Case Report

Compound heterozygote of Hb DIran [HBB: c.67G>C, β 22(B4) Glu>Gln] with β0-thalassemia [cds 41/42 (-CTTT)] from Eastern India

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A R T I C L E  I N F O

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Introduction

Hereditary hemoglobinopathies, the most common monogenic hemoglobin (Hb) disorders, result in a variety of clinical consequences. It has been observed that various Hb variants and thalassemias are found common to specific ethnic groups and regions. Hb DIran is a structural Hb variant resulting from the substitution of glutamine with glutamate at codon 22 (GAA>CAA, Glu>Gln) of the beta globin gene. This Hb variant was first reported by Rahbar in 1973 in a family from the central part of Iran.1 A deletion of four bases in codon 41/42 (-CTTT) is a rare β0-thalassemia mutation reported in India with a prevalence of 3–15%.2 The present report describes a rare combination of these two mutations for the first time in India.

Case report

A 45-year-old Sikh female from Sundergarh district of Odisha, India with a family history of β-thalassemia attended the Sickle Cell Institute, VIMSAR, Burla to screen her status. She was asymptomatic and had no history of blood transfusion or vaso-occlusive crisis. Ultrasonographic examination revealed normal spleen and liver. The various investigations of the proband and her daughter, including a complete blood count and biochemistry, are shown in Table 1. As evident, the index case had features suggestive of microcytic hypochromic anemia (mean corpuscular volume: 58.7 fl and mean corpuscular hemoglobin: 17.8 pg). An iron profile study indicated possible iron overload [iron 5.027 mg/dL (reference range – RR: 0.005–0.175 mg/dL); ferritin: 138.7 µg/L (RR: 20–200 µg/L) and transferrin: 490.05 mg/dL (RR: 212–360 mg/dL)].
Because of the endemcity of the sickle cell hemoglobinopathy and its combination with $\beta$-thalassemia in this region, the sickling test and alkaline agarose gel Hb electrophoresis were performed; the sickling test was negative and a single band in the Hb S/D position was observed by Hb electrophoresis (pH-8.6). Cation exchange high performance liquid chromatography (CE-HPLC) was performed using the VARIANT-II hemoglobin testing system (Bi-Rad Laboratories, Hercules, CA, USA) which showed a prominent peak in the Hb A$_2$ window (3.27–3.83 as per the manufacturer’s guide lines in the operating software): Hb Deer Lodge, Hb Lepore, Hb D$^{\text{Iran}}$, Hb E-Saskatoon, Hb G-Coushatta, Hb D-Granada, Hb G-Taipei and Hb Bury) and one $\beta^0$-thalassemia mutation [Codon 22 (G>T); GAA(Glu)>T AA (stop codon)] have been reported involving this codon. In Hb D$^{\text{Iran}}$, the change of glutamate to glutamine leads to an overall change of charge from negative to positive resulting in a protein that migrates to the position of Hb S in alkaline Hb electrophoresis.\(^6\)\(^{-10}\) This rare variant has heat stability with no effect on oxygen equilibrium, intracellular 2,3-diphosphoglycerate or the Bohr effect.\(^10\) The homozygous state of Hb D$^{\text{Iran}}$ reveals a milder phenotype even when Hb D$^{\text{Iran}}$ co-inherits with $\beta^0$-thalassemia.\(^5\)\(^{-9}\) The present case agrees with this as evidence from the clinical and hematological investigations show. Although Hb D$^{\text{Iran}}$ in combination with $\beta$-thalassemia produces a moderate microcytic and hypochromic red cell picture that is not transfusion dependent, the appearance of Hb D$^{\text{Iran}}$ in the position of Hb S in alkaline agarose gel electrophoresis can lead to significant confusion and might falsely be reported as a sickle cell hemoglobinopathy unless a sickling test and HPLC are read together with these findings. Hb S can easily be distinguished from Hb D$^{\text{Iran}}$ by performing CE-HPLC.

Reportedly in CE-HPLC, nine abnormal Hbs elute in the Hb A$_2$ window (3.27–3.83 as per the manufacturer’s guidelines in the operating software): Hb Deer Lodge, Hb Lepore, Hb D$^{\text{Iran}}$, Hb E, Hb Hamadan, Hb Osu-Christiansborg, Hb Tianshu, Hb G Honolulu and Hb G Copenhagen. Among these, Hb Deer Lodge, Hb Lepore and Hb D$^{\text{Iran}}$ elute prior to the standard RT of Hb A$_2$ (3.6 min) while others have higher RT to that of Hb A$_2$. Interestingly, Hb Lepore has the lowest average quantity (7–15%) followed by Hb G Honolulu (about 15% of total hemoglobin quantity) and Hb E (about 30% of total hemoglobin in absence of $\alpha$-thalassemias). All the other variants eluting in the Hb A$_2$ window have variant hemoglobin quantities higher than 30% on average under heterozygous conditions, making it difficult to distinguish in HPLC. Amongst these, Hb D$^{\text{Iran}}$ has been reported to elute in this window at

