



A COMPARATIVE STUDY OF QUANTITATIVE ESTIMATION OF HBA1C USING HPLC AND IMMUNOTURBIDIMETRY METHOD

Rosy Lekharu

Associate Professor, Department of Biochemistry, GCS Medical College, Ahmedabad

Jayshree Tolani*

Statistician, Department of Community Medicine, GCS Medical College, Ahmedabad

*Corresponding Author

Ramesh Pradhan

Professor, Department of Biochemistry, GCS Medical College, Ahmedabad

ABSTRACT

HbA1c is the most widely accepted parameter for the monitoring of long term metabolic control in individuals with diabetes mellitus. There are several methods for quantitative estimation of HbA1c. Thus, it is very important to compare the results from these methods which are used by different laboratories (Weykamp C. et al., 2004). The present study was conducted in the Central Clinical Laboratory of GCS Medical College Hospital & Research Centre, Ahmedabad to compare the results of HbA1c using two of the popular methods viz., HPLC and Immunoturbidimetry. A total of 137 samples of OPD patients were tested simultaneously by both methods. The difference found was significant though mean value of HbA1c by HPLC method was found to be higher than Immunoturbidimetric method. The correlation co-efficient between the two methods was found to be 0.83445. It could be concluded that both HPLC and immunoturbidimetry methods have been found to be accurate and the results of them were comparable, although having a significant differences in mean HbA1C levels was seen on comparing between the two methods.

KEYWORDS : HbA1c, diabetes mellitus, immunoturbidimetry, HPLC

Introduction:-

Hemoglobin A1c (HbA1c) assay is accepted as the most useful marker in determining the long-term glycemic control of diabetic patients. This marker has also been recommended for the diagnosis of diabetes mellitus when HbA1c levels are above 6.5% (Gillett MJ, 2009). The successful treatment of diabetes depends on maintaining blood glucose levels within normal limits over the long term. A single fasting blood glucose measurement is an indicator of the patient's immediate past condition (in hours), but may not represent the true status of blood glucose regulation.

Glycated hemoglobin (HbA1c) is considered as a gold standard in long term assessment of glycemic control in patients with diabetes mellitus (Larsen, M. L., 1990; Lenteris-Westra et al., 2008). HbA is converted to HbA1c by interaction of the amino group of its N-terminal valine with Glucose by a non-enzymatic amadori reaction (Banerjee S., 2014).

HbA1c is least affected by most factors and can be considered as the most dependable diagnostic marker of diabetes. According to classification of increased risk of diabetes by American Diabetes Association (ADA) 2016, 5.7%-6.4% HbA1c indicate pre-diabetes, and $\geq 6.5\%$ are diagnosed as diabetes.

The Diabetes Control and Complications Trial (DCCT) and The U.K. Prospective Diabetes Study (UKPDS), showed that the development and progression of diabetic complications can be delayed by monitoring the glycemic status of patients. According to their observations, a 1% decrement in HbA1c level complies with an approximate 30% reduction in developing risk of diabetic complications (Weykamp C. et al., 2009).

There are over 20 methods of HbA1c determination, based on differences in structure, charge and chemical reactivity. Most commonly used analytical methods are high performance liquid chromatography (HPLC), electrophoresis, other chromatographic methods, immune assays, etc. The reference method recommended and accepted in 2007 by the IFCC is Liquid Chromatography-Isotope Dilution-Mass Spectrometry (LC-ID-MS). Studies establish a difference in the results, which requires standardization and comparison of the methods used in practice. Accurate HbA1c results are essential for monitoring and appropriate treatment of diabetic patients.

Materials and Methods:-

The present study was conducted in Clinical Biochemistry Laboratory of GCS Medical College Hospital and Research Centre, Ahmedabad in January 2018. The study comprised of 137 whole blood samples randomly chosen from the out patients which included diabetic, pre-diabetic and non-diabetic patients who visited for either routine testing or control of the diabetic status. No further selection criteria were used. The age of the patients ranged from 25 to 75 years old (average: 45.8 years) of which 62.1% of all cases were female and 37.9% were male.

