Clinical cases on hemoglobin electrophoresis technologies

By Youssef Maakaroun, MD

6th International and 11th National Congress on Quality Improvement in Clinical Laboratories
Topics

• Epidemiology of Hemoglobin disorders
• Available Methods used for Hb investigations
• Clinical cases
• Rules for interpretation on CE
Topics

• Epidemiology of Hemoglobin disorders
  • Available Methods used for Hb investigations
  • Clinical cases
  • Rules for interpretation on CE
Births with a pathological hemoglobin disorder per 1,000 live births

Hemoglobinopathies are the most common, autosomal recessive disorder worldwide. 7% of the global population carry an abnormal hemoglobin gene. More than half a million affected children are born each year.

Global Epidemiology of Hemoglobin Disorders

- Around 7% of the global population carries an abnormal hemoglobin gene
- 300,000-500,000 children are born annually with clinically significant hemoglobin disorders
- About 80% of affected children are born in developing countries
- About 30% are born with Thalassemia Syndromes and the rest with Sickle Cell Disease
- 50,000-100,000 children with thalassemia major die each year in low and middle income countries
Thalassaemia distribution in Iran

Hassan Abolghasemi et al 2007,
Thalassaemia and other haemoglobinopathies

The Executive Board,

Having considered the report on thalassaemia and other haemoglobinopathies;¹

Recalling resolution WHA57.13 on genomics and world health, resolution EB117.R3 on sickle-cell anaemia and the recognition by the Executive Board at its 116th session of the role of genetic services in improving health globally and in reducing the global health divide;²

Concerned at the impact of genetic diseases, and of haemoglobinopathies (thalassaemia and sickle-cell anaemia) in particular, on global mortality and morbidity, especially in developing countries, and by the suffering of patients and families affected by the disease;

Recognizing that the prevalence of thalassaemia varies between communities, and that insufficient epidemiological data may hamper effective and equitable management;
Thalassaemia and other Haemoglobinopathies

EB118, May 2006 – Resolution EB118.R1

Urges Member States:

- Implement and reinforce national programs on HB disorders
- Evaluate the impact of national programs
- Intensify the training of all health professionals
- Promote community education
- Promote international cooperation
- Develop and strengthen medical genetic services
- Support basic and applied research

Requests the Director-General:

- provide technical support and advice to national programs
- expand the training and expertise of personnel
- support the further transfer of affordable technologies
- drafting guidelines on prevention and management
- fostering the establishment of regional groups of experts;
- support needed research
Topics

• Epidemiology of Hemoglobin disorders

• Available Methods used for Hb investigations

• Clinical cases

• Rules for interpretation on CE
Separation of Hemoglobin Fractions

• Traditional methods for separation of Hemoglobin fractions include electrophoresis (alkaline and acid), isoelectric focusing (IEF) or chromatography (HPLC).

• Recently Capillary Electrophoresis (CE)
1: β Thal
2: AS
3: AS + F
4: AC

5: AC + F (new born)
6: Hb Bart + F, α thalassemia
7: AE
Isoelectric Focusing - IEF
Comparison of Sebia Capillaries Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies

Dina N. Greene a,*, Amy L. Pyle b, Judy S. Chang a, Carolyn Hoke a, Thomas Lorey a

Fig. 1. Normal hemoglobin analysis by HPLC (A) and CE (B). *Peaks representing post-translationally modified Hb A.
Topics

• Epidemiology of Hemoglobin disorders
• Available Methods used for Hb investigations
• Clinical cases
• Rules for interpretation on CE
Case 1

Clinical context
26-yr old woman, not pregnant

Hematological parameters
- Hb 10 g/dL
- MCV 71 fL

Anemia, microcytosis

Iron balance parameters
- Iron 8 µmol/L (N: 10-30)
- Ferritin 8 µg/L (N: 10-200)

Iron imbalance

Low HbA2 in the presence of anemia and microcytosis. Check iron status

Reduced HbA2 must be rechecked after treatment of iron deficiency
Case 2

**Clinical context**

11-yrs old palestinian child
Exploration of a subclavicular adenopathy

**Hematological parameters**

- WBC 7.71 G/L
- RBC 5.18 T/L
- Hb 9.5 g/dL (11.5 - 15.5)
- Hct 29.6 % (35 - 45)
- MCV 57.1 fL (77 - 95)

**Iron status**

- Iron 15 µmol/L (N: 10-30)
- Ferritin 95 µg/L (N: 10-200)

Elevated HbA2, anemia and microcytosis indicate a beta-thalassemia
Case 3

Clinical context
9 yrs child from Laos with unknown family history

Hematological parameters
- WBC 5.62 G/L
- RBC 5.16 T/L
- Hb 8 g/dL
- Hct 27.9 %
- MCV 54.1 fL
- CCMH 15.5 pg

Iron status
- Iron 18 µmol/L (N: 10-30)
- Ferritin 115 µg/L (N: 10-200)

Presence of Hb H, reduced HbA2, anemia and microcytosis indicate an alpha-thalassemia
Case # 4

**Results**

<table>
<thead>
<tr>
<th>Test</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERY</td>
<td>%</td>
<td>43.2</td>
</tr>
<tr>
<td>HGB</td>
<td>g/L</td>
<td>87</td>
</tr>
<tr>
<td>HCT</td>
<td>12/L</td>
<td>32.9</td>
</tr>
<tr>
<td>MCV</td>
<td>fl</td>
<td>77.2</td>
</tr>
<tr>
<td>MCH</td>
<td>pg</td>
<td>20.1</td>
</tr>
<tr>
<td>MCHC</td>
<td>g/L</td>
<td>29.9</td>
</tr>
<tr>
<td>RDW</td>
<td>%</td>
<td>22.7</td>
</tr>
</tbody>
</table>

