Hb A                    | 95 - 97%                  |             |                |
| Hb A2                   | 2.0 - 3.5%                |             |                |
| Hb F                    | <1.5%                     |             |                |
| Abnormal Hemoglobin     |                           |             |                |
| TEST                    | PATIENT RESULT            | HEMOGLOBIN  | PATIENT RESULT |
| Hb S                    |                           | Hb J        |                |
| Hb C                    |                           | Hb O - Arab |                |
| Hb D                    |                           | Hb H        |                |
| Hb E                    |                           | Hb Barts    |                |
| Hb G                    |                           | Other Test  |                |
| Other Hb                |                           |             |                |

المشرف الفني بالوحدة: .....

ملاحظات: .....  
COMMENTS: .....

استشاري أمراض الدم بالمختبر: ..... التوقيع: .....

\* **ملاحظة هامة:** هذه الشهادة تبين نتيجة الفحص المخبري لمرضى الأنيميا المنجلية والثلاسيميا فقط، ولا تشمل أي

أمراض وراثية أخرى للطرفين المعنيين.



### نموذج فحص ما قبل الزواج

تاريخ سحب العينة: / / ١٤هـ رقم الملف الطبي: رقم المختبر  
اسم الطبيب المعالج: رقم التحويل/النداء: العيادة: P.C.C  
**البيانات الشخصية:**  
الاسم: .. الجنس: .. العمر: ( ) الجنس:  ذكر  دهر  
رقم السجل المدني/الإقامة: .. العنوان: .. الهاتف:  
الفحوصات المطلوبة:

- ١- تعداد الدم الكامل (CBC).
- ٢- اختبار الخلايا المنجلية (Sickling).
- ٣- الرحلان الكهربائي لخضاب الدم (Hb Electrophoresis).
- ٤- اختبارات أخرى (Other Tests).

#### LABORATORY RESULT

| TEST                    | NORMAL RANGE             | RESULT      | REMARKS        |
|-------------------------|--------------------------|-------------|----------------|
| RBCX10 <sup>12</sup> /L | M:4.7 - 6.1 ...F:4.2-5.5 | 4.5         |                |
| HBg/dL                  | M:13 -18 ....F:12-16     | 12.9        |                |
| Het%                    | M:42 - 52 ....F:37- 47%  | 37.8        |                |
| MCV fL                  | 80 - 94                  | 83.9        |                |
| MCH pg                  | 27 - 32                  | 28.6        |                |
| MCHCg/dL                | 32 - 36                  | 34.1        |                |
| RDW                     | 11.5 - 14.5%             | 13.6        |                |
| Retic                   | 0.5 - 2%                 |             |                |
| Sickling Test           | Positive or Negative     | Negative    |                |
| Hb A                    | 95 - 97%                 | 96.9        |                |
| Hb A2                   | 2.0 - 3.5%               | 2.6         |                |
| Hb F                    | <1.5%                    | <0.5        |                |
| Abnormal Hemoglobin     |                          |             |                |
| TEST                    | PATIENT RESULT           | HEMOGLOBIN  | PATIENT RESULT |
| Hb S                    |                          | Hb J        |                |
| Hb C                    |                          | Hb O - Arab |                |
| Hb D                    |                          | Hb H        |                |
| Hb E                    |                          | Hb Barts    |                |
| Hb G                    |                          | Other Test  |                |
| Other Hb                |                          |             |                |

المشرف الفني بالوحدة: .. ملاحظات: ..  
سالمه من أمراض الدم الوراثية

استشاري أمراض الدم بالمختبر: د. إبراهيم الحمد المشرفي  
التوقيع: 6-2-26

\* **ملاحظة هامة:** هذه الشهادة تبين نتيجة الفحص المخبري لمرضى الأنيميا المنجلية والثلاسيميا فقط، ولا تشمل أي أمراض وراثية أخرى للطرفين المعنيين.



