



PERFORMANCE CHARACTERISTICS OF DIRECT ENZYMATIC HBA1C ASSAY VERSUS HPLC IN HAEMOGLOBIN VARIANTS OF SUBHIMALAYAN POPULATION

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ABSTRACT **Background and Objective:** It is known that Hb structural variants and synthesis disorders, can affect the HbA1c measurement in different assays. Capillary electrophoresis or boronate affinity HPLC method is considered superior to ionic HPLC in case of variants. Due to inavailability of these methods in North Bengal owing to high cost, our aim of study was to compare the influence of variants in HbA1c estimation between ionic HPLC and enzymatic method. **Method:** HbA1c level estimation of 100 different haemoglobin variants attending OPD of North Bengal medical college was done using ionic HPLC BIORAD D10 and direct enzymatic method by Diazyme in automated analyser EM360 and grouped into diabetic and non diabetic. **Result :** Homozygous traits yielded no results by HPLC. Mean HbA1c levels by HPLC and enzymatic methods in the diabetic and non diabetic traits were 8.80 ± 2.28 , 9.30 ± 2.36 , 5.14 ± 0.79 , 6.12 ± 0.80 respectively. Pearson's correlation coefficient between HPLC and enzymatic in diabetic traits was 0.992 and that in non diabetic variants was 0.525. HbA1c by HPLC in non diabetic traits was found to be poorly correlated with their fasting and post prandial sugar. **Conclusion:** HbA1c estimation using HPLC in the presence of hemoglobin variants particularly in pre diabetics show relevant interference that can delay diagnosis and therapeutic monitoring which is not so with enzymatic assay.

KEYWORDS : HbA1c, HPLC, direct enzymatic assay, Capillary electrophoresis

INTRODUCTION

The global prevalence of diabetes mellitus is increasing rapidly. Measurement of glycosylated hemoglobin, predominantly HbA1c, is fundamental to the management of patients with diabetes¹. Hemoglobin A_{1c} (HbA_{1c}) is an important indicator of mean glycemia in patients with diabetes that has been shown to be strongly predictive of diabetes complications^{2,3,4}. The ADA has recently recommended HbA1c with a cut-point $\geq 6.5\%$ for diagnosing diabetes as an alternative to fasting plasma glucose (FPG ≥ 7.0 mmol/L) & HbA1c 5.7%–6.4% as pre diabetic⁵. Various methods are available for measuring HbA1c namely- HPLC- ionic and boronate affinity, direct enzymatic assay, immunoturbidometry, capillary electrophoresis etc. Cation-exchange HPLC is a well established and widely used method for HbA1c measurement, which enables reliable detection of the HbA1c fraction and can identify possible hemoglobin variants, even if they may interfere with the HbA1c^{6, 7,8}. Although enzymatic-photometric methods are relatively new, studies have shown good precision and accuracy^{6,9,10}. It has been evaluated in many studies that capillary electrophoresis or boronate affinity HPLC are superior to ion exchange HPLC as they are minimally interfered by Hb variants.

MATERIALS AND METHOD

The present study is an institutional based observational comparative study conducted among 100 diabetic as well as non diabetic haemoglobin variants above 35 years irrespective of gender attending North Bengal Medical College and hospital, a tertiary care centre in the sub Himalayan belt. 130 different Hb variants were selected based on Hb A₂, HbF chromatogram by HPLC Tosoh G8 showing variant peak/ window. Those with haemoglobin less than 7 g/dl, any acute illness, cardiac pathology, malignancy, pregnancy or on drugs that cause hyperglycaemia were excluded from the study. Whole blood samples were collected in EDTA vacutainer tubes and glycosylated haemoglobin was measured by HPLC on Biorad D10 and Diazyme kit for enzymatic assay. The principle for enzymatic assay bases on a proteolytic cleavage of the N-terminal fructosyl dipeptide from lysed whole blood and subsequent spectrophotometric measurement of the activity of a fructosyl dipeptide oxidase¹¹. Also fasting and post prandial blood glucose were measured. Total 30 among the study subjects were homozygous / diseased yielding no results by HPLC. The rest 100 subjects were divided into 2 groups- non diabetic and diabetic traits (based on their HbA1c values). Finally HbA1c values by both the methods were compared in the groups.

Statistical Analysis

Data were entered in Microsoft Excel and then to SPSS version 22 for analysis. Descriptive data was represented as mean and standard deviation. Comparison between the HbA1c values by each methods in the 2 groups done by student t test. Pearson's correlation analysis

was used to indicate the association between HbA1c values by HPLC, enzymatic assay, FBS and PPBS. Scatter plots of test data and reference methods were created and their linear relationship was detected.

