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Original Research Article

COMPARATIVE EVALUATION OF PARTICLE ENHANCED IMMUNOTURBIDIMETRY AND HPLC METHODS FOR HBA1C ESTIMATION IN CHRONIC KIDNEY DISEASE PATIENTS

J. Edith Samboornam[1], S. R. Saranya[2], K. Manjukarthikeyani[3]

[1]*Assistant Professor, Department of Biochemistry, Government Medical College, Pudukkottai, Tamil Nadu, India.*
[2]*Tutor, Department of Biochemistry, Government Medical College, Pudukkottai, India.*
[3]*Associate Professor, Department of Biochemistry, Government Medical College, Pudukkottai, India.*

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Corresponding Author:
Dr. J. Edith Samboornam,
Assistant Professor, Department of Biochemistry, Government Medical College, Pudukkottai, Tamil Nadu, India.
Email: jediths2000@gmail.com

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ABSTRACT

Background: Determination of HbA1c in patients with Chronic Kidney Disease (CKD) is a challenge because of the presence anemia and increased carbamylated hemoglobin both of which have an influence on HbA1c assay.
Aim: This study compares Particle Enhanced Immunoturbidimetric Assay (PETIA) with High-Pressure Liquid Chromatography (HPLC) to determine the analytical agreement and to assess the suitability of using PETIA method for glycemic monitoring among the CKD patients not on dialysis.
Materials and Methods: A cross sectional study of comparison was conducted with 75 CKD patients admitted in Government Medical College Hospital, Pudukkottai. HbA1c was estimated on the same day by 2 methods – HPLC and PETIA for all patients and the results compared. Blood sugar, urea, creatinine and Hemoglobin were measured using standard kits. CKD subjects were divided into subgroups (diabetic, anemic and presence of high CHb). Comparative results were computed separately for total group and subgroups. The two methods were compared by evaluating the concordance using the Bland-Altman plot and correlation using Spearmans's rank correlation coefficient (r) and linear regression.
Results: The two methods did not show significant difference of median (IQR) HbA1c values in the total group and subgroups except in severe anemia group(Hb<7g/dl). There was close concordance displayed by Bland-Altman plots with a mean bias of 0.63. Robust positive correlation (ρ=0.903; p<0.001) was observed between the measurements. In patients with severe anemia, the correlation was not significant (ρ=0.706; p=0.05). The relationship was linear with r^2 =0.83 and the linear regression equation was Y = 0.938x - 0.175. No interferences were found from carbamylated hemoglobin.
Conclusion: PETIA method is reliable for HbA1c estimation in CKD patients except in the case of severe anemia.
Keywords: HbA1c, immunoturbidimetric assay, HPLC, chronic kidney disease(CKD).

INTRODUCTION

Chronic kidney disease (CKD) is a significant health concern, affecting approximately 13-16 % of the Indian population, with greater occurrence among diabetic individuals.[1] Glycated haemoglobin (HbA1c) is a crucial biomarker for monitoring diabetes mellitus (DM), as it reflects blood glucose control over the preceeding 8-12 weeks.[2] Periodical HbA1c testing is mandatory to prevent the progression of renal damage and to avoid hypoglycemic episodes in CKD subjects.[3]

The HbA1c levels of samples can be reliably measured by using various methods such as High-Pressure Liquid Chromatography (HPLC), Immunoassay, Boronate Affinity Chromatography

and Enzymatic Assay.[4] Although HPLC is considered as the gold standard test method for HbA1c, it requires a separate instrument and has to be run in batches. It also consumes time and manpower. Moreover, in many of the secondary and tertiary care hospitals, HPLC apparatus is not available due to the high cost and lack of trained personnel for performing this intricate procedure.[5]

HbA1c can also be determined by an immunoassay technique called Particle Enhanced Immunoturbidimetric Assay (PETIA). PETIA is in use by many laboratories because this assay can be run in routine biochemistry analysers, does not need a separate equipment, easy to perform and gives quicker results compared to HPLC method.[6]

Measurement of Glycated hemoglobin can be commonly affected by altered RBC lifespan, level of hemoglobin and presence of Hb variants. Low Hb and presence of high carbamylated Hb are important interferences challenging the accuracy of HbA1c assay in CKD samples.[7] KDIGO guidelines state, HPLC is the preferred assay method for HbA1c in CKD patients.[8] Since HPLC apparatus is not available in every lab we are in need of another reliable method for testing glycated Hb among Chronic Kidney Disease patients.

Several studies have reported that the HbA1c results of immunoassays like PETIA are comparable to HPLC in the general population and among diabetic subjects.[9-11] But the accuracy and comparability of immunoturbidimetric method among CKD patients is not certain. Hence this research was done to compare the efficacy of PETIA against HPLC for HbA1c Estimation on non-dialysis CKD patients.

