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# HBA1C (GLYCOSYLATED HEMOGLOBIN) DISCORDANCE IN ESTIMATING GLYCEMIC CONTROL

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## Abstract

Glycated hemoglobin (HbA1c) is widely accepted as a standard biomarker for long-term glycemic control and prediction of diabetes-related complications; however, emerging clinical variables may influence its interpretation in routine practice. The present study was undertaken to evaluate the role and limitations of HbA1c measurement in the diabetic population and to analyse factors contributing to discrepancies in its reporting. This observational study included 100 whole blood samples collected from Medanta–The Medicity, Gurugram, India. Two milliliters of venous blood were collected in EDTA tubes, and HbA1c was estimated using High-Performance Liquid Chromatography (HPLC) on the Bio-Rad D100 analyser. Baseline HbA1c values were compared with repeat measurements obtained after an average follow-up period of 45 days. Correlation with blood glucose levels and associated comorbidities was also assessed. The study population had a mean age of 61 years. A statistically significant change in HbA1c levels was observed over the follow-up period (8.4% vs 8.1%, $p = 0.04$). Rapid availability of HbA1c results facilitated more frequent monitoring and influenced therapeutic decisions. However, clinically relevant discordance between HbA1c and glycemic status was noted in selected cases, highlighting potential analytical and biological interferences. These findings indicate that although HbA1c remains a valuable and reliable marker for monitoring glycemic control, its interpretation requires careful consideration of patient-specific factors and associated clinical conditions. Standard assessment norms may need contextual modification in selected scenarios to avoid misinterpretation and inappropriate treatment adjustments.

***Keywords:*** *HbA1c, Glycated hemoglobin, Diabetes mellitus, Glycemic control, HPLC, Diagnostic variability.*



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## 1. INTRODUCTION

Maintenance of optimal blood glucose levels is essential for effective immune function and prevention of infectious complications. Patients with diabetes mellitus (DM) are at increased risk of systemic infections and long-term vascular complications compared to non-diabetic individuals. Traditional complications of diabetes include diabetic kidney disease, retinopathy, peripheral neuropathy, coronary artery disease, stroke, and peripheral vascular disease. In addition, emerging associations with liver disease, cognitive dysfunction, and affective disorders further increase the burden of morbidity and mortality. Therefore, maintaining appropriate glycemic control is critical not only to reduce complications but also to optimize healthcare resource utilization [1].

Glycated hemoglobin (HbA1c) plays a central role in the diagnosis and monitoring of diabetes mellitus. It reflects the average glycemic status over the preceding 8–12 weeks and provides a convenient measure for long-term metabolic control. HbA1c is formed by non-enzymatic glycation of hemoglobin when glucose enters the erythrocyte through the GLUT1 transporter and binds to the N-terminal valine of the beta chain, forming a reversible Schiff base that undergoes rearrangement to a stable Amadori product [2]. HbA1c levels between 5.7% and 6.4% indicate prediabetes, while sustained reductions below 7% are associated with significant decreases in microvascular and macrovascular complications. However, intensive glycemic lowering may increase the risk of hypoglycemia, particularly in vulnerable populations. Recent meta-analyses also suggest that HbA1c variability, independent of mean HbA1c levels, is associated with increased risk of nephropathy, retinopathy, and cardiovascular events [3].

Despite its widespread acceptance, several clinical conditions may compromise the accuracy of HbA1c measurement. Chronic kidney disease, haemoglobinopathies, iron deficiency anemia, hemolytic states, pregnancy, and certain medications can alter erythrocyte lifespan and interfere with HbA1c estimation [2,4,5]. Conditions prolonging red blood cell survival, such as iron deficiency anemia and vitamin B12 deficiency, may falsely elevate HbA1c, whereas hemolysis, acute blood loss, and splenomegaly may lead to falsely reduced values. In advanced chronic kidney disease, glycated albumin has been shown to provide better glycemic assessment compared to HbA1c [6]. Similarly, HbA1c is not recommended for the diagnosis of gestational diabetes due to physiological alterations in red cell turnover during pregnancy.

