OPEN ACCESS

ORIGINAL ARTICLE

Comparison of HPLC with Tinia for hba1c measurement in diabetic and non-diabetic patient; a study from a tertiary care hospital of Baluchistan

Azeema Ahmed[1], Saima Bashir[2], Zeeshan Ayub[3], Javaria Ahsan[4] and Hamid Iqbal[5]

[1,2]Department of Chemical Pathology, CMH Quetta,

[3]Department of ENT, CMH Quetta,

[4]Department of Histopathology, CMH Quetta, [5]Department of Hematology, CMH Quetta

ABSTRACT

Background: Diabetes mellitus, a disease with serious implications on human health ,is on continuous rise. It is the need of hour to find and establish the markers to measure its long term effects. Diabetes Control and Complication Trial has confirmed role of HbA1c in effective blood glucose level monitoring .New methods are being developed for measurement of HbA1c .Before using these methods, it is essential to analyze these methods for reliability .Therefore in our study we will compare and evaluate correlation and agreement between Turbidimetric Inhibition Immunoassay TINIA and High Performance Liquid Chromatography HPLC for HbA1c measurement.

Methods: Three hundred and ninety-four samples were analyzed simultaneously on Roche Cobas 501 and D-10 HbA1c analyzer by Bio-Rad for HbA1c level estimation. The results were entered into SPSS version 26 for method validation. This is an observational study conducted at Department of Pathology CMH Quetta from January 2022 to August 2023.

Results: Inter and intra run coefficient of variation (CV) was within the allowable limit of 2 %. Mean difference between results of Turbidimetric Inhibition Immunoassay TINIA and High-Performance Liquid Chromatography HPLC was very low with HPLC results slightly on higher side. For low control TINIA had between run CV of 0.3% (Low control) 0.8 % (High control) and within run CV of 0.3% (Low control), 0.6% (High control); while HPLC had between run CV of 0.6% (Low control) 1.8% (High control), and within run CV of 0.3% (Low control), and 0.7%. Linear regression analysis displayed high concordance and agreement between the results of both methods.

Conclusion: Both methods have comparable results in HbA1c estimation.

Key words: Baluchistan, HbA1C, Method comparison.

This article may be cited as: Ahmed A, Bashir S, Ayub Z, Ahsan J, Iqbal H. Comparison of HPLC with Tinia for hba1c measurement in diabetic and non-diabetic patient; a study from a tertiary care hospital of Baluchistan Int J Pathol 2024; 22(3): 103-109. https://doi.org/10.59736/IJP.22.03.890

Introduction

Diabetes mellitus is a serious condition with implications on human health (1).Prevalence of diabetes is on continuous rise with an expected increase of 8.8 to 9.9% by the year 2045. Increase in prevalence has been ascribed to increase in life expectancy,

obesogenic lifestyle and use of refined foods. This increase in number of diabetic patients' calls for development of reliable and patient centered approaches for better monitoring of diabetes (2).

Diabetes Control and Complications trial has confirmed the role of effective blood glucose level monitoring in decreasing the rate of complications in diabetic patients.

CORRESPONDENCE AUTHOR
Saima Bashir
Department of Chemical Pathology
CMH Quetta
Email: saimabashir@live.com

Measurement of blood Glucose levels alone is not sufficient for determining the long-term control of diabetes.HbA1c is the test of choice for this purpose. It is the irreversible glycosylated subset of Hemoglobin at one or both N terminals of Beta Chains of Hemoglobin**.** After recommendation of international expert committee in 2009, HbA1c has already been used for diagnosis of diabetes at a cut off of 6.5 % (3).

Both long- and short-termglycemic variability's have been found to be associated with diabetic complications. These variabilities can be confirmed by serial measurement of HbA1c levels (4). For purpose of monitoring different cutoffs have been proposed by different committees including <7% by American Diabetes Association and <5% by IFCC [International Federation of Clinical Chemistry]. NGSP recommends twice yearly measurement of HbA1c (5). HbA1c is also helpful to predict complications and to enhance the cost effectiveness of diabetes prevention in the high-risk population suffering from prediabetes (6). For this purpose the cutoff of 5.7-6.4% can be used (7). International Federation of Clinical Chemistry (IFCC) has recommended two reference methods for measurement of HbA1c: HPLC with Mass spectrometry or capillary electrophoresis (5, 6). New methods are continuously being developed for HbA1c. All of them have both advantages and disadvantages .Before using HbA1c for monitoring diabetic patients, it is essential to check the reliability of the new method. Studies from different parts of the world have shown good correlation between TINIA and HPLC based methods (9, 10). There is a lack in data from Baluchistan regarding comparison of both these methods with large clinical material. The objective of this study is to compare and evaluate correlation and agreement between TINIA and HPLC for measurement of HbA1c (7).