**Alpha GPC MAP**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1a</td>
<td>1.0</td>
</tr>
<tr>
<td>A1b</td>
<td>0.7</td>
</tr>
<tr>
<td>Unknown</td>
<td>6.0</td>
</tr>
<tr>
<td>LA1c</td>
<td>0.9</td>
</tr>
<tr>
<td>A1c</td>
<td>4.1</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.4</td>
</tr>
<tr>
<td>P3</td>
<td>2.4</td>
</tr>
<tr>
<td>A0</td>
<td>75.6</td>
</tr>
<tr>
<td>A2</td>
<td>1.1</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Values outside of expected ranges:

- A1c: 4.1%
- A2: 1.1%

**Analysis comments:**

- HbA2 = 3.05%
- HbA2 = 1.36%
Fessas Bodies (Hb Inclusion bodies)

Brilliant Cresyl Blue Stain

NRBC
Case 5

Variant in the S zone, what to do?

The HbX value is higher than 35% (Hb S ~35 to ~40% for heterozygote A/S)

Heterozygous A/S

β6 Glu (negative) → Val (neutral)

Hb X value is higher than 35% (Hb S ~35 to ~40% for heterozygote A/S)

Hypochromic band of Hb S

Hb A

Hb S

Hb A2

Sickle cell test or Itano test positive
Case 6

Variant in the S zone, what to do?

The HbX value is below 35% (Hb S= ~35 to ~ 40% for heterozygote A/S)

Heterozygous A/non S beta variant

Sickle cell test or Itano test negative
- Hb S zone -

Potential variants:
- Hb Arya
- Hb Hasharon (Sinai)
- Hb Dhofar (Yukuhashi)
- Hb Shimonoseki (Hikoshima)
- Hb O-Indonesia (Buginese-X)
- Hb Ottawa (Siam)
- Hb Fort de France
- Hb Montgomery
- Hb G-Copenhagen
- Hb S-Antilles
- Hb Handsworth
- Hb S-Oman (peak 2)
- Hb Hamadan
- Hb Russ
- Hb Stanleyville-II
- "Lombard" Hb A2 variant
- "Tatras" Hb A2 variant
- "Cemenelum" Hb A2 variant
- "Jackson" Hb A2 variant
- "Hopkins-II" Hb A2 variant
- "J-Broussais" Hb A2 variant (alpha 2)
- Denatured Hb O-Arab

Alpha variant (14-19% heterozygote, major fraction)
Beta variant (15% heterozygote)

Alpha variant (15-25% heterozygote, major fraction)

Alpha variant (17% heterozygote, major fraction)

Alpha variant (9-32% heterozygote, major fraction)

Alpha variant (10-15% heterozygote, major fraction)
Beta variant (15-25% heterozygote)

Minor fraction of alpha variant

Database of known mutations for hemoglobin variants:
http://globin.cse.psu.edu
Case 7

Clinical Context
2 yrs old child with sickle cell syndrom under oracillin treatment

Hematological parameters
- WBC 10.48 G/L
- RBC 5.53 T/L
- Hb 10.6 g/dL
- Hct 30.8 %
- MCV 55.7 fL

Biochemical parameters
Ferritin 177 µg/L

Itano test: Positive

Elevated HbA2, Hb S > HbA, anemia and microcytosis indicate S- beta-thalassemia
Hemoglobin variant: β globin chain, heterozygote

α

β

δ

γ

α2β2: HbA

α2β2*: HbA2

α2γ2: HbF

α2δ2: HbA2
Hemoglobin variant: $\beta$ globin chain, homozygote

Absence of HbA + (\(\alpha_2\beta_2\))

\(\alpha_2\beta_2^*\)

\(\alpha_2\gamma_2: \text{HbF}\)

\(\alpha_2\delta_2: \text{HbA2}\)
Hb A + PRESENCE OF A MAJOR VARIANT (Hb S, Hb C, Hb E)

- **Hb A + Hb S**
  - Hb S = ~35 - 40%
  - Hb S < 35%
    - Hb S > Hb A
      - DoubleHeterozygote S/β⁺-thalassemia?
  - Hb S < 35%
    - Hb S < 35%
      - Hb S > Hb A
        - DoubleHeterozygote S/β⁺-thalassemia?

- **Hb A + Hb C**
  - Hb C = ~35 - 40%
  - Hb C < 35%
    - Hb C > Hb A
      - DoubleHeterozygote C/β⁺-thalassemia?
  - Hb C < 35%
    - Hb C > Hb A
      - DoubleHeterozygote C/β⁺-thalassemia?

- **Hb A + Hb E**
  - Hb E = ~25 - 30%
  - Hb E < 25%
    - Hb E > Hb A
      - DoubleHeterozygote E/β⁺-thalassemia?
  - Hb E < 25%
    - Hb E > Hb A
      - DoubleHeterozygote E/β⁺-thalassemia?

Vinatier I. CERBA recommandations (2010)
Case 8

Variant in the D zone, what to do?

The HbX value is below 35% (Hb D= ~ 35 to ~ 40% for heterozygote A/D)

Heterozygous A/non D beta variant

Hb A

Hb X non D
Hemoglobin Analysis

F Concentration = 1.2 %
A2 Concentration = 15.1 %

Values outside of expected ranges.

