A RARE HEMOGLOBIN VARIANT: HB TY GARD DETECTED IN AN INDIAN FAMILY

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Abstract: We report an Indian family case of Hb Ty Gard. A one year old male child presented with fever and was found to have low hemoglobin. Variant hemoglobin (Hb) was incidentally detected on HPLC electrophoresis as an unknown abnormal peak. Molecular analysis of β-globin gene showed presence of codon 124 Pro-Gln (CCA-CAA) variation or Hb Ty Gard. The family studies revealed presence of the same mutation in mother. Mutation analysis of β-globin gene serves as an important tool for confirmation of rare hemoglobinopathies.

Key Words: β-globin, β-thalassemia

Introduction

Hemoglobinopathies are a group of genetic disorders of hemoglobin and the commonest hereditary disorders in India (Madan et al. 2010, Verma et al. 2012; Mohanty et al. 2013). β-thalassemia, the most common single gene disorder, involve a diverse group of defects in hemoglobin synthesis, all of which result from reduced output of β-globin chains. Unlike the α-thalassemias, which are predominantly produced by deletions in the α-globin gene cluster, most β-thalassemias are caused by point mutations, small deletions or insertions within the β-globin gene or its immediate flanking sequences. Over 200 β-thalassemia alleles have been characterized worldwide. Due to the high diversity of mutations in the β-globin gene, mutations in one population will be different from others. However, in each affected ethnic group, a few common mutations together with a variable numbers of rare mutations account for most of the cases (Thedsawad et al. 2012). Most Hb variants are rare, but, due to the large number of variant hemoglobins, various rare Hb variants are found as unknown peaks during screening for hemoglobinopathies.

Case Report

A one year old male child presented with fever and upper respiratory tract infections (URTI) and was asked to do Complete blood count (CBC). The hemoglobin was found to be 8.6 gm/ dL and the sample was then sent to us for abnormal hemoglobin study. He had no previous history of anemia or blood transfusions. There were no other clinical symptoms and no thalassemia related tests were ever done on family members. High-performance liquid chromatography (HPLC) test was done using the Bio Rad Variant II for abnormal hemoglobin study. Abnormal Hb fraction (16.8%) with mobility close to the Hb A at retention time of 2.32 min was detected (Fig.
1) by HPLC. Capillary electrophoresis was also performed on Sebia CAPILLARYS™ 2, but it did not reveal presence of an additional peak (Fig. 2).

![Electrophoresis graph](image1)

**Figure: 1**

![HPLC graph](image2)

**Figure: 2**

We further sequenced the entire beta globin gene to identify the mutations in exons 1-3 and splice site junctions. PCR amplification of β-globin gene was done using primer pairs described earlier (Old et al. 2001, followed by bidirectional sequencing. The sequenced products were electrophoresed on 3500Dx Genetic
Analyzer; Applied Biosystems. The sequence was analyzed and Hbvar database (URL: http://globin.cse.psu.edu/globin/hbvar/) of human hemoglobin variants and thalassemia mutations was referred for identification of genetic variants (Burseaux et al. 2008). A mutation in heterozygous form was detected in codon 124, beta 124(H2) Pro-Gln, HBB:c. 374G-C was detected (Fig. 3). This variant was earlier reported as Hb Ty Gard. Hb Ty Gard, first reported in 1978 in a heterozygous patient by Burseaux et al. (Burseaux et al. 1978), is a stable high O2 affinity variant.

Subsequently, parent’s samples were collected and subjected to HPLC as well as molecular analysis. Mother’s sample showed same mutation with mild anemia and Father’s sample did not reveal any mutation. Table 1 shows the Hb electrophoresis and CBC profile of child and parents.

**Discussion**

Hb Ty gard is a rare form of hemoglobin and it has been reported twice in the literature (Burseaux et al. 1978; Badens et al. 2002). It was identified for the first time in 1978 as a stable high O2 affinity Hb in father and daughter living in France [8]. The second case was of a neonate from France, the molecular analysis of which showed compound heterozygosity with a mutation in codon 124 CCA->CAA (Hb Ty Gard) and a β-thalassaemia mutation IVS 2-654 T>C (Badens et al. 2002). The case described in this report presented with fever and CBC revealed lower hemoglobin. Hemoglobin study by HPLC showed an abnormal peak as a hump on HbA peak. This was later proved to Hb Ty Gard associated with CCA-CAA change at codon 124. Mother carrying the same mutation was clinically normal and had never displayed any related clinical symptoms. Both child and mother had low hemoglobin and low mean corpuscular volume (MCV). This can be due to iron deficiency which is very common in Indians. To the best of our knowledge this is the first report from India.
of Hb Ty Gard. Molecular methods like DNA sequencing provide comprehensive analysis of β-globin variants and aids in identification of rare variants.

Abbreviations
CBC – Complete Blood Count
Hb – Hemoglobin
HPLC - High-performance liquid chromatography
URTI - upper respiratory tract infections

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