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HbA1c Levels in Controlled Diabetics with and without Iron Deficiency Anemia: A Tertiary Care Experience

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A B S T R A C T

Introduction: Glycated hemoglobin (HbA_1c) is widely used to assess long-term glycemic control in diabetic patients. However, non-glycemic factors such as iron deficiency anemia (IDA) may alter HbA_1c levels, potentially affecting its accuracy. This study aimed to evaluate the effect of IDA on HbA_1c levels in diabetic patients with controlled fasting plasma glucose.

Materials & Methods: An analytical cross-sectional study was conducted on 80 diabetic patients aged 18–60 years, divided equally into IDA and non-IDA groups. IDA diagnosis was based on hemoglobin levels, red blood cell indices, serum ferritin, serum iron, and total iron-binding capacity (TIBC). HbA_1c was measured using high-performance liquid chromatography; while fasting plasma glucose was determined by the glucose oxidase-peroxidase method.

Results: Mean HbA_1c levels were 6.1 ± 0.59% in the IDA group and 6.2 ± 0.73% in the non-IDA group, with no statistically significant difference ($p = 0.6143$). Age and gender showed more influence on HbA_1c values than IDA status. No significant correlation was found between HbA_1c and hematological or iron parameters within the IDA group.

Conclusion: Iron deficiency anemia does not significantly affect HbA_1c levels in diabetic patients with well-controlled fasting glucose. Therefore, HbA_1c remains a reliable marker for glycemic control in these patients. Clinicians should still interpret HbA_1c cautiously in anemic individuals and consider additional assessments if necessary.

Keywords: glycated hemoglobin, anemia, iron deficiencies, diabetes mellitus, blood glucose

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Introduction

HbA_1c, the major component of HbA_1, is formed by the glycation of terminal valine on the beta chain of hemoglobin (1, 2). Its levels are directly proportional to plasma glucose concentrations and remain stable, reflecting the glycemic status of a patient over the preceding 8–12 weeks (3). HbA_1c is widely used for monitoring diabetes control and has been recognized since 2010 as a diagnostic and screening tool for high-risk individuals. The World Health Organization (WHO) supports its use for diagnosing diabetes (4). However, HbA_1c levels can be influenced by factors unrelated to glycemia, particularly conditions that affect red blood cell (RBC) turnover. These include hemoglobinopathies, hemolytic anemia, pregnancy, blood loss, and uremia (5). Iron deficiency anemia (IDA) is the most common form of anemia both globally and in India (6). Diabetes prevalence is increasing rapidly in India, with a 2023 ICMR study reporting an 11.4% diabetes rate, higher in urban than rural populations (7).

Research investigating the impact of IDA on HbA_1c has shown inconclusive results (8, 9). Some studies imply that people who don't get enough iron have higher HbA_1c levels, especially if their glucose levels are normal or pre-diabetic (10,11). However, other studies do not discover this link in people who already have diabetes (12). In light of these contradictory data, it is essential to delineate the effect of iron deficiency on HbA_1c, excluding the confounding variable of variable or uncontrolled blood glucose levels. Consequently, this research only included diabetic individuals with regulated glycemia (fasting plasma glucose <126 mg/dL). This method was used to guarantee that any fluctuations seen in HbA_1c levels may be more reliably ascribed to iron status rather than to changes in glycemic control. This strategy will improve the study's internal validity by reducing the variability caused by high blood sugar. It will also help us better understand how IDA could affect HbA_1c levels in people with diabetes.

So, the aim of the present study was to evaluate the effect of iron deficiency anemia (IDA) on HbA_1c levels in diabetic individuals with controlled glycemic status and to determine whether IDA significantly alters HbA_1c values independent of plasma glucose levels.

Materials and methods

Study Design

An analytical cross-sectional study was conducted from May to July 2021 by the Biochemistry Department in collaboration with Pathology and Endocrinology Departments at a tertiary care centre.

