Case Report

Compound heterozygote of Hb D\textsubscript{Iran} [HBB: c.67G>C, \(\beta\) 22(B4) Glu>Gln] with \(\beta^0\)-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 D\textsubscript{Iran} 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.\textsuperscript{1} A deletion of four bases in codon 41/42 (-CTTT) is a rare \(\beta^0\)-thalassemia mutation reported in India with a prevalence of 3–15\%.\textsuperscript{7} 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 \(\beta\)-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 endemicity of the sickle cell hemoglobinopathy and its combination with β-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 with low Hb A<sub>0</sub> and Hb F peaks (4.6% and 1.0%, respectively) (Figure 1). The possibility of homozygous Hb E was ruled out by the absence of a band in the position of Hb S in alkaline Hb electrophoresis. The presence of the characteristic of the present case was different from that of Hb Tianshui, Hb G Honolulu and Hb G Copenhagen. Among these, Hb D<sub>Iran</sub>-thalassemia (619 bp-deletion) and undefined β-thalassemia from India and Pakistan. Various studies have reported that the quantity of Hb D<sup>Iran</sup> eluting in the Hb A<sub>2</sub> 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<sup>Punjab</sup>, Hb S, β-thalassemia with the 619 bp deletion mutation and beta thalassemia with unknown mutation).<sup>6–10</sup>

Almost all these cases were mild in presentation with concomitant anemia.

Codon 22 (GAA), is a mutational hotspot in exon I of the human β globin gene, although it does not take part in α-β or protein-heme interactions, as this is an external residue positioned at the B4 site of the helix. To date, six Hb variants (Hb D<sup>Iran</sup>, Hb E-Saskatoon, Hb G-Coushatta, Hb D-Granada, Hb G-Taipei and Hb Bury) and one β<sup>0</sup>-thalassemia mutation [Codon 22 (G>T); GAA(Glu)>T AA (stop codon)] have been reported involving this codon. In Hb D<sup>Iran</sup>, 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.<sup>1,10</sup>

This rare variant has heat stability with no effect on oxygen equilibrium, intracellular 2,3-diphosphoglycerate or the Bohr effect.<sup>10</sup> The homozygous state of Hb D<sup>Iran</sup> reveals a milder phenotype even when Hb D<sup>Iran</sup> co-inherits with β<sup>0</sup>-thalassemia.<sup>5,9</sup>

The present case agrees with this as evidence from the clinical and hematological investigations show. Although Hb D<sup>Iran</sup> in combination with β-thalassemia produces a moderate microcytic and hypochromic red cell picture that is not transfusion dependent, the appearance of Hb D<sup>Iran</sup> 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<sup>Iran</sup> by performing CE-HPLC.

Reportedly in CE-HPLC, nine abnormal Hbs elute in the Hb A<sub>2</sub> window (3.27–3.83 as per the manufacturer’s guidelines in the operating software): Hb Deer Lodge, Hb Lepore, Hb D<sup>Iran</sup>, Hb E, Hb Hamadan, Hb Osu-Christiansborg, Hb Tianshui, Hb G Honolulu and Hb G Copenhagen. Among these, Hb Deer Lodge, Hb Lepore and Hb D<sup>Iran</sup> elute prior to the standard RT of Hb A<sub>2</sub> (3.6 min) while others have higher RT to that of Hb A<sub>2</sub>. 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 α-thalassemias). All the other variants eluting in the Hb A<sub>2</sub> window have variant hemoglobin quantities higher than 30% on average under heterozygous conditions, making it difficult to distinguish in HPLC. Amongst these, Hb D<sup>Iran</sup> has been reported to elute in this window at

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**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&lt;sup&gt;3&lt;/sup&gt;/L</td>
<td>7.4</td>
<td>6.9</td>
</tr>
<tr>
<td>Red blood cell count</td>
<td>×10&lt;sup&gt;12&lt;/sup&gt;/L</td>
<td>5.67</td>
<td>5.07</td>
</tr>
<tr>
<td>Hemoglobin g/L</td>
<td></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 fl</td>
<td></td>
<td>58.7</td>
<td>66.7</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin Pg</td>
<td></td>
<td>17.8</td>
<td>19.5</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration g/dL</td>
<td></td>
<td>30.3</td>
<td>29.3</td>
</tr>
<tr>
<td>Platelet count</td>
<td>×10&lt;sup&gt;12&lt;/sup&gt;/L</td>
<td>169</td>
<td>171</td>
</tr>
<tr>
<td>Serum creatinine μmol/L</td>
<td></td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Aspartate transaminase U/L</td>
<td></td>
<td>12.7</td>
<td>15.3</td>
</tr>
<tr>
<td>Alanine transaminase U/L</td>
<td></td>
<td>12.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Total bilirubin μmol/L</td>
<td></td>
<td>0.03</td>
<td>0.34</td>
</tr>
<tr>
<td>Lactate dehydrogenase U/L</td>
<td></td>
<td>198</td>
<td>189</td>
</tr>
<tr>
<td>Iron μmol/L</td>
<td></td>
<td>5.027</td>
<td>5.258</td>
</tr>
<tr>
<td>Transferrin g/L</td>
<td></td>
<td>490.05</td>
<td>462.09</td>
</tr>
<tr>
<td>Ferritin pmol/L</td>
<td></td>
<td>138.7</td>
<td>111.6</td>
</tr>
</tbody>
</table>

**Discussion**

The Hb D<sup>Iran</sup> trait and homozygous cases have been reported earlier.<sup>6</sup> However, few studies have reported compound heterozygotes of Hb D<sup>Iran</sup> with other Hb variants like Hb S and Hb D<sup>Punjab</sup>, β<sup>+</sup>-thalassemia IVS1-5 (G>C), β<sup>0</sup>-thalassemia (619 bp-deletion) and undefined β-thalassemia from India and Pakistan. Various studies have reported that the quantity of Hb D<sup>Iran</sup> eluting in the Hb A<sub>2</sub> 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<sup>Punjab</sup>, Hb S, β-thalassemia with the 619 bp deletion mutation and beta thalassemia with unknown mutation).<sup>6–10</sup>

be masked by the simultaneous presence of the cds 41/42 (-CTTT) mutation.
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}/ \beta^0 \text{thal} [\text{cds 41/42 (-CTTT)}].](image)

Further, as Hb D^{Iran} elutes in the Hb A_{2} window in HPLC masking elevated Hb A_{2}, it becomes difficult to suspect the presence of $\beta^0$-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 $\beta^0$-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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Figure 2 – (A) DNA sequence chromatogram showing HbD\text{\textsubscript{Iran}} mutation on 22 codon (GAA\textsubscript{AA}>GAG). (B) DNA sequence chromatogram showing \(\beta^0\) thal (4 bp del Cds 41/42 (-CTTT))

References