# Prenatal diagnosis of the haemoglobinopathies (Including thalassaemia)

## DNA Analysis

### A. Chorionic villus sampling

Transcervical approach (9 – 11 weeks of pregnancy)

Transabdominal approach (up to 15 weeks of pregnancy)

### B. Amniotic fluid cell analysis (16 – 20 weeks gestation)

### C. Fetal blood sampling (> 20 weeks gestation)

DNA analysis

Haematological parameters

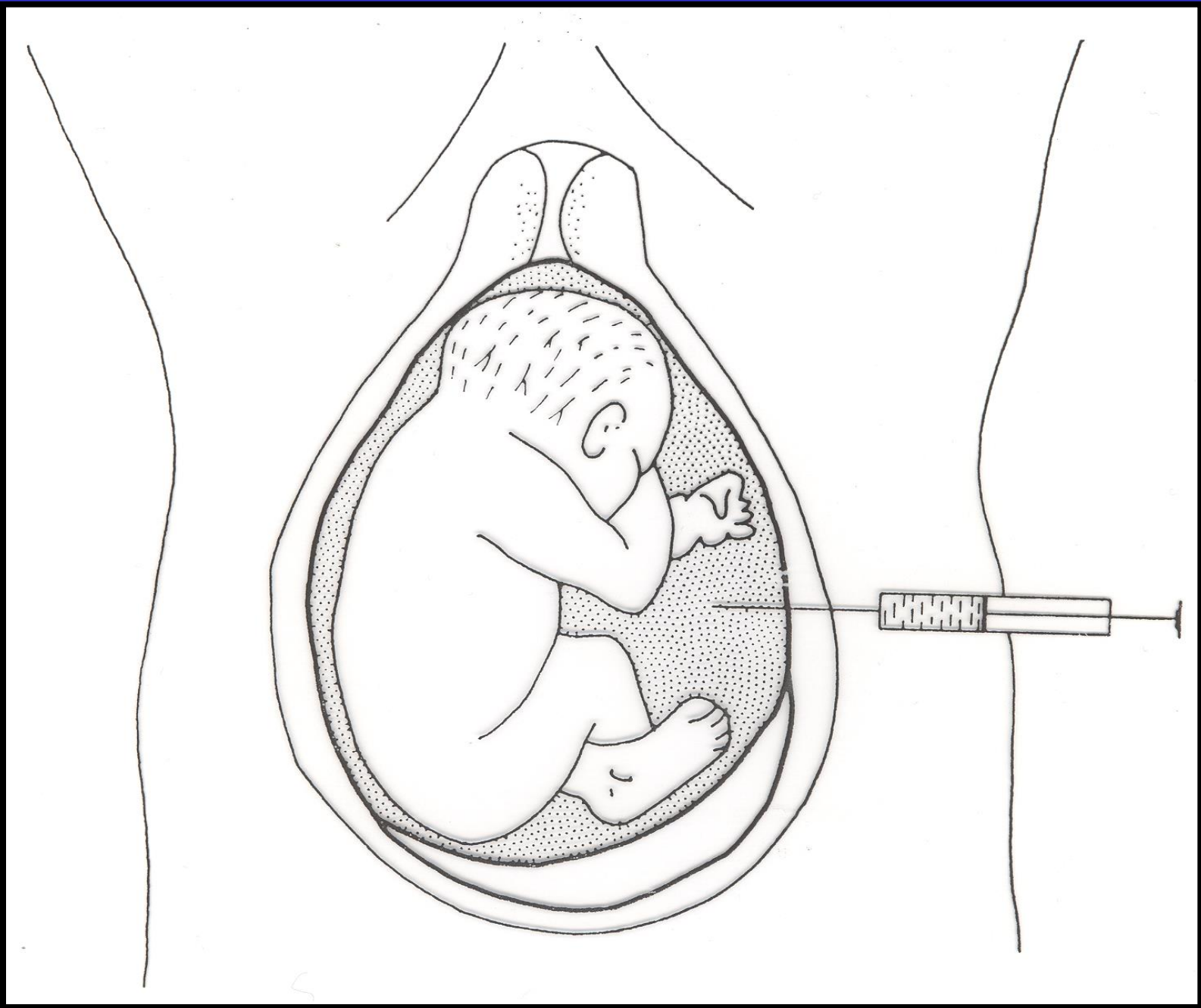
Biochemical analysis

Globin chain synthesis

$\alpha/\beta$  Ratio

$\alpha/\gamma$  Ratio

$\alpha/\delta$  Ratio



# DNA ANALYSIS

- 1. Gene mapping**
- 2. RFLPs linkage analysis**  
**(Restriction fragment length polymorphisms)**
- 3. Oligonucleotide probes**  
**(Using short gene probes 17 – 19 Nucleotide)**
- 4. Gene amplification**  
**(Enzymatic amplification of DNA sequences)**

**DNA polymerase chain reaction technique.**

# MANAGEMENT OF THE THALASSEMIAS

- **Blood Transfusion**
- **Iron chelation therapy**
- **Splenectomy**
- **Hormone replacement**
- **Bone marrow transplantation**
- **Gene therapy**