Setting and Participants

The study included diabetic individuals with controlled glycemic status, defined as fasting plasma glucose (FPG) levels below 126 mg/dL. To identify cases of iron deficiency anemia (IDA), participants were required to have hemoglobin levels below 13 g/dL in males and below 12 g/dL in females, as per WHO guidelines. Additional hematological indicators included hypochromic and microcytic red blood cell indices, specifically a mean corpuscular hemoglobin (MCH) less than 26 pg/cell and a mean corpuscular volume (MCV) less than 80 fL, along with a peripheral smear showing a microcytic hypochromic picture. Biochemical confirmation of IDA included serum ferritin levels under 30 ng/mL and serum iron levels less than 50 µg/dL in females and less than 65 µg/dL in males. Individuals were excluded if they had fasting plasma glucose above 126 mg/dL or random plasma glucose above 200 mg/dL, were taking iron supplementation, or if they had a history of conditions such as significant blood loss, hemoglobinopathies, hypothyroidism, coagulation disorders, or hemolytic anemia. Pregnant women and those with abnormal renal function, as indicated by elevated blood urea or serum creatinine, were also excluded. Female participants with amenorrhea for more than two months underwent urinary pregnancy testing, and those testing positive were excluded. Additionally, participants with a

reticulocyte count greater than 2.5% were not included in (13).

Sample Size

Based on Christy et al. (10), using SD = 1.4 and d = 1.22 with 5% type I error and 80% power, the sample size per group was calculated to be 21. Adding 10% for possible dropout, 24 participants per group is calculated. The study should include a total of 48 participants. But to increase the power of the study, we decided to select 40 participants per group.

Measurements & Validity and Reliability

Convenience sampling was used to recruit 80 diabetic patients (aged 18–60). Participants were divided into two groups: Group 1 (n=40) included diabetic patients with IDA, and Group 2 (n=40) included diabetic patients without IDA. HbA_1c was measured by high-performance liquid chromatography using the Bio-Rad D-10 analyzer. Glucose was measured by the glucose oxidase-peroxidase method. Additional hematological data (e.g., serum ferritin, MCH, MCHC, MCV) were retrieved from previous lab records.

Statistical and Data Analysis

Data were analysed using GraphPad Prism. Quantitative data were expressed as mean ± SD, and group comparisons were made using unpaired t-tests. Categorical variables were analysed using the chi-square test. A p-value <0.05 was considered statistically significant.

Results

The average age of the group was 49.99 years, with 39 women and 41 men. Seventy percent of IDA patients were female. Table 1 shows a comparison of several demographic and biochemical factors between diabetic individuals with iron deficiency anemia (IDA) and those without IDA (N = 40 in each group). The evaluated criteria include diabetic indices, hematological indices, and iron profile indicators. There were no statistically significant variations in fasting plasma glucose (FPG) and glycated hemoglobin (HbA_1c) levels between the two groups ($p = 0.7998$ and $p = 0.6143$, respectively), indicating similar glycemic management in diabetic patients regardless of IDA status. Nonetheless, significant discrepancies were seen in hematological and iron profile data.

The hemoglobin levels in IDA patients (11.4 ± 0.94 g/dl) were significantly lower than those in non-IDA patients (14.4 ± 1.42 g/dl), with a p-value of less than 0.0001. The IDA group had considerably lower red blood cell indices, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) ($p < 0.0001$ for all). This is in line with the microcytic hypochromic character of iron deficiency anemia. On the other hand, the red cell distribution width (RDW) was much greater in the IDA group (14.6 ± 2.21%) than in the non-IDA group (13.4 ± 1.24%, $p = 0.0267$). This shows anisocytosis, which is a symptom of iron-deficient erythropoiesis.

The iron profile analysis confirmed the diagnosis of IDA in the afflicted patients. In patients with IDA, serum ferritin and serum iron levels were considerably lower (28.1 ± 1.61 ng/dl and 47.8 ± 7.57 mcg/dl, respectively) than in patients without IDA (94.8 ± 31.99 ng/dl and 73.4 ± 10.90 mcg/dl), with p-values < 0.0001. Total iron-binding capacity (TIBC) was considerably higher in IDA patients (415.0 ± 25.45 mcg/dl vs. 324.2 ± 10.14 mcg/dl, $p < 0.0001$), which further supports the iron-deficient status.

