ANALYSIS OF HbA1c BY TINIA AND HPLC IN DIABETIC PATIENTS ATTENDING A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: To compare the analytical performance of reference & alternative method to measure glycated hemoglobin.

Material & Methods: This cross-sectional study was conducted at Fauji Foundation Hospital Rawalpindi from 1st March 2019 to 1st April 2019. Blood samples of 150 diabetic female patients were collected in potassium ethylene diamine tetra acetic (EDTA) vacutainers & measured for Glycated Haemoglobin (HbA1c) levels on Bio-Rad Variant II Turbo system by high-performance liquid chromatography (HPLC) and turbidimetric inhibitory immunoassay (TINIA) using Dimensions RXL Auto Analyzers. Mean ± standard deviation (SD) were calculated for quantitative variables. Correlation analysis and method comparison of the results obtained was done.

Results: The mean ± SD HbA1c values from TINIA and HPLC were 7.789% ± 2.106% and 7.797% ± 2.552%, respectively. The within-run coefficients of variation for TINIA and HPLC were 0.57% and 0.54% and for between-run were 0.87% and 0.83 respectively. Both methods showed good correlation (TINIA, r=.986, r^2 = .972, P <0 .001 and HPLC, r = .986, r^2= .972, P < 0.001).

Conclusion: Both methods showed accuracy and the results were comparable. A strong correlation existed between the two methods, which means these methods can be used equally and interchangeably. A developing country with limited resources requires an efficient and cost-effective solution to overcome the growing burden of diabetes. The turbimetric immunoassay is a more convenient and simple technique due to low cost, quick turnaround time of results compared to HPLC, sensitivity and specificity.

Key Words: Glycated hemoglobin, Diabetes Mellitus, Chromatography and immunoassay.

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INTRODUCTION

Diabetes is a non-communicable metabolic disorder characterized by underutilization of glucose leading to hyperglycemia. IDF estimates that 9.3% of adults age 20-79 years-a staggering 463 million are currently living with diabetes [1]. Asia is emerging as the epicenter of diabetes epidemic [2,3]. Hence there is a dire need of an efficient method of HbA1c estimation to cope with the growing global burden. Initially, Glucose diagnosis was based on a timed glucose sampling such as fasting, random and after a 75g glucose load. This timed measurement was tedious and also resulted in missed opportunity of screening [4].

Currently HbA1c assay stands as the most standardized marker to evaluate glycemia. HbA1c is formed by a non-enzymatic amadori reaction of N-terminal of amino group with Glucose [5]. HbA1c being the most efficient diagnostic tool with no time limitations and provides variation of average glucose levels over the last 2-3 months. HbA1c not only determines hyperglycemia but also correlates well with the complications. According to an observation 1% decrease in the level of HbA1c results in 30% reduction in diabetic complications [6].

At present more than 20 different analytical techniques are available to measure HbA1c which differ in either the structure , charge or the chemical reactivity[7, 8]. Most commonly used procedures are HPLC, electrophoresis, immunoassay. HPLC is the reference method of DCCT and gold standard of National glycohemoglobin standardization program [8,9]. Previous studies reported a disagreement in results and require standardization. Diagnostic accuracy of HbA1c is essential for efficient treatment and monitoring of diabetic patients [7]. The objective of this study is to compare TINIA with the gold standard HPLC.

MATERIAL AND METHODS

Present cross-sectional study was conducted after approval from Institutional Ethical Committee in Chemical Pathology Department of Fauji Foundation Hospital Rawalpindi from 1st March 2019-1st April 2019. Study consisted of 150 whole blood samples randomly chosen from the outpatients (OPD) after

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obtaining informed consent from the patients. The age of the patient ranged from 15 to 75 years, most of the patients were diabetic females since it is a family hospital of retired army personnel. Known cases of hemoglobinopathies and anemia (as it gives falsely low and/or high HbA1c results) [10, 11] and specimens received in the wrong Vacutainer were excluded. The analyzers and the reagents were used according to the instructions of the manufacturer. HbA1c levels were analyzed on Bio-Rad Variant II Turbo system by high-performance liquid chromatography (HPLC) and turbidimetric inhibitory immunoassay (TINIA) using Dimensions RXL Auto Analyzers.

