ISSN 2394-6784(Print)
e-ISSN 2394-6792(Online)

Indian Journal of Pathology and Oncology

IJPO

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

Pratiksha Chheda[1,*], Sandeep Warghade[2], Milind Chanekar[3], Yogita Salunkhe[4], Mayur Nigalye[5], Rajesh Bendre[6], Sameer Pandya[7] and Nilesh Shah[8]

[1,2,3,4,5,6,7,8] Research & Development, Metropolis Healthcare Ltd., Unit No. 409 to 416, 4th Floor, Commercial Building A, Kohinoor City, Near Kohinoor Mall, Kirol Road, Kurla-W, Mumbai-400 070.

***Corresponding Author:**
E-mail: pratiksha.chheda@metropolisindia.com

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 ß-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 ß-globin gene serves as an important tool for confirmation of rare hemoglobinopathies.*

Key Words: *ß-globin, ß-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).

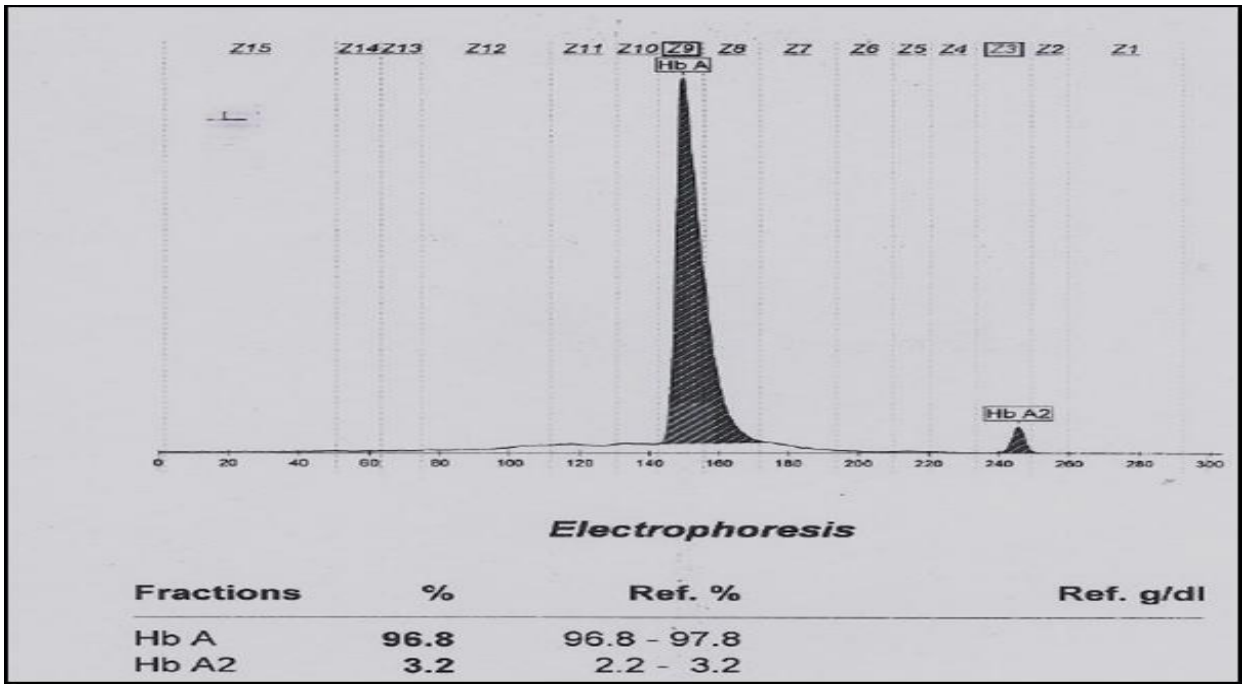

Figure: 1

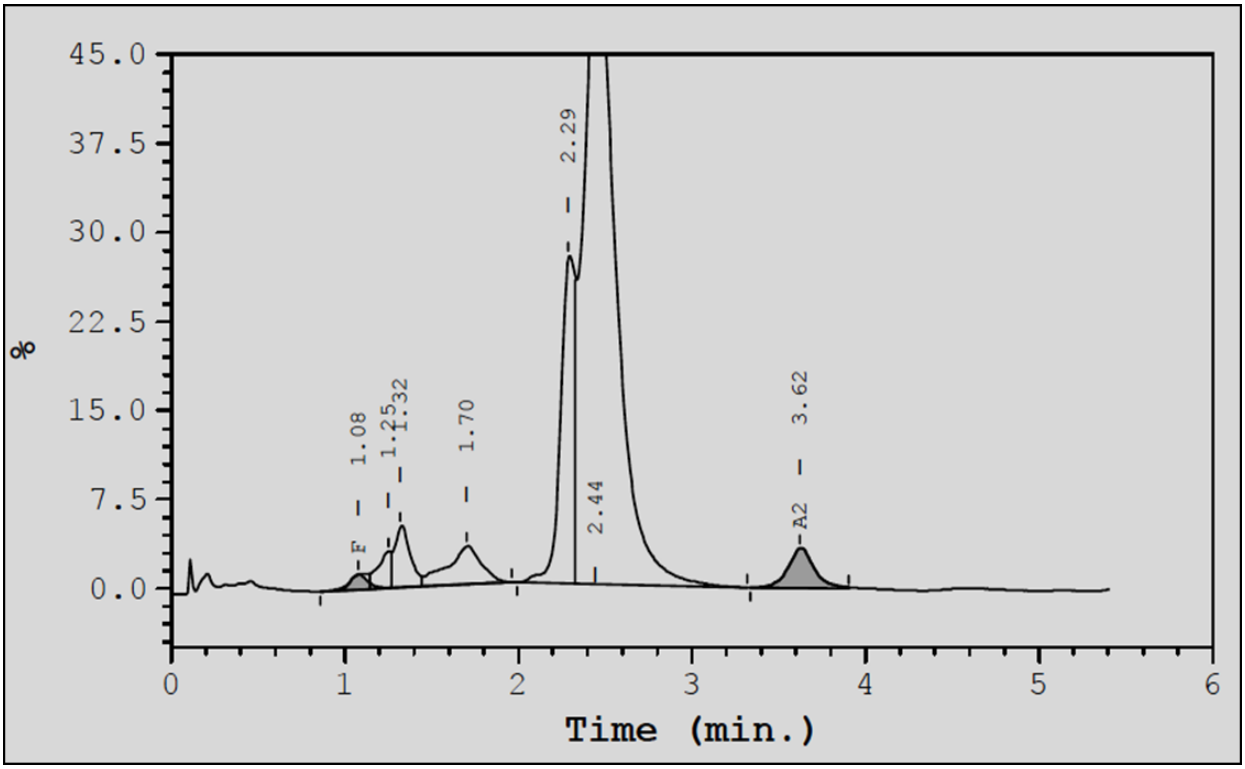

Figure: 2

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 O_2 affinity variant.

