

Comparison of the analytical techniques of hba1c estimation by immunoturbidimetric and HPLC methods in diabetic and pre-diabetics patients

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Abstract

Aims & Objectives: The aim of the study is to compare the available analytical methods of measurement of HbA1c like immunoturbidimetric and HPLC methods.

Materials and Method: Total of 150 pre-diabetic and diabetic patients are included in the study. HbA1c was measured by immunoturbidimetric and HPLC methods as these are the common methods used by the clinical laboratories.

Results: The HbA1c values of two methods have a significant difference. The value of immunoturbidimetric method was less than the HPLC method. The two methods correlated with the correlation coefficient of 0.972.

Conclusion: Both the methods are reliable and show a strong correlation. Even though the immunoturbidimetric values are low it is specific and more cost effective than the HPLC methods. HPLC is a sensitive method.

Keywords: HbA1C, Immunoturbidimetric, HPLC, Diabetics, Prediabetics.

Introduction

Normal human erythrocytes have >90% of total haemoglobin. Other than HbA and HbF there are sickle cell Hb and HbA1c which occurs as post translational modification. In the RBC HbA by interacting with the glucose at the amino group of the N-terminal valine is converted to HbA1c. It is a non-enzymatic reaction amadori reaction. HbA1c is more sensitive and specific. It is least affected by most factors and most dependable parameter.⁽¹⁾ HbA1c is used as a diagnostic marker of diabetes and classification of increased risk of diabetes by ADA 2016. 5.7%-6.4% HbA1c indicate pre-diabetes, and 6.5% are diagnosed as diabetes.^(1,2) In abnormal RBC turnover the values of HbA1c will be affected by the abnormal lifespan of RBCs. The changing trends in blood glucose level can be identified by the HbA1c values. Koeng et al. indicated the relation between diabetic control and HbA1c values. By 3 weeks the glycaemic control achieved is indicated by glycosuria and by HbA1c values it was lagging behind 3-4 weeks and ended by 7-8 weeks.⁽³⁾ Ditzel and Kjaerfaard indicated that after initiating the treatment in the newly diagnosed diabetes occurs after 2 weeks and ended by 7 weeks.⁽⁴⁾ Chantelau observed that 1% decrease of HbA1c per 10 days indicated a sudden and sustained decrease of blood glucose level.⁽⁵⁾ The HbA1c values will increase from 12.6% to 14.1% after 1 week of withdrawing sulphonyl ureas was indicated by Boden et al.⁽⁶⁾ There are a number of different methods for measuring HbA1c. Hence there arises a need to identify the accurate, easy and practical method for routine use in clinical laboratories. The clinician must be aware of the method of analysis of HbA1c before interpreting the results.⁽⁷⁾

Materials and Method

150 pre diabetic and diabetic patients of 25-70 years were enrolled in the study. Among them 605 are females

and 305 are males. Whole blood was collected in the EDTA vials. The sample was hemolysed by mixing with 1000µl tetradecyl trimethyl ammonium bromide containing hemolysing reagent and incubation according to the test procedure indicated by the methodology. The samples are preserved at 4°C. The samples were analysed by HPLC method also.

Immunoturbidimetric Method: The HbA1c values of hemolysed samples were measured by Meril kit following the kit manufacturer's instructions. In this method total Hb and HbA1c in hemolysed blood are attached to the latex particles with equal affinity. The monoclonal antibodies are used to detect HbA1c, next polyclonal antibodies against monoclonal antibodies can agglutinate the particles, and the turbidity is measured spectrophotometrically.

HPLC method: We used the automated HPLC analyzer (Aspen A1c analyser) with technology based on the principles of cation exchange with gradient elution, to separate human haemoglobin subtypes and variants from hemolysed whole blood. The glycosylation of haemoglobin will cause loss of cation from the haemoglobin molecule surfaces, resulting in different positive charges between the various subcomponents of the glycosylated and non-glycosylated haemoglobin. Glycosylated haemoglobin has less positive charges and lower binding force, while non-glycosylated haemoglobin has more positive charges and higher binding force. When the whole blood passes through the chromatography column, various haemoglobin will combine with the cation exchanger. Eluents with different pH and ionic strength are used to elute two kinds of hemoglobins with glucose successively. Later the non-glycosylated hemoglobin with high binding force is eluted. The separated hemoglobin eluent flows through the colorimetric cell. The separated haemoglobin fractions

are monitored by means of spectrometer absorption of light at 415 nm.

Statistical Analysis: The data analysed using Microsoft XL. Mean, SD and percentages are calculated. Pearson correlation (r) was used to identify the association between the HbA1c values by two methods.

Results

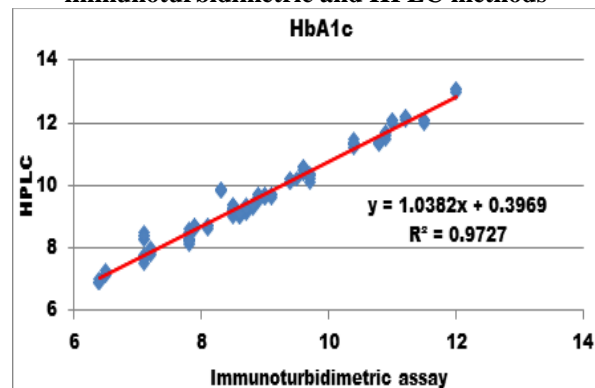
The analysis of the test results indicates that the mean HbA1c of immunoturbidimetric and HPLC methods are 8.90 and 9.64. The mean HbA1c of females in immunoturbidimetric method and HPLC methods are 8.90 and 9.60. The mean HbA1c of males in immunoturbidimetric method and HPLC methods are 8.88 and 9.67. Table 1 shows the mean HbA1c values by immunoturbidimetric method than HPLC method.

