DOI: 10.7860/NJLM/2016/14873:2086

Original Article

Biochemistry Section

Haemoglobin Hope: A Rare Hb Variant Causing Spuriously Elevated HbA1c Values on HPLC Assay

MRINAL GUPTA, PRIYANKA DATTA, PRAGNA RAO

ABSTRACT

Introduction: Hb Hope is a clinically asymptomatic β-chain variant [beta136 (H14) Gly→Asp (GGT→GAT)]. It is more prevalent in Mediterranean region of the world than in Asian countries and extremely rare in India.

Aim: To evaluate the interference of clinically silent and extremely rare variant; haemoglobin Hope (Hb Hope) on the final reported value of glycated haemoglobin (HbA1c) by high performance liquid chromatography (HPLC).

Materials and Methods: Eight EDTA blood samples showed very high values of HbA1c (>50 gm%) when processed by Bio-Rad Variant II turbo (HPLC). These were further processed for by capillary electrophoresis to look for the abnormal haemoglobin. Glycated Hb was also measured by immunoturbidimetry.

Results: On HPLC, single abnormally high peak was observed for all the samples in the chromatogram leading to very high glycated haemoglobin values while in immunoturbidimetric assay reportable values were obtained. Moreover, capillary electrophoresis showed the presence of haemoglobin Hope in all the samples.

Conclusion: Hb hope might lead to overestimation and possible misinterpretation of glycated haemoglobin results. Hb Hope is extremely rare in India but the possibility of interference by such clinically silent variant should always be kept in mind while interpreting the HbA1c values.

Keywords: Bio-Rad variant II turbo, Capillary electrophoresis, Immunoturbidimetric assay

INTRODUCTION

Glycated haemogobin (HbA1c) is a result of non-enzymatic glycation of hemoglobin with plasma glucose and is known to be clinically important in identifying the average plasma glucose concentration over periods of time. HbA1c is a measure of the beta-N-1-deoxy fructosyl component of haemoglobin. The extent of glycation and the relative involvement of β chains of haemoglobin remain unclear [1, 2]. HbA1c is expressed as the percentage of total haemoglobin which is 50% higher than HbA1c alone. Depending on the method used for estimating, the level of HbA1c is approximately 4–6% in healthy patients without diabetes. In clinical practice, the measurement of HbA1c serves as the marker the glycaemic control over the last three months and to determine whether the patient's blood glucose levels have remained within the target range [3]. Various methods and techniques such as high performance liquid chromatography (HPLC), immunoagglutination, boronate affinity assays, and electrophoresis have been developed based on different principles to measure HbA1C clinically but the designated DCCT comparison method is a cation exchange HPLC [4,5]. Despite advances in the measurement of glycated haemoglobin, an increasing number of hemoglobinopathies have been reported to cause falsely elevated or falsely low HbA1c results [3].

Hb Hope is a clinically asymptomatic [beta136 (H14) Gly→Asp (GGT→GAT)] unstable variant of the β-globin chain that is frequently found in the Thai population [6]. It is extremely rare in India. We report the interference of Hb Hope with glycated haemoglobin values in South India.

MATERIALS AND METHODS

It is a clinical study conducted in Department of Biochemistry, Kasturba Hospital, Manipal from April 2013 to January 2014. HbA1c measurements were performed on 16000 ethylenediamine tetra-acetic acid (EDTA) blood samples by Bio-Rad Variant II turbo, a cation-exchange HPLC system. Chromatograms of samples run in the months of April 2013 to January 2014, were visually inspected for presence of any abnormal patterns or peaks. Eight EDTA blood samples showed abnormally high values of HbA1c (>50 gm%) so the presence of variants was suspected. The presence of abnormal patterns on the chromatograms was confirmed by running the samples on another cation-exchange HPLC analyzer Biorad D10.

Glycated Hb was also measured by immunoturbidimetry for these samples. These 8 samples were further processed

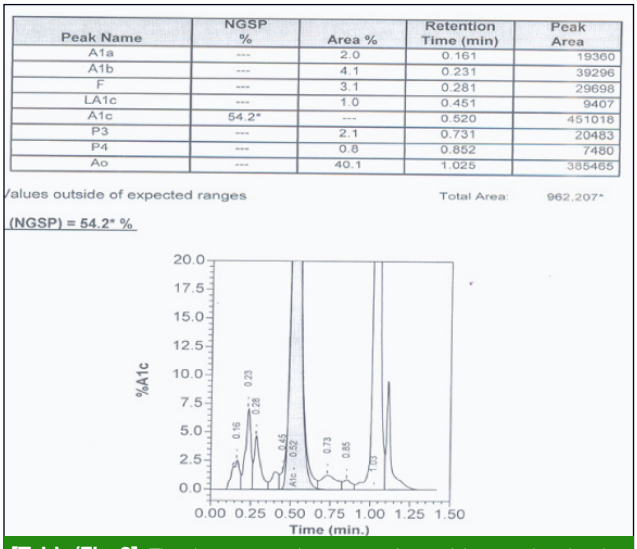
by capillary electrophoresis to look for the presence of any abnormal hemoglobin variants. Institutional ethical committee clearance was taken.