Blood samples were obtained through vein puncture and collected into EDTA vacutainers. HbA1c levels were measured by HPLC using Turbo Variant II hemoglobin system and by Immunoturbidimetry using Dimensions RXL Auto Analyzers. All pre-analytical, analytical, and post-analytical measures were considered to ensure the accuracy and precision of the test results. For standard deviations (SDs) and coefficients of variation (CVs) validation of instruments and procedures an internal quality control was carried out. Lyphochek diabetes control 1 and 2 (Bio-Rad) specimens were used as quality control materials throughout the evaluation of specimens by TINIA and HPLC. The controls used were human lyophilized whole blood.

All HbA1c levels were determined by using two methods according to the manufacturers' recommendation.

1. **Dimensions RXLMAX:** This method is based on turbidimetric inhibition immunoassay. The anti-HbA1c antibody reacts with a single binding site on HbA1c, forming soluble complex. Next polyclonal antibodies against monoclonal antibodies can agglutinate particles and resulting turbidity is measured spectrophotometrically.

2. **BIO RAD Variant II Turbo Hemoglobin System:** It utilizes the ion- exchange high performance liquid chromatography. The samples are automatically diluted and injected into analytical cartridge. At chromatographic station buffers of increase ionic strength are delivered, Hb are separated and pass through filter photometer where changes in absorbance at 415 nm measured.

Precision Study: The imprecision of TINIA and HPLC method was demonstrated as coefficient of variation (CV%) for within–run and between-run studies. Two levels of HbA1c control material (high and low levels) were studied 20 times within-run and 20 times on consecutive days.

Table-1: Results for the imprecision study

Specimen no.	Parameter	Method	Mean (%)	SD	CV (%)
1	HbA_{1c} within run, n = *20*				
	Low control	TINIA	5.4	0.031	0.57
		HPLC	5.3	0.029	0.54
	High control	TINIA	12.3	0.089	0.72
		HPLC	12.4	0.091	0.73
2	HbA_{1c} between run, n = *20*				
	Low control	TINIA	5.3	0.046	0.87
		HPLC	5.2	0.043	0.83
	High control	TINIA	12.2	0.110	0.90
		HPLC	12.0	0.106	0.88

Data Analysis: SPSS version 20 was used for statistical analysis. For correlation and linear equations, linear regression analysis was used. Bland-Altman plots were performed for method comparison data to see the distribution of bias on both the negative and positive sides. P value of < 0.05 was considered significant.

RESULTS

Out of 150 analyzed samples the mean ± SD age of all the patients included in the study was 47.15 years ± 12.15 years. The majority of the patients belonged to urban areas of Punjab. The mean HbA_{1c} levels from TINIA and HPLC were 7.789%±2.106% and 7.797%±2.552%, respectively (Table-2). The *P* value of both the methods was found to be <0.05. The results for the imprecision study are shown in Table-1. All of the methods show excellent precision.

The comparison of the 2 methods is shown in Figure-1 and shows good concurrence between TINIA with HPLC (r^2 = .972). The TINIA results are plotted on the x-axis and HPLC results are plotted on the y-axis. A significant value of < 0.001 was obtained. The correlation coefficient (r) was 0.986 and the coefficient of determination (r^2) was 0.972. Distribution of bias and agreement between two methodologies is shown in Bland-Altman plots Figure-2. The plot shows the presence of good agreement between the two methods, 95% of values are lying within the ± 2 SD range from the mean.

Table-2: HbA1c (%) measured by different methods.