RESULTS

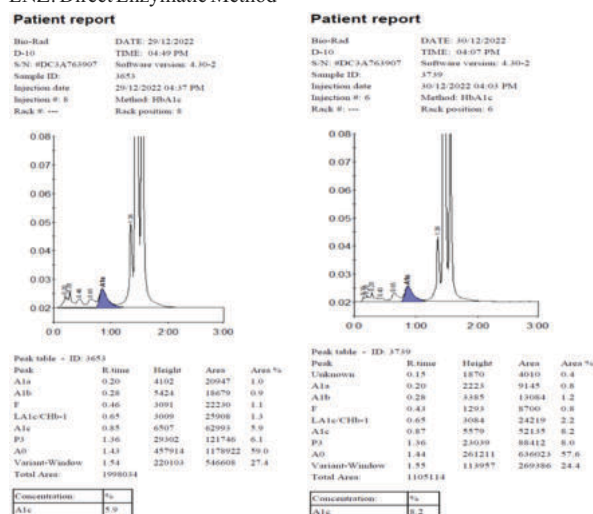
In the present Institution based Observational comparative study, the data collected from 100 Hb variants of age group (30-60) yrs was sorted, processed and analyzed with the help of statistical software SPSS (version 22) and Microsoft Office Excel 2010. Homozygous traits which yielded no results by HPLC were at times found to be profound diabetic with HbA1c values of 9.4% or 8.2% by enzymatic method which remains unexplained.

Table 1

VARIABLES	Mean \pm SD			
	Non Diabetic traits (N=50)		Diabetic traits (N=50)	
HbA1C (%)	HPLC	ENZ	HPLC	ENZ
	5.14 ± 0.79	6.12 ± 0.80	8.80 ± 2.28	9.30 ± 2.36

HPLC: High Performance Liquid Chromatography

ENZ: Direct Enzymatic Method



Non Diabetic Variant

Diabetic Variant

HPLC chromatogram showing HbA1c graph and values along with variant window.

Table 3: Pearson Correlation Coefficient(r) Of Hba1c Values Between HPLC, ENZ, PEIT, FBS,PPBS In The Non Diabetic Traits

Correlations		HPLC1	FBS	ENZYMATIC1	PPBS
HPLC1	Pearson Correlation	1	.347*	.525**	.284*
	Sig. (2-tailed)		.014	<.001	.046
	N	50	50	50	50
FBS	Pearson Correlation	.347*	1	.651**	.810**
	Sig. (2-tailed)	.014		<.001	<.001
	N	50	50	50	50
ENZY MATIC 1	Pearson Correlation	.525**	.651**	1	.744**
	Sig. (2-tailed)	<.001	<.001		<.001
	N	50	50	50	50
PPBS	Pearson Correlation	.284*	.810**	.744**	1
	Sig. (2-tailed)	.046	<.001	<.001	
	N	50	50	50	50

*. Correlation is significant at the 0.05 level (2-tailed).
 **. Correlation is significant at the 0.01 level (2-tailed).

HPLC1: HbA1c values by HPLC in group 1 ie. non diabetic traits

ENZYMATIC1: HbA1c values by Direct Enzymatic Method in group 1 ie. non diabetic traits

In group 2 ie. diabetic traits HbA1c values by HPLC showed very good correlation with FBS,PPBS and enzymatic method(p<.001)

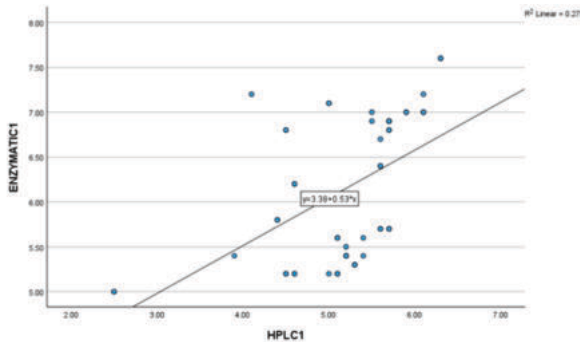


Fig 1: Graph Showing Correlation Of Hba1c Values Between Enzymatic And Hplc Method In Pre Or Non Diabetic Group:

DISCUSSION

The measurement of hemoglobin A1C (HbA1c) is an essential part of diabetes mellitus diagnostics and therapy monitoring¹². In some situations different methods of HbA1c estimation yield results with undesirable differences, hence it is very important to compare the methods used in most clinical laboratories¹³. In previous studies it has already been demonstrated that enzymatic method for HbA1c analysis method has a concordance and correlation with the most accepted reference method HPLC^{1,14}.

In our study, while enzymatic method evaluated did not show clinically significant interference with HbA_{1c} results in the presence of the tested variants, ion-exchange HPLC methods showed interference from homozygous as well as heterozygous variants. The homozygous or diseased variant did not yield any result by HPLC while among the diabetic and nondiabetic traits also significant differences in HbA1c values observed when compared to enzymatic method. Also HPLC unlike enzymatic method in pre or non diabetic traits showed poor correlation to FBS, PPBS. This can be a significant issue both in terms of clinical management and diagnosis of diabetes, especially in populations where there is a significant prevalence of one or more of prediabetic Hb variants. Though interfered values of HbA1c by HPLC may not cause any hindrance or change in the treatment of known or profound diabetes but in prediabetic variants this mismatch becomes a major factor in the delay of diagnosis, prevention or treatment of diabetes.

CONCLUSION

HPLC by ion exchange method particularly in case of prediabetic haemoglobin variants gives false or interfered HbA1c values that might hamper the diagnosis, prevention or treatment of diabetes in

undiagnosed patients. So in this ethnic population of subhimalayan belt, where different Hb variants specially Hb E are prevalent, enzymatic method for HbA1c analysis is the most precise, accurate, low cost preferable method compared to HPLC.

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