

A Comparative Evaluation of Estimation of Glycated Haemoglobin by Immunoturbidimetry and High Performance Liquid Chromatography in a Tertiary Care Hospital of Kolkata

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ABSTRACT

Glycated haemoglobin is a glycoprotein formed as a result of irreversible non-enzymatic addition of D-glucose to N-terminal valine residues of the Beta chain of haemoglobin. HbA1c has emerged as a reliable parameter both for diagnosing and assessing the effectiveness of treatment of Diabetes Mellitus. Various methods like immunoturbidimetry, HPLC, Boronate affinity chromatography are used to measure HbA1c. The aim of this study was to compare and analyse the two methods for measuring HbA1c – immunoturbidimetry and HPLC. Blood were collected from 110 patients in EDTA vials from the General Medicine OPD at NRS Medical College and Hospital for HbA1c estimation. The glycated haemoglobin was quantified by immunoturbidimetry method in Konelab 60i and by HPLC in Transasia Erba Hb-vario. The performance of both the methods was assessed using dedicated control. Values obtained by both the methods were tabulated in Microsoft Excel 2007 and statistical analysis was done using SPSS 22. The distribution of data was non-Gaussian and the values of two methods were found to be in agreement by Bland-Altman method and positively correlated. The HbA1c values are in agreement with each other by both the methods. However, the cut off limits of both the methods may be different and need to be determined with adequate sample size.

Keywords: Glycated haemoglobin, immunoturbidimetry, chromatography, HPLC.

INTRODUCTION

Diabetes mellitus is defined as a group of metabolic diseases characterized by hyperglycemia due to impaired insulin secretion or insulin action. Plasma glucose which is energy currency for metabolic homeostasis is regulated by Insulin; a hormone synthesized by Pancreas. In last two decades, there is an escalation in the burden of diabetic patients worldwide. A report by the American Diabetes Association suggests that India will have

highest incidence of patients diagnosed with diabetes by 2030. ^[1] With a burgeoning rise in the epidemic of diabetes, the cost incurred to the diagnosis and management is humongous. As per international consensus opinion, Fasting Plasma Glucose (FPG), Post Prandial Plasma Glucose (PPPG) and Glycated Haemoglobin (HbA1c) are the tools for the diagnosis and monitoring of diabetic patients. The Glycated haemoglobin is formed by non-enzymatic addition of glucose to the N-terminal valine

residue or ϵ -amino group of lysine residue of β -globin chain. Moreover, this analyte is unaffected by recent change in diet, exercise or drugs. The measurement of HbA1c is confounded by haemolytic anemia and iron deficiency anaemia. Recently, some novel analytes like plasma fructosamine, [2] plasma glycated albumin, [3] serum 1,5 anhydro-D-glucitol [4] and urinary myoinositol [5] have evolved as markers to monitor glycemic status but are very costly. So, glycated hemoglobin is the best and widely accepted analytical tool to monitor glycemic control. While HbA1c is fast emerging as the investigation of choice for both diagnosis and monitoring of diabetes mellitus, yet the methods are having interferences and are costly. Though different methods have been developed for quantification of glycated haemoglobin yet glycated haemoglobin should be reported as per NGSP or DCCT certified methods. [2] In this instance, the aim of this study was to compare and find the correlation, if any, between two methods for quantification of HbA1c.

MATERIALS AND METHODS

The present work is a hospital based cross sectional study based on the measurement of HbA1c by immunoturbidimetry and High performance liquid chromatography. For this purpose, blood samples were collected from 110 patients from the General Medicine OPD at NRS Medical College and Hospital. The study was conducted after receiving approval from the institutional ethical committee. The samples were collected after getting proper consent from both patients and control. Patients with history of hemoglobinopathies, iron deficiency anaemia and recent blood transfusion were excluded. Glycated haemoglobin was quantified using immunoturbidimetry and high performance liquid chromatography by Konelab 60i and Transasia Erba Hb-vario respectively. The methods were checked for in-house performance parameters like precision by analysis of dedicated control.

Statistical methods:

The values of glycated haemoglobin obtained by the two methods were extrapolated in Microsoft Excel 2007 and statistical analysis was done using SPSS 22. The data was checked for normal distribution using Kolmogorov-Smirnov test. The values were in non-Gaussian distribution as $P < 0.05$. The median values were assessed and compared using Mann-Whitney U test.

RESULTS

The median values of HbA1c were higher in HPLC (6.8 +1.78) than immunoturbidimetry (5.9+1.67) as shown in Figure 1. The Mann-Whitney U test between the HbA1c values was significantly higher in HPLC than immunoturbidimetry method. The p-value is 0.016101.

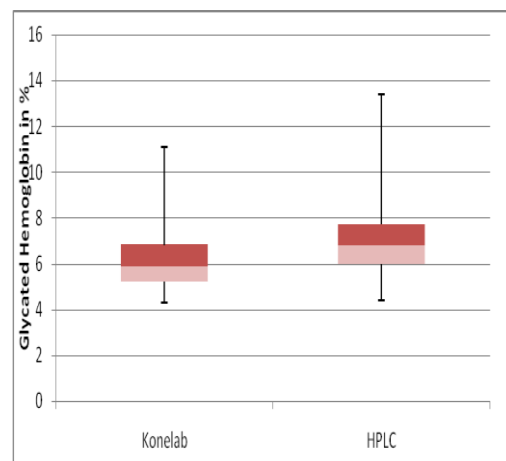


Figure 1: showing the box-whisker plot comparing the median values of glycated haemoglobin.