### Table 1 – Hematological and biochemical indices of proband and her daughter.

<table>
<thead>
<tr>
<th></th>
<th>Unit (SI)</th>
<th>Proband</th>
<th>Daughter</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count</td>
<td>×10$^9$/L</td>
<td>7.4</td>
<td>6.9</td>
</tr>
<tr>
<td>Red blood cell count</td>
<td>×10$^12$/L</td>
<td>5.67</td>
<td>5.07</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>g/L</td>
<td>10.1</td>
<td>9.9</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>%</td>
<td>33.3</td>
<td>33.8</td>
</tr>
<tr>
<td>Mean corpuscular volume</td>
<td>fl</td>
<td>58.7</td>
<td>66.7</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin</td>
<td>Pg</td>
<td>17.8</td>
<td>19.5</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration</td>
<td>g/dL</td>
<td>30.3</td>
<td>29.3</td>
</tr>
<tr>
<td>Platelet count</td>
<td>×10$^9$/L</td>
<td>169</td>
<td>171</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>µmol/L</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Aspartate transaminase</td>
<td>U/L</td>
<td>12.7</td>
<td>15.3</td>
</tr>
<tr>
<td>Alanine transaminase</td>
<td>U/L</td>
<td>12.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>µmol/L</td>
<td>0.03</td>
<td>0.34</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>U/L</td>
<td>198</td>
<td>189</td>
</tr>
<tr>
<td>Iron</td>
<td>µmol/L</td>
<td>5.027</td>
<td>5.258</td>
</tr>
<tr>
<td>Transferrin</td>
<td>g/L</td>
<td>490.05</td>
<td>462.09</td>
</tr>
<tr>
<td>Ferritin</td>
<td>pmol/L</td>
<td>138.7</td>
<td>111.6</td>
</tr>
</tbody>
</table>

**Discussion**

The Hb D$^{\text{Iran}}$ trait and homozygous cases have been reported earlier.\(^4\)\(^{-5}\) However, few studies have reported compound heterozygotes of Hb D$^{\text{Iran}}$ with other Hb variants like Hb S and Hb $\beta^0$-Punjab, $\beta^+$/thalassemia IVS1–5 (G>C), $\beta^0$-thalassemia (619 bp-deletion) and undefined $\beta$-thalassemia from India and Pakistan. Various studies have reported that the quantity of Hb D$^{\text{Iran}}$ eluting in the Hb A$_2$ window in HPLC varies from 36.0 to 47.7% in a heterozygous condition, while in compound heterozygous states, the quantity varies between 47.3 and 94.4% (with Hb D$^{\text{Punjab}}$, Hb S, $\beta$-thalassemia with the 619 bp deletion mutation and beta thalassemia with unknown mutation).\(^6\)\(^{-10}\) Almost all these cases were mild in presentation with concomitant anemia.

Codon 22 (GAA), is a mutational hotspot in exon I of the human $\beta$-globin gene, although it does not take part in $\alpha$-$\beta$ or protein-heme interactions, as this is an external residue. This change, however, results in a large shift in the charge distribution of the protein from negative to positive, which exhibits a small effect on the $\alpha$-$\beta$ interactions. However, in the context of the C-terminal region, this change results in an overall change of charge from negative to positive resulting in a protein that migrates to the position of Hb S in alkaline Hb electrophoresis.\(^1\)\(^{-10}\) The homozygous state of Hb D$^{\text{Iran}}$ reveals a milder phenotype even when Hb D$^{\text{Iran}}$ co-inherits with $\beta^0$-thalassemia.\(^5\)\(^{-9}\) The present case agrees with this as evidence from the clinical and hematological investigations show. Although Hb D$^{\text{Iran}}$ in combination with $\beta$-thalassemia produces a moderate microcytic and hypochromic red cell picture that is not transfusion dependent, the appearance of Hb D$^{\text{Iran}}$ in the position of Hb S in alkaline agarose gel electrophoresis can lead to significant confusion and might falsely be reported as a sickle cell hemoglobinopathy unless a sickling test and HPLC are read together with these findings. Hb S can easily be distinguished from Hb D$^{\text{Iran}}$ by performing CE-HPLC.
**Peak name** | **Calibrated area %** | **Area %** | **Retention time (min)** | **Peak area**
--- | --- | --- | --- | ---
Unknown | --- | 0.3 | 0.96 | 6736
F | 1.0 | --- | 1.09 | 17882
Unknown | --- | 0.8 | 1.60 | 16390
P3 | --- | 4.0 | 1.73 | 78869
A0 | --- | 4.6 | 2.20 | 90859
Unknown | --- | 1.0 | 2.50 | 20501
A2 | 82.8* | --- | 3.57 | 1740824

Total area: 1,972,061

F concentration = 1.0 %
A2 concentration = 82.8* %

*Values outside of expected ranges

Analysis comments:

![Figure 1 – CE-HPLC showing characteristic peak of HbD_{Iran}/β° thal [cds 41/42 (-CTTT)].](image)

Further, as Hb D_{Iran} elutes in the Hb A₂ window in HPLC masking elevated Hb A₂, it becomes difficult to suspect the presence of β-thalassemia and direct gene sequencing needs to be performed. To the best of our knowledge, this is the first report of Hb D_{Iran} with β°-thalassemia [cds 41/42 (-CTTT)] reported from Odisha, India.

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**Conflicts of interest**

The authors declare no conflicts of interest.

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REFERENCES