Five Rare β Globin Chain Hemoglobin Variants in India

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Abstract Thalassemias as well as structural hemoglobin (Hb) variants are common monogenic inherited disorders of Hb in India. In this paper we describe 5 rare β -chain Hb variants identified in the Indian population on the basis of high performance liquid chromatography (HPLC). Of these 3 were identified during antenatal screening of β -thalassemia while the other 2 cases were referred to us for a diagnostic work up. These 5 Hb variants were Hb British Columbia (β CD 101 GAG $\rightarrow$ AAG), Hb Saint Louis (β CD28 CTG $\rightarrow$ CAG), Hb G Coughatta (β CD 22 GAA $\rightarrow$ GCA), Hb Pyrgos (β CD 83 GGC $\rightarrow$ GAC) and Hb Agenogi (β CD 90 GAG $\rightarrow$ AAG). Hb Saint Louis and Hb G Coughatta eluted in the HbA₂ window, Hb British Columbia and Hb Agenogi eluted in the Hb C window while Hb Pyrgos eluted in an unknown window on HPLC. They were all identified by DNA sequencing. The child having Hb St. Louis had hepatosplenomegaly and anemia while the individuals with the other 4 variants were asymptomatic. Rare Hb variants are diagnostic curiosities that may be encountered by laboratories. Correct identification requires the application of more than one technique to avoid misdiagnosing them as more common variants (e.g. St. Louis and G Coughatta as E or D Iran on HPLC. Some, like G Coughatta may interfere with HPLC-based HbA_{1c} estimation).

Keywords Rare β chain variants · HPLC · India

Introduction

The inherited disorders of hemoglobin (Hb), both the thalassemias due to reduced synthesis of globin chains as well as structural Hb variants are common monogenic disorders in India. [1] Every centre involved in screening and diagnosis of the hemoglobinopathies encounters rare or unusual Hb variants which are mostly picked up as abnormal peaks on high performance liquid chromatography (HPLC) or electrophoresis. We describe five rare β chain Hb variants identified in the Indian population.

Material and Methods

Five unusual β globin chain Hb variants were encountered over a period of 5 years. The complete blood counts were done on a Sysmex K1000 automated Hematology Analyzer Hb A₂, Hb F and other Hb variants were quantitated by HPLC (Variant Hb Testing System or Variant II, Biorad Laboratories, Hercules CA, USA). Hb electrophoresis on cellulose acetate membranes at alkaline pH and the heat stability test were done wherever possible. The variants were identified by DNA sequencing on the ABI 3130 XL 16 capillary genetic analyzer (Applied Biosystem, CA, USA). The study was approved by our Institutional Ethics Committee.

Results

3 of the 5 Hb variants were identified during antenatal screening for β thalassemia while the other 2 cases were referred to us for a diagnostic work-up. The hematological findings are shown in Table 1 and the HPLC profiles in Fig. 1 and the DNA sequence analysis in Fig. 2.

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Table 1 Unusual β chain variants identified

Variant	Age/sex origin	Clinical presentation	Hb (g/dl)	RBC ($\times 10^6/\mu\text{l}$)	MCV (fl)	MCH (Pg)	MCHC (g/dl)	RDW (%)	HbA ₂ (%)	HbF (%)	Variant Hb (%) elution window	Cellulose acetate Electrophoresis test at pH 8.9	Heat stability test
Hb British Columbia β CD 101 (GAG $\rightarrow$ AAG) (HBB:C.304 G $\rightarrow$ A)	24 years/F Punjab	Identified during antenatal Screening	12.0	5.10	73.0	23.2	31.6	17.6	3.9	1.0	32.7 % Eluted in HbC window (RT–5.05 min)	–	–
Hb Saint Louis β CD 28 (CTG $\rightarrow$ CAG) (HBB:C.86 T $\rightarrow$ A)	5 years/F Maharashtra										Hepatosplenomegaly & anemia	9.4	3.70
86.8	25.3	29.2	19.6	17.7	4.3		17.7 % Eluted in HbA ₂				window (RT – 3.59 min)	–	Positive
Hb G Coughatta β CD 22 (GAA $\rightarrow$ GCA) (HBB: c 68 A $\rightarrow$ C)	30 years/F Maharashtra	Asymptomatic	12.4	4.07	86.2	30.5	35.3	15.8	41.4	0.1	41.4 % Eluted in Hb A ₂ window (RT—3.46 min)	A slow moving band was seen at HbS/D position.	Negative
Hb Pyrgos β CD 83 (GGC $\rightarrow$ GAC) (HBB: c 251 G $\rightarrow$ A)	28 years/F Punjab	Identified during Antenatal Screening	11.7	4.35	85.9	27.0	31.4	12.0	2.3	0.6	53.4 % Eluted in unknown window (RT—1.44 min)	–	–
Hb Agenogi β CD 90 (GAG $\rightarrow$ AAG) (HBB: c 271 G $\rightarrow$ A)	31 years/F Gujarat	Identified during Antenatal Screening	11.0	4.10	82.0	26.6	32.4	46.1*	3.2	6.4	42.6 % Eluted in Hb C window (RT – 4.92 min)	A slow moving band was seen at HbS/D position.	Negative

RDW: SD Red cell distribution width; Standard Deviation, RT retention time

Fig. 1 HPLC analysis of the 5 variants

Case 1

A 24 year old female screened during pregnancy showed microcytosis and hypochromia with a borderline Hb A₂ level of 3.9 % and an abnormal peak of 32.7 % in the Hb C window on HPLC. No β thalassemia mutation was identified but DNA sequencing showed the presence of a β chain variant, Hb British Columbia (β CD 101 GAG $\rightarrow$ AAG, HBB: c.304 (G $\rightarrow$ A) in the heterozygous condition.