Analysis comment:

[Graph showing hemoglobin distribution]
Frequency – localization:
Lepore (- Boston – Washington): Found mainly in Italian families, Middle East, Romania, Australia, Mexico...
Characterization:

β et δ chains recombination by crossing over.
Alkaline buffer: Decrease of the total charge → Migration slowed down like Hb S on agarose gel, more anodic than S (D Zone) on Capillarys/Minicap

<table>
<thead>
<tr>
<th>Homozygous form:</th>
<th>Heterozygous form:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb Lepore fraction: about 30%</td>
<td>Hb Lepore fraction: 5 – 15 %</td>
</tr>
<tr>
<td>Clinical signs of homozygous β</td>
<td>Clinical signs of minor β thalasemia</td>
</tr>
<tr>
<td>thalassemia</td>
<td></td>
</tr>
</tbody>
</table>
Case 9

2 beta variant and HbA, what to do?

3 beta globin chains Hb A, Hb S, Hb C but only 2 genes beta on chromosomes

Check for blood transfusion history (suspected double heterozygote S/C). Request another sample before transfusion if transfused patient

Result must not be reported

Heterozygote S/C transfused with normal blood
Very elevated HbA2, what to do?

HbA2 value >8%, suspicion of a variant co-migrating with HbA2

Heterozygous A/0-Arab

Heterozygote A/0-Arab
<table>
<thead>
<tr>
<th>Also Known as</th>
<th>Egypt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematology</td>
<td>Normal in heterozygote; mild anemia in homozygotes</td>
</tr>
<tr>
<td>Agarose</td>
<td>Moves like Hb C</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>Moves close to Hb S at acidic pH</td>
</tr>
<tr>
<td>Function studies</td>
<td>Normal</td>
</tr>
<tr>
<td>Occurrence</td>
<td>Found mainly in Gypsies and in Pomaks (a population group in the Balkan countries) and also in Arabian, Egyptian and black families. Quantity in the heterozygote 30 – 40%; found in combination with Hb S, Hb C, beta-thal and alpha-thal; found in the homozygous condition in Bulgaria and Yugoslavia. Causes severe sickle cell anemia in combination with Hb S</td>
</tr>
</tbody>
</table>
Case 11

Clinical context
30-yrs old asian women

Hematological parameters
- Hb  13.2  g/dL
- MCV  89.5  fL
- MCH  31.1  pg

Normal parameters

Elevated HbA2 in presence of HbE but without anemia and microcytosis


<table>
<thead>
<tr>
<th>Phenotype</th>
<th>HbA2</th>
<th>HbE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygote A/E</td>
<td>3.5%±0.4%</td>
<td>25.6%±1.4%</td>
</tr>
</tbody>
</table>
# Hb A2 with Hb E on CE

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>Hb AE</td>
<td>57</td>
<td>3.50</td>
<td>0.42</td>
<td>2.4-4.3</td>
</tr>
<tr>
<td>Hb EE</td>
<td>5</td>
<td>4.66</td>
<td>NA</td>
<td>3.6-6.1</td>
</tr>
<tr>
<td>Hb Eβ</td>
<td>2</td>
<td>4.9</td>
<td>NA</td>
<td>4.5-5.3</td>
</tr>
</tbody>
</table>

**One patient with E/E and $-^{SEA}/\alpha\alpha = 6.1\%$**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
<th>Mean</th>
<th>+/-</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb AE</td>
<td>85</td>
<td>3.5</td>
<td>0.4</td>
<td>3.1-3.9</td>
</tr>
<tr>
<td>Hb EE</td>
<td>56</td>
<td>4.1</td>
<td>0.8</td>
<td>3.1-4.9</td>
</tr>
<tr>
<td>Hb Eβ</td>
<td>48</td>
<td>4.9</td>
<td>1.6</td>
<td>3.3-6.5</td>
</tr>
</tbody>
</table>

Other example Hb E trait

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.2</td>
<td>0.105</td>
<td>3919</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.4</td>
<td>0.174</td>
<td>9415</td>
</tr>
<tr>
<td>A1a</td>
<td>---</td>
<td>0.4</td>
<td>0.214</td>
<td>9670</td>
</tr>
<tr>
<td>A1b</td>
<td>---</td>
<td>0.6</td>
<td>0.271</td>
<td>13319</td>
</tr>
<tr>
<td>F</td>
<td>1.8</td>
<td>---</td>
<td>0.473</td>
<td>30212</td>
</tr>
<tr>
<td>LA1c</td>
<td>---</td>
<td>0.5</td>
<td>0.735</td>
<td>11865</td>
</tr>
<tr>
<td>A1c</td>
<td>3.9*</td>
<td>---</td>
<td>0.981</td>
<td>27960</td>
</tr>
<tr>
<td>P3</td>
<td>---</td>
<td>4.1</td>
<td>1.534</td>
<td>91831</td>
</tr>
<tr>
<td>A0</td>
<td>---</td>
<td>57.6</td>
<td>1.776</td>
<td>1292986</td>
</tr>
<tr>
<td>A2</td>
<td>33.1*</td>
<td>---</td>
<td>2.888</td>
<td>745402</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.3</td>
<td>3.571</td>
<td>6065</td>
</tr>
</tbody>
</table>

*Values outside of expected ranges

F Concentration = 1.8 %
A1c Concentration = 3.9% *
A2 Concentration = 33.1% *

Analysis comments:

