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Comparative study of various methods in the estimation of blood HbA1c and its outcome

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ABSTRACT

Diabetes mellitus is a major health problem all over the world. Blood glucose measurement has limited value in assessing the long term glycaemic control. Estimation of HbA1c is now routinely used in clinical laboratories for long term assessment of glyceamic control. The result of different methods of HbA1C estimation has a lot of variations and hence it is essential to compare their results. This study is aimed to assess the accuracy and reliability of estimation of HbA1c levels by PEITT and Column Chromatography with ion-exchange resin by comparing it with the hba1c levels of High-performance liquid chromatography (HPLC) which is a reference method. We have included 50 patients of type 2 diabetes mellitus in our study. HbA1c was determined using BIO RAD D-10 HbA1c Analyzer, Immuno-turbidimetric assay in ERBA-Chem semi automated analyzer and Column chromatography with Ion-exchange resin method in ERBA-Chem semi automated analyzer. The results obtained from Immuno-turbidimetric assay and Ion-exchange resin methods were compared with the results of BIO RAD D-10 HbA1c Analyzer. The results obtained from Particle Enhanced Immuno-Turbidimetric Test method showed a better correlation with BIO RAD D-10 HbA1c Analyzer than Column chromatography with Ion-exchange resin (CCG-IER). Hence, Particle Enhanced Immuno-Turbidimetric Test method is more reliable and accurate and can be used as an alternative method to HPLC in clinical laboratories.

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Type I diabetes is characterized by inappropriate hyperglycemia, primarily a result of pancreatic islet β –cell destruction and a tendency to ketoacidosis. Type I diabetes mellitus is a result of cellular mediated auto-immune destruction of the β –cells of the pancreas, causing an absolute deficiency of insulin secretion. Type II diabetes, in contrast, includes hyperglycemia cases that result from insulin resistance with an insulin secretory defect. This resistance results in a relative, not an absolute, insulin deficiency (Michael *et al.*, 2018). Complications include micro vascular problems such as nephropathy, neuropathy, and retinopathy (Nathan *et al.*, 1993; Goldstein *et al.*, 2004; Bennett *et al.*, 2007). The successful treatment of diabetes mellitus depends on keeping blood glucose level as close as possible to normal level to minimize the complications of diabetes mellitus. A plasma glucose level

in diabetes tells about the glucose level at that particular date/hour. It cannot speak the true status of long term blood glucose regulation. Fasting Plasma Glucose level is used for the diagnosis of Diabetes. Estimation of fasting plasma glucose has some disadvantages like Fasting for 8-12 hours, large biological variations, altered by stress, acute illness. Fasting blood glucose is less tightly linked to diabetic complications (than A1C). Blood glucose values are not useful to know about long term glycemic control. HbA1c level reveals the mean glucose level over the previous 10-12 weeks (Nathan *et al.*, 1993; Goldstein *et al.*, 2004; Bennett *et al.*, 2007). Estimation of HbA1c is recommended by the ADA and others for monitoring long-term glycemic control (8-12 weeks) (Bodor *et al.*, 1992; Halwachs-Baumann *et al.*, 1997) predictor of diabetic complications. The chronic complications of diabetes like nephropathy, neuropathy and retinopathy are reduced when the HbA1c level is maintained below 7% (Sacks, 2011; Banerjee, 2014; McCarter *et al.*, 2006). Glycated hemoglobin can be used to know the effectiveness of therapy as it tells about the long term glycemic control (Reddy *et al.*, 2013). The longer hyperglycemia occurs in blood, the more glucose binds to haemoglobin in the red blood cells and the higher the glycated haemoglobin. The lifespan of RBC is 120 days and the build-up of glycated haemoglobin within the red cell, therefore, reflects the average level of glucose to which the cell has been exposed during RBC's life-cycle. During hyperglycemia, glucose molecules itself attaches to hemoglobin. An accurate index of the HbA_1c level is proportional to average blood glucose concentration over the previous three months (Nathan *et al.*, 1993; Goldstein *et al.*, 2004; Bennett *et al.*, 2007). Many methods are available for the measurent of HbA1c. But the results varied between assay types and manufacturers due to the fact that glycohemoglobin are heterogeneous and that different methods measure different glycated species with different reliability and often without standardization (McCarter *et al.*, 2006). The use of different units is also a cause of variable results (Hoelzel *et al.*, 2004). The Diabetes Control and Complications Trial research group (DCCT) and the National Glycohemoglobin Standardization Programme (NGSP) have recommended HPLC as an acceptable standard method (Özçelik *et al.*, 2010). The effects of abnormal and minor Hb fractions are reduced in BIO-RAD D-10 by using modern chromatographic materials (Özçelik *et al.*, 2010). Measurement of HbA1c by Capillary electrophoresis and Electrospray mass spectrometry are cumbersome and costly (Joslin *et al.*, 2005). Measurement of HbA1c by HPLC Method is based on the charge of the globins component of haemoglobin [Hb]. HPLC measures all types of Hb and is affected by abnormal and minor Hb fractions. Measurement of HbA1c by HPLC Method is based on the charge of the globins component of hemoglobin [Hb]. HPLC measures all types of Hb and is affected by abnormal and minor Hb fractions, more expensive and demands experienced technical staff.