# **SUMMARY OF RECOMMENDATIONS FOR THE TREATMENT OF THALASSEMIA MAJOR TRANSFUSION**

**Transfusion, in the absence of cardiopathy:**

- **Blood-type the patient completely;**
- **Vaccinate hepatitis B negative patients against hepatitis;**
- **Transfuse when the Hb remains consistently below 8 g/dL, or earlier if there are other indications;**
- **Keep the pretransfusion Hb between 10.5 and 11 g/dL;**
- **Give 10-15 mL/kg of blood preparation in 2 h;**
- **Do not raise the posttransfusion Hb above 16 g/dL;**
- **Choose a 3-4 week transfusion interval.**

# SUMMARY OF RECOMMENDATIONS FOR THE TREATMENT OF THALASSEMIA MAJOR (Continued)

## TRANSFUSION

**Transfusion in the presence of cardiopathy, or when the Hb is less than 5 g/dL:**

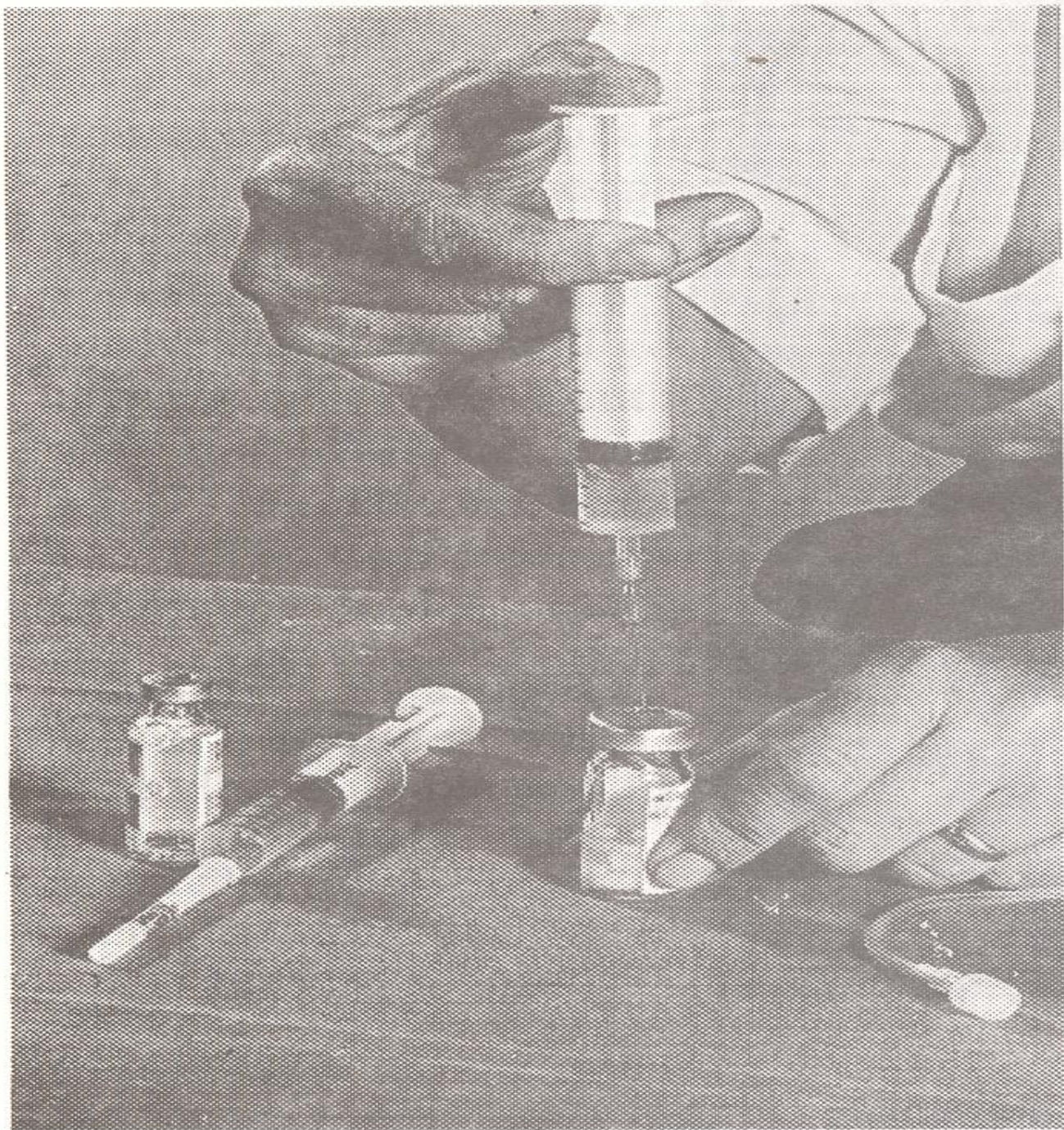
- **Inject furosemide 1-2 mg/kg;**
- **Preferably use fresh blood;**
- **Do not transfuse more than 5 mL/kg of blood;**
- **Do not transfuse faster than 2 mL/kg, or for more than 4 h;**
- **If necessary, divide the blood among 2 or more bags;**
- **Use very short intertransfusion intervals.**