![Graph of Hb E trait analysis](image)
Case 12
Heterozygote delta variant

Clinical context
42-yr-old man

Hematological parameters
- Hb 15.7 g/dL
- MCV 89 fL

Normal parameters

Iron balance parameters
- Iron 19 μmol/L (N: 10-30)
- Ferritin 156 μg/L (N: 30-300)

Normal iron status

Presence of an additional peak in zone Z1
Reduced HbA2 with normal hematological and iron parameters
True HbA2 value = HbA2 value + peak in Z1
Hemoglobin variant: δ globin chain, heterozygote

α

β

δ

γ

α2β2: HbA

α2γ2: HbF

α2δ2: HbA2

α2δ2*
Case 13

Clinical context
38-yr-old man

Hematological parameters
- Hb 15.1 g/dL
- MCV 90 fL

Normal parameters

Iron balance parameters
- Iron 22 µmol/L (N: 10-30)
- Ferritin 180 µg/L (N: 30-300)

Normal iron status

Presence of an additional peak in zone Z1 and reduced HbA2 indicated the presence of delta variant.
HA2 value on ZA2 (Z3) < Delta variant value on Z1 with normal hematological parameters indicated the presence of delta-thalassemia.
Hemoglobin variant: δ globin chain, heterozygote

\[\begin{align*}
\alpha & : \text{HbA} \\
\beta & : \text{HbF} \\
\delta & : \text{HbA2} \\
\gamma & : \text{HbA2*}
\end{align*}\]
Case 14
Heterozygote alpha variant

**Clinical context**
27-yrs old man

**Hematological parameters**
- Hb 15 g/dL
- MCV 87 fL

**Normal parameters**

**Iron balance parameters**
- Iron 27 µmol/L (N: 10-30)
- Ferritin 203 µg/L (N: 30-300)

**Normal iron status**

**Alpha variant**

**Hb A2**

Presence of 2 additional peaks in zones D and Z1
Reduced HbA2 with normal hematological and iron parameters
True HbA2 value = HbA2 value + peak in Z1
Heterozygote α chain globin hemoglobin variant

α

β

δ

γ

α2β2: HbA

α2*β2

α2γ2: HbF

α2*γ2

α2δ2: HbA2

α2*δ2
Search between zones if a similar name is found

### Hb D-Punjab zone

- **Hb Memphis**
- **Hb Leiden**
- **Hb Muravera**
- **Hb D-Bushman**
- **Hb C-Norfolk**
- **Hb S-Oman (peak 1)**
- **Hb Matsue-Oki**
- **Hb Osu Christiansborg**
- **Hb D-Punjab (D-Los Angeles)**
- **Hb C-Waimanalo (Aida)**
- **Hb Muskegon**
- **Hb D-Ibadan**
- **Hb Buenos Aires (minor peak)**
- **Hb Q-India**
- **Hb Lepore (Lepore-BM)**
- **Hb Q-Iran**
- **Hb Sumner Hill**
- **Hb G-Philadelphia**
- **Hb D-Ouled Rabah**
- **Hb Yaizu**

### Z1 zone

- **Hb Sante Ana free alpha chain**
- **Hb Mizuho** (minor peak)
- **Hb delta A2**
- **Hb alpha A2**
- **Hb T-Canindia**
- **Hb San Antonio**
- **Hb Watts**
- **Hb Ferrara**
- **Hb Koln (Ube-1)**
- **Hb Fort Worth**
- **Hb Korle-Bu (G-Accra)**
- **Hb G-Taipe**
- **Hb D-Iran**
- **Hb St. Lulu’s**
- **Hb G-Coushatta (G-Saskatoon)**
- **Hb Inkster**
- **Hb Zurich**
- **Hb G-Pest**
- **Hb Queens (Ogi)**
- **Hb Setif**
- **Hb P-Nilotic**
- **Hb Sunshine Seth**
- **Hb Titusville**

Hb Memphis = Major fraction of an alpha variant

Hb Winchester = Major fraction of an alpha variant

'Memphis' Hb A2 variant = Minor fraction of an alpha variant

'Memphis' Hb A2 variant = Minor fraction of an alpha variant
Case 15

Clinical context
21-yrs old pregnant woman

Hematological parameters
- Hb  13.1 g/dL
- MCV  85.1 fL
- MCH  30.2 pg

Normal parameters

Elevated Hb F due to pregnancy
Cases of increased foetal Hb

1< Hb F <5%
Normal HbA2
MCH >27 pg

Diabetes
Pregnancy
Hyperthyroidism
Chemotherapy
Dyserythropoiesis
Anemia stress

Moderate hereditary persistence of Hb F

5< Hb F <35%
Normal or ↓ HbA2
MCH >27 pg

Heterozygote δβ-thalassemia
Hb F % (5-15%), MCH<27 pg

Heterozygote hereditary persistence of Hb F (HPFH)
Hb F % (15-35%), normal MCH
Case 16

Clinical context
45-yrs old man

Hematological parameters
- Hb 14 g/dL
- MCV 86.2 fL
- MCH 29.2 pg

Normal parameters

Heterozygote hereditary persistance of Hb F (HPFH)
Cases of increased foetal Hb

- **Hb F >35% MCH <27pg**

- **Homozygote hereditary persistance of Hb F**
  - No HbA, HbF 100%, Microcytosis, hypochromia

- **Homozygote \( \delta \beta \)-thalassemia**
  - No HbA, HbF 100%, Anemia, microcytosis