On the contrary, in turbidimetric immunoassay (PEITT) method, HbA1c antibody used react only with HbA1c. It is cheaper in cost and easier to adapt to biochemical analyzers. Column chromatography with Ion-exchange resin method is also cheap and easier to adapt to biochemical analyzers. Hence this study is designed to compare the HbA1c levels obtained by Particle Enhanced Immuno Turbidimetric Test (PEITT) and column chromatography – ion exchange resin with High performance liquid chromatography (HPLC) which is a "gold standard" method for the estimation of blood HbA1c to find out which method is accurate and reliable and can be practiced in clinical laboratories.

MATERIALS AND METHODS

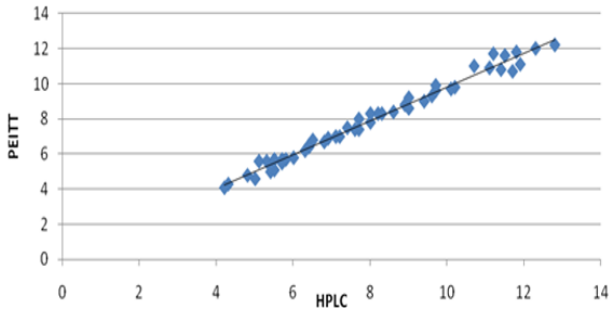

Figure 1: Scatter diagram of HbA1c levels by HPLC and PEITT for 50 patients

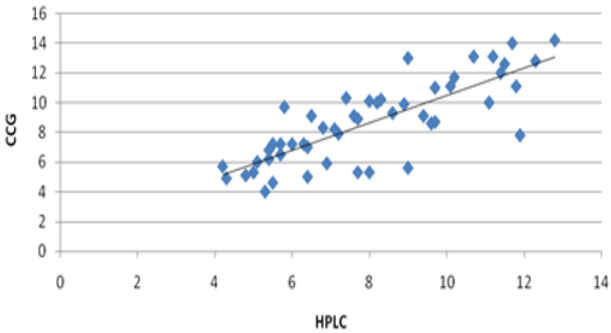

Figure 2: Scatter diagram of HbA1c levels by HPLC and CCG for 50 patients

Institutional Ethical committee approval was obtained to do the study. The study was carried out in the Clinical Biochemistry Lab, Saveetha Medical

Table 1: HbA_{1c}values by HPLC, PEITT and Column chromatography with Ion-Exchange resin

Method	Sample size(N)	HbA1c Values		
		Mean + SD	Minimum	Maximum
HPLC	50	8.01+ 2.39	4.2%	12.8%
PEITT	50	7.90 + 2.31	4.1%	12.2%
Ion-Exchange resin method	50	8.65 + 2.76	4.0%	14.2%

Table 2: Correlation of HbA1c levels between PEITT and HPLC Methods

Methods	Sample size (N)	HbA1c values	
		Pearson correlation (r)	p value
HPLC & PEITT	50	0.992	<0.001 Highly significant

