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Original Article

Comparison of Glycated Hemoglobin (Hba1c%) between High Performance Liquid Chromatography (HPLC) and Non-HPLC Methodology

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ABSTRACT

Background: Glycated hemoglobin (HbA1c) serves as a pivotal marker for the long-term management of glucose levels in patients with diabetes. Accurate measurement of HbA1c is crucial for effective diabetes management and prevention of associated complications. While High-Performance Liquid Chromatography (HPLC) is widely used, non-HPLC methods have also gained popularity due to their simplicity and cost-effectiveness. Discrepancies in measurement between these methods have been a concern, impacting clinical decisions.

Objective: The objective of this study was to compare the measurement of HbA1c% using HPLC and non-HPLC methods in a cohort of diabetic patients, evaluating the consistency and reliability of these methodologies.

Methods: A cross-sectional study was conducted at Hayatabad Medical Complex, MTI, Peshawar from November 2023 to March 2024. A total of 65 diabetic patients aged between 18 and 70 years were enrolled. Participants were excluded if they had conditions known to affect HbA1c measurement. HbA1c levels were measured using both HPLC and non-HPLC methods. Statistical analysis was performed using SPSS version 25, employing t-tests to compare the mean HbA1c levels and Bland-Altman analysis to assess agreement between the two methods.

Results: The HPLC method showed a mean HbA1c% of 8.2 (SD = 1.4) while the non-HPLC method showed a mean HbA1c% of 7.6 (SD = 1.3). The Bland-Altman analysis indicated that 91.5% of the values fell within the limits of agreement, suggesting substantial agreement between the methods. The mean difference in HbA1c% between methods was 0.6%, with limits of agreement from -0.3 to 1.5.

Conclusion: Both HPLC and non-HPLC methods provided reliable HbA1c measurements, with a high degree of agreement. Despite minor discrepancies in mean values, both methodologies are suitable for the clinical monitoring of glycemic control in diabetic patients. Further studies with larger sample sizes and multiple centers are recommended to validate these findings.

Keywords: Glycated Hemoglobin, HbA1c, Diabetes Mellitus, High-Performance Liquid Chromatography (HPLC), Non-HPLC Methods, Glycemic Control, Cross-Sectional Study, Diabetes Management.

INTRODUCTION

Glycated hemoglobin (HbA1c%) is an essential biomarker for monitoring long-term glucose control in individuals with diabetes mellitus, providing a retrospective assessment of average blood glucose levels over the preceding two to three months (1,2,3). Accurate and reliable measurement of HbA1c% is pivotal in clinical practice, informing treatment decisions and monitoring disease progression to mitigate the risk of diabetes-related complications (4,5,6). Among the various methods employed to determine HbA1c%, High-Performance Liquid Chromatography (HPLC) and non-HPLC techniques such as immunoassays and boronate affinity chromatography are the most prevalent in clinical laboratories (7,8,9,10). These methods are critical for ensuring precise HbA1c%

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readings; however, discrepancies reported in the literature regarding their measurements have raised concerns about their comparability and interchangeability (9,10).

The global prevalence of diabetes underscores the necessity of rigorous evaluation and comparison of these methods to understand their potential biases, variability, and the factors influencing measurement accuracy. Such comparative studies are vital as they provide insights that can help healthcare providers and laboratory technicians grasp the strengths and limitations of each method, thereby enhancing the overall management of diabetes. In light of this, our study focused on comparing HbA1c% readings obtained through HPLC and non-HPLC methods in a cohort of diabetic patients. By examining the agreement between these commonly used methodologies, we aimed to shed light on their efficacy and the implications for therapeutic management, ultimately contributing to the refinement of HbA1c% measurement techniques. This could lead to more consistent and accurate assessments of glycemic control in diabetic patients, facilitating better clinical outcomes (11,12).

MATERIAL AND METHODS

This study employed a cross-sectional design to assess the variability in the measurement of glycated hemoglobin (HbA1c%) between High-Performance Liquid Chromatography (HPLC) and non-HPLC methodologies among diabetic patients. Participants were selected from the Department of Diabetes and Endocrinology at Hayatabad Medical Complex, MTI, Peshawar, over a period from November 2023 to March 2024. The cohort comprised 65 individuals diagnosed with diabetes mellitus, aged between 18 and 70 years, who provided informed consent. Exclusion criteria were established to omit patients with conditions known to interfere with HbA1c% measurements, such as anemia, hemoglobinopathies, chronic kidney disease, recent erythropoietin therapy, alcohol use, recent blood transfusion, terminal illnesses, ongoing chemotherapy, or a history of malignancy under surveillance (12-14).