Table 1. Comparison of Haematological and Glycemic Parameters Between Controlled Diabetic Patients With and Without IDA.

Parameter (Normal range) [13]	Diabetics with IDA (N = 40) Mean ± SD	Diabetics without IDA (N = 40) Mean ± SD	P value
Fasting Plasma Glucose (mg/dL) (<100)	112.7 ± 12.05	113.6 ± 14.23	0.7998
HbA_1c (%) (<5.6%)	6.1 ± 0.59	6.2 ± 0.73	0.6143

Hemoglobin (g/dL) (12.0–17.2)	11.4 ± 0.94	14.4 ± 1.42	<0.0001*
MCV (fL) (80–100)	77.7 ± 2.84	89.3 ± 3.91	<0.0001*
MCH (pg) (26–33)	24.6 ± 1.05	30.6 ± 1.80	<0.0001*
MCHC (g/dL) (33–37)	32.1 ± 1.83	34.2 ± 0.92	<0.0001*
RDW (%) (11.5–14.5)	14.6 ± 2.21	13.4 ± 1.24	0.0267
Serum Ferritin (ng/mL) (30–100)	28.1 ± 1.61	94.8 ± 31.99	<0.0001*
Serum Iron (µg/dL) (>50)	47.8 ± 7.57	73.4 ± 10.90	<0.0001*
TIBC (µg/dL) (300–360)	415.0 ± 25.45	324.2 ± 10.14	<0.0001*

*Statistically significant ($p < 0.05$). Values are presented as mean ± standard deviation (SD). FPG = Fasting Plasma Glucose, HbA_1c = Glycated Hemoglobin, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, RDW = Red Cell Distribution Width, TIBC = Total Iron-Binding Capacity

Tables 2a, b, and c present the mean HbA_1c levels among 80 diabetic patients, stratified by age, gender, and presence of iron deficiency anemia (IDA). Overall, patients older than 50 years exhibited higher HbA_1c levels (6.33 ± 0.66%) compared to those under 50 years (5.96 ± 0.61%), indicating poorer glycemic control with advancing age. When analyzed by gender within age groups, both males and females showed an increase in HbA_1c with age, although the rise was more pronounced in males—from 6.05 ± 0.68% in those under 50 to 6.59 ± 0.68% in those over 50. In contrast, the corresponding increase in females was from 5.95 ± 0.61% to 6.22 ± 0.49%. This suggests a gender-related variation in glycemic trends, with older males having the highest HbA_1c levels overall.

When stratified by IDA status, non-IDA patients had slightly higher mean HbA_1c levels (6.27 ± 0.73%) compared to IDA patients (6.17 ± 0.59%). Among females, the IDA group had higher HbA_1c (6.11 ± 0.57%) than the non-IDA group (5.94 ± 0.59%), whereas among males, HbA_1c was higher in the non-IDA group (6.40 ± 0.75%) compared to those with IDA (6.31 ± 0.66%). Further, age-based comparison within IDA and non-IDA groups showed that HbA_1c increased with age regardless of anemia status. Notably, non-IDA patients over 50 years recorded the highest mean HbA_1c (6.56 ± 0.67%), reinforcing the influence of age on glycemic control. While some differences in HbA_1c across groups appear modest, the patterns suggest that age and gender exert stronger effects on HbA_1c than IDA status alone.

