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HbA1c ESTIMATION- IS IMMUNOTURBIDIMETRY A RELIABLE SUBSTITUTE FOR HPLC?

Clinical Biochemistry

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ABSTRACT

Background: Glycated haemoglobin (HbA1c) has been gaining importance over the past few decades as the test of choice for diabetes. The gold standard for its estimation is HPLC, which is most often not cost effective and requires skilled personnel to perform. Our mission hospital is a tertiary care institution run in a rural area of Kerala, India. Patients who are most often economically backward come from afar in the hope of getting their tests done and starting treatment if any on the same visit. The hospital laboratory already has the Ortho clinical diagnostics Vitros 5600 machine with an immunoturbidimetric method for HbA1c assay. We explore here the possibility of using this machine to carry out routine HbA1c tests with accurate and comparable results.

Methods: HbA1c assay was done in 387 blood samples by both methods Immunoturbidimetry and HPLC. The agreement of HbA1c measurements between HPLC and Immunoturbidimetry were studied using Kappa statistic and Bland Altman analysis. A simple linear regression was constructed to predict the HPLC values based on Immunoturbidimetry values.

Results: There is a high agreement [Kappa=0.85] in the HbA1c categories between HPLC and Immunoturbidimetry. The agreement based on Bland Altman analysis for the numerical scale measurements of between HPLC and Immunoturbidimetry not found to be satisfactory. However the predictive ability of the constructed linear regression model to predict the HPLC values based on Immunoturbidimetry values was found to 97% [R2 =0.977]

Conclusion: There is a high agreement between the HPLC and Immunoturbidimetry values for the classification of diabetic status. The numerical values of HbA1c can be predicted based on the Immunoturbidimetry measurements with same credibility as that of HPLC.

KEYWORDS

Glycated haemoglobin, HPLC, Immunoturbidimetry, Diabetes Mellitus

INTRODUCTION

Glycated haemoglobin (HbA1c) is a glycated protein formed by the non-enzymatic addition of D- Glucose to the N-terminal valine of the β- chain of haemoglobin. This is purely an intracellular process and is influenced by the concentration of glucose inside the RBC. Blood HbA1c level is an indicator of long-term glycemic status[1] and is also a reliable predictor of vascular complications in diabetic patients[2,3], so that it is currently considered as the test of choice for diagnosis, monitoring and management of diabetes[4,5]. Although glycation depends on the life span of RBC which is normally 120 days, considering the average age of all RBCs in circulation at a given point of time, it is accepted that HbA1c reflects glycemic status of the previous 90 days [6]. HbA1c estimation is also useful to differentiate between stress hyperglycemia and pre-existing undetected diabetes in emergency situations when insulin therapy is needed[7]. As per American Association of Clinical Endocrinologists recommendation 2007, HbA1c value is also helpful to plan therapy in newly detected Type II DM. If HbA1c is >10%, insulin should be started from the onset of detection, if between 8% and 10% combinations of oral antidiabetic should be the choice and if <8%, treatment should be started with a single drug [8].

Inconveniences in the measurement of plasma glucose like requirement of fasting state, preparations and duration for performing an OGTT, day-to-day variability in blood glucose levels have posed the need of an alternative to glucose measurement for the diagnosis of diabetes. HbA1c test can be performed at any time of the day and does not require any special preparation such as fasting. These properties have made it the preferred test for assessing glycemic control in diabetic patients. More recently, it is gaining wide acceptance as a diagnostic test for diabetes and as a screening test for persons at high risk of diabetes [9]. HbA1c has now been recommended by an international committee and by the American Diabetes Association (ADA) as a means to diagnose diabetes [9]. ADA 2010 recommends that persons with HbA1c value ≥6.5% are to be diagnosed as diabetic and HbA1c values in the range 5.7-6.4 to be considered as category for increased risk of diabetes [10].

However, HbA1c measurement has some disadvantages too. Firstly, HbA1c value may be affected by a variety of genetic, hematologic and illness- related factors most common among them being hemoglobinopathies [1, 11,12,13], certain anemias and disorders associated with accelerated red cell turnover such as malaria [14]. The second factor is the relatively high cost and availability of HbA1c assays. A WHO expert consultation held on March 2009 recommended that HbA1c can be used as diagnostic test for diabetes, provided that stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values and there are no conditions present which preclude its accurate measurement [15].