RESULTS

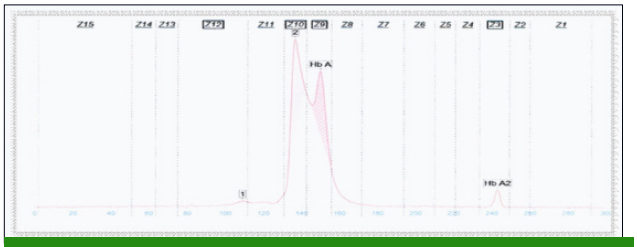
All the eight samples showed elevated (>50gm%) glycated haemoglobin values on HPLC platform [Table/Fig-1]. A single abnormally high peak was observed for all the samples in the chromatogram leading to very high glycated haemoglobin values (>50 gm%) [Table/Fig-2]. These samples were further processed using immunoturbidimetric assay of glycated haemoglobin estimation where, reportable values were obtained. Furthermore, capillary haemoglobin electrophoresis was performed on these samples which showed the presence of rare haemoglobin variant, haemoglobin Hope in all the samples [Table/Fig-3].

Samples	Glycated hemoglobin by HPLC (gm%)	Glycated hemoglobin by immunoturbidimetry (gm %)	Hb (A2) (%)	Fasting blood sugar (mg/dl)
Sample 1	56.3	8	6.5	156
Sample 2	54.2	5.6	7.2	104
Sample 3	52.4	5.2	5.5	151
Sample 4	51.9	5.1	5.2	130
Sample 5	56.0	5.5	7.7	109
Sample 6	57.3	5.4	7.4	106
Sample 7	54.2	6.3	6.2	96
Sample 8	56.7	5.9	6.8	102

[Table/Fig-1]: Glycated hemoglobin values of different samples as measured by different methods.



[Table/Fig-2]: The chromatography pattern of one of the samples showing abnormally high HbA1c by high performance liquid chromatography.



[Table/Fig-3]: Capillary electrophoresis pattern of the one of the samples showing the presence of Hb Hope.

DISCUSSION

Several methods are available for glycated haemoglobin estimation like affinity-binding chromatography, cation-exchange chromatography, and immunoassays. BIORAD Variant turbo /D-10 utilizes the principle of HPLC to estimate glycated haemoglobin. Hb species elute from the cation-exchange column at varying times with the application of buffers of increasing ionic strength and the eluted fraction is estimated spectrophotometrically. Ion exchange HPLC method is an NGSP certified method for estimating glycated haemoglobin and it separates Hb species based on charge differences. Presence of variants was suspected in all the samples showing abnormally high HbA1c values (>50gm %). Number of Hb variants like Hb D, Hb Punjab substitute a neutral amino acid for a positively charged amino acid on the α or β chain. This charge alteration decreases the retention time of the non glycated variant causing them to co-elute with glycated fraction leading to a substantial overestimation of HbA1c.

These samples when processed using immunoturbidimetry and values were found to be reportable. Immunoturbidimetric assay methods use antibodies that recognize the N-terminal glycated amino acids of the beta-globin chain of the haemoglobin. Therefore, haemoglobin variants with mutations only in specific region will affect HbA1c values by immunoassay. For variant evaluation, samples were sent for capillary electrophoresis and showed the presence of rare haemoglobin variant, Hb Hope. The single high peak by HPLC might be due to the elution of Hb Hope in the same window as HbA1c leading to the masking of Hb Hope peak and thus the overestimation.

The interference of Hb hope with estimation of glycated haemoglobin by HPLC is very rarely reported in India. HPLC method for the HbA1c estimation is based on charge differences; haemoglobin variants with amino acid substitutions on globin chains lead to the charge difference on haemoglobin which gives rise to overestimation. These charge differences vary the retention time of the non glycated variants fraction causing them to co-elute with glycated fraction causing overestimation of HbA1c according to Alla Joutovsky et al., [7]. HPLC methods can detect the presence of haemoglobin variant in the sample, but they lack the ability to specify or quantitate the variant present.

Variant haemoglobins which lead to alteration in charges in the haemoglobin molecule are expected to interfere only in the assays that are based on charge but not the assays which are based on antigenic characteristics thus, when we estimated haemoglobin using immunoturbidimetry which

uses antibodies that target N-terminal glycated amino acids on the β-chain to quantify Hb A1c, the Hb A1c values were found to be in reportable range in these 8 samples.

Capillary electrophoresis has the ability to completely separate and identify the haemoglobin variants. In our samples, Hb Hope peak was found in zone 10 of the electrophoretogram. Additionally, these samples showed increased Hb A2 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 [8]. 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 [9]. Hb Hope [β136 (H14)Gly→Asp (GGT→GATOMIM: 141900.0112; dbSNP: 33949486)] 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 [10].