# IRON CHELATION THERAPY

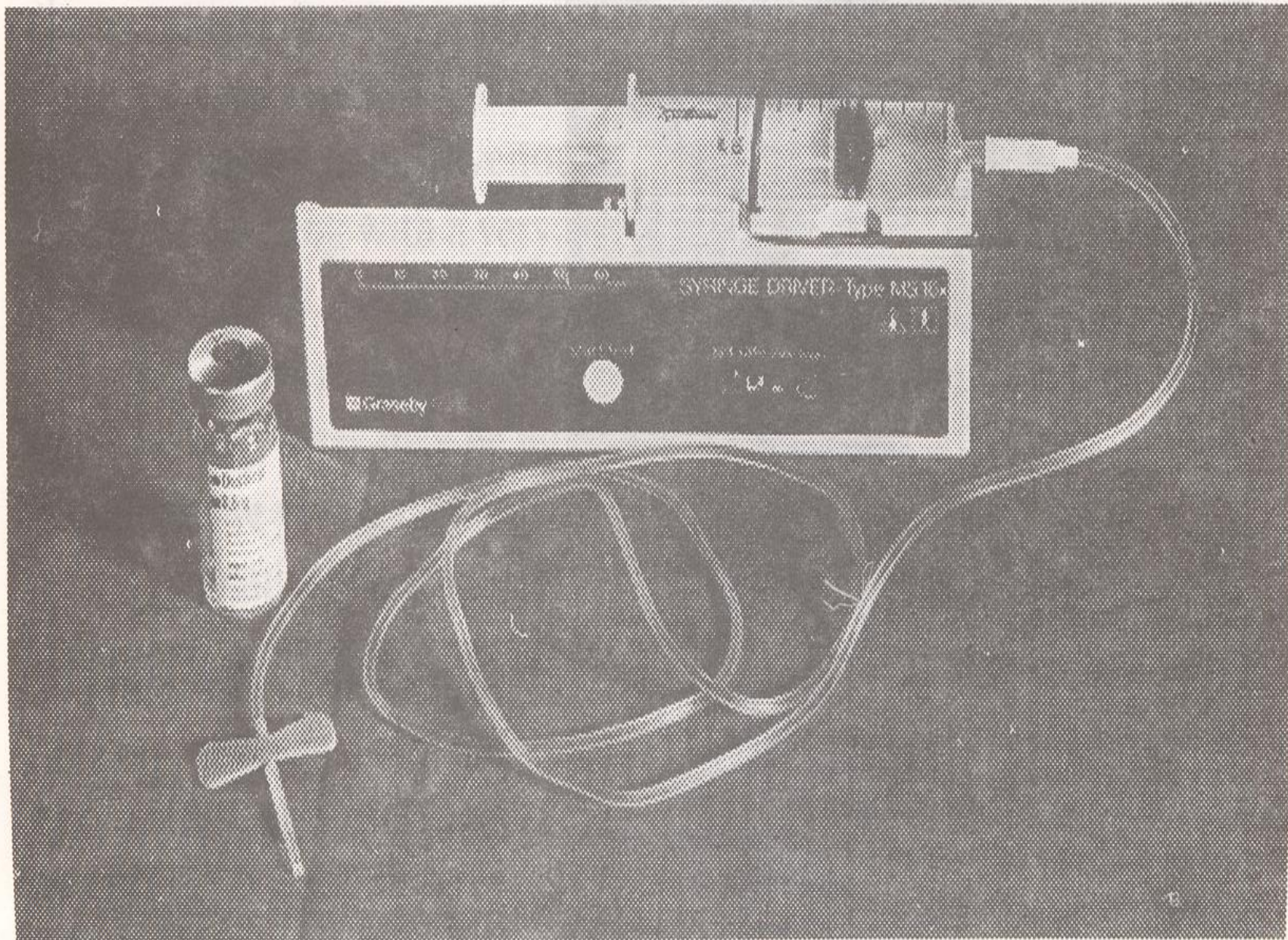
- 1) **Desferrioxamine S.C. 20-60 mg/kg/day in 8 h (average 40 mg/kg/day, or 280 mg/kg/ week).**
- 2) **In selected subjects, give desferrioxamine i.v. in high dose, maximum 100 mg/kg over 8 h, only on the days of transfusion.**