T-Test	HPLC	TINIA	P-value
Mean	7.797%	7.789%	
STD	2.552%	2.106%	<0.001***

Note: ***Significance at 0.001, **Significance at 0.01 level, *Significance at 0.05 level

Table-3: Correlation of HbA1c (%) value by different methods.

n=150	R	R^2	P value
HPLC	.986	.972	<0.001***
TINIA	.986	.972	<0.001***

Note: ***Significance at 0.001, **Significance at 0.01 level, *Significance at 0.05 level

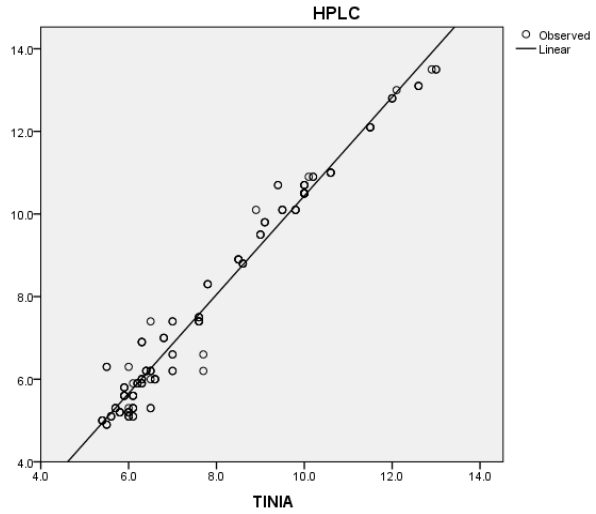

Figure-1: Method comparison plot for the determination of HbA1c(%) using TINIA and HPLC (r^2=.986) calculated by linear regression analysis.

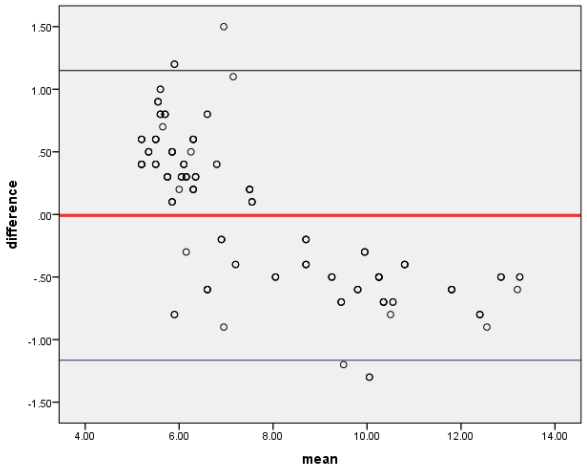

Figure-2: Bland-Altman plot between TINIA and HPLC. Mean= mean of TINIA and HPLC. Difference= difference between TINIA and HPLC.

DISCUSSION

The relevance of HbA1c in diabetes management is well recognized. The level of glycosylated hemoglobin is an accurate index of integrated blood glucose for the recent months. Clinicians must be well aware of the different methods required for measuring HbA1c values before interpreting results.

In our study a comparison between gold standard HPLC and commonly used TINIA was performed among 150 patients with HbA1c levels ranging from 4.4-16.7%(24.6-159mmol/mol). Our study showed good concordance between the two methods (r^2=.972). Similar study was conducted by Lekharu *et al*. in 2018 which also showed good correlation between the two methods (r^2 = .83) [7]. Genc *et al* in their study showed that TINIA method correlated well with HPLC (r^2 = 0.96) [12]. Gilani *et al*. in 2020 also showed that TINIA correlated well with HPLC (r^2 = .994) [13].

Although studies conducted earlier highlight the superiority of HPLC but many studies have documented good concordance between the two assay techniques, which was the case in our study as well [12, 14-16].

The precision (CV %) for HbA1c assays recommended by IFCC is <2.5%. Our study also showed CV% less than the IFCC recommended values. TINIA and HPLC methods in our study for within-run and between-runs were also in good agreement with the reference CVs of previous reports [13, 17-19].

CONCLUSION

TINIA and HPLC show similarity in terms of high imprecision and good accuracy as well as excellent correlation. The results of HbA1c obtained by both methods were in good agreement which shows that either method can be used. A developing country with limited resources requires an efficient and cost-effective solution to overcome the growing burden of diabetes.

The turbidimetric immunoassay is a more convenient and simple technique due to low cost rapidity, sensitivity and specificity.

AUTHORS CONTRIBUTION

Sami Saeed: Conception and design of the research, proof reading
Mehnaz Khattak: Literature review, Statistical and result analysis, proof reading
Saima Syed: Literature review, Data collection
Jawwad Anis Khan: Critical review of the article
Muhammad Amir: Final review
Afshan Khattak: Data collection

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