BRIEF RESEARCH COMMUNICATION

Comparison of HbA1c Estimation by Enzymatic and HPLC Methods

Surya Kantha Bugge[1], Tahniyath Fathima[2]

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Abstract

Introduction and objective: There are methods of HbA1c estimation that depend on different physical, chemical, immunological features of glycated hemoglobin. Numerous analytical techniques have been developed for assessing HbA1c in diabetes mellitus (DM), including immunoturbidimetry, boronate affinity chromatography, enzymatic assays, and high-performance liquid chromatography (HPLC) immunoassay. Notably, different estimation methods may yield disparate results. This study aims to conduct and compare two analytical techniques—specifically, the enzymatic method and HPLC method—to observe and analyze any variations in results within the same set of samples.

Materials and methods: This is an observational cross-sectional study involving 100 EDTA samples. The study focused on the analysis of HbA1c using two distinct methods: Atellica CH 930 enzymatic hemoglobin A1c (HbA1c_E) from Siemens Healthineers and cation exchange HPLC from Bio-Rad Laboratories.

Results: A strong robust correlation was observed between the two methods, as evidenced by a Pearson's Coefficient of 0.983. The Bland–Altman plot demonstrated a high level of agreement between the two techniques, with 95% of values falling within ±SD (standard deviation), indicating a strong concordance.

Conclusion: This study establishes that both methods, Atellica CH 930 enzymatic hemoglobin A1c (HbA1c_E) and cation exchange HPLC, produced comparable results for HbA1c. Therefore, both analytical techniques are deemed suitable for the effective management of diabetes.

Keywords: Diabetes mellitus, Glycated hemoglobin, High performance liquid chromatography, Hemoglobin A1c by enzymatic method.

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Introduction

Hemoglobin, an iron-containing protein integral to oxygen transport in erythrocytes, primarily manifests as HbA in adults, composed of α and β chains (α2β2), and constituting about 97% of adult hemoglobin. Approximately 6% of HbA is glycated, with the primary component being hemoglobin A1c (HbA1c) (5%), alongside minor components HbA1a and HbA1b (1%).

Formation of HbA1c occurs via the nonenzymatic glycation process, wherein glucose covalently binds to the N-terminal valine of the hemoglobin β-chain. This process is influenced by both blood glucose levels and the lifespan of erythrocytes, approximately 120 days or 8–12 weeks, establishing HbA1c as a valuable indicator for long-term glycemic control.[1] Recognized factors affecting HbA1c measurements include blood transfusion, hemoglobin variants, hemorrhage, iron deficiency, anemia, and red blood cell lifespan.

The hemoglobin A1c (HbA1c) serves as the primary biomarker for evaluating long-term glycemic control in individuals with diabetes, and its levels correlate with the risk of developing complications. Initially excluded from diabetes diagnosis, HbA1c gained recognition in 2010 when advancements in assays prompted the American Diabetes Association (ADA) to endorse its use as a diagnostic criterion for diabetes, with a cutoff of ≥6.5%. It also identified pre-diabetes at levels between 5.7 and 6.4%, and normal levels as <5.7%.[2]

Over the years, as HbA1c has found expanded applications for monitoring glycemia and, more recently, for diagnosing diabetes, there has been a growing demand for even greater accuracy and precision in the results obtained. This underscores the ongoing efforts to enhance the reliability of HbA1c measurements to meet the evolving needs of clinical practice.

The National Glycohemoglobin Standardization Program (NGSP) was founded in 1996 with the primary objective of standardizing HbA1c measurements. This standardization aimed to ensure that routine clinical results could be traced back to those obtained in the Diabetes Control and Complications Trial (DCCT), and subsequently, the UK Prospective Diabetes Study (UKPDS).[1,3] This alignment enabled physicians and patients to work toward achieving glycemic targets recommended by clinical diabetes societies.

The NGSP's commitment to standardization, exemplified by its collaboration with manufacturers of HbA1c methods,[3] plays

[1,2]Department of Biochemistry, Government Medical College, Nizamabad, Telangana, India

Corresponding Author: Surya Kantha Bugge, Department of Biochemistry, Government Medical College, Nizamabad, Telangana, India, Phone: +91 8106631153, e-mail: suryakanthabugge@gmail.com

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a crucial role in standardizing proficiency testing using whole blood. This partnership fosters accuracy-based assessments within individual clinical laboratories, ensuring the consistency of HbA1c measurements. These concerted efforts not only contribute to ongoing improvements in HbA1c assessment but are also supported by initiatives of the International Federation of Clinical Chemists (IFCC) network. The combined endeavors of the NGSP, manufacturers, and IFCC sustainably enhance HbA1c methods.[3] Clinicians commonly diagnose diabetes by assessing fasting plasma glucose (FPG) values, 2-hour plasma glucose (2-h PG) during a 75 gm oral glucose tolerance test (OGTT), or hemoglobin A1c (A1c or HbA1c). Laboratories are advised to use NGSP-certified methods traceable to the DCCT reference assay.[1]

This study aims to compare two analytical techniques for HbA1c—enzymatic and HPLC methods—on the same samples to discern any differences in results. Understanding the nuances between these methods contributes to the ongoing refinement of HbA1c measurement in clinical practice.