Several analytical methodologies are available for HbA1c estimation, including ion-exchange high-performance liquid chromatography (HPLC), boronate affinity chromatography, immunoassays, and enzymatic assays. Each method has specific advantages and limitations, particularly in the presence of hemoglobin variants such as HbS and HbC. Standardization of HbA1c measurement was established through the National Glycohemoglobin Standardization Program (NGSP) and later aligned with the International Federation of Clinical Chemistry (IFCC) reference system. A change of ≥0.5% in HbA1c is considered clinically significant in NGSP-certified laboratories, and approximately 1% change in HbA1c corresponds to an estimated 30 mg/dL variation in mean plasma glucose [2,7].

Emerging evidence indicates that HbA1c alone may not adequately reflect an individual's glycemic profile, as similar HbA1c values may correspond to widely different glucose patterns. Continuous glucose monitoring (CGM) has demonstrated that HbA1c may overestimate or underestimate actual mean glucose concentrations in certain patients. Therefore, integration of HbA1c with CGM data and patient-specific characteristics may improve therapeutic decision-making and personalised diabetes management [8,9].

Accurate interpretation of HbA1c is crucial, particularly when comorbid conditions may influence its reliability. Therefore, this study was conducted to examine the clinical utility and limitations of HbA1c in diabetic patients and to explore factors responsible for variations in its interpretation.

## 2. MATERIALS AND METHODS

**Study Design and Setting**

This observational study was conducted at Medanta – The Medicity, Gurugram, India, over a period of three months from October to December 2024. Patients attending both the Outpatient Department (OPD) and the Inpatient Department (IPD) with a clinical requisition for HbA1c testing were considered eligible for inclusion.

**Study Population**

A total of 100 patients were enrolled in the study. Baseline demographic characteristics, including age and gender, were recorded. Relevant clinical details, including behavioral and lifestyle factors, history of diabetes mellitus, Hypertension (HT), cardiovascular disease (CVD), and other comorbidities, were documented from medical records. Patients with incomplete clinical data were excluded from the analysis.

**Sample Collection and Laboratory Analysis**

Approximately 2 mL of venous blood was collected aseptically in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes. Samples were processed as per standard laboratory protocols. Glycated hemoglobin (HbA1c) was measured using ion-exchange High-Performance Liquid Chromatography (HPLC)7 on the Bio-Rad D-100 analyser. Internal quality control procedures were followed in accordance with laboratory guidelines to ensure analytical reliability.

**Study Variables**

Baseline HbA1c values were recorded and compared with repeat HbA1c measurements obtained after an average follow-up period of 45 days. Correlation with concurrent blood glucose values and associated comorbid conditions was analysed. Discordance between HbA1c and glycemic status was evaluated.

**Statistical Analysis**

Data were analysed using SPSS software. Continuous variables were expressed as mean ± standard error (SE). The chi-square ($\chi^2$) test was used for comparison of categorical variables. A two-tailed paired t-test was applied to determine statistically significant differences between baseline and follow-up HbA1c values. Multiple logistic regression analysis was performed to identify factors contributing to discordance in HbA1c values. A p-value < 0.05 was considered statistically significant.

## 3. RESULTS

A total of 100 subjects were included in the study. The mean baseline and follow-up HbA1c values were analysed. A statistically significant reduction in HbA1c levels was observed during follow-up.

Table 1: Gender Distribution

| Gender | Number (n) | Percentage (%) |
|---|---|---|
| Male | 64 | 64.0 |
| Female | 36 | 36.0 |

Table 2: Comparison of Baseline and Follow-up HbA1c Levels

***Statistically significant (p < 0.05)**

| Comorbidity | N (100) | Baseline HbA1c (Mean ± SE) | Follow-up HbA1c (Mean ± SE) | t value | P value |
|---|---|---|---|---|---|
| Hypertension (HT) | | | | | |
| Present | 48 | 8.72 ± 0.24 | 7.63 ± 0.22 | 3.41 | 0.001* |
| Absent | 52 | 7.94 ± 0.21 | 6.98 ± 0.19 | — | — |
| **Cardiovascular Disease (CVD)** | | | | | |
| Present | 32 | 8.89 ± 0.27 | 7.75 ± 0.24 | 3.96 | <0.001* |
| Absent | 68 | 8.05 ± 0.18 | 7.08 ± 0.17 | — | — |
| **Chronic Kidney Disease (CKD)** | | | | | |
| Present | 18 | 9.02 ± 0.33 | 7.88 ± 0.29 | 2.87 | 0.005* |
| Absent | 82 | 8.14 ± 0.19 | 7.15 ± 0.18 | — | — |

There was a statistically significant reduction in HbA1c levels between baseline and follow-up measurements (8.31 ± 0.20% vs 7.28 ± 0.19%; paired t = 5.827, p < 0.001).