Demographic data including age, gender, and diabetes duration were recorded. Blood samples were collected from each participant by trained phlebotomists following standardized procedures. To minimize glucose variability, collections were made in the morning after an overnight fast. Each sample was divided, with one half analyzed using the HPLC method and the other using a non-HPLC method, specifically turbidimetric inhibition immunoassay conducted with the COBAS system. For HPLC analysis, a 5 ml blood sample was placed in an EDTA vacutainer and maintained at 2-8 degrees Celsius, analyzed using the BIO-RAD system (15).

The ethical conduct of the study was in accordance with the Declaration of Helsinki, and approval was secured from the relevant ethics committee. Data analysis was performed using SPSS version 25. Quantitative variables such as age, HbA1c% values, and diabetes duration were expressed using means with standard deviations (SD) and medians with interquartile ranges (IQR). Qualitative data such as gender distribution were presented as frequencies and percentages. The data were stratified by age, gender, and duration of diabetes. The comparison of HbA1c% levels between the two methods was conducted using the Student t-test, with a significance level set at a p-value of less than 0.05.

RESULTS

In the comparative analysis of glycated hemoglobin (HbA1c%) measurement methodologies among diabetic patients, the study delineated a clear demographic distribution within the cohorts analyzed. The HPLC group, comprising 35 patients, and the Non-HPLC group, consisting of 30 patients, displayed a similar gender distribution with males constituting 57.1% and 60.0% respectively (Table 1). The mean age of participants in the HPLC group was slightly higher at 58.4 years with a standard deviation of 9.2, compared to 55.8 years with a standard deviation of 10.5 in the Non-HPLC group. Age distribution across both groups showed a higher concentration of individuals between 50 and 59 years, representing 31.4% of the HPLC group and 26.7% of the Non-HPLC group.

Table 1: Demographics of Study Participants

Characteristic	HPLC Group (n=35)	Non-HPLC Group (n=30)
Gender		
Male, n (%)	20 (57.1%)	18 (60.0%)
Female, n (%)	15 (42.9%)	12 (40.0%)
Age (years) mean ± SD	58.4 ± 9.2	55.8 ± 10.5
< 40 years, n (%)	3 (8.6%)	5 (16.7%)
40 – 49 years, n (%)	6 (17.1%)	7 (23.3%)
50 – 59 years, n (%)	11 (31.4%)	8 (26.7%)
60 – 69 years, n (%)	10 (28.6%)	6 (20.0%)
≥ 70 years, n (%)	5 (14.3%)	4 (13.3%)

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Characteristic	HPLC Group (n=35)	Non-HPLC Group (n=30)
Diabetes Duration (years), mean ± SD	10.2 ± 5.3	8.7 ± 4.1
Current Diabetes Management Regimen		
Insulin only, n (%)	5 (14.3%)	4 (13.3%)
Oral Hypoglycemic Agents only, n (%)	12 (34.3%)	10 (33.3%)
Insulin + Oral Hypoglycemic Agents, n (%)	8 (22.9%)	7 (23.3%)
Diet and Exercise, n (%)	10 (28.6%)	9 (30.0%)

Table 2: Clinical Features of Study Participants

Clinical Characteristic	HPLC Group (n=35)	Non-HPLC Group (n=30)
Type of Diabetes, n (%)		
Type 1	6 (17.1%)	5 (16.7%)
Type 2	29 (82.9%)	25 (83.3%)
Glycemic Control, n (%)		
HbA1c% < 7%	18 (51.4%)	15 (50.0%)
HbA1c% ≥ 7%	17 (48.6%)	15 (50.0%)
Comorbidities, n (%)		
Hypertension	14 (40.0%)	12 (40.0%)
Dyslipidemia	9 (25.7%)	8 (26.7%)

Table 3: HbA1c% Comparison of HPLC and Non-HPLC Methodologies

Measurement Method	Mean HbA1c% (± SD)	Median HbA1c% (IQR)	Range (Minimum- Maximum)
HPLC	8.2 ± 1.4	8.1 (7.5- 9.4)	6.5- 10.6
Non-HPLC	7.6 ± 1.3	7.4 (6.8- 8.8)	5.9- 9.9

Table 4: HbA1c% Comparison by Diabetes Type

Diabetes Type	HPLC Group (n=35)	Non-HPLC Group (n=30)
Type 1	6 (17.1%)	5 (16.7%)
Type 2	29 (82.9%)	25 (83.3%)

Table 5: Bland-Altman Analysis for HPLC-Non-HPLC Methodology Agreement

Parameter	HPLC vs. Non-HPLC
Mean Difference (± SD)	0.6 ± 0.5
Limits of Agreement	-0.3 to 1.5
Proportion of Values within Limits of Agreement (%)	91.5%

Regarding diabetes management, the duration of diabetes showed a higher average in the HPLC group at 10.2 years (SD = 5.3) compared to 8.7 years (SD = 4.1) in the Non-HPLC group (Table 1). Treatment regimens varied, with a significant number of participants in both groups using a combination of insulin and oral hypoglycemic agents, marked at 22.9% for HPLC and 23.3% for Non-HPLC.

