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Research Article

Impact of Hemoglobin Variants on HbA1c Results in Diabetic Patients: A Study from Pakistan

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Abstract
Background: The accuracy of HbA1c readings may be greatly impacted by hemoglobin variations, which may cause diabetes patients' glycemic control to be misclassified.

Objective: To assess the impact of hemoglobin variants on HbA1c results in diabetic patients in Pakistan and determine potential discrepancies in HbA1c measurement methodologies.

Methodology: This cross-sectional study was conducted at the University of Sialkot, from January 2023 to December 2023. The HbA1c values of 148 diabetic individuals were determined by high-performance liquid chromatography (HPLC). Variants in hemoglobin were found, and their effects on HbA1c levels were examined. Independent t-tests were used for statistical comparisons, and p-values less than 0.05 were deemed significant.

Results: Hemoglobin variations were present in 36 (24.32%) of the 148 patients, with the most prevalent being HbD (8.11%), HbS (6.76%), HbC (5.41%), and HbE (4.05%). In contrast to patients with HbS, HbC, and HbE, who had considerably lower values of 7.10 ± 1.20% ($p < 0.05$), 6.90 ± 1.00% ($p < 0.05$), and 7.20 ± 1.10% ($p < 0.05$), respectively,

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patients with normal hemoglobin had a mean HbA1c of 8.30 ± 1.40%. Compared to patients with normal hemoglobin (25%, $p < 0.05$), those with variations had a higher chance of being categorized as well-controlled (50%).

Conclusion: Variants in hemoglobin may cause HbA1c levels to be underestimated, which might misclassify glycemic control in diabetic patients. This emphasizes the need of using different glucose monitoring techniques in those who are impacted.

Introduction

A popular biomarker for evaluating the long-term glycemic management of individuals with diabetes mellitus is glycated hemoglobin (HbA1c) [1]. It reflects average blood glucose levels over the preceding two to three months and is an essential tool for diagnosing and managing diabetes [2]. However, a number of variables, such as hemoglobinopathies, red blood cell turnover, and analytical interferences, may affect how accurate HbA1c tests are [3]. Because they may change the glycation process or tamper with the methods used to quantify HbA1c, hemoglobin (Hb) variations are particularly concerning among these [4].

In many parts of the world, particularly South Asia, hemoglobinopathies—including structural variations like HbS, HbC, HbD, and HbE—as well as thalassemias are common [5]. Pakistan has a significant burden of hemoglobinopathies due to high consanguinity rates and genetic predisposition [6]. These variations may result in differences in HbA1c readings, which might misclassify glycemic status and lead to improper clinical judgment [7]. Certain hemoglobin variations change the longevity of erythrocytes, affecting the exposure of hemoglobin to circulating glucose and, subsequently, the HbA1c level [8]. Furthermore, certain analytical techniques used to quantify HbA1c may not discriminate between glycated normal hemoglobin and glycated variant hemoglobin, thus confounding the interpretation of data [9].

In places like Pakistan, where hemoglobinopathies are very frequent, it is vital to examine the influence of these variations on HbA1c levels to provide appropriate diabetes monitoring and treatment. Ignorance of these interferences may result in poor treatment choices, raising the risk of complications from diabetes [10,11]. There hasn't been much research done in Pakistan on this topic, which emphasizes the need for a thorough investigation to assess how hemoglobin variations affect HbA1c readings and spot any inconsistencies in widely used measurement methods.

Research Objective

To assess the impact of hemoglobin variants on HbA1c results in diabetic patients in Pakistan and determine potential discrepancies in HbA1c measurement methodologies.

Materials and Methods

Study Design and Setting

This cross-sectional study was conducted at the University of Sialkot, over a period of one year, from January 2023 to December 2023.

Inclusion and Exclusion Criteria

Diagnosed diabetes patients (Type 1 or Type 2) having regular HbA1c testing, aged 18 years and above, and willing to grant informed permission were included in the research. Patients who were pregnant, had chronic hemolytic anemia or other hematological conditions that affect RBC turnover, or had received a recent blood transfusion (within three months) were not included.

Sample Size

Convenient sampling was used to include 148 diabetic patients in total. The sample size was determined based on feasibility and resource availability. This sample was judged enough to examine the effect of hemoglobin variations on HbA1c levels since hemoglobinopathies are common in Pakistan.

Data Collection

Every registered member had venous blood samples taken. High-performance liquid chromatography (HPLC), regarded as the gold standard for HbA1c testing and competent of spotting hemoglobin variations, was used to measure HbA1c levels. Common hemoglobinies were found by further hematological testing. Recorded were demographic

and clinical data including age, gender, diabetes duration, and history of blood problems.

Statistical Analysis

SPSS version 25 helped one to examine data. Clinical and demographic aspects were gathered using descriptive statistics. Independent t-tests allowed one to evaluate how hemoglobin variations affected HbA1c levels. Considered statistically significant was a p-value of 0.05.