Values are expressed as mean ± SEM *p<0.05 –Significant, **p<0.01-Highly significant, ***p<0.001-Extremely significant

Table 3: Correlation of Hba1c levels between HPLC and column chromatography with ion exchange resin method

Methods	Sample size (N)	HbA1c values	
		Pearson correlation (r)	p value
HPLC & Ion-exchange resin method	50	0.803	<0.001 Highly significant

Table 4: Sensitivity and Specificity of HbA_1c level by PEITT and HPLC

PEITT	HPLC		Total
	Diabetic with control	Diabetic without control	
Diabetic with control	32	1	33
Diabetic without control	0	17	17
Total	32	18	50

Table 5: Sensitivity and Specificity of HbA_1c level by CCG and HPLC

CCG-IER	HPLC		Total
	Diabetic with control	Diabetic without control	
Diabetic with control	28	8	36
Diabetic without control	4	10	14
Total	32	18	50

College & Hospital. 50 Diabetic individuals of both genders in any age group were selected for the study. Subjects were chosen from out-patient and in-patient department of Saveetha Medical College & Hospital. HbA1c was measured by using BIO RAD D-10 HbA1c Analyzer, which is based on cation exchange HPLC.

The Immuno-turbidimetric assay was performed in ERBA-Chem semi automated analyzer. The Column chromatography with ion-exchange resin method was performed in ERBA-Chem semi automated analyzer. Statistical analysis between the three methods for measurement of HbA_1c values by column chromatography with ion exchange resin, HPLC and PEITT methods were Correlation between HbA_1c values were assessed by Karl-Pearson correlation coefficient method, which explains the degree or extent of the linear relationship between two variables. The analysis were carried out using the SPSS Software package. The data were expressed as mean ± standard deviation and in the entire test P (probability) value <0.001 was taken as statistically highly significant and value <0.05 was taken to be statisti-

cally significant.

RESULTS AND DISCUSSION

The HbA_1c levels of 50 diabetics of any age and gender were estimated by three different methods, namely HPLC, PEITT and Column chromatography with Ion-Exchange resin are tabulated in Table 1 shows the mean HbA1c was slightly lower for PEITT (7.90%) method than HPLC (8.01%). The mean HbA1c was slightly higher for column chromatography with ion exchange resin (8.65%) method than HPLC (8.01%).

Table 2 shows the correlation of HbA_1c levels between PEITT and HPLC methods. The results show a good positive correlation between PEITT and HPLC methods with a (r) value of (0.992) and high statistical significance with a value of ($p<0.001$). The data represented in Figure 1 clearly showed a good positive correlation of the HbA_1c levels between HPLC and PEITT. Table 3 shows the correlation of HbA1c values between HPLC and Column Chromatography with Ion exchange resin methods. The results show a Positive correlation between HPLC and Ion exchange resin methods with a (r) value of (0.803) and high statistical significance with a (p) value of (<0.001). The data represented in Figure 2 clearly showed a positive correlation of the HbA1c levels between HPLC and CCG.

When scattered diagrams in Figures 1 and 2 are compared, the correlation between HbA_1c levels of HPLC and PEITT is found to be better (0.992) than the correlation between HbA_1c levels of HPLC and column chromatography with Ion-Exchange Resin method (0.802). Table 4 shows a sensitivity of 100% and specificity of 94.4% between PEITT and HPLC method. Table 5 shows a sensitivity of 87.5% and specificity of 55.6% between CCG and HPLC method. A comparison of the sensitivity and specificity of both the methods PEITT and CCG showed that PEITT correlates with HPLC better than CCG and hence PEITT is more reliable than CCG to assess the HbA1c level compared to the Gold Standard method HPLC.

Estimation of HbA_1C is very useful in the management of diabetes and its measurement has become an integral part for the management of diabetes. The result of different methods of HbA1c estimation has lot of variations and hence it is essential to compare their results. Measurement of HbA_1c by Capillary electrophoresis and Electrospray mass spectrometry are cumbersome and costly (Joslin *et al.*, 2005). Measurement of HbA_1c by HPLC Method is based on the charge of the globins component of haemoglobin [Hb]. HPLC measures all types of Hb and is affected by abnormal and minor Hb fractions. The effects of abnormal and minor Hb fractions are reduced in BIO-RAD D-10 by using modern chromatographic materials. HPLC measures all types of Hb and is affected by abnormal and minor Hb fractions, more expensive and demands experienced technical staff.

