Original Research Article

Comparison of HbA1c values by immunoturbidimetric and HPLC methods

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Abstract

Glycated hemoglobin or hemoglobin A1C is stated by the international federation of clinical chemistry working group (IFCC) as Standard of Care (SOC) which is used for assessing and monitoring of history of blood glucose level past 2-3 month of test. In current study we have compared immunoturbidimetric method which is light scattering immunoassay and analytical performance of D10 hemoglobin (HPLC) testing system. In this study total 152 patients of were included from Kasturba Hospital Manipal. The selected patients were between 20 to 80 years of age of both sexes. HbA1c was estimated by both the Immunoturbidimetric and HPLC methods. Both the Immunoturbidimetric and HPLC methods showed no significant difference in the HbA1c values. There is no significant difference in the values between the males and females, and age below and above 50 years. The comparison of Bio-Rad quality control values of both level 1 and level 2 between HPLC method and Immunoturbidimetric method were not significant. The study concluded that both the methods are reliable for the estimation of HbA1c and can be recommended for the management of diabetic patients.
Key words: HbA1c, HPLC, Immunoturbidimtric, Quality Control

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INTRODUCTION

Glycated hemoglobin or hemoglobin A1c is stated by the international federation of clinical chemistry working group (IFCC) [1] as Standard of Care(SOC) which is used for assessing and monitoring of history of blood glucose level past 2-3 month of test.[2] Huisman was the first person isolated HbA1c in 1958[3] and which is characterized by Bookchin and Gallop in 1968,[4] then HbA1c levels are elevated in diabetic patients were described by Rabbar in 1969.[5] This pathway is identified by the Bunn and Koenig,[6] they proposed HbA1c as a biomarker for monitoring the levels of glucose among diabetic patients. [7] Erythrocytes life span is about 120 days that enables HbA1c which is used as an index of glycemic control [8] Glycated hemoglobin is an irreversible non enzymatic addition of a sugar residue to the hemoglobin, the rate of production is directly proportional to the glucose concentration. [9] HbA1c are obtained from red blood cells, being separated on the basis of cation exchange chromatography. [10,11],[12]. Monitoring of HbA1c is suggested by the American Diabetic association, American diabetic federation, and European association for the management of diabetes. [13] So HbA1c is now routinely obtained as the most prominent, single and independent parameter of metabolic control. [14] It is a risk factor for the growth and development of diabetic complications and significantly used in treatment and management. [15,16] Analysis of HbA1c in blood gives evidence about individual's average blood glucose levels in the period of two to three months; it is specified as half-life of red blood cells. [17] The different methods for resolution of HbA1c have been developed. These methods depend on different physical, chemical or immunological feature of the glycated hemoglobin. [18,19] For patient monitoring, management and control of diabetes, cardiovascular disease,

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pancreatitis, nephropathy, renal failure and other diseases - HbA1c is used. So its measurement should be precise and accurate. Estimation of HbA1c regularly may produce illogical results in different methods. [20,21] It has long been recognized that regular glyco hemoglobin measurement in patients with diabetes mellitus provides a valuable tool in the assessment of long-term metabolic control [22-24] Diabetes Control and Complications Trial (DCCT) results gave sufficient proof of the significance of keeping excellent metabolic control in decreasing the risk of late growth and development. [25]. As dissimilarity methods for its measurements produce results with unwanted differences, it has become significant to compare the results of different methods used by different laboratories. In current study we have compared immunoturbidimetric method which is light scattering immunoassay and analytical performance of D10 hemoglobin (HPLC) testing system which is an analytical method helps to identify, separation and to determine the mixture containing components. [26], [27] The motive was to assess a technique with higher precision and enhanced accuracy. The aim is to compare the two methods to know the variations in the results obtained using the same sample. We also correlated Glycated hemoglobin results with different biochemical parameters namely Age, Sex, Lipid profile, Fasting blood glucose, Urea and Creatinine.