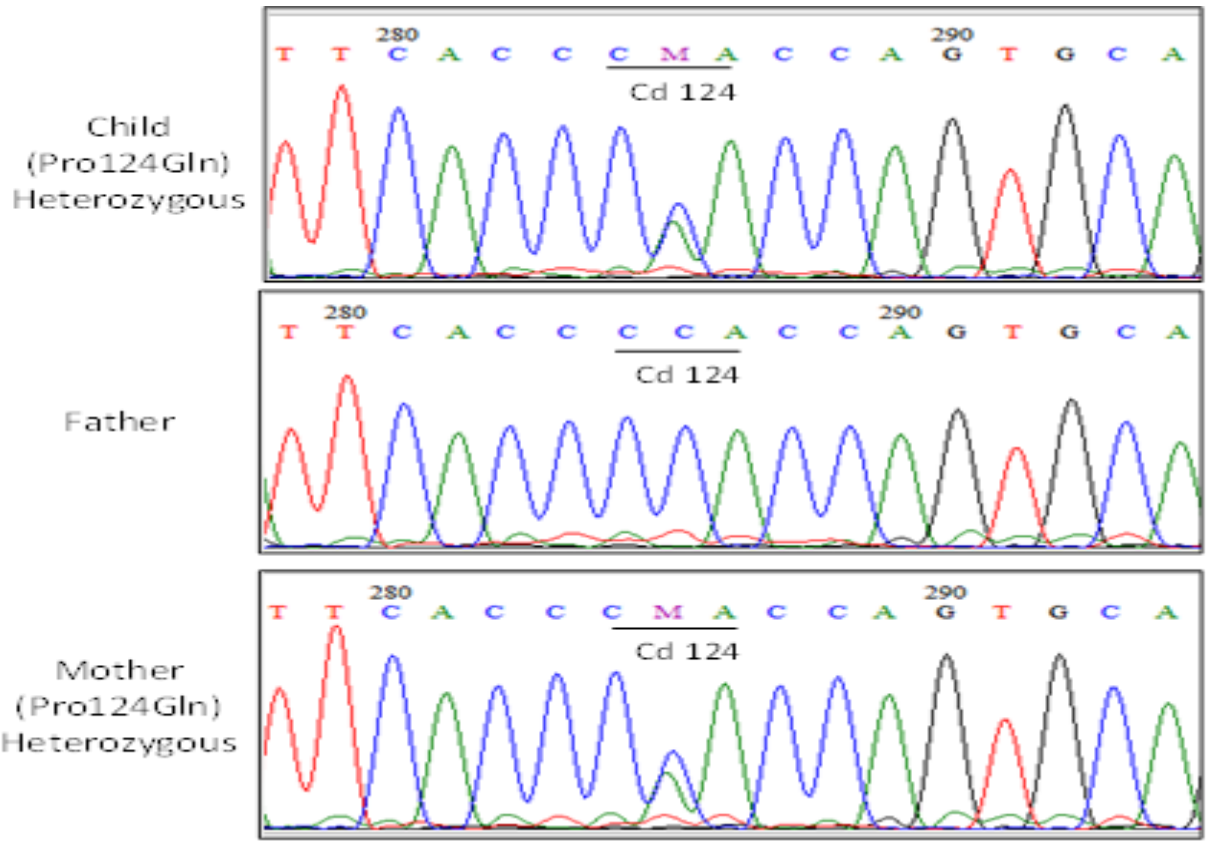

Figure: 3

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 O_2 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

Financial disclosure statement: This was a non-funded project and no grants were received from any international or national bodies.

References:

1. Badens C, Merono F, Martini N, Lena-Russo D, Gulbis B, Thuret I. 2002 Four globin gene defects in a healthy child. *Haematologica* **87,** (11) ELT42.
2. Burseaux E, Blouquit Y, Poyart C, Rosa J. 1978 Hemoglobin Ty GARD (alphaA2beta2 124 (H2) Pro replaced by Gln). A stable high O2 affinity variant at the alpha1beta1. *FEBS Lett.* **88,** 155-9.
3. Database of human Hemoglobin Variants and Thalassemia Mutations. URL: http://globin.cse.psu.edu/globin/hbvar/.
4. Hardison R.C, Chui D.H, Giardine B, Riemer C, Patrinos G.P, Anagnou N, Miller W, Wajcman H. 2002 HbVar: A relational database of human hemoglobin variants and thalassemia mutations at the globin gene server. *Hum Mutat.* **19**, 225-33.
5. Madan N, Sharma S, Sood S.K, Colah R, Bhatia LH. 2010 Frequency of β-thalassemia trait and other hemoglobinopathies in northern and western India. *Indian Journal of Human Genetics,* **16**, 16-25.
6. Mohanty D, Colah R.B, Gorakshakar A.C, Patel R.Z, Master D.C, Mahanta J, et al. 2013 Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: a multicentre study. *J Community Genet.* **4**, 33–42.
7. Old J.M, Khan S.N, Verma I, Fucharoen S, Kleanthous M, Ioannou P, et al. 2001 A multi-center study in order to further defines the molecular basis of β-thalassemia. *Hemoglobin* **25**, 397-407.
8. Verma IC, Saxena R, Kohli S. 2012 Hemoglobinopathies in India--clinical and labo
9. Thedsawad A, Jindadamrongwech S, Chuncharunee S, Butthep P. 2012 Multiplex ARMS-PCR Analysis for Nineteen b-Thalassemia Mutations. *J Hematol Transfus Med.* **22**, 31-4.0.
10. ratory aspects. *Clin Lab Med.* **32**, 249-62.