Table 2. HbA_1c Stratified by Age, Gender, and IDA Status in Controlled Diabetic Patients.

a) By Age and Gender			b) By IDA and Gender			c) By Age and IDA Status		
Group	<50 Years Mean ± SD	>50 Years Mean ± SD	Group	IDA Mean ± SD	Non-IDA Mean ± SD	Group	<50 Years Mean ± SD	>50 Years Mean ± SD
Total (N = 80)	5.96 ± 0.61	6.33 ± 0.66	Total (N = 80)	6.17 ± 0.59	6.27 ± 0.73	IDA (N = 40)	6.01 ± 0.58	6.31 ± 0.58
Females (N = 39)	5.95 ± 0.61	6.22 ± 0.49	Females (N = 39)	6.11 ± 0.57	5.94 ± 0.59	Non-IDA (N = 40)	5.98 ± 0.68	6.56 ± 0.67
Males (N = 41)	6.05 ± 0.68	6.59 ± 0.68	Males (N = 41)	6.31 ± 0.66	6.40 ± 0.75			

able 3 shows the connection analysis of HbA_1c levels and several blood parameters in people with diabetes who also have iron deficiency anemia (IDA). None of the factors exhibited a statistically significant connection with HbA_1c levels ($p > 0.05$). Among the factors examined, serum iron had the most robust positive connection with HbA_1c ($r = +0.34$), but this correlation did not achieve statistical significance (p

= 0.10). There was a modest negative association between hemoglobin concentration and HbA1c (r = –0.25, p = 0.24), which means that when hemoglobin levels go up, HbA1c levels may go down. Likewise, red cell distribution width (RDW) and mean corpuscular hemoglobin concentration (MCHC) exhibited minor negative relationships (r = –0.24 and –0.23, respectively), however these findings were not statistically significant. Other parameters, such as serum ferritin (r = –0.13), total iron-binding capacity (TIBC) (r = +0.07), mean corpuscular volume (MCV) (r = +0.09), and mean corpuscular hemoglobin (MCH) (r = –0.12), exhibited negligible correlations with HbA1c, with all p-values exceeding 0.05, signifying a lack of significant statistical association. Age exhibited no significant correlation with HbA1c levels (r = +0.18, p = 0.41). In general, our results indicate that among diabetes patients with iron deficiency anemia, changes in hematological parameters, such as iron status, do not have a significant effect on HbA1c levels. Nonetheless, the identified tendencies, especially with serum iron and hemoglobin, need further examination in more extensive cohorts.

Table 3. Correlation of HbA1c with Hematological Parameters in Diabetic Patients with IDA.

Parameter	Correlation Coefficient (r)	P value
Age (years)	+0.18	0.41
Hemoglobin (g/dL)	-0.25	0.24
Serum Iron (µg/dL)	+0.34	0.10
Serum Ferritin (ng/mL)	-0.13	0.56
TIBC (µg/dL)	+0.07	0.73
MCV (fL)	+0.09	0.69
MCH (pg)	-0.12	0.58
MCHC (g/dL)	-0.23	0.28
RDW (%)	-0.24	0.31

Discussion

The World Health Organization (WHO) endorses HbA1c as a key biomarker since it is very reliable, useful, and closely linked to issues connected to diabetes. Nonetheless, other demographic and clinical factors—such as age, gender, ethnicity, genetic predisposition, and specific medical conditions—can affect HbA1c levels (14–16).

Anemia is regarded as one of the most prevalent variables affecting HbA1c levels. Many research have looked at this link, which seems to rely on what caused the anemia in the first place. Iron deficiency anemia is the most prevalent form of nutritional anemia. It accounts for almost 50% of the global load. There has been a recent surge of interest in the scientific community about the examination of HbA1c values in commonly seen anemias, such as iron deficiency anemia (IDA) (9). The precise processes remain ambiguous, and the ideas suggested by various investigations to far are speculative. This encouraged us to do a research to assess the effect of IDA on HbA1c levels in diabetes individuals with regulated glucose. In line with previous studies by Mitchell et al. (17) and Van Heyningen et al. (18), our results indicated no significant difference in HbA1c levels between managed diabetic individuals with and without IDA, and the absence of association remained unaccounted for. Grossman et al. (19) found that HbA1c levels were related to several things, such hematocrit, hemoglobin, and dietary variables that cause anemia in older persons. Our results on HbA1c levels in well-controlled diabetic individuals, both with and without iron deficiency anemia (IDA), diverge from findings in numerous other studies. A comprehensive review and meta-analysis conducted by Alharbi et al. in 2023 reaffirmed the finding that iron deficiency may cause inaccurately high HbA1c values, particularly in those without diabetes. A research conducted in India by

Dutta et al. (21) indicated elevated HbA_1c levels in people with iron deficiency, suggesting that iron therapy may have corrected these levels.