Materials and Methods

This observational cross-sectional research was carried out in the Department of Biochemistry at the Government General Hospital (GGH) located in Nizamabad, Telangana, India. A total of 100 samples were included encompassing all HbA1c samples received at GGH, with exclusion criteria applied to samples with very low volumes. Two milliliters of whole blood were collected in dipotassium ethylenediaminetetraacetate (K2-EDTA) tubes and processed within 6–12 hours.

Hemoglobin A1c measurements were initially performed using the Siemens Healthineers Atellica CH 930 Enzymatic Hemoglobin A1c (A1c_E) method, followed by the HPLC method on the BioRAD D10 analyzer. Internal quality controls were conducted before sample analysis. The Siemens Healthineers Atellica CH 930 Enzymatic Hemoglobin A1c (A1c_E) assay and the HPLC method on the BioRAD D10 analyzer employ different methodologies for measuring HbA1c.

The A1c_E assay, designed exclusively for the Atellica CH 930 Analyzer, involves pretreatment steps, a protease reaction, and subsequent stages to quantify mmoL/moL HbA1c (IFCC) and %HbA1c (DCCT/NGSP) in human whole blood.

The A1c_E assay generates two distinct measurements: glycated hemoglobin (A1c_E), measured at 658/805 nm and total hemoglobin (tHb_E), measured at 478/694 nm. These measurements allow the determination of either %HbA1c (NGSP units) or the hemoglobin A1c_E/tHb_E ratio in mmoL/moL (IFCC units).

Results from the Atellica CH 930 Analyzer are reported in NGSP equivalent units (%HbA1c) or IFCC equivalent units (mmoL/moL). The conversion formula for %NGSP is calculated as (0.09148 × IFCC) + 2.152.

On the other hand, the HPLC method on the BioRAD D10 utilizes ion-exchange HPLC principles. The process begins with automatic dilution, injection into the analytical cartridge, and separation of hemoglobins based on their ionic interactions, with absorbance changes measured at 415 nm. The D-10 software, crucial for data analysis, employs a two-level calibration approach. It generates sample reports and chromatograms, shading the A1c peak. The area of this peak is calculated using an exponentially modified Gaussian (EMG) algorithm, excluding labile A1c components.

Both methods comply with IFCC and DCCT/NGSP standards for HbA1c measurements, ensuring their clinical suitability. The Atellica CH 930 A1c_E assay and the HPLC method offer distinct results, with absorbance measurements at different wavelengths, providing valuable information for diabetes management.

Table 1: Means of HbA1c% by the HPLC and enzymatic methods

HbA1c assay method	*Mean*	*Number of samples*
HPLC	6.78 ± 2.03	100
Enzymatic method	6.68 ± 2.02	100

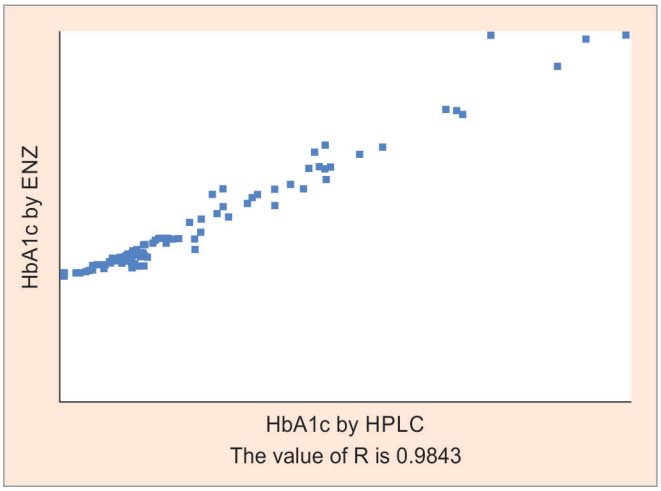

Fig. 1: Coefficient of variation on comparing the two methods $r = 0.98$

Statistical Analysis

The data analysis employed Excel and SPSS version 20. Descriptive statistics, including mean (±SD) and percentages, were applied. The degree of linear correlation between HbA1c measurements obtained from both methods was assessed through Pearson correlation (*r*). Furthermore, a Bland–Altman plot was created to determine the mean difference and examine the agreement between the two methods.

Ethical Consideration

The study adhered to ethical principles outlined in the Helsinki Declaration on medical research ethics. To protect participant confidentiality, samples were coded and kept anonymous. Furthermore, to validate the accuracy of results, a recheck of the samples was conducted at the laboratory in GGH, Nizamabad, India. This rigorous approach ensures the ethical conduct of the study and safeguards the integrity of the research process.

Results

The mean HbA1c level obtained through the enzymatic method was slightly lower (6.78 ± 2.03) compared to the HPLC method (6.68 ± 2.02), as shown in the Table 1. Statistical analysis using a student *t*-test revealed that there was no significant difference between these two methods ($p = 0.785$). The results indicate a high level of agreement between the enzymatic and HPLC methods.