Ethical Approval

The Institutional Review Board (IRB) of University of Sialkot, approved ethically this work. Before enrolling each subject had informed permission.

Results

The research included 148 diabetes patients with almost similar gender distribution: 78 men, 52.7%, and 70 women, 47.3%. Participant mean age was 52.4 ± 10.5 years. Of them, 106 (71.62%), had Type 2 diabetes; 42 (28.38%) had Type 1 diabetes. With an 8.10 ± 1.50 overall mean HbA1c score, several patients had inadequate glycemic control (table 1).

Table 1: Demographic and Clinical Characteristics of Study Participants

Category	Characteristic	Number of Patients (n; %)
Gender	Male	78 (52.70)
	Female	70 (47.30)
Age	Mean Age (years)	52.4 ± 10.5
Diabetes Type	Type 1 Diabetes	42 (28.38)
	Type 2 Diabetes	106 (71.62)
HbA1c Levels	Mean HbA1c	8.10 ± 1.50

Of the 148 patients, 36 (24.32%), had hemoglobin variations and 112 (75.68%) had normal hemoglobin. HbD (12 patients, 8.11%) followed by HbS (10 patients, 6.76%), HbC (8 patients, 5.41%), and HbE (6 patients, 4.05%) was the most often occurring variety. This emphasizes (Figure 1) the frequency of hemoglobinopathies in the investigated population.

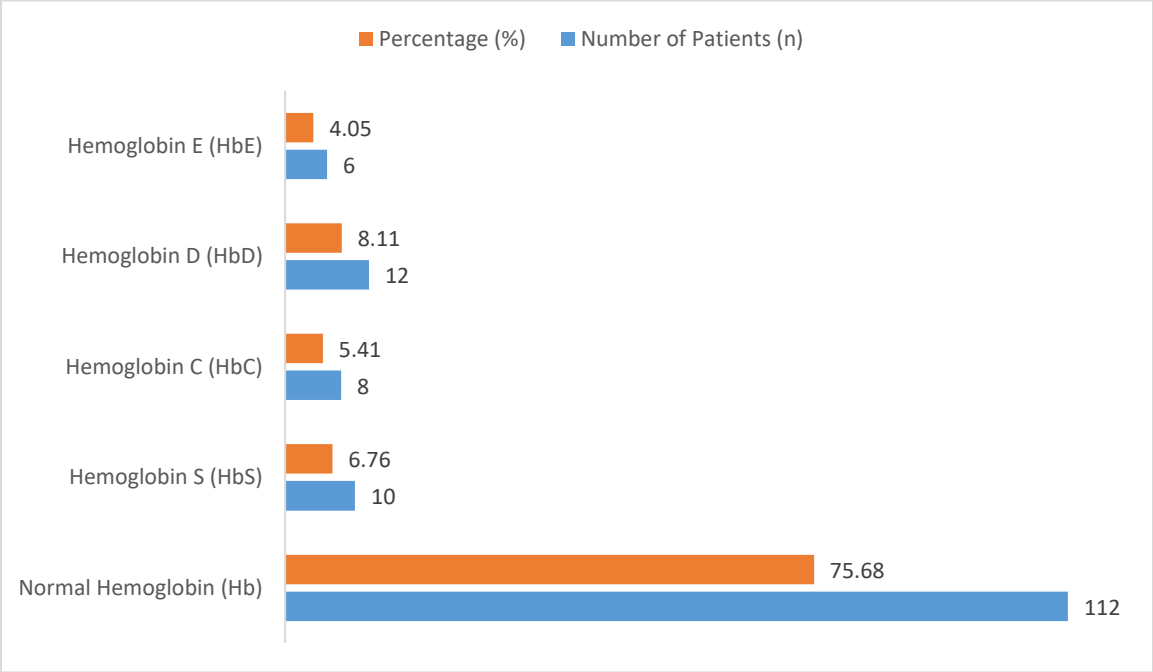

Figure 1: Distribution of Hemoglobin Variants in Study Participants

Normal hemoglobin patients' mean HbA1c was 8.30 ± 1.40% (table 2). With HbS at 7.10 ± 1.20% ($p < 0.05$), HbC at 6.90 ± 1.00%, and HbE at 7.20 ± 1.10%, those with hemoglobin variations had noticeably reduced mean HbA1c values. With a mean HbA1c of 7.50 ± 1.30%, HbD patients differed statistically insignificantly ($p = 0.08$).

Table 2: Mean HbA1c Levels in Patients with Different Hemoglobin Variants

Hemoglobin Variant	Mean HbA1c (%)	p-value
Normal Hb	8.30 ± 1.40	Ref.
HbS	7.10 ± 1.20	<0.05
HbC	6.90 ± 1.00	<0.05
HbD	7.50 ± 1.30	0.08
HbE	7.20 ± 1.10	<0.05

Patients with hemoglobin variations were more likely to be categorized as well-controlled (18 out of 36, 50%) than those with normal hemoglobin (28 out of 112, 25%) ($p < 0.05$). On the other hand, compared to normal Hb patients (34 out of 112, 30.36%) less variant Hb patients (6 out of 36, 16.67%) were categorized as poorly managed ($p < 0.05$). This implies that hemoglobin variations might cause reduced HbA1c values, thereby perhaps misclassifying glycemic control in diabetes individuals (table 3).