- **Major \( \beta \)-thalassemia**
  - HbA \( \downarrow \downarrow \) or absent
  - Hb F% \( \uparrow \uparrow \), HbA2 \( \uparrow \) or N,
  - Anemia (7< g/dl), microcytosis, hypochromia

- **Composite heterozygote HPFH/\( \beta \)-thalassemia**
  - Hb F% (70%), normal HbA2
  - Anemia, microcytosis

- **Composite heterozygote \( \beta \)-thalassemia/\( \delta \beta \)-thalassemia**
  - Hb F% \( \uparrow \uparrow \), HbA \( \downarrow \downarrow \)
  - Anemia, microcytosis

- **Intermediate \( \beta \)-thalassemia**
  - Hb F% \( \uparrow \), HbA2 \( \uparrow \), HbA \( \downarrow \)
  - Anemia (6-9g/dl), microcytosis
Case 17
Heterozygote Camperdown

Unusual shape of HbA

Normal sample
**Hb Camperdown**

\((\beta_{104} \text{Arg} \rightarrow \text{Ser})\)

<table>
<thead>
<tr>
<th>Hematology</th>
<th>Normal in heterozygote</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis</td>
<td>Moves slightly faster than Hb A on alkalin gel. At acidic pH, moves between Hb A and Hb F, close to Hb F. Moves on HbA on capillary electrophoresis</td>
</tr>
<tr>
<td>Function studies</td>
<td>Normal</td>
</tr>
<tr>
<td>Occurrence</td>
<td>Quantity in the heterozygote about 50%</td>
</tr>
</tbody>
</table>
Case 18

Unstable Hb with multiple fractions

Köln variant
### Hb Köln (β98 Val→Met)

<table>
<thead>
<tr>
<th>Also Known as</th>
<th>San Francisco (Pacific); Ube-1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematology</strong></td>
<td>Mild hemolytic anemia in the heterozygote; reticulocytosis; Heinz body formation</td>
</tr>
<tr>
<td><strong>Agarose Electrophoresis</strong></td>
<td>Moves as a multiple Hb component between Hb A and Hb A2 at alkaline pH</td>
</tr>
<tr>
<td><strong>Function studies</strong></td>
<td>Increased oxygen affinity</td>
</tr>
<tr>
<td><strong>Occurrence</strong></td>
<td>Found in various racial and ethnic groups; It is the most common unstable Hb. Quantity in the heterozygote not accurately determined</td>
</tr>
</tbody>
</table>
Case 19

Presence of an additional peak in zone Z10
Incomplete quantification of Hb A and Hb X peaks
Overestimation of Hb A2 peak

Requantification of Hb A and Hb X peaks using Phoresis manual quantification
Case studies: #20 - Mother

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
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<td>---</td>
<td>0.3</td>
<td>0.102</td>
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<tr>
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<td>15079</td>
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<tr>
<td>A1a</td>
<td>---</td>
<td>1.0</td>
<td>0.215</td>
<td>22212</td>
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<tr>
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<td>1.0</td>
<td>0.269</td>
<td>22424</td>
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<tr>
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<td>---</td>
<td>0.507</td>
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<td>0.8</td>
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<td>1.372</td>
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<td>78.1</td>
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<td>1716507</td>
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<tr>
<td>A2</td>
<td>6.4*</td>
<td>---</td>
<td>2.991</td>
<td>138177</td>
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</table>

*Values outside of expected ranges

F Concentration = 5.7%  
A1c Concentration = 4.8%  
A2 Concentration = 6.4%

Analysis comments:
**Case #20 - Father**

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
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<tr>
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<td>---</td>
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<tr>
<td>A1a</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
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<td>A1b</td>
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<td>0.3</td>
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</table>

*Values outside of expected ranges*

F Concentration = 0.8 %  
A1c Concentration = 4.4 %  
A2 Concentration = 21.8%  

Analysis comments:

---

**Graph and Diagram**

---
### Genotyping

<table>
<thead>
<tr>
<th></th>
<th>Mom: $\beta^N$</th>
<th>$\beta^0$</th>
<th>$\alpha\alpha$</th>
<th>$\alpha\alpha$</th>
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<tbody>
<tr>
<td>Dad:</td>
<td>$\beta^N$</td>
<td>$\beta^N$</td>
<td>$\alpha\alpha$</td>
<td>$\alpha\alpha$</td>
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<tr>
<td>$\beta^N$</td>
<td>$\beta^N$</td>
<td>$\beta^0$</td>
<td>$\alpha\alpha$</td>
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<tr>
<td>$\beta^E$</td>
<td>$\beta^N$</td>
<td>$\beta^E$</td>
<td>$\alpha^{cs}\alpha$</td>
<td>$\alpha^{cs}\alpha$</td>
</tr>
</tbody>
</table>

### Worst case

- $\beta^E / \beta^0$
- $\alpha^{cs}\alpha / \alpha\alpha$
Case studies: #20-son

1:1 MIX IS NEXT SAMPLE (#16); FOR BJORAD; 2 peaks coming off after A2 peak
AF control
Pedigree

