Case Report

Dealing with Rare Beta Chain Hb Variants – Diagnostic Dilemmas

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Abstract

The diagnosis of inheritance of Hb Hope [b136(H14)Gly ? Asp, GGT[GAT] by high performance liquid chromatography (HPLC) is difficult because Hb Hope has a HPLC elution pattern similar to that of Hb Pyrgos, Hb New York, Hb Kodaira, and Hb Phimai. This variant hemoglobin (Hb) is mildly unstable and has reduced oxygen affinity, but is generally innocuous clinically. We report the use of a capillary electrophoresis (CE) for diagnosis of a rare beta chain variant Hb Hope in two young female patients of Indian origin and mild normocytic anaemia. Hb Hope eluted with a retention time of 125–140 s (Zone 10) of CE electrophoregram. Thus, the CE method provides an accurate diagnosis of Hb Hope which is useful in genetic counseling, prevention and control programs for these hemoglobinopathies.

Keywords:

Capillary electrophoresis, Hb Hope, High performance liquid chromatography, beta chain variant, hemoglobinopathy

Introduction

Hb Hope [beta136(H14)Gly-->Asp (GGT-->GAT)] was first described in an African-American family in 1965. Since then, it has been found in combination with several different globin gene mutations in many other families of divergent ethnic backgrounds [10]. Hb Hope has been reported in several African-American families, as well as in Japanese, Thai, Laotian, Cuban and Mauritanian families but is extremely rare in Indian population or is under reported. One study showed prevalence of 0.002% Hb Hope in Western Indian population [12]. Hb Hope ($\beta^{136GGT\text{-}GAT,\ Gly\text{-}Asp}$) was one of the most prevalent β-globin chain variants (19.0% of the cases) in the Thai population [11].It is more prevalent in Mediterranean region of the world than in Asian countries. Hb Hope is one of the 700 haemoglobin variants identified till date that is clinically silent or is a silent variant that does not present with any clinical symptoms usually [1]. Capillary electrophoresis has the ability to completely separate and identify the

haemoglobin variants [1]. We bring forth two cases of probable Hb Hope peaks eluted in zone 10 of the electrophoretogram assayed on capillary electrophoresis (Sebia Capillarys Octa 3) and also show the comparative peaks on HPLC on same patients. Additionally, both the samples showed increased HbA2 which might have resulted from the decreased synthesis of the abnormal β globin chain that allows increased binding between the excess α and γ globin chains.

Case Report

We bring forth two cases of Indian origin who were clinically asymptomatic. CBC findings and patient demographics of the two cases are captured in the Table 1 below.

Table 1: Patient demographics, presentation and relevant CBC parameters in the two Hb Hope cases

	Age/Gender	**Ethnicity**	**Clinical Presentation**	**Hb (g/dl)**	**MCV (fL)**	**RDW-CV (%)**	**BT History**
Case 1	29 yr / F	Indian ,R/O Pune	Asymptomatic Mild anaemia	10.6	84.9	13.5	No
Case 2	27 yr / F	Indian, R/O Patna	Asymptomatic Mild anaemia	10.6	91.7	17.5	No

Both cases were young females, Hb Variant analysis requested as part of antenatal screen investigations with mild normocytic anaemias and no relevant clinical history of blood transfusion or detected hemoglobinopathies in the family. The clinical presentation is in concordance to other studies of Hb Hope cases which show minimal clinical significance and are sometimes discovered during a systematic study performed within program for prevention of thalassemia or sickle cell disease. In several regions, these are found during a premarital screening or neonatal screening program [11]. Peripheral smears of both cases show normocytic picture with few hypochromic microcytes. The EDTA samples received in both cases were run on HPLC at another laboratory and referred to our reference laboratory for the assay on capillary electrophoresis (CE).

The CBC findings along with CE and HPLC graphs are shown below in Figures 1 & 2.

Presumptive diagnosis:

Presumptive diagnosis of a rare beta chain variant – probability of Hb Hope. Confirmation by a molecular study along with family study was advised to the patient.

Discussion

Hb Hope , being a very rare beta chain variant in India , these two cases detected by the classical X (or inverted V) electrograph formation of Zone 10 and Hb A , raises a line of interest and also makes one wonder the extent of undetected cases in the Indian sub-continent population. Rare Hb Variants detection in screening methods are dependent upon the technology being used. Both HPLC and Capillary electrophoresis are complementing technologies that should be available in a reference laboratory setup hemoglobinopathy segment. The differential diagnosis between Hb Hope and some variants like Hb Pyrgos, Hb New York, Hb Kodaira, Hb J-Bangkok etc. is difficult because these hemoglobinopathies are b-globin chain variants which have similar HPLC elution pattern and elute in the P2 / P3 peaks on HPLC. However, they can be classified by the CE system.