Table 1: HbA1c measured by different methods

Study group	HbA1c	
	Immunoturbidimetric method	HPLC method
Total study group	8.90	9.64
Females	8.90	9.60
Males	8.88	9.67

The HbA1c values of both methods correlated well with correlation coefficient of 0.972 as shown on the Graph 1.

Graph 1: correlation of HbA1c values between immunoturbidimetric and HPLC methods



Discussion

The diabetic care of patients are enhanced by HbA1c analysis. HbA1c has become an integral part of management of diabetes. Relation between the improved glycemic control and risk of diabetic complications has been established.^(8,9) Various methods of HbA1c measurement are HPLC, immunoassay, boronate affinity chromatography and enzymatic assay. Various studies have indicated an observed difference between HbA1c measurement based on different techniques, as the methods were standardised using reference model and calibrated with the calibrator.^(10,11) In some situations these two methods yield results with undesirable differences, hence it is very important to compare these methods used in most clinical laboratories.⁽¹²⁾ In this

study the comparison between the above mentioned methods was performed among 150 patients with HbA1c levels ranging from 4.9% to 14%. The protocol for measuring HbA1c was as per the Diabetes Control and Complications Trial (DCCT) and National Glycohemoglobin Standardization Program Standards (NGSP).⁽¹³⁾ HPLC method can identify the abnormal haemoglobin and has the reproducibility, CV <1%. Moreover HPLC needs advanced equipment's and it is a time consuming procedure. HPLC method needs only trained technicians to do the tests and maintain the equipment.^(12,13,15) Immunoassay can be done by an auto analyser, hence this method not takes a long time for measuring a large number of samples. The total haemoglobin needs to be assessed by an additional measurement.^(12,16) The HbA1c values should be monitored periodically by QC and each laboratory is responsible to determine the accurate reference values and correction factors for more reliable results. American Diabetes Association (ADA) suggested an important issue with test is the lack of available and adequate assay to manage diabetes in developing countries.⁽¹⁷⁾ Immunoturbidimetric assay is easy to use and commonly available in most of the developing countries in considerable rural populations where there is limited accessibility to advanced devices.^(10,18)

In the within run and between –run imprecision studies there was an effective reproducibility by the immunoturbidimetric HbA1c immunoassay. But the immunoturbidimetric assay is better than the commonly used methods eventhough it is less precise than the HPLC method.^(19,20,21) Because the reproducibility of the immunoturbidimetric is more better than the common methods.⁽²¹⁾ As most of the HbA1c values fall in the range of 5-15%, the immunoassay and HPLC are linear in this range. If Hb values are less than 3g/L or 50g/L, the manufacturer advised to repeat the sample with twice the sample volume. The immunoturbidimetric assay shows a higher value in anaemic patients and lower value in polycythemic patients was indicated by Tiran and colleagues.⁽²²⁾ Both methods had good results with control samples. It is better to assess the results of immunoassay and HPLC methods as by Goldstein et al.⁽²³⁾ The use of different calibration and standards caused the bias of the immunoassay.^(24,25) The advantage of immunoassay over the HPLC method is because of the insensitivity towards the labile factor. The immunoassay is not affected by the removal of the Schiff base but the removal of the Schiff base is necessary for the accurate results of the HPLC methods.⁽²⁶⁾ The immunoassay values are not decreased after the incubation in saline (5 hours, 37°C) to remove the labile factors.⁽²⁷⁾ The typing of the haemoglobins should be done in cases of haemoglobinopathy as the immunoassay cannot interpret the haemoglobin variants. As the HPLC method can identify the Hb variants, it is better to use the HPLC method in case of suspected Hb variants.⁽²⁸⁾

Conclusion

The immunoturbidimetric assay is better than the common methods used in the laboratories. Moreover the immunoassay can be used in the common clinical labs and avoids the need for the trained staffs and is cost effective as well. To be a final alternative for ion-exchange HPLC methods, the precision of the immunoturbidimetric assay should be further improved toward the clinically desirable intralaboratory CV of <3.5%.⁽²⁹⁾ In a study by Farideh razi they observed a sensitivity and specificity of 78% and 88% for the immunoturbidimetric method in HbA1c assay as compared to the HPLC method.⁽³⁰⁾ Deterioration of reagents during lengthy use periods may contribute to imprecision. The new homogeneous immunoturbidimetric assay to determine HbA1c can be easily applied to routine chemistry analyzers. The fully automated immunoturbidimetric HbA1c assay will be preferable for the high volume reference laboratories, using the fully automated immunoassay as compared to the HPLC as immunoturbidimetric assay measures HbA1c more accurately in diabetic patients who have Hb variants, and it needs less time. HbA1c values are altered by the medications taken for the associated illness. Clinicians should be aware of the method of analysis of HbA1c before interpreting the values and initiating the treatment as the FBS monitoring is of short duration and has limited value.⁽³¹⁾

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