MATERIALS AND METHODS

Study design and participants

An analytical cross sectional study was conducted at the Department of Biochemistry among 75 CKD patients admitted in Government Medical College, Pudukkottai during April 2026 - June 2026. Informed consent was obtained from all patients. All procedures concerning patients were permitted by the Institutional Ethics Committee. Inclusion criteria were patients above 18yrs with chronic kidney disease on regular follow up under a physician and not on dialysis. Exclusion criteria were patients undergoing dialysis, renal transplant recipients and patients who are not willing to participate. All study participants were explained about the study protocol. Patients were divided into subgroups based on the presence of diabetes, anemia and high carbamylated hemoglobin.

Sample collection

5ml blood was collected from patients satisfying the inclusion criteria after their consent. Out of the total 5ml of blood sample, 2ml was collected in anticoagulant (K2 EDTA) containing tube and analysed for Hb and HbA1c levels. Remaining 3ml of blood was collected in plain tube and utilized for analysis of basic biochemical parameters – urea, creatinine and sugar. Serum was separated after centrifuging the sample at 3000rpm for 10 minutes and the following investigations were performed.

Biochemical estimation

Blood sugar was estimated by GOD-POD method, urea by GLDH-urease method and creatinine by Jaffe's compensated method in Erba XL 640 fully automated analyzer.

HbA1c estimation techniques

PETIA technique on the Erba XL-640 fully automated analyzer

In this technique, HbA1c was analysed without measuring total hemoglobin. EDTA sample is mixed with hemolysing solution. Total Hb and HbA1c in hemolysed blood bind with the same affinity to particles in reagent. The amount of binding is proportional to the relative concentration of both substances in the blood. Mouse anti-human HbA1c monoclonal antibody (R2a) binds to particle bound HbA1c. Goat anti-mouse IgG polyclonal antibody (R2b) interacts with the monoclonal mouse anti-human HbA1c antibody and agglutination takes place. The measured absorbance is proportional to the HbA1c bound to particles, which in turn is proportional to the percentage of HbA1c in the sample. The technique is standardized according to IFCC reference method. It is NGSP certified and traceable to the DCCT reference method.[12]

HPLC technique on the Biorad-D10 HbA1c analyzer

The technique used was a chromatographic separation of the analytes by ion exchange high pressure liquid chromatography. Hemoglobins were separated based on their ionic interactions. The absorbance was measured at 415 nm wavelength. A chromatogram was generated and the area of the HbA1c was calculated using an exponentially modified Gaussian algorithm. This Technique is NGSP certified and traceable to the DCCT reference method.[12]

Data Analysis

Data were analysed using SPSS software version 32. Baseline characteristics of the subjects were expressed as numbers, median and interquartile range (IQR). The measurements of the two methods HPLC and PETIA were compared using Mann-Whitney U test and p-value of less than 0.05 was considered statistically significant. The two methods were compared by evaluating the concordance using the Bland-Altman plot. HPLC method was considered as the gold standard or the X method while PETIA was considered as the Y method. Spearman's rank Correlation coefficient (ρ) was utilized for determining the strength of association between HbA1c measurements by the two mentioned methods. Linear regression r2 value was calculated. Regression equation was obtained.

RESULTS

HbA1c was estimated by HPLC and PETIA method for all the 75 CKD samples. CKD cases were divided into subgroups – diabetic group and non-diabetic group, patients with and without anemia, patients with Carbamylated Hb less than 2 or greater than 2.

Table 1: Status of demographical, biochemical and hematological variables in chronic kidney disease (CKD) patients

S. No.	Demographic Variables	CKD (N=75)
1.	Gender Male	58
	Female	17
2.	CKD Severity Stage 2	1
	Stage 3	30
	Stage 4	26
	Stage 5	18
3..	Non-diabetic	22
	Diabetic	53
4.	Non-anemic	24
	Anemic i) Mild anemia (N=9) ii) Moderate anemia (N=34) iii) Severe anemia(N=8) (Hb<7g/dl)	51
5.	Less CHb(<2%)	35
	High CHb(>2%)	40
6.	Age (years)	60(16.5)
	Biochemical Variables	**Median(IQR)**
7.	eGFR	27(25.5)
8.	Urea (mg/dl)	56(33.5)
9.	Creatinine (mg/dl)	2.5(1.9)
10.	RBS (mg/dl)	178(120.5)
11.	HPLC-HbA1c (%)	6.4(2.7)
12.	PETIA-HbA1c (%)	6.1(2.35)
13.	Hb(g/dl)	9.8(3.15)
14.	CHb	1.95(1.5)