Mother

Father

Aunt

Further DNA investigation
Case studies: #21

Capillary Electrophoresis  Gel Electrophoresis
**CBC**

**MIXING (1 Vol. sample + 1 Vol. Hb A2 NC)**

---

**CBC**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Unit</th>
<th>Normal Range</th>
<th>Differential</th>
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<tbody>
<tr>
<td>R.B.C</td>
<td>4.94</td>
<td>x10^6/mm^3</td>
<td>4.2 - 5.4</td>
<td>Monocyte 4%</td>
</tr>
<tr>
<td>W.B.C</td>
<td>0.17</td>
<td>x10^9/l</td>
<td>4000 - 11000</td>
<td>Lymphocyte 32%</td>
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<tr>
<td>Hemoglobin</td>
<td>13.2</td>
<td>g/dL</td>
<td></td>
<td>Neutrophils 62%</td>
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<tr>
<td>Hematocrit</td>
<td>38.9</td>
<td>%</td>
<td>36 - 46</td>
<td></td>
</tr>
<tr>
<td>M.C.V</td>
<td>78.8</td>
<td>fl</td>
<td>80 - 97</td>
<td></td>
</tr>
<tr>
<td>M.C.H</td>
<td>26.7</td>
<td>pg</td>
<td>27 - 32</td>
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<tr>
<td>M.C.H.C</td>
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<td>g/dL</td>
<td>32 - 36</td>
<td></td>
</tr>
<tr>
<td>R.D.W</td>
<td>12</td>
<td>%</td>
<td>11 - 14.7</td>
<td></td>
</tr>
<tr>
<td>MPV</td>
<td>9.2</td>
<td>fl</td>
<td>7-10</td>
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</tr>
<tr>
<td>Reticulocyte</td>
<td>0.6</td>
<td>%</td>
<td>Adult 0.5-1.5%</td>
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</tr>
<tr>
<td>Platelets</td>
<td>183</td>
<td>10^3/mm^3</td>
<td>140-450 *1000</td>
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<tr>
<td>Hb elect (HPLC)</td>
<td>-</td>
<td></td>
<td>&lt;2</td>
<td></td>
</tr>
<tr>
<td>Hb A1</td>
<td>-</td>
<td>%</td>
<td>94.5 - 98</td>
<td></td>
</tr>
<tr>
<td>Hb A2</td>
<td>-</td>
<td>%</td>
<td>&lt;3.5 Normal</td>
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**Hemoglobin Electrophoresis**

<table>
<thead>
<tr>
<th>Fractions</th>
<th>%</th>
<th>Ref. %</th>
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<tr>
<td>Z9</td>
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</tr>
<tr>
<td>Z6</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>Z3</td>
<td>5.2</td>
<td></td>
</tr>
</tbody>
</table>

---

**Address:** Sari, Keshavarz Blvd  
**Tel:** 3236691

---

**ازامایشات خونی مختصری - ماهدی میلادی - بررسی علائم خونی آزمایشات بالینی آزمایشات مشترک و مشاوره‌های علمی شما با این موسسه در بهبود کیفیت آزمایش پیام مورا است.**

**Signature:**

[Footer details not legible]
FATHER

MOTHER
double mutation with beta variant and alpha variant
## Case studies: # 22

### Table

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
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<tbody>
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<td>0.3</td>
<td>0.110</td>
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<td>9.6</td>
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<td>A2</td>
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<td>3.022</td>
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</tbody>
</table>

*Values outside of expected ranges

1896642

F Concentration = %
A1c Concentration = %
A2 Concentration = 5.0% %

### Analysis comments:

![Graph](image.png)
Case # 22: Mother and Father
Case # 22 : Sister
β–Thalassemia Intermedia

- Requires ongoing transfusion therapy
- At risk for Iron overload
Beta-thalassemia

- Heterozygote $\beta^+$ Thal (Silent $\beta$ Thal)
- Homozygote $\beta^+$ Thal (Intermediate $\beta$ Thal)
- Heterozygote $\beta^+ / \beta^0$ Thal (Intermediate $\beta$ Thal)
- Homozygote $\beta^0$ Thal (Major $\beta$ Thal)
- Heterozygote $\beta^0$ Thal (Minor $\beta$ Thal)
- Deletion
- Reduced synthesis
Case studies: # 23

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>--</td>
<td>0.1</td>
<td>0.101</td>
<td>4097</td>
</tr>
<tr>
<td>A1a</td>
<td>--</td>
<td>0.7</td>
<td>0.176</td>
<td>21737</td>
</tr>
<tr>
<td>A1b</td>
<td>--</td>
<td>0.6</td>
<td>0.212</td>
<td>18545</td>
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<tr>
<td>Unknown</td>
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<td>0.7</td>
<td>0.264</td>
<td>20172</td>
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<tr>
<td>F</td>
<td>3.4*</td>
<td>--</td>
<td>0.492</td>
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<td>LA1c</td>
<td>--</td>
<td>1.2</td>
<td>0.727</td>
<td>35566</td>
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<tr>
<td>A1c</td>
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<td>0.930</td>
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<td>3.001</td>
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<td>C</td>
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<td>0.1</td>
<td>4.428</td>
<td>4217</td>
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</table>

*Values outside of expected ranges*

F Concentration = 3.4%  
A1c Concentration = 5.0%  
A2 Concentration = 2.6%

Analysis comments:
Case studies: # 24

Potential variants:
- Hb Barta
- Hb J-Providence
- Hb J-Breuissa
- Hb J-Terente
- Hb J-Hong Kong (J-Bangkok)
- Hb J-Mexico
- Hb J-Baltimore
Hemoglobin variant: $\alpha$ globin chain, heterozygote