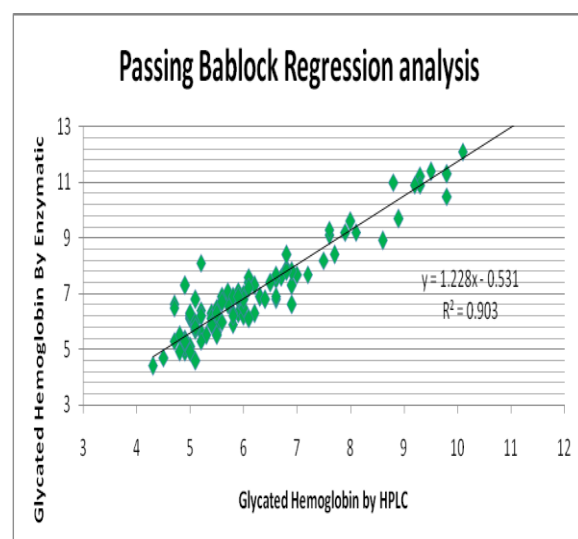


Figure:2 Showing the Regression analysis of the two methods.

The regression analysis showed the linearity of glycated haemoglobin values as evident by Figure 2, where the regression coefficient is 0.90. The values of glycated haemoglobin are in agreement with each other.

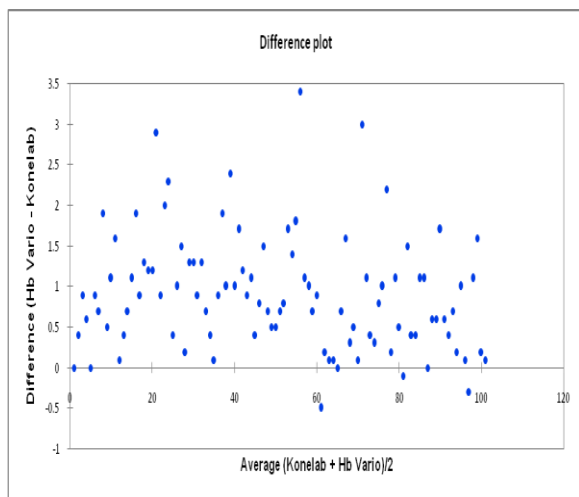


Figure 3: Showing the Bland-Altman plot comparing the values of HbA1c by HPLC and immunoturbidimetry method.

DISCUSSION

Literature reviews have suggested a perfect concordance between HbA1c values obtained by various methods. The comparative study by Ozcelik et al., [6] for measurements of HbA1c in Turkey suggested that the mean HbA1c measured by high performance liquid chromatography (HPLC) was statistically higher than particle-enhanced immunoturbidimetric assay (PEITT) and turbidimetric inhibition immunoassay (TINIA). They concluded that these higher values may be due to interference of HbA1c by other silent Hemoglobin variants because they elute at the same time with glycated haemoglobin. These findings are similar to our study. This is further supported by the fact that in 10 study subjects haemoglobin could not be detected by HPLC but the values could be estimated by immunoturbidimetry. However, these subjects were excluded from statistical analysis. Similar findings were noted by Sudhakar et al. [7] and they recommended PEITT as a better and reliable method for estimation of glycated haemoglobin.

However, the findings of similar study by Khan et al. [8] concluded that there was no significant difference between the mean values of glycated haemoglobin upto 10% but significant variation was noted when HbA1c was more than 10%. They also concluded that HbA1c can be measured by immunological method in an automated chemistry analyser more cost effectively.

A comparative study by Thvarajahet et al. [9] between two HPLC (boronate affinity and cation exchange chromatography) and immunoturbidimetric methods found good correlation between the methods. Similarly, our study also found good agreement between the methods. However, there was a positive bias on the higher range of HbA1c by HPLC method as revealed by Bland-Altman analysis. In our study, statistically and clinically significant differences between median may be due to interferences from silent variants of haemoglobin which warrants further evaluation. The major advantage of HPLC is fast turn-around time, which is usually less than 5mins. [10] The scattering of light due to patient's HbA1c binding with latex coated antibody is measured in immunoturbidimetry. [11,12] However, the antibodies do not identify or react with labile intermediates or other Glycated Haemoglobins because both the ketamine with glucose and the specific amino acid sequences are required for avidity. Similarly, other haemoglobin variants, such as HbF, HbA2, HbS and carbonylated haemoglobin do not interfere. [13]

CONCLUSION

Despite the limitations imposed by different limits of quantification and linearity, the values of glycated haemoglobin estimated by both the methods are in agreement. However, to exclude the interference from silent haemoglobin variants and effects of temperature warrants study with larger population. Moreover, using NGSP certified assays is essential in measuring HbA1c. So, errors in pre-analytical, analytical, and post-analytical

phases must be monitored and method performance must be clinically acceptable as per standard recommendations.

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