Homozygous Hemoglobin D Disease: A Case Report
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Citation

Abstract
Homozygous Hb D disease is a rare disease and usually presents with mild hemolytic anemia and mild to moderate splenomegaly. Heterozygous form of Hb D is clinically silent, but coinheritance of Hb D with Hb S or thalassemia produces clinically significant conditions like sickle cell anemia and chronic hemolytic anemia of moderate severity. The main differential for homozygous Hb D disease is Hb D-beta zero thalassemia. Hb D has also been reported to be associated with hematological malignancies.

INTRODUCTION
Hemoglobin D (Hb D), a hemoglobin variant, occurs mainly in north-west India, Pakistan and Iran. Encountered by Itano, in 1951, Hb D differs structurally from normal Hemoglobin A at 121 position on beta chain, where glutamine replaces glutamic acid.

The electrophoretic mobility of Hb D is identical to that of Hb S at alkaline pH in cellulose acetate, but HbD can be distinguished from Hb S by its normal solubility as well as different mobility from Hb S on citrate agar electrophoresis at pH 6.2. Hb D gene can be detected by DNA amplification and globin chain analysis. Prenatal diagnosis can also be used for the detection of Hb D in high risk couples. There are several hemoglobin D variants, amongst them Hb D-Punjab (also known as Hb D-Los Angeles) is by far the commonest.

Hemoglobin D-Punjab occurs with greatest prevalence (2%) in Sikhs in Punjab, India, whereas Gujarat, the province in the west from where the case was reported, has a prevalence rate of 1%. It is also found sporadically in Blacks and Europeans, the latter usually coming from countries that have had close associations with India in the past.

Hb D occurs in four forms: heterozygous Hb D trait, Hb D-thalassemia, Hb S-D disease and the rare homozygous Hb D disease, which is usually associated with mild hemolytic anemia and mild to moderate splenomegaly.

CASE REPORT
Our patient was a 13-year-old Indian girl from the state of Gujarat, India. She presented with the complaint of a gradually increasing painless lump in the left upper quadrant of abdomen for last two years. There were no other significant complaints. There was no history of blood transfusions in the past. Her family history was not contributory.

Physical examination revealed marked pallor. Spleen was palpable up to the right iliac fossa and was nontender. No other organomegaly or lymphadenopathy were present. No skeletal deformities were evident.

Blood samples were collected in 5 ml vacutainer containing EDTA as an anticoagulant. Complete blood count and red cell indices were measured by Abacus Haematology Analyzer (Diatron Messtechnik Ges.m.b.H., Austria). Thin and thick blood smears were examined using the Leishman stain and Geimsa stain respectively. 1% BCB stain was used for the reticulocyte count. Zinc protoporphyrin (ZPP) level was measured using ProtoFluor-Z hematofluorometer (Helena Laboratories) for the detection of iron deficiency, whereas solubility test using Sodium dithionate powder was done for the sickling. Hemoglobin electrophoreses were obtained using cellulose acetate at pH 8.4 and citrate agar at pH 6.0. Hb A2 levels were estimated using the microcolumn chromatography with glycine-potassium cyanide developers. Fetal hemoglobin (Hb F) levels were estimated using alkali denaturation technique. The results are mentioned in the table.

The patient's peripheral thin smears showed microcytic hypochromic red cells with few target cells. A mild degree of anisopoikilocytosis was noticed. No Hb H inclusions were...
seen in the red cell smears. Normal ZPP level ruled out the iron deficiency. She had red cell indices consistent with microcytic hypochromic anemia, but inconsistent with thalassemia minor. The solubility test for the sickling was negative. Her HbA2 and Hb F levels were in the normal range. The electrophoretic pattern on cellulose acetate at pH 8.4 showed bands at the positions of Hb A2 and Hb S or D (figure 1), the latter was confirmed to be Hb D on citrate agar electrophoresis at pH 6.0.

**Figure 1**
Figure 1: Hb electrophoresis on cellulose acetate at pH 8.4 showing hemoglobin mobility of samples from patient, father, mother, and a control from a known case of sickle cell trait.

Following the patient's tests, her parents were investigated. Both of them showed the heterozygote state for Hb D. Neither their red cell indices nor their Hb A2 were in thalassemic range. Solubility tests were negative for sickling. The results are shown in the table.

**Figure 2**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hb A</th>
<th>Hb S/D</th>
<th>Hb A2</th>
<th>Hb F</th>
<th>HbA2</th>
<th>Hb S</th>
<th>Hb D</th>
<th>HbA2%</th>
<th>Hb S%</th>
<th>Hb D%</th>
<th>ZPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>4.3</td>
<td>3.9</td>
<td>22.6</td>
<td>86</td>
<td>16.4</td>
<td>20.4</td>
<td>34.3</td>
<td>4.3</td>
<td>0.4</td>
<td>96.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Father</td>
<td>4.4</td>
<td>4.1</td>
<td>42.6</td>
<td>96</td>
<td>20.7</td>
<td>22.4</td>
<td>32.2</td>
<td>1.3</td>
<td>0.5</td>
<td>96.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Mother</td>
<td>4.4</td>
<td>4.3</td>
<td>30.7</td>
<td>76</td>
<td>28.4</td>
<td>22.4</td>
<td>34.3</td>
<td>1.3</td>
<td>0.5</td>
<td>96.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Though Hemoglobin D is not very uncommon in India, its homozygous form is very rare and very few case reports have been reported. Hb D in the form of heterozygote Hb D trait, Hb S-D disease and Hb D-thalassemia are commoner forms. Hb D has a prevalence of 1% in Gujarat; India. By applying the Hardy-Weinberg formula, the expected frequencies of the homozygous Hb D disease can be estimated. The expected incidence for the homozygous Hb D disease is 0.000025%, or 1 case per 40,000 births (considering the 1% prevalence). When considering the almost negligible prevalence of Hb D in the Western Population, the number of cases of homozygous Hb D disease would at the most number a handful.

Heterozygous state of Hb D does not produce any clinical or hematological symptoms, but its association with Hb S produces clinically significant, but less severe condition mimicking sickle cell anemia. Even the different Hb D variants seem to produce different severity of disease with Hb S. Hb D-Punjab produces clinically significant condition like sickle cell disease, whereas Hb D Iran and Hb D Ibadan are non-interacting and produce benign conditions like sickle cell trait.

The main differential for the diagnosis of homozygous Hb D disease is with Hb D-beta zero thalassemia. These two conditions should be differentiated by using the parameters like red cell indices, Hb A2 & Hb F levels and family studies. The major concern for ruling out Hb D-beta zero thalassemia is that homozygous Hb D disease causes mild hemolytic anemia, but co-inheritance of beta zero thalassemia seems to give deleterious effects on the presentation of Hb D disease, leading to chronic hemolytic anemia of moderate severity. The association between Hb D and hematological malignancies has also been reported.

In our case, the patient had a microscopic picture of microcytic hypochromic anemia with normal ZPP levels suggestive of the non-iron deficient status. Her solubility test for sickling was negative, but electrophoretically hemoglobins showed mobility at the position of Hb D and Hb A2 without the band in the position of Hb A at alkaline pH which were also confirmed by citrate agar electrophoresis. Neither the red cell indices nor the Hb A2 and Hb F levels were in the thalassemic range. Both of her parents were proved to be heterozygous for Hb D without any clinical manifestations.

In today’s ever changing population demographics with racial inter mixing, hemoglobin D disease should no longer be considered as an entity confined to the south Asian region. It is important for a hematologist to keep this important differential in mind when dealing with any
suspected hemoglobinopathy.

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