Changes in the lifetime of red blood cells (RBCs) have a big effect on HbA_1c levels. For example, RBCs with shorter lifespans have lower HbA_1c levels even when blood sugar levels are high (22, 23). This corroborates prior research indicating that iron shortage, which extends RBC lifespan, might increase HbA_1c levels. Moreover, discrepancies in HbA_1c readings have been ascribed to variations in analytical methodologies (24). When looking at HbA_1c values, these things should be taken into account. Iron deficiency pathophysiologically impacts the structure and function of hemoglobin. Sinha et al. (9) posited that modified hemoglobin structure in iron deficiency anemia (IDA) may enhance its vulnerability to glycation, irrespective of glucose concentrations. Additionally, iron shortage has been linked to prolonged RBC life resulting from diminished erythropoiesis, which increases Hb glycation over time (25). On the other hand, iron treatment boosts erythropoiesis, which means there are more younger RBCs with less glycation. This lowers HbA_1c (26). Chronic inflammation, which is frequent in people with diabetes, makes iron metabolism even more difficult. Elevated cytokines, such as interleukin-6 (IL-6), stimulate hepatic hepcidin production, hindering iron uptake and mobilization, which may worsen anemia and influence glycation dynamics (27). Fernandez-Real et al. (28) highlighted the interplay between iron metabolism and diabetes, suggesting that imbalances in iron levels may indirectly influence HbA_1c measurements. In general, we can say that there might be significant confounders in HbA_1c tests, as we have shown, not only iron deficiency.

Inconsistent findings among studies may also be caused by differences in the way HbA_1c is measured. Certain tests, particularly those predicated on charge differentials, may be influenced by modified hemoglobin variants in iron deficiency anemia (IDA), resulting in erroneous HbA_1c measurements (29). Our research did not detect a strong link between iron deficiency anemia and HbA_1c levels in diabetes individuals who were well-controlled. However, these null results are still scientifically important. They challenge established beliefs, augment the current research base, and highlight the intricacy of glycation processes in the context of hematological diseases. Publishing this kind of information is very important for getting a full picture and for improving and informing future research in this area.

No further evaluation was conducted to determine changes in HbA_1c post-iron treatment. The research only included individuals with regulated glycemia, excluding those with uncontrolled diabetes. Furthermore, the research did not juxtapose HbA_1c with other glycemic indicators such as fructosamine or glycated albumin. Also, since it was a fundamental research project for undergraduates, it didn't include any complicated theories.

Conclusion

Further prospective studies are required to observe the impact of iron therapy on HbA_1c status in larger populations, with emphasis on various confounding factors. However, alternatively, an oral glucose tolerance test could be considered important to avoid misdiagnosing diabetes in all subjects with or without IDA. Clinicians should interpret HbA_1c cautiously in anemic patients, especially in borderline cases, and consider adjunct markers like fructosamine and glycated albumin that are less influenced by RBC turnover and iron status.

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Ethical Considerations

Ethical approval was obtained from the Institutional Ethics Committee (EC/OA-116/2020). The study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

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Competing Interests' Disclosure

No conflict of interest was declared by authors.

Authors' contributions

Conceptualization: AC, JK, Methodology: MB & SM, Validation: AC, Data Curation, Formal Analysis: SM, Investigation: JK & SK¸ Writing– Original Draft Preparation: JK, Writing– Review & Editing: SM & AC, Visualization: AC, Supervision, Software: MB, Resources, Project Administration: SK.

Writing Disclosure

According to the writers, they wrote and prepared this work on their own, without using a professional writing service. The authors are the only ones who have contributed original work to the material.

Data Availability Statement

The corresponding author is readily available to provide the data that substantiates the findings of this study upon a reasonable request.

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