# SPLENECTOMY

- 1) **Is indicated when the blood consumption is more than 1.5 times normal.**
- 2) **Give anti-pneumococcal vaccine to children more than 2 years old prior to splenectomy.**
- 3) **Inform the patients and their family doctors of increased risk of serious infections.**
- 4) **Give prophylactic penicillin, and a platelet anti-aggregant when there is thrombocytosis.**









# ORAL IRON CHELATION THERAPY

---

- **Deferiprone [ Ferriprox ]**
- **Oral Tablet or Syrup 5 to 10 mg /kg /day divided in 3 doses.**
- **More effective than desferoxamine in chelating cardiac iron.**
- **Total iron excretion with deferiprone is less than with desferoxamine.**
- **Major adverse effect especially in children include -  
Gastrointestinal symptoms, joint pain, liver dysfunction,  
neutropenia in 27% of patients.**

## ORAL IRON CHELATION THERAPY (CONTINUE)

---

- ✓ **Deferasirox (EXJADE, NOVARTIS)**
- ✓ **The dose is 20-30 mg/kg/day once daily.**
- ✓ **Approved by FDA.**
- ✓ **Reduction of liver iron to 50%, reduction of serum ferritin to 70% after 1 year treatment.**

### *Side effects:*

- **Nausea, vomiting, diarrhea, abdominal pain, skin rash.**
- **Mid increase in serum creatinine in 30% of patients as with Desferoxamine ocular and auditory disturbance have been reported.**
- **Increase in serum transaminases in 10% of patients.**
- **Reduction of the dose in steps 5-10mg/kg/day every 3-6 months depending on serum ferritin level.**

# Assessment of Iron Stores

- **Serum ferritin**
- **Serum iron and percentage saturation of transferrin (iron-binding capacity)**
- **Bone marrow biopsy (Perl's stain) for reticuloendothelial stores**
- **DNA test for mutation resulting in Cys282 Tyr in the HFE gene**
- **Liver biopsy (parenchymal and reticuloendothelial stores)**
- **Liver CT scan or MRI**
- **Cardiac MRI**
- **Desferrioxamine iron excretion test (chelatable iron)**
- **Repeated phlebotomy until iron deficiency occurs**

# Assessment of tissue damage caused by iron overload

|                  |  |
|------------------|--|
| <b>Cardiac</b>   | <b>Clinical; chest X-ray; ECG; 24-h monitor; echocardiography; radionuclide (MUGA scan to check left ventricular ejection fraction at rest and with stress</b>   |
| <b>Liver</b>     | <b>Liver function tests; liver biopsy; CT scan</b>   |
| <b>Endocrine</b> | <b>Clinical examination (growth and sexual development) glucose tolerance test; pituitary gonadotrophin release tests; thyroid, parathyroid, gonadal, adrenal function, growth hormone assays; radiology for bone age; isotopic bone density study</b> |

---

**CT, computed tomography; ECG, electrocardiography; MRI, magnetic resonance imaging; MUGA, multiple gated acquisition.**

# INVESTIGATIONS AND FOLLOW-UP

- Prior to treatment:** Study the case, and do complete red cell typing.
- Before each transfusion:** Hb, cross-match and red cell antibody detection, serum transaminases (in areas with a high incidence of hepatitis). Record the date of transfusion, net weight and mean hematocrit of the blood preparation, and the Hb of the patient
- After each transfusion:** Measure the posttransfusion Hb.
- Every 3 months:** Measure height and weight
- Every 6 months:** Ferritin estimation.
- Every year:** Evaluate growth and development.  
Calculate the transfusion indices.  
Evaluate iron balance.  
Complete evaluation of the case.
- Variable intervals:** Cardiac and endocrinological investigations according to the clinical state of the patient.

A tropical beach scene at sunset. The sky is a mix of deep blue, purple, and orange, with large, dark clouds. Several palm trees are silhouetted against the sky. The ocean is visible in the background, and the foreground shows a sandy beach. The text "Thank you !!!" is written in a yellow, cursive font across the bottom of the image.

*Thank you !!!*