Furthermore, the correlation analysis demonstrated a strong and positive correlation between the two methods, with a correlation coefficient (*r*) of 0.9843 as shown in (Fig. 1). This high correlation suggests that the HbA1c measurements obtained through the enzymatic method are closely associated with those

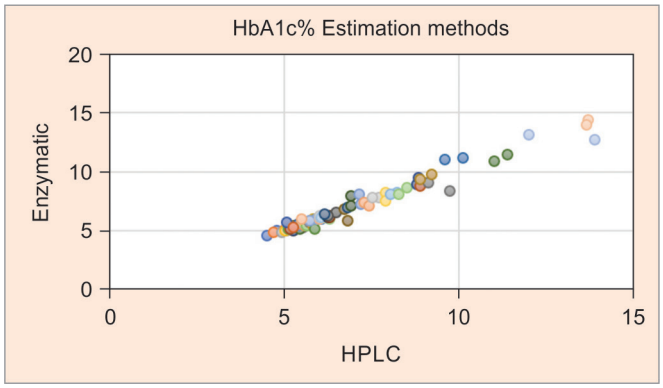

Fig. 2: All the HbA1c% values by the enzymatic and HPLC methods of the same sample

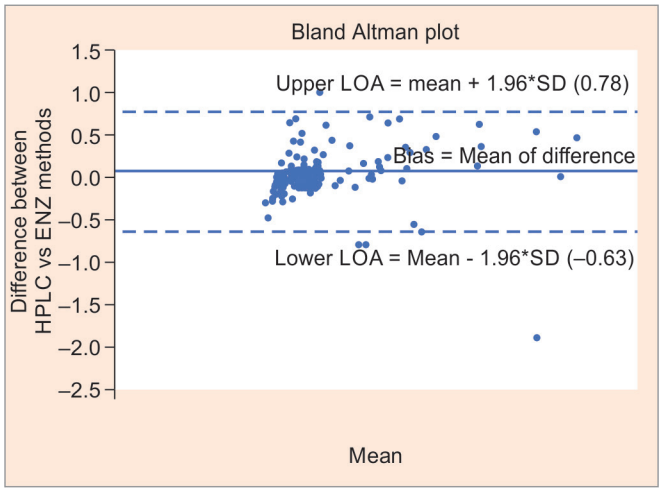

Fig. 3: Bland–Altman plot differences between the two techniques are plotted against the averages of the two techniques

obtained through the HPLC method. The robust correlation coefficient reinforces the reliability and consistency of the results obtained from both methodologies.

The mean difference of the methods was between 4.64% (4.5–4.78) and 14.16% (14.4–13.92) of HbA1c as seen in (Fig. 2).

Analysis of the Bland–Altman plot for the 100 samples revealed a high number of points clustered close to zero as observed in (Fig. 3). This observation indicates a strong agreement between the measurements obtained from both methods for the same set of samples. In other words, the enzymatic and HPLC methods exhibit good concordance in their measurements.

Moreover, the outcomes observed in the Bland–Altman plots align with the HbA1c levels determined by both methods, resulting in a 95% confidence interval (CI). This suggests that the differences between the measurements by the enzymatic and HPLC methods are within a narrow range, reinforcing the agreement observed in the Bland–Altman plot as observed in (Fig. 3).

Discussion

The purpose of this study was to compare HbA1c analysis outcomes through the analyzers currently in use, namely Siemens Healthineers Atellica CH 930 (Enzymatic) and BIORAD D10 (HPLC). Both methods are NGSP certified, and recommended for clinical evaluation. The study results indicate no significant difference between their mean values. The mean HbA1c by BIORAD D10 was 6.78%, while the Siemens healthineers Atellica CH 930 yielded 6.68%.

These findings align with a study by Mitchai et al. where diabetic HbA1c was measured with hemoglobin variants, supporting the reliability of both methods in assessing HbA1c levels.[4] Graphical representation further substantiates the strong correlation between the two methods, with a correlation coefficient of 0.9843 (Fig. 1). Similar approaches utilizing correlation coefficients for comparing HbA1c methods have been employed by other authors as well,[5–7] emphasizing the consistency of the current study's methodology.

Various measurement techniques often yield undesirable variations. Comparing outcomes across different laboratories is crucial. This study thoroughly compared two techniques, demonstrating no significant variation in mean quality control values. The lack of significant differences in coefficients of variation, bias, and sigma matrix suggests both methods are highly accurate and precise. In conclusion, the study indicates no significant difference between the Siemens healthineers Atellica CH 930 (Enzymatic) and BIORAD D10 (HPLC) methods.[8]

Conclusion

Both the Bio-Rad D10 and Siemens Healthineers Atellica CH 930 methods demonstrated comparable HbA1c results, suggesting their interchangeable utility in diabetes management. Clinicians can confidently choose either method for monitoring HbA1c levels in diabetic patients. Notably, the Bio-Rad D10 offers an additional advantage with its ability to identify Hb variants through chromatograms.

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Orcid

Surya Kantha Bugge https://orcid.org/0000-0003-2366-6449

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