Table 3: Impact of Hemoglobin Variants on Glycemic Classification

Glycemic Category (Based on HbA1c)	Normal Hb (n=112)	Variant Hb (n=36)	p-value
Well-controlled (<7.0%)	28 (25.00)	18 (50.00)	<0.05
Moderately controlled (7.0-8.5%)	50 (44.64)	12 (33.33)	0.12
Poorly controlled (>8.5%)	34 (30.36)	6 (16.67)	<0.05

Discussion

The results of our research underline the major influence of hemoglobin variations on HbA1c values in diabetic patients, which has important consequences for glycemic control evaluation. 24.32% of patients (n=36) in our research had hemoglobin variations; HbD was the most often occurring (8.11%), followed by HbS (6.76%), HbC (5.41%), and HbE (4.05%). The frequency of hemoglobinopathies in our analysis is in line with other studies where strong consanguinity rates and genetic inclination clearly record the burden of hemoglobin variations [12,13].

Patients with hemoglobin variations had notably lower HbA1c levels than those with normal hemoglobin (8.30 ± 1.40%), according to data. Indicating that these variations may cause underestimating of glycemic control, individuals with HbS, HbC, and HbE had mean HbA1c levels of 7.10 ± 1.20% (p Similarly, using high-performance liquid chromatography (HPLC), a research by Jaisson et al. revealed that HbS and HbC variations produced notably lower HbA1c levels, hence possibly contributing to misdiagnosis of diabetes management status [14].

With a mean HbA1c of 7.50 ± 1.30%, ($p = 0.08$), HbD did not reveal a statistically significant difference in our research. This result is consistent with other studies showing that HbD variations may not regularly change HbA1c levels, presumably because of changes in glycation processes or different effects of analytical techniques used for HbA1c estimate [15].

Our investigation clearly showed that hemoglobin polymorphisms affected glycemic categorization; 50% of patients with variants were categorized as well-controlled (HbA1c < 7.0%) compared to only 25% of

those with normal hemoglobin ($p < 0.05$). Comparatively, fewer patients with variations (16.67%) were categorized as poorly managed (HbA1c > 8.5%), than those with normal hemoglobin (30.36%, $p = 0.05$). This implies that in these people, conventional HbA1c tests might understate long-term glucose intake. This result is consistent with other studies as several hemoglobinopathies interact with HbA1c tests, thereby producing differences in glycemic categorization [16,17].

These results confirm the requirement of alternate glucose monitoring techniques, like fructosamine or continuous glucose monitoring, in patients with established hemoglobinopathies to prevent possible misclassification and improper treatment choices. Assay-specific interferences must be evaluated and consistent guidelines for HbA1c interpretation in various populations must be developed via further study.

Study Strengths and Limitations

This study's emphasis on a Pakistani diabetic population—where hemoglobinopathies are common but their effect on HbA1c values is still understudied—is a great strength. The dependability of our results is improved by the use of high-performance liquid chromatography (HPLC), a gold standard technique adept of identifying hemoglobin variations. Furthermore, our work emphasizes the clinical consequences of variable hemoglobin on diabetes control, therefore adding important local evidence to the international debate on HbA1c accuracy. Still, many restrictions have to be admitted. With a quite small sample size (n=148), our findings were not generally very generalizable. The investigation was carried out at one tertiary care facility, which may not fairly represent the variety of the general community. Moreover, we did not evaluate other glycemic indicators such fructosamine or continuous glucose monitoring, which would have given a more all-encompassing assessment of glycemic state in hemoglobinopathies.

Conclusion

Our work shows that hemoglobin variations greatly affect HbA1c readings, thereby causing possible misdiagnosis of glycemic management in diabetes patients. Patients with HbS, HbC, and HbE had lower HbA1c results, which draw attention to the danger of underestimating long-term glucose levels that can lead to less than ideal diabetes control. Given the great frequency of hemoglobinopathies in Pakistan, doctors should be aware of these interferences and take alternate glucose monitoring under consideration for afflicted people. Accurate diabetes monitoring and treatment choices in different groups depend on future research using bigger multicenter cohorts with uniform protocols.

Ethical Considerations

Approved by the Board of Advance Studies and Research, University of Sialkot, Sialkot, Pakistan.

Authors' contributions

Conceptualization and supervision: ZA, MT; Methodology: TZ, UL, SZ; Investigation, writing original draft and review: All authors; Data collection: SM, TZ, UL, SZ; Data analysis: ZA, MT.

Conflict of interest

The authors declared no conflict of interest.

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