Case Report

Compound heterozygote of Hb D\textsuperscript{Iran} \([\text{HBB: c.67G>C, }\beta^{22(4)} \text{Glu>Gln}]\) with \(\beta^{0}\)-thalassemia \([\text{cds 41/42 (-CTTT)}]\) from Eastern India

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

### Discussion

The Hb D\text{Iran} trait and homozygous cases have been reported earlier.\textsuperscript{4,5} However, few studies have reported compound heterozygotes of Hb D\text{Iran} with other Hb variants like Hb S and Hb D\text{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\textsubscript{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).\textsuperscript{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 positioned at the B site of the helix. To date, six Hb variants (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)>TAA [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.\textsuperscript{1,10} This rare variant has heat stability with no effect on oxygen equilibrium, intracellular 2,3-diphosphoglycerate or the Bohr effect.\textsuperscript{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.\textsuperscript{5,9} The present case agrees with this as evidenced 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\textsubscript{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 Tian- shu, 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\textsubscript{2} (3.6 min) while others have higher RT to that of Hb A\textsubscript{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\textsubscript{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>(\times 10^9/L)</td>
<td>7.4</td>
<td>6.9</td>
</tr>
<tr>
<td>Red blood cell count</td>
<td>(\times 10^9/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>(\times 10^9/L)</td>
<td>169</td>
<td>171</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>(\mu)mol/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>(\mu)mol/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>(\mu)mol/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>
<tr>
<td>Peak name</td>
<td>Calibrated area %</td>
<td>Area %</td>
<td>Retention time (min)</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------</td>
<td>--------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.3</td>
<td>0.96</td>
</tr>
<tr>
<td>F</td>
<td>1.0</td>
<td>---</td>
<td>1.09</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.8</td>
<td>1.60</td>
</tr>
<tr>
<td>P2</td>
<td>---</td>
<td>4.0</td>
<td>1.73</td>
</tr>
<tr>
<td>A0</td>
<td>---</td>
<td>4.6</td>
<td>2.20</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>1.0</td>
<td>2.50</td>
</tr>
<tr>
<td>A2</td>
<td>82.8*</td>
<td>---</td>
<td>3.57</td>
</tr>
</tbody>
</table>

Total area: 1,972,061

F concentration = 1.0 %
A2 concentration = 82.8% %

*Values outside of expected ranges

Analysis comments:

![CE-HPLC showing characteristic peak of HbD\textsuperscript{Iran}/\beta\textsuperscript{0} thal [cds 41/42 (-CTTT)].](image)

Figure 1 – CE-HPLC showing characteristic peak of HbD\textsuperscript{Iran}/\beta\textsuperscript{0} thal [cds 41/42 (-CTTT)].

Further, as Hb D\textsuperscript{Iran} elutes in the Hb A\textsubscript{2} window in HPLC masking elevated Hb A\textsubscript{2}, it becomes difficult to suspect the presence of \beta-thalassemia and direct gene sequencing needs to be performed. To the best of our knowledge, this is the first report of Hb D\textsuperscript{Iran} with \beta\textsuperscript{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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