MATERIALS AND METHODS

After approval from Institutional Ethical Committee, total 152 patient samples were collected from Clinical Biochemistry Laboratory of Kasturba Hospital Manipal. All the patients are from 20 to 80 years of age. 5ml of blood was collected in EDTA vacutainer tube and stored at 2-8 degree. Roche Immunoturbidimetric methods (Roche Hitachi 902) and D10 hemoglobin testing system (Bio RAD Laboratories) are the two methods which are used to measure HbA1c .The newly established fully automated analyzer that is Bio RAD D10 hemoglobin testing system from cation exchange HPLC. The double kit recorder pack carry blood primer, elusion buffer 1 and 2, Calibrator 1 and 2, diluents calibrator, wash reagent and cartridge. The manufactures instructions were followed for the analysis of quality control and calibrations. Both the technique requires manual handling of patients sample or predilution. The samples in the primary tubes were introduced first followed by both control samples and calibrators. Samples were spontaneously mixed, diluted and introduce into the cartridge. The analyzer transports a buffer gradient which help in increasing ionic strength and helps in separate on the basis of their cartridge material and their ionic interactions. So that the hemoglobin's which are separated and they passed through the photometer filter absorbance at 415nm. Time of run is about 3min per sample with in every part of 20 samples per hour so that for each sample chromatogram and reports are produced. On the Roche Cobas 6000 analyzer the immunoturbidimetric assay was performed as per the manufacturer's instructions. Initially, by using hemolysing agent the sample is diluted and kept at room temperature for about 10 min. Depend on turbidimetric Inhibition immunoassay the HbA1c is determined. In starting stage the sample contain glycohemoglobin reacts with anti- HbA1c antibody to form antigen soluble – anti body complexes. After that R2 (polyhaptens) is added. So that polyhaptens react with anti-HbA1c antibodies to form an antibody Insoluble – polyhapten complex by turbidimetrically. The immunoturbimetric method is standardized through IFCC reference system and results are described in m mol/mol conversion factor is installed in the analyzer for the conversion of results to % HbA1. The Bio-Rad Laboratories Lypocheek Diabetic controls (high and low HbA1c concentration)were used.

OBSERVATIONS and RESULTS

By the use of standard SPSS Software version 16 (SPSS In, Chicago) the data were analyzed. For quantitative variables mean (+SD) was given and for qualitative variables percentage and frequency were given and for determining the power of direct relationship between HbA1c measurement by two methods Pearson correlation was used. The significance of threshold was 0.001 for two-tailed test. And to calculate the mean difference Bland and Altman plots were used and agreement between two techniques. It was considered that 95% of all values lying within (±SD) indicate a good agreement. For analysis, the results of HbA1C values are divided into male and female, above and below 50 years of age. Both the Immunoturbidimetric and HPLC methods showed no significant difference there is no significant difference in the values between the males and females, age below and above 50 yr. The comparison of quality control values of both lavel 1 and level 2 between HPLC method and Immunoturbidimetric method were not significant. There were not significant difference between the coefficient of variation, bias and sigma matrix. The results of HbA1c value by immunoturbidimetric method with other parameter like age, lipid profile, urea, and creatinine are not significant.

Table 1: Comparison HbA1c values by Immunoturbidimetric and HPLC methods

Methods	Mean±SD (N±152)	* P value
Immunoturbidimertic (%)	6.97±2.98 0.884	0.884
HPLC (%)	6.93±3.10	

Independent sample test

Table 2: Comparing Quality control 1 values by Imminoturbidimetric and HPLC methods

Methods	Mean±SD	CV	Bias	Sigma	* P value
Immunoturbidimertic(%)	5.15±0.15	2.9	-0.05	3.0	0.010
HPLC (%)	5.01±0.10	2.1	-2.0	2.0	

Independent sample test

Table 3: Comparing Quality control 2 values by Immunoturbidimetric and HPLC methods

Methods	Mean±SD	CV	Bias	Sigma	* P value
Immunoturbidimertic(%)	9.69±0.22	2.2	4.4	-0.6	0.289
HPLC (%)	9.63±0.16	1.7	4.4	-0.8	

Independent sample test

Table 4: Correlating GlycoHb of Immunoturbidimetric method with other parameters

Parameters	N	r value	*P value
Age	152	0.09	0.91
FBS	150	0.22	0.06
PPBS	22	0.38	0.07
Total cholesterol	65	0.02	0.82
Triglyceride	63	0.18	0.13
HDL	63	0.03	0.80
LDL	63	0.05	0.65
Urea	62 0.	10	0.40
Creatinine	68	0.00	0.95

Independent sample test

DISCUSSION

Various techniques tend to produce outcomes with undesirable variations for its measurement. Comparing the outcomes of distinct techniques used by distinct laboratories has become very crucial. In terms of accuracy, both techniques were compared and their general correlation was also evaluated using correlation analysis. In some circumstances, these two techniques produce outcomes with undesirable variations, so comparing these techniques in most clinical laboratories is very crucial. Both techniques with control samples had excellent outcomes. Our study results showed no significant difference in the values between the two methods may be because of the standardization and traceability. Both methods are National Glycohemoglobin Standardization Program (NGSP) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) certified. There is also no significant difference in the comparative values between gender and age group. This also indicates high precision and accuracy of methods across gender and different age groups. The study also showed that there is no significant correlation between the GlycoHb estimated by the immunoturbodimetric method value and other parameters like FBS, PPBS, lipid profile, Urea and Creatinine. The mean quality control values between the two methods were not significant which indicates that both the methods are highly accurate and precise. Even there is no significant variation in the coefficient of variation, bias and sigma matrix. Over all the study indicates no significant difference between the two methods.

CONCLUSION

There is no significant difference found between the two methods i.e. Immunoturbidimetric and HPLC method for Glycated hemoglobin estimation. So we conclude from our study that both the methods are reliable for the estimation of HbA1c. So it can be recommended that both methods can be used for the management of diabetic patients.

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