Hb Hope is characterized by a comparable charge altering mutation (β 136 Gly→Asp) and the intra chain salt bridge so formed between the carboxyl group of β136 Asp and the charged α amino group (NH2+) of β1 valine leads to altered behavior of Hb Hope. This salt bridge neutralizes the positive charges to the extent that HbA1c and Hb hope co-elute from the HPLC column [11]. Since, at pH 8.6 no charge difference can be detected between Hb Hope and Hb A. So in HPLC method both elute in same window leading to probable overestimation [12]. As capillary electrophoresis was done at pH 9.4, Hb Hope and Hb A can be differentiated from each other. There are numerous studies indicating the interference by variant haemoglobins in glycated haemoglobin but studies illustrating the interference by Hb Hope specifically on glycated Hb in Indian perspective are very few as the prevalence is documented to be very less in our sub continent. Accidental discovery of this abnormal haemoglobin emphasises 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.

LIMITATIONS

Samples involved in our study were from single strata of population (i.e) South India and prevalence of variants is known to be region dependent so, further studies with larger sample sizes should be taken up for generalizing the result for the Indian sub continent.

CONCLUSION

As the effect of hemoglobin variants on HbA1c measurements is highly method-dependent whenever erroneous results are obtained on HPLC, the presence of haemoglobin variant Hb Hope can be suspected even in Indian scenario and efforts should be made to identify the variant by electrophoresis before taking any clinical decisions or planning the management.

REFERENCES

[1] Kilpatrick E. Glycated haemoglobin in the year 2000. *Journal of Clinical Pathology*. 2000;53(5):335-39.
[2] John WG. Glycated Haemoglobin analysis. *Annals of Clinical Biochemistry.* 1997;34(1):17-31.
[3] Schnedl W, Liebminger A, Roller R, Lipp R, Krejs G. Hemoglobin variants and determination of glycated hemoglobin (HbA1c). *Diabetes/Metabolism Research and Reviews*. 2001;17(2):94-98.
[4] Bry L, Chen PC, Sacks DB . Effects of hemoglobin variants and chemically modified derivate on assays for glycohaemoglobin [review]. *Clin Chem*. 2001;47:153-63.
[5] Little RR, Rohlfing CL, Wiedmeyer H-M et al. 4. The national glycohaemoglobin standardization program: a five-year progress report. *Clin Chem*. 2001;47(11):1985-92.
[6] Ingle J, Adewoye A, Dewan R, Okoli M, Rollins L, Eung S et al. Hb Hope [β136(H14)Gly→Asp (G G T→G A T)]: Interactions with Hb S [β6(A3)Glu→Val (G A G→G T G)], other variant hemoglobins and thalassemia. *Hemoglobin*. 2004;28(4):277-85.
[7] Joutovsky A. HPLC Retention time as a diagnostic tool for hemoglobin variants and hemoglobinopathies: a Study of 60000 samples in a clinical diagnostic laboratory. *Clinical Chemistry*. 2004;50(10):1736-47.
[8] Panyasai S, Sukunthamala K, Jaiping K, Wongwiwatthananukit S, Singboottra P, Pornprasert S. Interference of hemoglobin hope on - thalassemia diagnosis by the capillary electrophoresis method. *American Journal of Clinical Pathology.* 2011;136(1):14-18.
[9] Alphabetical hemoglobin variant list. Hemoglobin. 1995;19(1-2):125-49.
[10] Beneitez D, Carrera A, Ramón Duran-Suárez J, Paz V, León A, García Talavera J. Heterozygous Hb Hope [β136(H14) Gly→Asp] in Association with heterozygous β 0 -thalassemia with apparent homozygous expression, in a spanish patient. *Hemoglobin*. 2006;30(1):45-49.
[11] Thillet J, Caburi J, Brun B, Cohen-Solal M, Garel M, Minh M et al. Abnormal functional properties of Hb hope αCA2β2 (H14) Gly → Asp: A low oxygen affinity hemoglobin with decreased DPG effect. *FEBS Letters*. 1974;47(1):47-52.
[12] Enoki Y, Ohga Y, Furukawa K, Takaya A, Sakata S, Kohzuki H et al. HB hope, β136(H14)Gly→Asp, in a diabetic japanese female and its functional characterization. *Hemoglobin*. 1989;13(1):17-32.

AUTHOR(S):

1. Dr. Mrinal Gupta
2. Dr. Priyanka Datta
3. Dr. Pragna Rao

PARTICULARS OF CONTRIBUTORS:

1. Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal, Karnataka, India.
2. Department of Biochemistry, Kasturba Medical College, Manipal University Manipal, Karnataka, India.
3. Department of Biochemistry, Kasturba Medical College, Manipal University Manipal, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Pragna Rao,
Department of Biochemistry, Kasturba Medical College, Manipal University Manipal-576104, Karnataka, India.
E-mail: drpragnarao@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Publishing: **Jan 05, 2016**