The HbA1c values ranged from 4.5% to 16.2%. Blood samples were obtained through venipuncture into EDTA vacutainers. Hemolysates were prepared using proper instructions and were kept at +4°C until studied. HbA1c levels were measured by HPLC method using Bio-Rad D-10 fully automated system and by Immunoturbidimetry using XL-640 autoanalyser. Compliance of Bio-Rad D-10 HPLC instrument with the latest Diabetes Control and Complications Trial (DCCT) reference method has been documented by the National Glycohemoglobin Standardization Program (NGSP) (Hoelzel W et al. 2004). The assays were completed within four hours following blood sampling. HbA1c determination with the D-10 Dual Program has been optimized to eliminate interferences from hemoglobin variants, labile A1c, and carbamylated hemoglobin. In Immunoturbidimetric Method, total Hb and HbA1c in hemolyzed blood are attached to the latex particles with equal affinity.

In the next step, monoclonal antibodies are used to detect HbA1c, next polyclonal antibodies against monoclonal antibodies can agglutinate the particles, and the resulted turbidity is measured spectrophotometrically (Goldstein DE et al., 1995).

Results:-

The study consisted of 137 people who fulfilled the inclusion criteria.

The mean HbA1c was found to be slightly higher by HPLC method than by the immunoturbidimetric method.

Results depict that there is a significant difference between these two mean numbers ($p=0.025$).

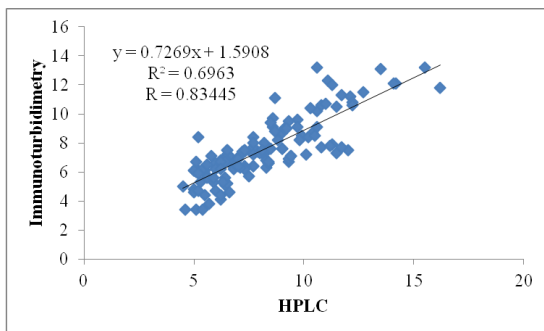
Table 1: HbA1c measured by different methods

T-test	HPLC method	Immunoturbidimetric method	P-value
Mean	8.079562	7.4635	0.025
Variance	5.786344	4.390422	

Data was tabulated in Microsoft Excel and analyzed using IBM SPSS 20.0 and Microsoft Excel 2007. Students T-test was used to test the significance between the two methods HPLC and Immunoturbidimetry. P value less than 0.05 was considered significant.

From the table, differences in the mean HbA1c measured with HPLC method and Immunoturbidimetric method were statistically significant ($p=0.025$).

Though both the methods show statistically significant differences in the mean HbA1c levels, the HbA1c values of both methods have correlated well with correlation coefficient of 0.83445 as shown in the Graph 1.

Graph 1: Correlation of HbA1c values between Immunoturbidimetric and HPLC methods**Discussion and Conclusion :-**

The availability of the hemoglobin A1c test has enhanced diabetic care and its measurement has become an integral part in the management of diabetes. Also the relationship between the improved glycemic control and risk of diabetic complications has been established (Roszyk L. et al., 2007).

Physician should be acquainted with different assay methods before interpreting the results. DCCT adopted a standardisation of the assay methods and that should be followed everywhere.

In our study the comparison between the above mentioned methods was performed among 137 patients with HbA1c levels ranging from 4.5 % to 16.2 %. Though some studies reported that the HPLC method can detect abnormal hemoglobin with favorable reproducibility and a $CV < 1\%$, this technique needs a large dedicated devices and rather a time consuming procedure. In addition, many trained staffs are needed to maintain the instrumentation (Sakurabayashi I. et al., 2003; Shidfar F. et al., 2014). The immunoassay can be performed by an automated analyzer, thus this method does not take a long time for measuring a large number of samples.

The turbidimetric immunoassay is easy to use and more available in most developing countries especially in considerable rural populations where limited accessibility to advanced devices and laboratories performing the proper assays is still an unsolved problem (Metus P. et al, 1999).

In addition, both methods have been shown to be accurate and the results of them were comparable in our study.

Further studies by the interference analysis are needed to examine the effect of such factors on the glycated hemoglobin measurements.

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