Table 2: Comparison of HbA1c levels by two methods in total and subgroups of CKD

Group/Subgroup	HPLC-HbA1c (%) Median(IQR)	PETIA-HbA1c (%) Median(IQR)	Mann Whitney U test p value
Total CKD patients (N=75)	6.4(2.7)	6.1(2.35)	0.173
Non-diabetic (N=22)	5.5(0.93)	5.35(2.3)	0.444
Diabetic (N=53)	7.5(3.7)	6.7(3.2)	0.144
Non-anemic (N=24)	7(2.1)	7(2.5)	0.662
Mild anemia (N=9)	7.2(3.2)	6.6(3)	0.232
Moderate anemia (N=34)	6.4(2.7)	5.9(2.2)	0.293
Severe anemia(Hb<7g/dl) (N=8)	4.85(1.15)	3.45(1.48)	0.036 (p<0.05)
CHb (<2) (N=35)	6(1.75)	5.9(1.7)	0.371
High CHb (>2) (N=40)	6.95(4.15)	6.4(3.17)	0.268

Test of significance

Quantitative data were summarized using the median and Inter Quartile Range. [Table 1] There was no statistically significant difference between the HbA1c values obtained by PETIA and HPLC methods in the total CKD group and across all the subgroups of study participants based on diabetic status, presence of mild or moderate anemia and high level of CHb as shown in the Table 2. The HbA1c value median(IQR)=3.45(1.48) obtained through the PETIA method was significantly lower, compared to the HPLC method median(IQR)= 4.85(1.15) in the severe anemia group (p=0.036).

Concordance

Analysis of the Bland–Altman plot of all the 75 samples revealed a high number of points clustered close to zero as observed in (Fig. 3). 95% of the values were within the range of mean ± 2 standard deviations (SD) around the study mean. This observation indicates a strong agreement between the two methodologies. In other words, PETIA and HPLC methods exhibit good concordance in their

measurements. The mean bias for the total CKD group was 0.63. The Bland–Altman plots for the non-diabetic and diabetic group is shown in Fig 4, Fig 5. The mean biases in the non-diabetic and diabetic group are 0.54 and 0.67 respectively.

Correlation

Spearman's rank correlation analysis of the HbA1c values obtained by two methods for the same set of samples demonstrated a statistically significant robust positive correlation between the two analytical methods, with a correlation coefficient (ρ) of 0.903 in total CKD group. The correlation between the measurements was not statistically significant in the severe anemia(Hb<7g/dl) subgroup (ρ=0.7; p=0.05). Across all other subgroups, the correlation was significant and positive as given in Table 3.

Linear regression

Linear regression between HPLC and PETIA values among total CKD cases yielded an r^2 value of 0.83; r=0.911. The regression equation obtained was Y = 0.938x - 0.175 (Slope = 0.938; Intercept = -0.175) which is interpreted as presence of minimal constant bias and the test method(PETIA) gives lower values as concentration increases (proportional underestimation). The linearity was maintained in subgroups too; r values are given in Table 4.

Figure 1: Box plot of HbA1c values by two methods in diabetic cases

Figure 2: Box plot of HbA1c values by two methods in non-diabetic cases

Figure 3: Bland Altman plot for HPLC vs PETIA method in all CKD cases(N=75)

Mean of the difference = 0.63 SD=0.98
Agreement limits (95% confidence interval limit) = -1.29 to 2.55

Figure 4: Bland Altman plot for HPLC-HbA1c and PETIA- HbA1c method in diabetic cases (N=53)

Mean of the difference = 0.67 SD=0.88
Agreement limits (95% confidence interval limit) = -1.05 to 2.39

Figure 5: Bland Altman plot for HPLC-HbA1c and PETIA- HbA1c method in non- diabetic cases (N=22)

Mean of the difference = 0.54 SD=1.20
Agreement limits (95% confidence interval limit) = -1.81 to 2.89

Table 3: Spearman's rank correlation of HbA1c measurements by two methods in total CKD group and subgroups of CKD

Correlation of HbA1c values HPLC vs PETIA	ρ value	p value	Interpretation
Total CKD cases (N=75)	0.903	<0.001	significant strong positive correlation

Diabetic (N=53)	0.939	<0.001	significant strong positive correlation
Mild anemia (N=9)	0.701	0.035	significant positive correlation
Moderate anemia (N=34)	0.909	<0.001	significant positive correlation
Severe anemia(Hb<7g/dl) (N=8)	0.706	p=0.05	positive correlation NOT statistically significant
High CHb (>2) (N=40)	0.802	<0.001	significant strong positive correlation

Table 4: Linear regression of HbA1c measurements by two methods in total CKD group and subgroups of CKD