Table 3 Age-wise Comparison of Baseline and Follow-up HbA1c Levels with ANOVA Analysis (n = 100)

| Age Group (Years) | N (100) | Baseline HbA1c (Mean ± SE) | Follow-up HbA1c (Mean ± SE) | F value | P value |
|---|---|---|---|---|---|
| < 40 | 12 | 7.82 ± 0.31 | 6.95 ± 0.28 | | |
| 40 – 49 | 24 | 8.15 ± 0.26 | 7.12 ± 0.23 | | |
| 50 – 59 | 30 | 8.48 ± 0.22 | 7.39 ± 0.21 | | |
| 60 – 69 | 22 | 8.67 ± 0.24 | 7.54 ± 0.22 | | |
| ≥ 70 | 12 | 8.90 ± 0.33 | 7.71 ± 0.30 | | |
| ANOVA (Baseline) | — | — | — | 3.21 | 0.016* |
| ANOVA (Follow-up) | — | — | — | 2.87 | 0.026* |

*Statistically significant (p < 0.05)

aseline HbA1c levels showed a statistically significant difference across age groups (F = 3.21, p = 0.016). Similarly, follow-up HbA1c values also differed significantly among age categories (F = 2.87, p = 0.026). Higher HbA1c levels were observed in older age groups.

**Table 4 Comparison of Baseline and Follow-up HbA1c Levels According to Comorbidities**

| Comorbidity | N (100) | HbA1c (Mean ± | up HbA1c (Mean ± | t value | P value |
|---|---|---|---|---|---|
| Hypertension (HT) | | | | | |
| Present | 48 | 8.72 ± 0.24 | 7.63 ± 0.22 | 3.41 | 0.001* |
| Absent | 52 | 7.94 ± 0.21 | 6.98 ± 0.19 | — | — |
| **Cardiovascular Disease (CVD)** | | | | | |
| Present | 32 | 8.89 ± 0.27 | 7.75 ± 0.24 | 3.96 | <0.001* |
| Absent | 68 | 8.05 ± 0.18 | 7.08 ± 0.17 | — | — |
| **Chronic Kidney Disease (CKD)** | | | | | |
| Present | 18 | 9.02 ± 0.33 | 7.88 ± 0.29 | 2.87 | 0.005* |
| Absent | 82 | 8.14 ± 0.19 | 7.15 ± 0.18 | — | — |

*Statistically significant (p < 0.05)

Patients with hypertension, cardiovascular disease, and chronic kidney disease demonstrated significantly higher baseline and follow-up HbA1c levels compared to those without comorbidities (p < 0.05). The highest HbA1c values were observed in patients with CKD (Table -4).

Table 5: Comparison of HbA1c levels following the intervention across both genders

| | Pre HbA1c (Mean ± SD) | Post HbA1c (Mean ± SD) | Mean ± SD of Difference | 95% C.I. of the Difference | | t - value | p - value |
|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | |
| **Male (n = 64)** | 8.1±2 | 7.3±1.8 | 0.9±1.8 | 0.4 | 1.3 | 3.9 | < 0.001* |
| **Female (n = 36)** | 8.6±2.1 | 7.3±2 | 1.3±1.7 | 0.7 | 1.9 | 4.6 | < 0.001* |
| **Overall (n = 100)** | 8.3±2 | 7.3±1.9 | 1±1.8 | 0.7 | 1.4 | 5.8 | < 0.001* |

**C.I.: Confidence Interval; *p – value < 0.05, statistically significant**

Table 5 observed that the paired comparison analysis demonstrated a statistically significant reduction in HbA1c levels following the intervention across both genders and in the overall study population. Among male participants (n = 64), the mean HbA1c decreased from 8.1 ± 2.0% at baseline to 7.3 ± 1.8% at follow-up, with a mean reduction of 0.9 ± 1.8%. This reduction was statistically significant (t = 3.9, *p*< 0.001), and the 95% confidence interval (CI) for the mean difference ranged from 0.4% to 1.3%, indicating a consistent improvement.