Methods

This observational study was conducted at Combined Military Hospital Quetta from January 2022 to August 2023. Non probability consecutive sampling method was used for sample collection. Our study included 394 samples collected from patients of both the genders between ages 18-65 years, coming for different blood tests after taking informed verbal consent. Pregnant females, patients with known hemoglobinopathies and anemia, patients with history of recent massive blood loss or blood transfusion, those taking steroids or insulin, known patients of Chronic Kidney Disease, Chronic Liver Disease, and cancer were excluded from study. This study is approved by Ethical review committee ERB No 10-2023 dated: 09-01-2023. Venous blood was collected in tubes containing EDTA. Samples for between run studies were refrigerated at temperature between 2-8^{o} C. Rest of analysis was done within 06

hours of collection simultaneously on both instruments.

All samples were analyzed simultaneously on Roche Cobas 501 and D-10 HbA1c analyzer by Bio-Rad. The Cobas c501 module is an automated analyzer for HbA1c measurement via the principle of Turbidimetric Inhibition Immunoassay. It is a large analyzer with physical dimensions of 120 cm (width), 98 cm (depth), 130 cm (height) and weight of 330 kg. It can be placed on the floor for ergonomic use. The D-10 Hemoglobin A1c Program utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). Physical dimensions of the instrument are: Height 49.5 cm, width 40.2 cm and length 53.4 cm.

Imprecision for both methods is calculated as CV. For this purpose 02 levels of controls were used. Controls were run 20 times in one day for within run precision and daily for 20 days for between run studies. .Intra run CV was calculated by performing 20 assays on same control on same day. The inter run CV was calculated by measuring HbA1c in controls materials in duplicate for 20 days. Linear regression analysis was used to determine the correlation between 02 methods. Quantitative data was expressed as mean with standard deviation and percentages. P-value of less than 0.05 was considered significant. All data was analyzed on SPSS version 26.

Results

Samples were collected from 394 patients out of these 104 (26.4%) were females and 290 (73.6%) were males. Average age for males was 55.4 ± 5.2 years and for females it was 52.3 ± 4.7years. Table I shows HPLC Method showed within run CV of 0.3% for low control and 0.7% for high control while TINIA Showed within run CV of 0.3% for low control as and 0.6%for high control. The between run CV was below 1% for TINIA and <2% for HPLC. The mean values of HbA1c low level controls were 3.20 ± 0.07% and 3.24 ± 0.06 for Turbidimetric Inhibition Immunoassay TINIA and High-Performance Liquid Chromatography HPLC respectively and 10.48 ± 0.08 and 10.58 ± 0.07 for high level controls for the same methods. Difference between both methods was found to be insignificant i.e. ($p>0.05$). Distribution of bias between Turbidimetric Inhibition Immunoassay TINIA and High-Performance Liquid Chromatography HPLC results with majority of values lie within the total allowable limit of 5%.

Comparison of both methods is shown in figure 1. It shows good agreement between both the methods with $r^2 = 0.989$ the slopes and intercept between both methods were, A= -0.114 (CI = -0.199 - -0.029), B=1.04 (CI = 1.029 – 1.051) and y = 0.11+1.04×. The Pearson correlation coefficient value 0.994 showed significant agreement between results of both the methods with $p<0.001$.

Table 1: Comparison of mean and SD values between TINIA and HPLC

Sr. No.	Method	Mean	SD	CV
Within Run				
Low Control	TINIA	3.24	0.01	0.3%
	HPLC	3.20	0.01	0.3%
High Control	TINIA	10.58	0.07	0.6%
	HPLC	10.48	0.08	0.7%
Between Run				
Low Control	TINIA	3.24	0.01	0.3%
	HPLC	3.19	0.02	0.6%
High Control	TINIA	10.59	0.09	0.8%
	HPLC	10.48	0.19	1.8%

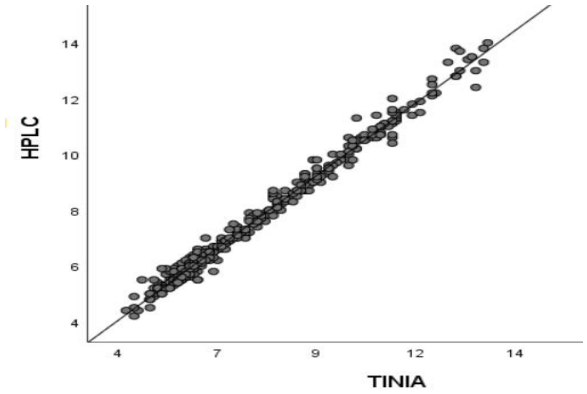

Fig-1: Method comparison (Linear regression plot) for HbA1c using HPLC and TINIA WITH $r^2 = 0.989$