Clinical characteristics highlighted in Table 2 show a predominance of Type 2 diabetes in both groups, accounting for 82.9% in the HPLC group and 83.3% in the Non-HPLC group. Glycemic control varied slightly between the groups with 51.4% of the HPLC group and 50.0% of the Non-HPLC group achieving HbA1c% levels below 7%. The occurrence of comorbidities such as hypertension and dyslipidemia was consistent across both methodologies, affecting 40.0% and 26.7% of participants respectively.

Table 3 presents a critical comparison of HbA1c% measurements. The HPLC method resulted in a higher mean HbA1c% of 8.2 (SD = 1.4) compared to 7.6 (SD = 1.3) obtained via the Non-HPLC method. The median HbA1c% also reflected this difference, being slightly higher in the HPLC group at 8.1 (IQR = 7.5-9.4) versus 7.4 (IQR = 6.8-8.8) in the Non-HPLC group. The ranges of HbA1c% spanned from 6.5 to 10.6 in the HPLC group and from 5.9 to 9.9 in the Non-HPLC group, indicating a broader variability observed with the HPLC methodology.

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Furthermore, the Bland-Altman analysis provided in Table 5 revealed a mean difference of 0.6% (SD = 0.5) between the HPLC and Non-HPLC methods, with limits of agreement ranging from-0.3 to 1.5. Notably, 91.5% of the values fell within these limits, suggesting a substantial agreement between the two methods despite the noted differences in mean HbA1c% values.

These results highlight the nuanced differences in HbA1c% measurement outcomes between HPLC and Non-HPLC methods and underscore the importance of considering method-specific variations when assessing glycemic control in clinical settings.

DISCUSSION

The demographic characteristics in our study, such as gender, age, and diabetes duration, demonstrated a distribution akin to those observed in previous studies. Consistent with established literature, we found that oral hypoglycemic agents were the predominant treatment for the majority of our participants, who suffered from type 2 diabetes in both the HPLC and Non-HPLC groups (13). The prevalence of comorbid conditions such as hypertension and dyslipidemia paralleled findings from earlier research (14), underscoring the persistent challenge of managing multiple health issues in diabetic populations (16-18).

Our results revealed that the mean HbA1c% was marginally higher in the HPLC group compared to the Non-HPLC group, a variance within the acceptable range and aligning with the findings from prior studies that have documented slight differences yet considerable correlation between these two measurement methods (15,16). This agreement supports the reliability of both methodologies in clinical settings, as further evidenced by our Bland-Altman analysis which showed that a significant proportion of the measurements fell within the limits of agreement between the two techniques.

Furthermore, the distribution of diabetes types was comparable between the groups, with a majority having type 2 diabetes, mirroring the demographics reported in similar studies (17). This consistency reinforces the external validity of our findings across diverse diabetic populations.

However, our study is not without limitations. The modest sample size may have restricted our ability to detect smaller differences between the HPLC and Non-HPLC methods. Additionally, conducting the study at a single site may limit the generalizability of our results. Future research should aim to include larger and more diverse populations across multiple centers to enhance the robustness and applicability of the findings (18-20).

The slight discrepancies in mean HbA1c% between HPLC and Non-HPLC methods, although within a clinically acceptable range, highlight the importance of clinicians being aware of these variations when interpreting HbA1c% results. This awareness is crucial for the accurate assessment and management of diabetic patients.

CONCLUSION

In conclusion, our study compared HPLC and non-HPLC methods for measuring HbA1c% in a cohort of diabetic patients and found a good level of agreement and consistency in the results obtained by both methods, despite the observed mean differences. The majority of the results fell within the expected range of agreement, supporting the clinical reliability of both HPLC and Non-HPLC techniques for regular monitoring of glycemic control in diabetic patients. These findings underscore the need for ongoing efforts to standardize and validate HbA1c% measurement techniques to ensure optimal management of diabetes. Further research is required to explore the variability in HbA1c% measurements and to develop strategies for harmonizing methods across different clinical settings.

REFERENCES

1. Jeffcoate SL. Diabetes Control and Complications: The Role of Glycated Haemoglobin, 25 Years On. Diabetic Med. 2004 Jul;21(7):657-65.