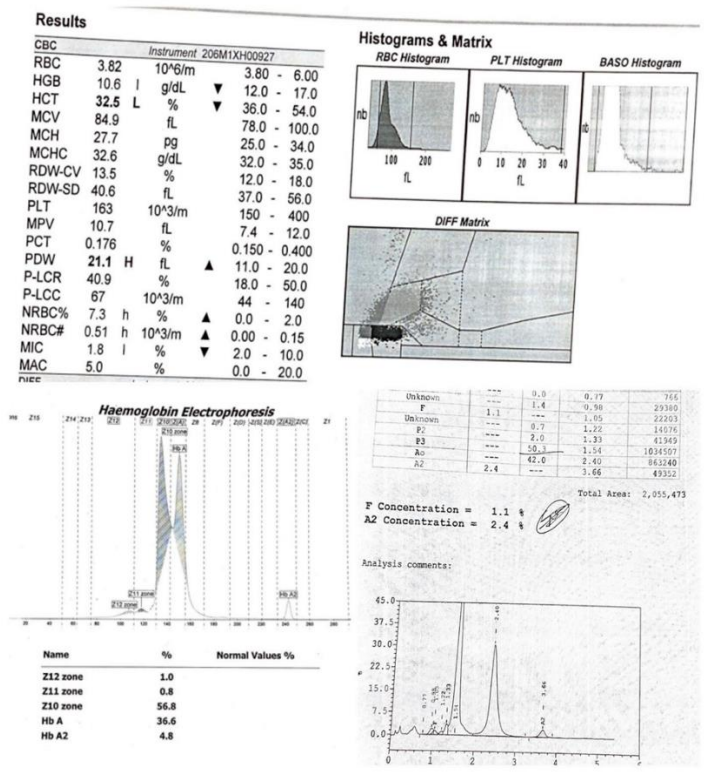

Figure 1: CBC (a), capillary electrophoresis (b) & HPLC (c) graphs of case 1

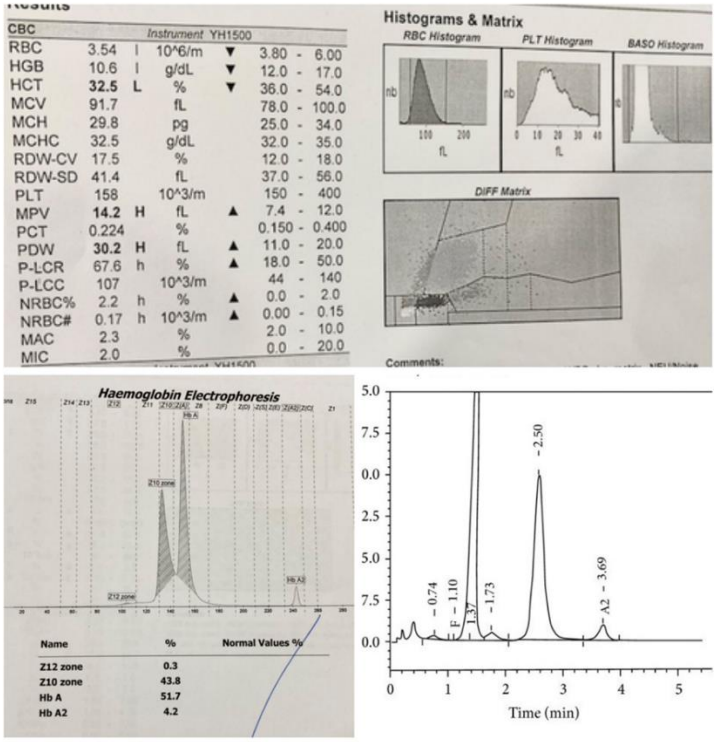

Figure 2: CBC (a), capillary electrophoresis (b) & HPLC (c) graphs of case 2

Another interesting known fact is which should be known to laboratorians as well as clinicians is that in cation exchange HPLC method, Hb Hope may be mistaken as erroneously high HbA1c, as it elutes in the same position as HbA1c [2]. And so was the case with both these patient samples. Hb Hope is characterized by a comparable charge altering mutation (β136 Gly→Asp) and the intra chain salt bridge so formed between the carboxyl group of β136 Asp and the charged α-amino group of β1 valine. This salt bridge neutralizes the positive charges to the extent that HbA1c and Hb Hope co-elute from the HPLC column. So Hb Hope and HbA1c elute in the same window leading to probable overestimation [3]. Accidental discovery of this abnormal hemoglobin emphasizes the importance of testing glycated haemoglobin by several methods whenever very high values are obtained which are not in line with the blood glucose levels of the patient. The possibility of interference by silent variant like Hb Hope should always be kept in mind while interpreting the abnormally elevated HbA1c values.

A third point of interest is the fact that in both our probable Hb Hope cases there seems to be a falsely elevated Hb A2 values of 4.8% and 4.2% whereas HPLC technique on same samples had normal Hb A2 levels 2.4% and 2.5% respectively. An elevated HbA2 level in samples containing Hb Hope measured by CE may lead to an incorrect diagnosis of coinheritance of β-thalassemia with Hb Hope – in case of unawareness of this fact by the laboratorian. Thus, the results of the RBC indices (MCV), the osmotic fragility test, and molecular analysis should be considered as they provide great value for β-thalassemia investigations [4]. In reviewing the electrophoretogram in our cases, we noted that the Hb Hope and HbA overlap in the CE pattern. Because of this, the software shifts the baseline up to approximate the percentage of HbA and Hb Hope. However, while this provides an estimate of the relative amounts of HbA and Hb Hope, it does not account for the overlapping haemoglobin below it in the pattern. Because the percentage of HbA2 is calculated as a percentage of the total haemoglobin, by not accounting for the overlap area, the percentage of HbA2 is falsely elevated [5].

Conclusion

With increasing global awareness and mass screening programs undertaken at various levels by health care systems, the responsibility for laboratory personnel has greatly enhanced in detection and prevention of this problem. Awareness of the diagnostic problems as well as their solutions is very important so that one does not miss a single case. However, one must be aware of the limitations and problems associated with the diagnostic methods to avoid false negative diagnosis in day-to-day practice. Genetic studies are indicated to confirm borderline cases and to detect silent carriers of beta thalassemia, alpha thalassemia, and rare and novel variants in routine practice.

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Competing Interests: The authors report no conflicts of interest

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