Linear regression of values HPLC vs PETIA	r	r^2	Slope	Intercept
Total CKD cases (N=75)	0.911	0.83	0.938	-0.175
Diabetic (N=53)	0.928	0.861	0.911	0.025
Mild anemia (N=9)	0.808	0.653	0.676	1.849
Moderate anemia (N=34)	0.954	0.910	0.861	0.381
Severe anemia(Hb<7g/dl) (N=8)	0.90	0.81	1.184	-2.360
High CHb (>2) (N=40)	0.924	0.854	0.891	0.117

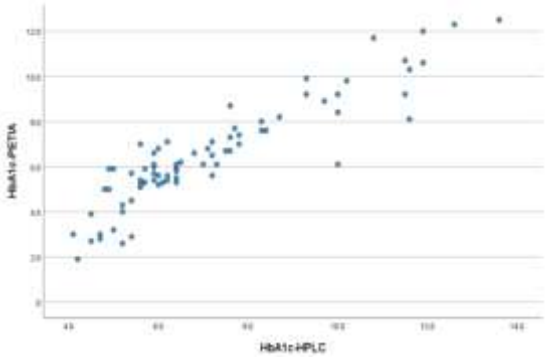

Figure 6: Scatter plot HPLC vs PETIA for all CKD cases (N=75)

DISCUSSION

In our study we compared the HbA1c values of PETIA method against HPLC values for the same set of CKD samples. Comparative values were not significantly different among the total CKD cases and subgroups of CKD. Our results are consistent with the results of previous studies.[6,9-11] But HbA1c measurements of patients with severe anemia showed significant difference between PETIA and HPLC methods. PETIA reported significantly lower HbA1c values [median(IQR)=3.45(1.48)] compared to HPLC [median(IQR)=4.85(1.15). This result was noted by Dhingra et al also in 2023 in the comparative study of Immunoturbidimetric Inhibition Assay and HPLC on CKD patients in which the immunoassay did not give values for anemic patients below the hemoglobin level of 7.2g/dl.[13]

In the present study, most of the values were within the agreement limits and good concordance was observed by Bland-Altman plot of the values obtained by the two methods. The Bland-Altman plot shows an increase in mean bias observed, 0.54 in non-diabetic patients compared to a mean bias of 0.67 among diabetic individuals. The same result was observed by Faralahy et al in a study conducted in Madagascar on patients coming for HbA1c testing.[6]

The high correlation coefficient (ρ=0.903, p<0.001) suggests that the HbA1c measurements obtained through the PETIA method are closely associated with those obtained through the HPLC method. Sabarinathan et al in 2020 estimated HbA1c levels in 50 diabetic patients by using same methods and showed a good positive correlation with *r* value of 0.992.[14] Koyako Goya et al also reported strong significant correlation (r=0.954; p<0.001) of immunoassay to HPLC.[15] This close agreement with HPLC makes PETIA method, a highly specific and sufficiently precise assay for HbA1c analysis.

We observed in our study, that among the CKD patients with severe anemia (Hb<7g/dl), the HbA1c values obtained by PETIA did not correlate significantly with HPLC values, suggesting that PETIA values may not reflect the exact glycemic status in CKD patients with Hb less than 7g/dl. PETIA method exhibited good linearity (r=0.911; r^2=0.83; Y = 0.938*x* - 0.175) with HPLC with a minimal constant bias and slight proportional under-estimation of glycated Hb values by PETIA. Paolo Metus et al. also reported good linearity of PETIA and HPLC with *r* = 0.98.[16] The constant bias was highest for patients with severe anemia (Y = 1.1847*x* - 2.3604; intercept -2.3604) adding to the fact that PETIA cannot be relied in severe anemia.

Positive correlation between the two methods was not affected (ρ=0.802, p<0.001) even in patients with high Carbamylated Hemoglobin (CHb in the range of 2 to 3.5%). Since we did not include patients on dialysis, this result is less valued. Nevertheless this indicates that PETIA result is not

influenced by the level of carbamylated hemoglobin in CKD patients not on dialysis.[16]

As inferred from the results of the present study the relationship and concordance between these two methods support the reliability of PETIA method in CKD patients but Hemoglobin level less than 7g/dl affects the HbA1c values obtained by PETIA.

CONCLUSION

The Particle Enhanced Immunoturbidimetric method is easier to perform and available in most of the laboratories. This method shows high correlation and close agreement with HPLC results and therefore it can be used as a reliable alternative to HPLC method for estimation of HbA1c among CKD patients in regions with limited accessibility to advanced devices. But this method is not suitable for CKD patients with severe anemia.

Limitations

The limitations of the study are small sample size and the study did not include CKD patients on dialysis. Further studies with wider sample size are required to get deeper insights on this topic of research.

Conflict of interest

The authors of this study declare that there is no conflict of interest with regard to the content of the manuscript.

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