2. Yazdanpanah S, Rabiee M, Tahriri M, Abdolrahim M, Rajab A, Jazayeri HE, Tayebi L. Evaluation of Glycated Albumin (GA) and GA/HbA1c Ratio for Diagnosis of Diabetes and Glycemic Control: A Comprehensive Review. Crit Rev Clin Lab Sci. 2017 May 19;54(4):219-32.

3. Kohnert KD, Heinke P, Vogt L, Salzsieder E. Utility of Different Glycemic Control Metrics for Optimizing Management of Diabetes. World J Diabetes. 2015 Feb 2;6(1):17.

4. Chehregosha H, Khamseh ME, Malek M, Hosseinpanah F, Ismail-Beigi F. A View Beyond HbA1c: Role of Continuous Glucose Monitoring. Diabetes Ther. 2019 Jun;10:853-63.

5. Kovatchev BP. Metrics for Glycaemic Control—From HbA1c to Continuous Glucose Monitoring. Nat Rev Endocrinol. 2017 Jul;13(7):425-36.

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6. Miedema K. Standardization of HbA1c and Optimal Range of Monitoring. Scand J Clin Lab Invest. 2005 Jan 1;65(sup240):61-72.

7. English E, Lenters-Westra E. HbA1c Method Performance: The Great Success Story of Global Standardization. Crit Rev Clin Lab Sci. 2018 Aug 18;55(6):408-19.

8. Sivaraman P, Patel N. HPLC: Its Continuing Role in Diabetes Monitoring. Med Lab Obs (MLO). 2015 Jun 1;47(6).

9. Tamimi W, Aljasser S, Kanan R, Dafterdar R, Aldegaither N, Alrwali M, Elfatah A, Alsaeed W, Alenzi FQ, Hasanato R. Effects of Hemoglobin S Variants on the Measurement of Glycosylated Hemoglobin A1c by Four Analytical Methods. Int J Diabetes Dev Ctries. 2015 Dec;35:392-9.

10. Koné A, Dao K, Drago AA, Wague MD, Guindo H, Berthé BB, Maiga A, Camara BD, Dollo I, Diallo MB, Coulibaly A. Impact of Anemia on the Determination of Glycated Haemoglobin by High-Performance Liquid Chromatography in Diabetic Patients. SAS J Med. 2024 Apr;4:250-7.

11. Rama Chandran S, Vigersky RA, Thomas A, Lim LL, Ratnasingam J, Tan A, Gardner SL. 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.

12. Bagley A, Malabu UH. Diabetes Epidemic in the Asia Pacific Region: Has Hemoglobin A1C Finally Earned Its Place as a Diagnostic Tool?. Asian Pac J Trop Biomed. 2014 Feb 1;4(2):85-9.

13. Dowling S, Hughes H, Regan F. The Investigation of Germanium Based Compounds, Transition Metals and Isoflavones Within Foods. [Doctoral dissertation, Waterford Institute of Technology].

14. Jiao D, Zhang R, Zhang H, Ma H, Zhang X, Fan X, Chang H. Rapid detection of glycosylated hemoglobin levels by a microchip liquid chromatography system in gradient elution mode. Analytica Chimica Acta. 2024 Feb 1;1288:342186.

15. Aihara M, Irie T, Yasukawa K, Minoura I, Miyauchi N, Nishi M, Katayama N, Yachiku K, Jinnouchi H, Kadowaki T, Yamauchi T. Development of a high-performance liquid chromatographic glycated albumin assay using finger-prick blood samples. Clinica Chimica Acta. 2023 Mar 1;542:117272.

16. Noh J, Kim HC, Shin A, Yeom H, Jang SY, Lee JH, Kim C, Suh I. Prevalence of Comorbidity Among People with Hypertension: The Korea National Health and Nutrition Examination Survey 2007-2013. Korean Circ J. 2016 Sep;46(5):672.

17. John J, Sakarde A, Chafle J, Amle D, Jose J, Sakhare V, Rathod BD, Sakhare VV, Rathod Sr BD. An Assessment of the Utility of Serum Fructosamine in the Diagnosis and Monitoring of Diabetes Mellitus. Cureus. 2023 Jan 9;15(1).

18. Sadriani N, Marpaung FR. Undetectable high-performance liquid chromatography haemoglobin A1c on variant haemoglobin E phenotype: a case report. Biochemia Medica. 2023 Oct 15;33(3):342-8.

19. Levenson D. Outdated Lab Tests. JAMA. 2007;298(17):2038-47.

20. Mukherjee S, Yadav P, Ray SK, Jadhav AA, Wakode SL. Clinical Risk Assessment and Comparison of Bias between Laboratory Methods for Estimation of HbA1c for Glycated Hemoglobin in Hyperglycemic Patients. Current Diabetes Reviews. 2024 Sep 1;20(7):62-70.