Similarly, female participants (n = 36) showed a greater absolute decline in HbA1c, with mean values reducing from 8.6 ± 2.1% pre-intervention to 7.3 ± 2.0% post-intervention. The mean reduction of 1.3 ± 1.7% was highly significant (t = 4.6, *p*< 0.001), with a 95% CI of 0.7% to 1.9%, reflecting a robust glycaemic improvement.

When considering the overall cohort (n = 100), HbA1c levels declined from 8.3 ± 2.0% to 7.3 ± 1.9%, corresponding to a mean reduction of 1.0 ± 1.8%. This change was also statistically significant (t = 5.8, *p*< 0.001), with the 95% CI of the difference spanning 0.7% to 1.4%. Collectively, these findings indicate a clinically and statistically meaningful improvement in glycaemic control, observed consistently across males, females, and the overall study population.

## DISCUSSION

The present study demonstrated a statistically significant reduction in HbA1c levels following the intervention, with mean values decreasing from 8.31 ± 0.20% at baseline to 7.28 ± 0.19% at follow-up (p < 0.001). The mean reduction of approximately 1% is both statistically and clinically significant. Evidence from the United Kingdom Prospective Diabetes Study showed that each 1% reduction in HbA1c is associated with nearly 35% reduction in microvascular complications and a significant decline in diabetes-related mortality 10. Similarly, the Diabetes Control and Complications Trial confirmed the long-term benefits of intensive glycemic control 11. Thus, the magnitude of reduction observed in our study suggests meaningful clinical benefit.

Age-wise analysis revealed significantly higher HbA1c values among older age groups at both baseline and follow-up. This may reflect longer duration of diabetes, progressive β-cell dysfunction, increased insulin resistance, and higher comorbidity burden in elderly individuals. Similar observations were reported in the Action to Control Cardiovascular Risk in Diabetes, where intensive glycemic strategies in older patients required cautious individualization due to increased risk of adverse outcomes 12.

Patients with hypertension, cardiovascular disease, and chronic kidney disease exhibited significantly higher HbA1c levels compared to those without comorbidities, with the highest values observed in CKD patients (Table 4). This aligns with findings from the ADVANCE trial, which emphasized the close association between persistent hyperglycemia and vascular complications 13. Poor glycemic control may accelerate organ damage, while established vascular or renal disease may further impair metabolic regulation.

Gender-based comparison demonstrated significant HbA1c reduction in both males and females, with slightly greater absolute decline among females (Table 5). However, the therapeutic benefit was consistent across genders, supporting the universal effectiveness of glycaemic optimization strategies.

Despite these encouraging findings, certain limitations must be critically considered. The study was conducted in a relatively small sample from a single center, limiting generalizability. The absence of a randomized control group restricts definitive causal inference. Potential confounders such as duration of diabetes, treatment modifications during follow-up, medication adherence, dietary compliance, and body mass index were not independently adjusted, which may influence glycaemic outcomes.Furthermore, HbA1c measurement itself has inherent analytical and biological limitations. Conditions such as anemia, hemoglobinopathies, iron deficiency, altered erythrocyte lifespan, and chronic kidney disease can result in falsely elevated or reduced HbA1c values independent of true glycemic status.

## CONCLUSION

HbA1c remains the gold standard for monitoring glycemic control and is widely used as a diagnostic criterion for diabetes due to its standardized measurement and strong correlation with long-term complications. However, clinicians must recognize that certain clinical conditions can falsely elevate or lower HbA1c values. In such situations, alternative or complementary markers such as fructosamine, glycated albumin, 1,5-anhydroglucitol, or continuous glucose monitoring may be useful, as they reflect shorter-term glycemic status. Despite these limitations, HbA1c remains the primary and most validated predictor of diabetes-related outcomes, and results should be interpreted carefully, especially when discordant with self-monitoring blood glucose levels or during acute changes in glycaemia.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## INFORMED CONSENT

Informed consent was obtained from participants.

## ETHICAL STATEMENT

Not applicable.

## AUTHOR CONTRIBUTION

Dr. Sarita Choudhary contributed to study design, data interpretation, and manuscript drafting. Dr. Anuj Prakash supervised the study and critically revised the manuscript. Kamini Vinayak performed laboratory analysis and data acquisition. Dr. Ankana Debnath and Dr. Ravi Singhal assisted in data collection and manuscript preparation. Dr. Charu Yadav contributed to clinical coordination and manuscript review.

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