HbA1c level can be reliably measured using various methods such as ion-exchange chromatography, High performance liquid chromatography (HPLC), immunoassay, boron affinity chromatography and enzymatic assay. Each of these methods has some advantages and drawbacks so that the choice depends on individual needs and laboratory possibilities. Fulfilment of clinical and analytical quality criteria should be taken into account during application of these methods. To achieve a uniform international standardization, the IFCC established a working group on HbA1c standardization in 1995. Two reference methods developed by this working group- HPLC and either mass spectrometry or capillary electrophoresis were approved by the IFCC in July 2001 [16]. Ion exchange HPLC remains the gold standard due to minimizing interferences and its ability to identify variants and derivatives. But the disadvantage is that the HPLC device is very expensive and not cost-effective for all laboratories and most often run as batch analysers and so becomes time consuming. Our hospital is a tertiary care unit in Kerala, India. In our setting, many patients come from afar with the hope of getting their diagnosis and subsequent treatment if any on the same day making a random access analyser the need of the hour. Hence there is the necessity for replaceable methods whose reports are closely and strongly correlated to those of HPLC reports.

In the present study we aimed to study the agreement between HPLC and Immunoturbidimetry (IT) in the measurement of HbA1c in terms of both categorical (diabetic status) and numerical data and to predict the HbA1c values attainable by HPLC using the values obtained by Immunoturbidimetry. The study was carried out in the Central Clinical Laboratory of MOSC Medical College Kolenchery, a tertiary health care hospital located in a rural area of Kerala State in South India.

MATERIALS AND METHODS

387 blood samples referred to the Central Clinical laboratory of MOSC Medical College Kolenchery for HbA1c estimation were included in the study. Whole blood collected in EDTA vacuutainer was the sample used. Exclusion criteria were clotted samples and samples insufficient in volume. HbA1c measurement was performed in all samples by 2 methods simultaneously - Immunoturbidimetry (Done using Ortho Clinical Diagnostics Vitros 5600) and HPLC (carried out by Biorad D10). HbA1c values obtained were divided into 3 categories:-[10]

(i) Normal (<5.7%)
(ii) Pre- diabetic (5.7-6.4%)
(iii) Diabetic (≥6.5%)

On the basis of a pilot study conducted, the minimum required sample size was estimated to be around 51 based on the equation for Bland Altman analysis (at 5% α, 90% power and clinically allowable difference of 0.1). In the present study we included 387 blood samples in order to increase the precision of the findings.

Statistical analysis: Agreement in the HbA1c categories between HPLC & Immunoturbidimetry was analysed using kappa statistics. The agreement of numerical scale measurement of HbA1c between HPLC and Immunoturbidimetry was analysed using Bland Altman analysis. Also a simple linear regression equation was constructed to predict HPLC value based on Immunoturbidimetry values as data follows normal distribution. The entire statistical analysis was performed using EZR software.

RESULTS:

HbA1c values obtained by HPLC and Immunoturbidimetry methods were categorized into Normal (<5.7), Pre -diabetic (5.7-6.4) and Diabetic (≥6.5)[17] and the extent of agreement between the HPLC and Immunoturbidimetry values to classify the subjects into normal, pre diabetic and diabetic was studied using kappa statistic (Table 1).

Table 1: Agreement Between HPLC And Immunoturbidimetry To Classify Subjects Into Normal, Pre Diabetic And Diabetic Using Kappa Statistic.

		HPLC			Kappa Statistic	P value
		Normal	Pre diabetic	Diabetic		
Immunoturbidimetry	*Normal*	48	10	0	0.852	P<0.001
	Pre-diabetic	4	78	14		
	Diabetic	0	3	230		

The estimated kappa was around 0.852 (p<0.001) showing that there is a statistically significant high agreement between the HPLC and Immunoturbidimetry values for the classification of glycemic status.