Case 2

This 5 year old female child presented with high grade fever, hepatosplenomegaly (Liver-2 cm, spleen-1 cm) and anemia with a reticulocyte count of 3.0 %. Her Hb at the time of investigation was 9.4 g/dl with marginally reduced MCH and normal MCV. Hemoglobin analysis showed a peak of 17.7 % in the Hb A₂ window with slight elevation

in the fetal Hb level. This variant was identified as Hb Saint Louis (β CD28 CTG $\rightarrow$ CAG; HBB: c.86 T $\rightarrow$ A).

Case 3

This was a 30 year old lady who was asymptomatic but her husband had sickle cell trait. Her hematological indices were normal but on Hb analysis she had an abnormal peak in the Hb A₂ window of 41.4 % DNA sequencing showed a β chain variant Hb G Couthatta (β CD 22 GAA $\rightarrow$ GCA; HBB: c:68 A $\rightarrow$ C).

Case 4

A 28 year old female was screened for β thalassemia during an antenatal check-up. Her red cell indices were normal. Hemoglobin analysis revealed a normal Hb A₂ and Hb F but a large unknown peak of 53.4 % eluting at

Fig. 2 DNA sequencing of 4 variants

1.44 min. This β chain variant was identified as Hb Pyrgos (β CD 83 GGC → GAC; HBB: c.251 G → A).

Case 5

This 31 year old female was also screened for β thalassemia during her antenatal visit and although she was not a carrier of β thalassemia, on HPLC she showed a peak of 42.6 % in the Hb C window. On DNA sequencing, this variant was identified as Hb Agenogi (β CD 90 GAG → AAG; HBB: c. 271 G → A).

Discussion

The population of India is extremely heterogeneous with racial, cultural and religious diversity. We identified 5 rare β globin chain variants in individuals originating from different states. Hb British Columbia has been described earlier in a 26 year old east India male from Canada and a Caucasian family in the USA, and was associated with mild erythrocytosis [2, 3]. Our case had a Hb of 12.0 g/dl and an RBC count of $5.1 \times 10^6/\mu\text{l}$ but had no clinical manifestations.

Hb Saint Louis has been associated with a chronic hemolytic anemia and cyanosis and occasional cases have been reported from the French, Slovakian and Yugoslavian population [4–7]. Our case had a similar presentation although the percentage of the variant was relatively low (17.7 %). We are not aware of any history of blood transfusion in this case which could have led to an erroneous low percent of the variant. As this variant was unstable, it is also possible that some of the haemoglobin could have precipitated leading to a lower percentage seen on HPLC.

Hb G-Coughatta also known as Hb G Hsim Chu, Hb Saskatoon or Hb G Taegu has been reported from many countries including Algeria, Japan, Korea, Thailand, Egypt and recently from China, Turkey and Sri Lanka [4, 8]. This variant has had no clinical presentation in the heterozygous or homozygous condition or when inherited with β⁺ thalassemia. Our case with this variant was also asymptomatic.

The first report of Hb Pyrgos was in a Greek in combination with HbS leading to no clinical manifestations. The second was is an African from the Republic of Mali, also not associated with any clinical consequences. Hb Pyrgos is a relatively common Hb variant in the South East Asian region particularly in North East and Central

Thailand. Hb Pyrgos has been co-inherited with HbH with no deleterious effect of the variant on the clinical presentation of Hb H disease [4, 9]. As we identified this variant during antenatal screening for β thalassemia, the lady had no clinical presentation but the percentage of this variant was 53.4 %.

Hb Agenogi is a mildly unstable β chain variant seen in the African American, Hungarian, Italian, Japanese and Argentinian populations and has also been co-inherited with β^0 thalassemia in a Sicilian family [4, 10]. In our case, this variant did not have any clinical consequences and was identified during antenatal screening. Except for Hb Agenogi, the other 4 variants are being reported for the first time in the Indian population.

Although many of these variants may not have clinical consequences, it is important to differentiate them from other clinically significant variants. Hb St. Louis and Hb G Coughatta elute in the Hb A₂ window and could be mistaken for the common variants like Hb E and Hb D^{Iran} which also elute in the same window on HPLC. Hb British Columbia and Hb Agenogi elute in the Hb C window and may be misclassified as Hb C, which is not a common Hb variant in the Indian population. Thus they need to be interpreted with caution. If a simple cellulose acetate electrophoresis at alkaline pH is also run when such variants are encountered, some of these problems may be resolved. Current guidelines too stipulate that at least 2 techniques based on different principles should be applied for presumptive identification of any Hb variants found [11]. The presence of some of these Hb variants may affect the estimation of HbA_{1c} by HPLC [12, 13]. HbG Coughatta interferes with the quantitation of HbA_{1c}, the HbA_{1c} level measured by HPLC being reduced to about half the actual level thereby leading to a pitfall in diabetic care [13]. These 5 rare Hb variants add to the repertoire of hemoglobinopathies seen in the Indian population.

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Authorship Contribution RC designed the study and wrote the manuscript. AN and AG supervised the laboratory work. PS, MG, PM and MS undertook the laboratory investigations. KG reviewed the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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