On the contrary, in turbidimetric-immunoassay (PEITT) method, HbA_1c antibody used react only with HbA_1c. It is cheaper in cost and easier to adapt to biochemical analyzers. Column chromatography with Ion-exchange resin method is also cheap and easier to adapt to biochemical analyzers. Hence, this study is designed to compare the HbA_1c levels obtained by Particle Enhanced Immuno Turbidimetric Test (PEITT) and column chromatography – ion exchange resin with Bio-Rad D10- HPLC method for the measurement of blood HbA_1c and to find out which method is accurate and reliable and can be practiced in clinical laboratories. In this study, the HbA_1c levels of 50 diabetics of any age and genders were estimated by three methods, namely BIO-RAD D- 10 HPLC analyzer, PEITT and column chromatography with ion-exchange resin (CCG-IER) method.

The HbA_1c levels ranged between a minimum 4.2% to maximum 12.8%. The mean with SD for BIO-RAD D- 10 HPLC , PEITT and CCG-IER methods are respectively (8.01+ 2.39),(7.90 + 2.31), and(8.65 + 2.76). When HbA_1c levels of PEITT method was compared with BIO-RAD D- 10 HPLC (cation-exchange HPLC) method , a good positive correlation with an **(r)** value of (0.992) was obtained with high statistical significance with a p value (<0.001). This is in consistent with the studies done by (Özçelik *et al.*, 2010; Matteucci *et al.*, 2001; Metus *et al.*, 1999). In PEITT method labile intermediates, HbA1a, HbA1b, HbF, HbA2, HbS and carbamylated hemoglobin are not detected. When HbA_1c levels of Column chromatography with ion-exchange resin method was compared with BIO-RAD D- 10 HPLC method a good positive correlation with an r value of (0.802) was obtained with statistical significance with a p value (<0.001). These two comparisons are represented in scatter Figure 1 &Figure 2. Though both PEITT and Column chromatography with ion-exchange resin method have positive correlation with HPLC, PEITT method has more Positive Correlation with HPLC than Column chromatography with ion-exchange resin method, revealed by (r) values are (0.992) and (0.802). The HbA_1c levels of 50 diabetics of any age and gender estimated by three different methods namely HPLC, PEITT and Column chromatography with Ion-Exchange resin (CCG-IER) are divided into Diabetic's with control and Diabetic's without control by taking HbA_1c value 6.5% as the cutoff value to find out the sensitivity and specificity. The sensitivity and specificity of PEITT

when compared to HPLC are 100 % and 94.4% respectively and the sensitivity and specificity of CCG-IER when compared to HPLC are 87.5 % and 56.5% respectively. In PEITT method was found more reliable than CCG to assess the HbA1c level by comparing the sensitivity and specificity of these methods.

CONCLUSION

From the results and discussion held so far and by the comparison of HbA_1c levels measured by PEITT, CCG-IER with BIO-RAD D- 10 HPLC method the following are concluded. The HbA_1c levels estimated by BIO-RAD D- 10 HPLC method, PEITT, Column chromatography with ion exchange resin method ranged between 4.0% - 14.2%. Particle Enhanced Immuno Turbidimetric Test (PEITT) showed good positive correlation with high statistical significance and 100% of sensitivity and 94.4% of specificity obtained when compared with BIO-RAD D- 10 HPLC method. Column chromatography with ion exchange resin (CCG -IER) showed positive correlation with high statistical significance and 87.5% of sensitivity and 55.6% of specificity obtained when compared with BIO-RAD D-10 HPLC method. Hence, it is concluded the Particle Enhanced Immuno Turbidimetric Test (PEITT) method is a more reliable and accurate method than Column chromatography with ion exchange resin (CCG -IER) and it can be used as an alternative method to HPLC in clinical laboratories.

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