Also the agreement of numerical scale measurements of HbA1c between HPLC and Immunoturbidimetry was analysed using Bland Altman analysis. The differences between HPLC and Immunoturbidimetry values were plotted against the mean of the two measurements using Bland Altman plot (Figure 1) which showed that average of the difference between HPLC and Immunoturbidimetry values was -0.022 with an upper limit of 0.584 and lower limit -0.628. As these limits of agreements are beyond the clinically allowable difference of 0.1, it was concluded that there is no agreement between the measurements of HPLC and Immunoturbidimetry

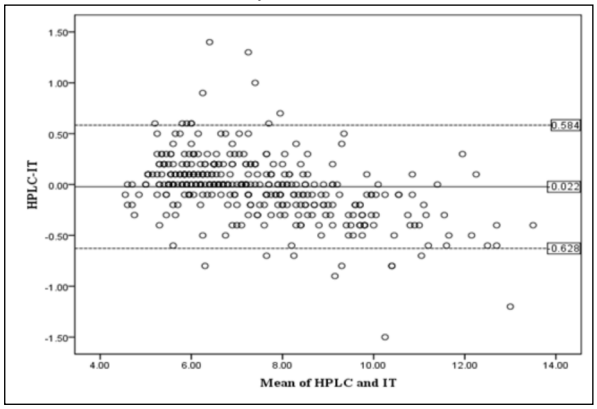

Figure 1: Bland And Altman Plot Comparing The HbA1c Values Obtained Using HPLC And Immunoturbidimetry Methods

Hence, a simple linear regression was fitted to predict HPLC values based on Immunoturbidimetry values (Figure 2).

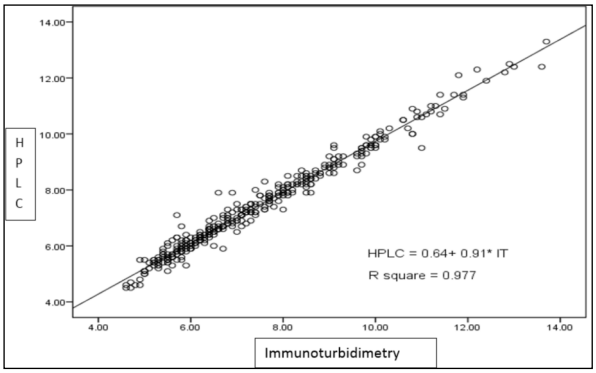

Figure 2: Relationship Between HbA1c Values Obtained By HPLC And IT

There was a high positive correlation (Pearson's correlation, r=0.98) between HbA1c measurements by two methods. The fitted simple linear regression equation is:

HPLC value = 0.64 + 0.91 × Immunoturbidimetry value.

The predictive ability of the model is found to be 97% (R^2 of 0.977) and also the model is found to be statistically significant (p<0.05). Hence HbA1c values obtained using this equation will have the same credibility as that of HPLC.

DISCUSSION

In the current clinical scenario HbA1c is gaining wide acceptance as a test of choice for diagnosis, monitoring and follow-up of Diabetes[4,5]. Thus precise measurement of HbA1c by a suitable laboratory method is essential[5]. Because employing a reference method is not affordable for all laboratories[17], there is the need for a reliable replaceable method. In the present study, we aimed to find out whether Immunoturbidimetry can be used as a reliable substitute for HPLC in HbA1c estimation. Kappa statistics with an estimated kappa around 0.852 (p<0.001) shows that there is a high agreement between the HPLC and Immunoturbidimetry values for the classification of diabetic status. This has the advantage of monitoring the therapeutic effect in diabetic patients. Bland Altman plot showed that there is no agreement between the measurements of HPLC and Immunoturbidimetry. However as there is high positive correlation between these two methods, the constructed linear regression model can be used to predict HPLC values.

Apart from these statistical observations there exist certain practical aspects that favour Immunoturbidimetry over HPLC. Primary reason being the cost of the test. HbA1c being a reliable parameter for the follow-up of diabetes, there is need for frequent check of HbA1c values and many patients cannot afford the cost of HbA1C by HPLC method. As far as clinical laboratories are concerned, HPLC device is very expensive, time consuming to work with and needs professional personnel exclusively to handle the HPLC machine[17]. No other biochemical test can be carried out in that machine. On the other hand, a machine like Vitros 5600, a random access analyser, which is being used in the Clinical laboratory of our Medical College carries out HbA1c assay by Immunoturbidimetry method is a random access analyser and many other biochemical tests can be done in that machine. It is cost-effective, time saving and needs no additional laboratory personnel. The regression line formula derived from present study can be employed to convert HbA1c values obtained by immunoturbidimetry to that of HPLC thereby elevating the reliability of the HbA1c report issued from the clinical laboratory to the gold standard value and making it feasible for all category of patients.

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