Discussion

HbA1c has gained the reputation of reliable therapeutic guiding marker for diabetic patients leading to development of different methods for its measurement and their detailed studies to get reliable results .Serial measurements of HbA1C have predictive value in risk stratification including increase in complications related to renal ,ophthalmological and neurological systems in both type 1 and type 2 diabetes patients (7).A recent study shows role of HbA1c in GDM although not to replace Oral Glucose Tolerance Test (OGTT) but use of HbA1C can reduce cost and inconvenience of Oral Glucose Tolerance Test OGTT for patients (8). More than 100 methods have already been developed for measurement of HbA1 c. These methods measure HbA1c on basis of either charge or structure. Charge based methods include capillary electrophoresis ion exchange and affinity chromatography. Enzymatic and immunoassays are the tests based on chemical reactions (9). For best results, there are few prerequisites including use of standardized method, proper internal and external quality control program and accommodation of pre and posttest variables effect. Any new method introduced needs to be standardized by IFCC along with certification by National Glycohemoglobin Standardization Program NGSP. The National Glycohemoglobin Standardization Program NGSP guideline sets the Total allowable error (TEa) limit of HbA1c measurement at ±6% (3). Even after all these steps significant differences can be found in results of the tests done by different methods (9).

In our study we compared Turbidimetric Inhibition Immunoassay TINIA and High-Performance Liquid Chromatography HPLC for measurement of HbA1c. Our study shows that there is very good correlation and hardly any variability in the results of these two methods we used TINIA as the reference method for our laboratory as it is the method in practice for last 05 years and HPLC based method has just been introduced here. Our results are consistent with two different studies by Koyoka and Mehwish et al (10, 11). Ghaith et al evaluated HPLC based instrument against TINIA for measurement of HbA1c in both normal and in blood of patients with Hemoglobin variants. HPLC based instrument showed high analytical performance adequate for routine clinical use (12).Mean difference between both methods is very small and insignificant .These results are comparable with the results of Wilaiwan et al study conducted in Thailand and another multicenter study conducted in Andalusia (14, 15).Both of these studies showed good concordance between Turbidimetric Inhibition Immunoassay TINIA and High Performance Liquid Chromatography HPLC based methods (16). Although the results of HPLC in our study were comparatively higher than Immunoassay, similar results shown in a Japanese study conducted in 2020.Another study by Saeed et al showed high comparability with r=0.986 between the

results of both methods with conclusion that these methods can be used interchangeably (16) . Different results were found in another study done by Juang et al who compared performance of HPLC and capillary electrophoresis in samples with differences in blood indices. Their results showed statistically significant difference (14).A study conducted by Cihan et al (17) showed non concordance in HbA1c results of HPLC and TINIA. These differences could be attributed to differences in sample preparation, internal quality control rules etc.

The independent performance of both the methods also remained satisfactory. CV values of both the methods were within the allowable limit of 2% as recommended by IFCC (10). Our CV is close to the results of Wilaiwan et al study in which they compared 03 methods including Roche Turbidimetric Inhibition Immunoassay TINIA and Arkray High Performance Liquid Chromatography HPLC and Mindray Enzymatic Assay with Capillary Electrophoresis for analysis of HbA1C (15). Turbidimetric Inhibition Immunoassay TINIA and High Performance Liquid Chromatography HPLC results of CV for low level control were 1.13 and 0.46 and for high level controls 0.90 ,0.52 respectively.

Conclusion

Our study showed strong correlation, high comparability between the two methods along with consistent individual performance, making both the methods are interchangeable and backup of each other.

Conflict of interest: None.

Funding: No funding was received for this project

References

1. Rama Chandran S, A. Vigersky R, Thomas A, Lim LL, Ratnasingam J, Tan A, SL Gardner D. Role of composite glycemic indices: A Comparison of the Comprehensive Glucose Pentagon Across Diabetes Types and HbA1c Levels. Diabetes Technol Ther.2020 Feb 1; 22(2):103-11.
2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Res Clin Pract. 2019 Nov 1; 157:107843. doi: 10.1016/j.diabres.2019.107843
3. Klonoff DC, Aron D, Cohen RM, Home P, John WG, Little RR, et al.The need for accuracy in hemoglobin A1C proficiency testing: why the proposed CLIA rule of 2019 is a step backward. J Diabetes Sci Technol. 2019 May; 13(3):424-7.
4. Ceriello A, Monnier L, Owens D. Glycaemic variability in diabetes: clinical and therapeutic implications. Lancet Diabetes endocrinol. 2019 Mar 1; 7(3):doi: 10.1016/S2213-8587(18)30136-0. Epub 2018 Aug 13. PMID: 30115599.
5. Greiner GG, Emmert-Fees KM, Becker J, Rathmann W, Thorand B, Peters A, et al. Toward targeted prevention: risk factors for prediabetes defined by impaired fasting glucose, impaired glucose tolerance and increased HbA1c in the population-based KORA study from Germany. Acta diabetol. 2020 Dec; 57(12):1481-91. doi: 10.1007/s00592-020-01573-x.
6. Jang JY, Moon S, Cho S, Cho KH, Oh CM. Visit-to-visit HbA1c and glucose variability and the risks of macrovascular and microvascular events in the general

