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Evaluation of HbA1c measurement by HPLC andenzymaticmethodmeasuring N-terminal fructosyl dipeptide of β–chain in sickle cellpatients with Diabetes mellitus

KEYWORDS

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Introduction

Hemoglobin Variants are one of the designated factors which may influence the accuracy of HbA1c level according to what type of assay method is used . Prevalence of sickle cell trait in Gujarat is quite high (6.54%) [1]. This common clinically silent hemoglobin variant may interfere in proper analysis of HbA1c. [2–6] But no systematic study available in this respect in tertiary care hospital in Ahmedabad. Sheth V S General hospital a tertiary care teaching hospital. This hospital caters patients from different ethnic of life. After years of using an automated enzymatic method which measures N-terminal fructosyl dipeptides of the β-chain of HbA1c in our Clinical Biochemistry laboratory, we recently changed to a HPLC-based method which is based on ion exchange chromatography. The change of method by HPLC enabled us to identify the presence of HbS in diabetic patients from the abnormal peaks in the chromatograms, which was not possible when we were using the enzymatic-based method. This may cause underreport or over report of HbA1c by our laboratory. This may ultimately alter treatment plan and effective therapy of Diabetes mellitus patients.

Considering the above mentioned facts we have undertook present our study is to compare the HbA1c values measured on HPLC as well as enzymatic method in patients who were detected with HbS.

Materials and Methods

Samples received in the laboratory for HbA1c measurement were included in the study. All the patient results evaluated in this study were known stable cases of type 2 diabetes mellitus whose medical therapy had been unaltered over the last 12 months. HbA1c measurements were performed from ethylene diamine tetra-acetic acid (EDTA) blood samples using ion-exchange HPLC on Bio-Rad D-10, Diabetes Mode. Now HbA1c values of the patients diagnosed with HbS measured using HPLC were compared with the HbA1c values derived using the enzymatic method on Architect C-8000, which was obtained from the laboratory information system (LIS).

Results

A total of numbers of 350 patient's samples having diabetes mellitus were received in the laboratory during August 2014 to July 2015 for HbA1c testing. Out of 21 diabetic patients were identified to have hemoglobin variants: elevated heterozygous HbS (n=20), homozygous HbS (n=1).

Table

Hemoglobin variant	% HbA1c (mean + SD)		*P* value
	HPLC (Bio-Rad D10)	Enzymatic (Architect C-8000)	
HbS trait (n = 20)	7.82± 2.34	9.88 ± 3.37	<0.05
Homozygous HbS (n = 1)	Undetectable	10.7	N/A

NA – Not Applicable

Patients with heterozygous HbS showed significantly higher HbA1c by enzymatic than HPLC (mean ± SD of 9.94 ± 3.36% and 7.99 ± 2.42%, respectively, *P* <0.05). The significance of the HbA1c differences between HPLC and enzymatic method for patients with homozygous HbS was not able to be computed due to the limited data available.

Discussion

At present more than 1000 hemoglobin variants have been identified. [7] Most of them are clinically silent. HbA1c deviation of 1% reflects a change of 1.4 – 1.9 mmol/L in average blood glucose concentration.[2-6] So, if there is a presence of clinically silent hemoglobin variant this causes a falsely high or low HbA1c value that can give falsely high or low blood sugar concentration. And this will lead to over- or under-treatment of diabetic patients. Ion-exchange high performance liquid chromatography (HPLC) is one of the methods that is affected by presence of hemoglobin variants on HbA1c measurements. [8–10] Since last year we have started analyzing HbA1c by Bio-Rad D10, which is a dedicated HPLC system for analysis of hemoglobin variants along with HbA1c. This enables us to detect HbS during measurement of HbA1c. In this ion-exchange HPLC system separates hemoglobin species based on charge differences. Co-elution of the hemoglobin variant with HbA1c will cause gross overestimation of HbA1c, while co-elution of the hemoglobin variant with HbA, with resolution of the glycated hemoglobin variant from HbA1c, will underestimate the HbA1c results. When the glycated derivatives of the hemoglobin variant co-elute with HbA1c, and the non-glycated hemoglobin variant is resolved from HbA, overestimation of HbA1c will occur. Hemoglobin variants with mutations in this susceptible region will affect HbA1c measurements by enzymatic method. [11]. This scope was not fulfilled by enzymatic analysis, which was the only method in our laboratory. But now we have both the methods available in our laboratory. HbS is comparatively common haemoglobin variant in Ahmedabad population. Whether this factor influence HbA1c no hospital based systematic studies has been done in this regard in Ahmedabad.

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But between HPLC and enzymatic methods there may be possibility of calibration bias. So for correction of this bias, we have compared result from homozygous HbA (normal adult hemoglobin) sample and corrected the bias in this present study. Linear regression and correlation coefficient were used to determine whether the presence of hemoglobin variants caused a statistically significant difference ($P < 0.05$) in HbA1c results measured by HPLC relative to enzymatic as the comparison method.

We found significantly higher HbA1c results when we measured with enzymatic than with HPLC, in patients with an HbS trait. The effect of the HbS trait on HPLC varies depending on the method and platform used.[11] Studies by Roberts *et al*. show that HA-8160 is not affected by the HbS trait, but Cobas Integra shows a clinically significant positive bias.[14,15] This is probably because the amino acid substitution alters the shape of the protein and binding characteristic of protease enzyme, causing interference with HbA1c estimation. This finding shows that when we use an enzymatic method to measure HbA1c, there is a probability that patients with the HbS trait have been overtreated due to the higher HbA1c results produced, without us realizing it, as we are not able to identify the presence of hemoglobin variants with the enzymatic method.

In our patient with homozygous HbS, HbA1c by HPLC was undetectable, while the enzymatic was able to produce HbA1c results, which was above the reference range.

A case study by Higgins *et al.* reported that HPLCs (Tosoh G7 and Bio-Rad Variant II) showed no HbA1c result in a patient with homozygous HbS, while enzymatic (Siemens DCA 2000) gave a falsely low HbA1c result due to decreased red cell survival and high HbF. [13] The measurement of HbA1c to monitor glycemic control in diabetic patients with homozygous HbS has limited value and should be interpreted with caution, as factors such as anemia, decreased red cell survival causing decreased glycated hemoglobin values, increased HbF, and transfusion requirements may affect the HbA1c results.[11-13]

Conclusion

Routine analysis by the ion-exchange chromatograms can identify the presence of HbS, which are clinically silent and further investigations can be done when necessary, including family studies and genetic counseling. Knowledge and awareness of hemoglobin variants like HbS affecting HbA1c measurements is essential, especially in areas with a high prevalence of hemoglobinopathy, in order to avoid mismanagement of diabetic patients.

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