$\alpha$ chain:
- $\alpha_{2} \beta_{2}$: HbA
- $\alpha_{2}^{*} \beta_{2}$

$\beta$ chain:
- $\alpha_{2} \gamma_{2}$: HbF
- $\alpha_{2}^{*} \gamma_{2}$

$\delta$ chain:
- $\alpha_{2} \delta_{2}$: HbA2
- $\alpha_{2}^{*} \delta_{2}$

$\gamma$ chain:
### Zone 12 – β Variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Zone 1</th>
<th>Zone 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb J-Baltimore</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>81</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>82</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Hb J-Bankok</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Hb K-Ibadan</td>
<td>81</td>
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</tr>
</tbody>
</table>

### Zone 12 – α Variants

<table>
<thead>
<tr>
<th>Variant</th>
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<th>Zone 2</th>
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</thead>
<tbody>
<tr>
<td>Hb Suresnes</td>
<td>98</td>
<td>204</td>
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</table>

Other Hb J

<table>
<thead>
<tr>
<th>Variant</th>
<th>Zone 1</th>
<th>Zone 2</th>
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<tbody>
<tr>
<td>Hb J-Kaohsiung</td>
<td>121</td>
<td>J2 219</td>
</tr>
<tr>
<td>Hb J-Rovigo</td>
<td>68</td>
<td>J2 195</td>
</tr>
</tbody>
</table>
Case studies: #25

Sample No. 32

Fraction values:
- Hb A: 32.1%
- Hb F: 18.1%
- Hb S: 47.5%
- Hb A2: 2.3%

Minimum mode:
- O.D. Max: 0.155
- Peak No.: 2
- Rack No.: 2
- Read time: 10:42

Peaks:
- Hb A: 32.1%
- Hb F: 18.1%
- Hb S: 47.5%
- Hb A2: 2.3%

Attached Card:
- Pathological
- View

Comments:
- THIS IS A 1:1 MIX
Hb D Range = 205-209
Case studies # 26: Baby JT

• Baby boy born at 38 weeks gestational age
• Parents from Vietnam, no consanguinity
• Both parents were carriers for alpha thalassemia trait
  – 27 year old mother
  – 34 year old father
<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
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</thead>
<tbody>
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<tr>
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<td>A1b</td>
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<td>1.4</td>
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<td>15649</td>
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<td>4.415</td>
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*Values outside of expected ranges

<table>
<thead>
<tr>
<th>F Concentration</th>
<th>0.5 %</th>
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<tbody>
<tr>
<td>A1c Concentration</td>
<td>6.1 %</td>
</tr>
<tr>
<td>A2 Concentration</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

Analysis comments:

Hemoglobin Electrophoresis

<table>
<thead>
<tr>
<th>Fraction</th>
<th>%</th>
<th>Ref %</th>
</tr>
</thead>
<tbody>
<tr>
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<td>2.1</td>
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</tr>
<tr>
<td>Hb Barts</td>
<td>55.2</td>
<td></td>
</tr>
<tr>
<td>Hb A</td>
<td>37.0</td>
<td></td>
</tr>
<tr>
<td>Hb F</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Hb A2</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

Comments

Hb X @261 Barts @106
Single cell with H inclusion bodies:
Can be seen in any form of alpha thalassemia

“Hemoglobin H” disease
HPLC

Hb Bart’s
Hb H

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
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<tbody>
<tr>
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<td>89.5</td>
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<td>---</td>
<td>2.6</td>
<td>0.353</td>
<td>53574</td>
</tr>
</tbody>
</table>

Total Area: 2079554

F Concentration =
A1c Concentration =
A2 Concentration =

Analysis comments:
Baby JT’s Older Sibling

- Ultrasound at 19 weeks GA showed unexplained fetal hydrops and limb defects
- Mother presented at 28 weeks in labour.
- Baby delivered by cesarean section
- Severely hydropic, died at 3 hours of age
- Homozygous alpha thalassemia diagnosed on autopsy
Baby JT’s Prenatal Counselling

• After first pregnancy, parents followed by...
  – Genetics Clinic at McMaster University Medical Centre referred to High Risk Obstetrics at Mount Sinai Hospital

• Both parents found to carry the Southeast Asian gene deletion (-^{SEA}/\alpha\alpha)
  – 1 in 4 of normal baby (\alpha\alpha/\alpha\alpha)
  – 2 in 4 chance baby carrier of deletion (-^{SEA}/\alpha\alpha)
  – 1 in 4 chance that baby has homozygous alpha thalassemia (-^{SEA}/-^{SEA})
Baby JT’s Pedigree
Case studies # 27

HPLC seems to split Constant Spring into separate subspecies

Heterozygote Constant Spring
Case studies # 28

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (Min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>0.3</td>
<td>1.97</td>
<td>5878</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>1.27</td>
<td>4690</td>
<td></td>
</tr>
<tr>
<td>Fx</td>
<td>---</td>
<td>1.36</td>
<td>93196</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>1.54</td>
<td>6050</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>2.1</td>
<td>1.75</td>
<td>42160</td>
<td></td>
</tr>
<tr>
<td>A0</td>
<td>92.3</td>
<td>2.45</td>
<td>1453166</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>1.7</td>
<td>3.68</td>
<td>42088</td>
<td></td>
</tr>
<tr>
<td>C-window</td>
<td>1.1</td>
<td>5.00</td>
<td>22911</td>
<td></td>
</tr>
</tbody>
</table>

Total Area: 2,115,146

F Concentration = 0.3 %
A2 Concentration = 1.7 %

*Values outside of expected ranges

Analysis comments:

Haemoglobin Electrophoresis

<table>
<thead>
<tr>
<th>Name</th>
<th>%</th>
<th>Normal Values %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb H</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Hb Barts</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Hb A</td>
<td>88.6</td>
<td></td>
</tr>
<tr>
<td>Hb A2</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Constant Spring</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

Hb H/ Constant Spring disease
Table II. Concentrations of Hb Bart’s, Hb H, and Hb CS levels measured by CE in various phenotypes of thalassemia

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Number</th>
<th>Hb Bart’s (%)/undetected number</th>
<th>Hb H (%)/undetected number</th>
<th>Hb CS (%)/undetected number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb CS trait</td>
<td>21</td>
<td>–</td>
<td>–</td>
<td>0.52 ± 0.52 / 4</td>
</tr>
<tr>
<td>Hb CS homozygote</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>3.5 ± 2.5 / 0</td>
</tr>
<tr>
<td>Hb H-CS</td>
<td>9</td>
<td>4.2 ± 4.1 / 3</td>
<td>11.3 ± 6.5 / 3</td>
<td>2.6 ± 1.4 / 1</td>
</tr>
<tr>
<td>Hb H</td>
<td>26</td>
<td>1.1 ± 0.7 / 14</td>
<td>6.7 ± 4.8 / 0</td>
<td>–</td>
</tr>
</tbody>
</table>
Guangdong Province- South China

**Screening for Hb Constant Spring in South China**

Hb Constant Spring (Hb CS; $\alpha$142, TAA $\rightarrow$ CAA in $\alpha_2$) is a variant characterized by an elongated $\alpha$ globin chain of 31 amino acids due to a mutation that alters the mRNA termination codon. Individuals with Hb H disease caused by Hb CS and $\alpha^0$-thalassemia ($\alpha^\theta$-thal) deletional defects ($\alpha^\theta$/$\alpha^\theta$/--) have a more severe disease than those with three deleted $\alpha$-globin genes ($\alpha$--/--/--) (1,2). Therefore, it is important to screen for Hb CS in areas.

Out of a total of 23,842 individuals, Hb CS was detected in 71 cases (0.3%); of these, only one was a compound heterozygote for Hb CS and $\alpha^0$-thal ($\alpha^\theta$/$\alpha^\theta$/--). The values of Hb, MCV, and MCH in the 70 carriers of Hb CS were 11.6 ± 1.3 g/dL, 79.2 ± 2.2 fl, and 26.5 ± 0.6 pg, respectively. The levels of Hb CS in the 70 heterozygotes ranged from 0.1–1.0%, with an average of 0.6 ± 0.1% (Figure 1); whereas the percentage of Hb CS in the one case in association with $\alpha^0$-thal was 22%. All of the Hb CS carriers were confirmed by molecular analysis using DNA sequencing. Four partners of the 71 individuals who were Hb CS heterozygotes were $\alpha^0$-thal carriers, making these couples at-risk of conceiving fetuses with Hb H-Hb CS. Prenatal four couples. Indeed, two pregnancies were affected, and were terminated at the parents’ request.

**FIGURE 1** Representative chromatogram of Hb Constant Spring (Hb CS) with a Sebia capillary 2 pattern of a Hb CS trait patient. A: Hb CS = 0.1%; B: Hb CS = 1.0%.
Topics

• Epidemiology of Hemoglobin disorders
• Available Methods used for Hb investigations
• Clinical cases
• Rules for interpretation on CE
Information required for interpretation

- Hemoglobin electrophoresis profile must always be associated with other essential information to consider the clinical context in case of thalassemias, elevated HbF or hemoglobin variants:
  - Hematological parameters (Hb, median cell volume (MCV), mean corpuscular hemoglobin (MCH)....) to relate anemia, microcytosis or hypochromia
  - Iron balance (in particular for reduced Hb A2 due to iron deficiency)
  - Patient’s age, geographical origin and any possible family history or transfusion

<table>
<thead>
<tr>
<th>Age</th>
<th>Hb (g/dl)</th>
<th>RBC (T/l)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child 2-6yrs</td>
<td>12.2 ± 0.7</td>
<td>4.67 ± 0.3</td>
<td>77.6 ± 3.3</td>
<td>26.3 ± 1.3</td>
</tr>
<tr>
<td>Child 6-12yrs</td>
<td>12.7 ± 0.8</td>
<td>4.68 ± 0.3</td>
<td>80.4 ± 3.4</td>
<td>27.3 ± 1.3</td>
</tr>
<tr>
<td>Child 12-16yrs</td>
<td>13.5 ± 1.1</td>
<td>4.74 ± 0.4</td>
<td>83.8 ± 4</td>
<td>29.2 ± 1.5</td>
</tr>
<tr>
<td>Adult woman</td>
<td>11.5-15</td>
<td>4-5</td>
<td>82-98</td>
<td>27-32</td>
</tr>
<tr>
<td>Adult man</td>
<td>13-17</td>
<td>4.5-5.5</td>
<td>82-98</td>
<td>27-32</td>
</tr>
</tbody>
</table>
Prevention of Hemoglobin disorders

**Screening tests**
- MCV
- OF/DCIP
- R/O Iron deficiency Anemia

**GOAL:**
- Avoid marriages between carriers
- Monitoring High risk Pregnancies
- Keeping National Thalassemia Registry

**Confirmation**
- Hb electrophoresis
- Genotyping

[Diagram of prevention cycle]

- Health Education and Public Awareness
- Genetic Counselling
- Screening
- Management of Patients
Thank you for your attention...