population. Sci Rep. 2019 Feb 4;9(1):1-8. doi: 10.1038/s41598-018-37834-7.
7. Lee S, Zhou J, Wong WT, Liu T, Wu WK, Wong IC, Zhang Q, Tse G. Glycemic and lipid variability for predicting complications and mortality in diabetes mellitus using machine learning. BMC Endocr Disord. 2021 May 4; 21(1):1-5. Doi: 10.1186/s12902-021-00751-4.
8. Sriwimol W, Choosongsang P, Choosongsang P, Treerut P, Muenniam B, Makkong P, et al. Strong correlation and high comparability of capillary electrophoresis and three different methods for HbA1c measurement in a population without hemoglobinopathy. Scand J Clin Lab Invest. 2020 Feb-Apr; 80(2):139-150. Doi: 10.1080/00365513.2019.1703213.
9. Goya K, Tanaka S, Makio T, Kanno M, Tabuchi Y, Fukuzawa K,et al. HbA1c levels Measured by Enzymatic Assay and Immunoassay during Off-Site Health Checkups Are Both Lower than Those Measured by On-Site HPLC Assay. Kobe J Med Sci. 2021 Mar 25; 66(5):E170-E174.
10. Okeyo HM, Biddle M, Williams LB. Impact of Diabetes Self-Management Education on A1C Levels among Black/African Americans: A Systematic Review. Sci Diabetes Self Manag Care. 2024 Feb; 50(1):87-95. Doi: 10.1177/26350106231213400.
11. Gilani M, Aamir M, Akram A, Haroon ZH, Ijaz A, Khadim MT. Comparison of turbidimetric inhibition immunoassay, high-performance liquid chromatography, and capillary electrophoresis methods for glycated hemoglobin determination. Lab Med. 2020 Nov 2;51(6):579-84. Doi: 10.
12. Altawallbeh G, Makky VF, Saenger AK, Peters JM, Killeen AA. Evaluation of an ion-exchange HPLC device for HbA1c measurement. J Appl Lab Med. 2020 Jul 1; 5(4):695-703. Doi: 10.1093/jalm/jfaa042.
13. Juang YJ, Wu HM, Cheng YW, Eng HL, Lin TM. Influence of RBC Indices on HbA1c Measurement by Capillary Electrophoresis and HPLC Methods. Clin Lab. 2022 Aug 1; 68(8). Doi: 10.7754/Clin.Lab.2021.211053.
14. Sriwimol W, Choosongsang P, Choosongsang P, Treerut P, Muenniam B, Makkong P,et al. Strong correlation and high comparability of capillary electrophoresis and three different methods for HbA1c measurement in a population without hemoglobinopathy. Scand J Clin Lab Invest. 2020 Feb-Apr; 80(2):139-50. Doi: 10.1080/00365513.2019.1703213.
15. Fırat Oğuz E, Eren F, Neşelioğlu S, Akbıyık F, Erel Ö. Comparison of three different HbA1c measurement methods–the Atellica® CH930, Capillary 3 Tera, and BioRad Variant Turbo II. Scand J Clin Lab Invest. 2022 May; 82(3):218-25. doi: 10.1080/00365513.2022.2051070.
16. Saeed S, Khattak M, Syed S, Khan JA, Amir M, Khattak A. Analysis of HBA1C by TINIA and HPLC in diabetic patients attending a tertiary care Hospital. Pakistan Journal of Pathology. 2020; 31(2):42-5.
17. Cihan M, Üner A, Kılınçkaya MF, Noyan T. Comparison of high performance liquid chromatography and turbidimetric inhibition immunoassay methods for measurement of hemoglobin A1c. Journal of Health Sciences and Medicine. 2022 Sep 9; 5(5):1281-5.1

HISTORY	
Date received:	22-05-2024
Date sent for review:	19-07-2024
Date received reviewers comments:	20-08-2024
Date received revised manuscript:	12-09-2024
Date accepted:	27-09-2024

CONTRIBUTION OF AUTHORS	
AUTHOR	**CONTRIBUTION**
Azeema Ahmed	A,B,C
Saima Bashir	A,B,C
Zeeshan Ayub	A,B
Javaria Ahsan	A,B
Hamid Iqbal	A,B

KEY FOR CONTRIBUTION OF AUTHORS:

A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion