ENDOCRINOLOGY RESEARCH AND PRACTICE

SOCIETY OF ENDOCRINOLOGY AND METABOLISM OF TÜRKİYE

Effect of Iron Deficiency Anemia on HbA1c in Non-Diabetics: An Analytical Study from Eastern India

ORIGINAL ARTICLE
Endocrinol Res Pract. 2024;28(4):216-223

ABSTRACT

Objective: Hemoglobin A1c (HbA1c), a key indicator of glycemic status over the last 3 months, is increasingly favored by clinicians to diagnose diabetes. Iron deficiency anemia (IDA), the most prevalent anemia worldwide, may alter HbA1c levels. However, data from eastern India are scarce, and existing studies offer conflicting results. This study aimed to investigate the impact of IDA on HbA1c levels in nondiabetics and to explore the correlation of HbA1c with hemoglobin (Hb), parameters of the iron profile, and red blood cell (RBC) indices.

Methods: This cross-sectional analytical study compared 60 euglycemic patients with IDA, aged 18 to 60, to 60 age- and sex-matched euglycemic individuals without anemia. Data analysis was performed using SPSS version 25, with significance set at $P < .05$. Spearman's rho correlation coefficients examined correlations between Hb, serum iron, serum ferritin, total iron binding capacity (TIBC), transferrin saturation, packed cell volume (PCV), reticulocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) with HbA1c. Multinomial logistic regression analyzed the relationships of HbA1c (dependent variable) with RBC indices and iron profile parameters (independent variables).

Results: The mean (SD) HbA1c value for IDA patients was 6.5 (0.5)% compared to 5.13 (0.80)% for controls ($P < .001$). HbA1c showed significant negative correlations with Hb, PCV, serum iron, serum ferritin, transferrin saturation, MCV, MCH, and MCHC, while TIBC was positively correlated with HbA1c. Each unit decrease in Hb increased the likelihood of HbA1c values in the prediabetic and diabetic range by 2.61 times (95% CI = 1.65-3.50) and 2.40 times (95% CI = 1.81-3.77), respectively ($P < 0.001$).

Conclusion: The study highlights a significant increase in HbA1c levels with the worsening of IDA in non-diabetics, indicating that IDA should be addressed before interpreting HbA1c results accurately.

Keywords: Eastern India, hemoglobin A1c (HbA1c), iron deficiency anemia, non-diabetics

Introduction

Anemia is a significant public health issue, affecting approximately 22.8% of the global population, and disproportionately impacts low and middle-income countries (LMICs), leading to serious health, social, and economic burdens. Globally, iron deficiency accounts for 50% of anemia cases.[1,2] Iron deficiency is viewed as a spectrum, ranging from iron depletion and iron-deficient erythropoiesis to iron deficiency anemia (IDA).[3] In IDA there is a gradual decline in serum iron, serum ferritin, and transferrin saturation, while total iron-binding capacity (TIBC) increases. Other valuable laboratory tests for diagnosing IDA include assays for serum transferrin receptor, reticulocyte hemoglobin (Hb) content, percentage of hypochromic erythrocytes, and erythrocyte zinc protoporphyrin.

HbA1c, the most prevalent form of glycohemoglobin, is produced by the glycation of the terminal valine on the beta chain of Hb. It indicates a patient's glycemic control over the past 3 months. HbA1c is increasingly favored for use in population-based screenings because it does not require fasting and exhibits low inter-user variability. The American Diabetes Association (ADA) recently endorsed HbA1c levels of ≥6.5% as a diagnostic marker for diabetes mellitus and levels between 5.7% and 6.4% as indicating prediabetes.[4] The HbA1c level is influenced by 3 primary factors: the Hb concentration in reticulocytes at the time of their release from the bone marrow, the average lifespan of RBCs in circulation, and the rate at which Hb undergoes glycation.[5] Therefore, conditions that reduce RBC turnover and increase the glycation rate of Hb, such as IDA, hemoglobinopathies, and altered red cell survival, can affect HbA1c values.[6] HbA1c levels are also linked to erythrocyte indices like Hb, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH), independent of glucose levels, in premenopausal

Nebedita Dutta[1]
Bilkis Khatun[1]
Indrani Das[1]
Arindam Ghosh[2]
Soumit Roy[3]

[1]*Department of General Medicine, Medical College and Hospital, Kolkata, West Bengal, India*
[2]*Department of Paediatrics, Medical College and Hospital, Kolkata, West Bengal, India*
[3]*Department of Community Medicine, IQ City Medical College, Durgapur, West Bengal, India*

Corresponding author:
Arindam Ghosh
✉ arindamghosh.mck@gmail.com

Received: June 30, 2024
Revision Requested: July 12, 2024
Last Revision Received: August 1, 2024
Accepted: September 2, 2024
Publication Date: October 1, 2024

Cite this article as: Dutta N, Khatun B, Das I, Ghosh A, Roy S. Effect of iron deficiency anemia on HbA1c in non-diabetics: an analytical study from eastern India. *Endocrinol Res Pract.* 2024;28(4):216-223.

DOI: 10.5152/erp.2024.507

women, even if they are not anemic.[7] Moreover, other factors such as age, smoking, genetics, and ethnicity were also found to influence HbA1c levels.[8]

Studies have found that IDA is associated with higher HbA1c levels in both diabetic and non-diabetic individuals. A few studies have also demonstrated that treating IDA can reduce HbA1c.[9-11] On the other hand, other researchers have found no such significant change. In some cases, they even reported a lower HbA1c levels in non-diabetic individuals with IDA.[13-15]

Amidst these controversies and the scarcity of data from eastern India, despite its high prevalence of IDA, our study aimed to investigate the impact of IDA on HbA1c levels in non-diabetics and to explore the correlation of HbA1c with Hb, parameters of the iron profile and RBC indices.

Materials and Methods

Study Design, Settings, and Population

A cross-sectional analytical study was carried out in the Department of General Medicine at a tertiary care teaching hospital in eastern India, spanning from January 2020 to July 2022. Sixty euglycemic patients with IDA were selected as cases, and sixty euglycemic, age- and sex-matched healthy individuals were chosen as controls after considering inclusion and exclusion criteria.

Inclusion Criteria for Cases

Euglycemic individuals aged 18-60 years, diagnosed with IDA, were selected as cases after having provided written informed consent.

Inclusion Criteria for Controls

Euglycemic, non-anemic, age- and sex-matched healthy subjects accompanying the patients were recruited as controls after providing written informed consent.

Exclusion Criteria for Cases and Controls

Patients were excluded if they had glucose tolerance abnormalities (impaired glucose tolerance or diabetes mellitus), hemolytic anaemias, hemoglobinopathies, acute blood loss, vitamin B12/folate deficiency, infestations, chronic smoking, heavy alcohol use, chronic renal failure, chronic obstructive pulmonary disease, or were undergoing erythropoietin therapy. Additionally, those with hypothyroidism, pregnancy, or those taking medications that can affect HbA1c levels, such as corticosteroids, antiretrovirals, and ribavirin, were also excluded.

MAIN POINTS

- The presence of IDA elevates the HbA1c levels in euglycemic individuals toward the prediabetic-diabetic range.
- HbA1c was negatively and significantly correlated with Hb, PCV, serum iron, serum ferritin, transferrin saturation, MCV, MCH, and MCHC.
- HbA1c had a positive and significant correlation with TIBC and reticulocyte count.
- For each unit decrease in Hb below its normal level, the risk of having an HbA1c value in the prediabetic and diabetic range increased by about 2.4 times and 2.61 times, respectively.
- Clinicians must address IDA before interpreting HbA1c results accurately.

Calculation of Sample Size

The sample size (N) was calculated using the standard formula for a comparative study:

$$N = \frac{\left[\left(Z_{\left\{1-\frac{\alpha}{2}\right\}} + Z_{\{1-\beta\}}\right)^2 \cdot 2 \cdot \sigma^2\right]}{d^2}$$

(where $Z_{1-\alpha/2} = 1.96$ at a two-sided 95% CI, $Z_{1-\beta} = 0.84$ for the power of 80%, σ = SD of the population, d = effect size, which is the mean difference to be detected in the outcome variable).

$$\text{The SD } (\sigma) \text{ of the population} = \frac{\{(N_1-1)S_1 + (N_2-1)S_2\}}{N_1+N_2-2}$$

(where N_1 and S_1 = sample size and SD of 1 group (based on the previous study), and N_2 and S_2 = sample size and SD of another group (based on the previous study)).

In a similar previous study, N_1 and N_2 were 46 and 40, respectively, and S_1 and S_2 were 0.91 and 0.6, respectively.[16] Using these values, the SD (σ) was calculated to be 0.76. Applying this SD of 0.76 and a margin of error (d) of 0.4 to the sample size (N) calculation formula, the required sample size for each group was calculated to be 57. Rounding off, we included 60 samples in each group.

Sampling Technique and Data Acquisition

A systematic random sampling method was employed to select cases until the required sample size was reached, with the first sample chosen using a simple random technique. Controls were randomly chosen and matched with cases based on age and gender to ensure an optimally representative sample.

Participants aged 18 to 60 attending the General Medicine department, either as inpatients or outpatients, underwent screening for glucose tolerance abnormalities (impaired glucose tolerance/diabetes mellitus) via fasting plasma glucose (FPG) and postprandial plasma glucose (PPG) testing. Non-diabetic patients meeting the selection criteria were included after securing informed written consent. Detailed demographic and clinical data, including medical history, smoking habits, alcohol consumption, exercise, and family disease history, were recorded using a pre-designed proforma. Five milliliters of peripheral venous blood was collected from each participant after an 8-10 hour fast. Two milliliters of blood was transferred to an EDTA anticoagulant-containing vial for immediate analysis, while the remaining sample was collected in additive-free clot vials, allowed to clot for 30 minutes, and then centrifuged at 2000 rpm for 15 minutes. The serum was separated and stored at −8°C until further analysis. Those who were found to be anemic underwent further testing for IDA by measuring serum iron, TIBC, serum ferritin, and transferrin saturation (Figure 1).

Laboratory Measures

The FPG and PPG levels were measured using the Hexokinase method on the Cobas c501 analyzer to assess glycemic status. The HbA1c levels were determined via the latex agglutination method on the Randox Mondena platform. Serum ferritin was quantified using an immuno-chemiluminescence assay (CLIA) on the Advia Centaur XP system. Serum iron and TIBC were measured through spectrophotometric analysis on the Thermo Scientific Indiko analyzer. Transferrin saturation was calculated with the formula: [(Serum

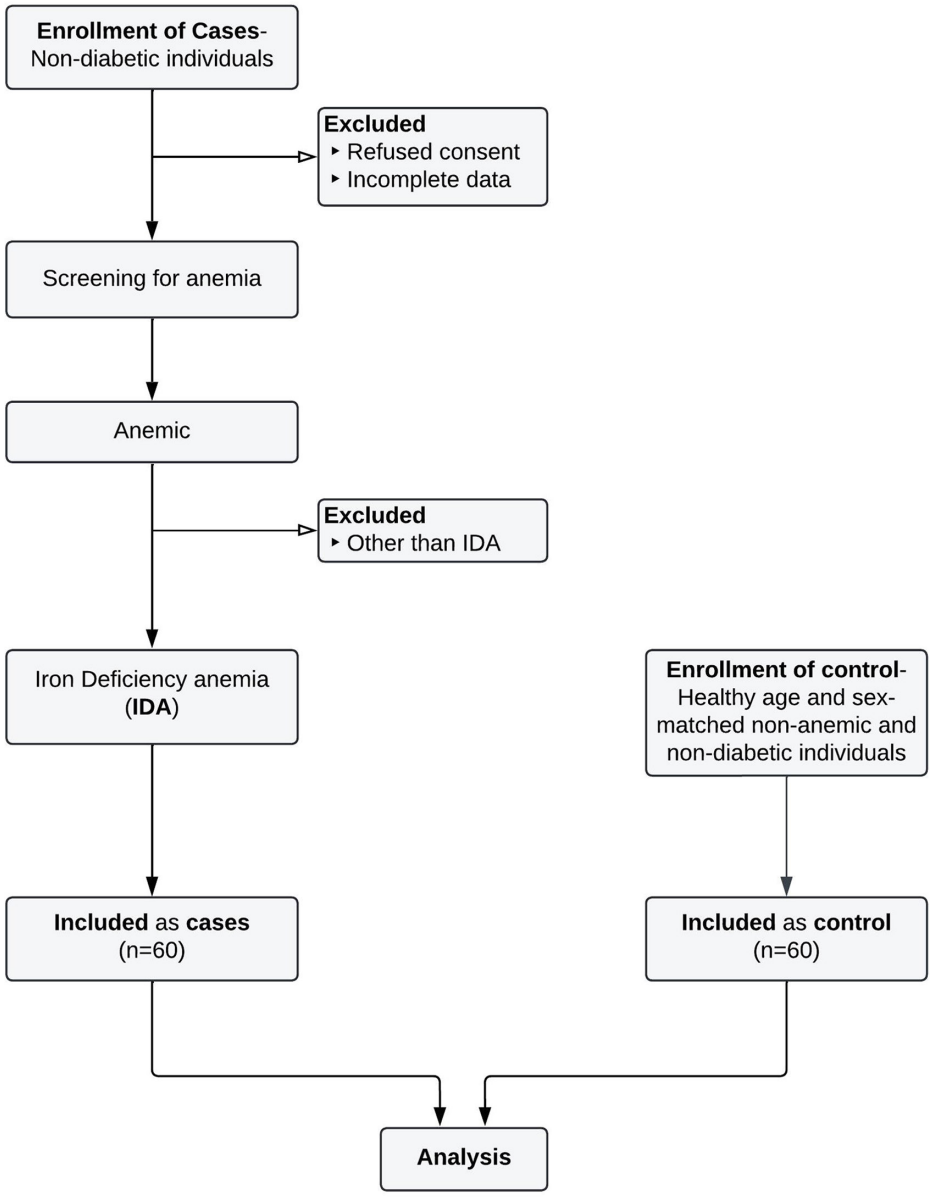

Figure 1. Flow diagram illustrating the recruitment process for study participants.

iron / TIBC) × 100]. The Hb, PCV, MCV, MCH, and MCHC were measured by flow cytometric analysis using the Sysmex 6-part analyzer. The reticulocyte count was performed by examining a peripheral blood smear.

Operational Definitions

Non-diabetics: Individuals having FPG < 100 mg/dL and PPG < 140 mg/dL.[4]

Anemia: Hb concentration < 130g/L in men over 15 years of age, <120g/L in non-pregnant women over 15 years of age, and <110g/L in pregnant women.[2]

IDA: Anemia with iron deficiency (serum iron ≤30 µg/dL, serum ferritin ≤15 µg/L, TIBC ≥360 µg/dL, and transferrin saturation ≤15%).[17]

Adequate exercise: Moderate-intensity exercise of ≥30 minutes duration with ≥5 exercise sessions per week.

Statistical Analysis

Categorical variables were presented as numbers (%), while continuous variables were reported as mean ± SD or as median ± interquartile range (IQR) for non-normally distributed data, as assessed by the Kolmogorov–Smirnov test. Statistical Package for the Social Sciences version 25 (IBM SPSS Corp.; Armonk, NY, USA) was utilized for analysis. The clinical and laboratory parameters between the 2 groups were compared using the chi-square test for categorical variables and either the independent *t*-test or Mann–Whitney *U*-test for continuous variables, depending on the nature and distribution of data. Spearman Rho correlation coefficients (*r*) were calculated to investigate the correlations between Hb, serum iron, serum ferritin, TIBC, transferrin saturation, PCV, reticulocyte count, MCV, MCH, and MCHC with HbA1c values. Relationships between dependent (HbA1c) and independent variables (RBC indices and iron profile parameters) were analyzed using a multinomial logistic regression model. No

significant multicollinearity was detected among covariates included in the final model (variance inflation factor < 5). All tests were -tailed, with a *P*-value of less than .05 regarded as statistically significant.

Ethical Considerations

The study received approval from the Institutional Ethics Committee of Medical College, Kolkata (approval number: IEC/NON-SPON/508/12/2019; date: December 21, 2019). Written informed consent was obtained from all the participants, ensuring the confidentiality and anonymity of their responses and identity. All procedures adhered to the principles outlined in the 1964 Helsinki Declaration and its subsequent amendments.

Results

Among the IDA individuals, 68.33% were female. Table 1 summarizes the demographic and clinical characteristics of the study population. No statistically significant differences were observed regarding age, gender, comorbidities, exercise habits, FPG, and PPG values between the 2 groups. However, the mean (SD) value of HbA1c was 6.5 (0.5), ranging from 5.04 to 7.30 in the IDA group, and 5.13 (0.8), ranging from 4.03 to 7.20 in the control group ($P < .001$). Furthermore, individuals with IDA exhibited significantly lower median (IQR) values of Hb and other RBC indices, serum iron, serum ferritin, and transferrin saturation, while TIBC was significantly higher compared to the control group ($P < .001$).

A negative and statistically significant ($P < 0.001$) correlation of HbA1c was noticed with Hb ($r = -0.638$), serum iron ($r = -0.609$), serum ferritin ($r = -0.522$), transferrin saturation ($r = -587$), MCV ($r = -0.570$), MCH ($r = -0.637$), and MCHC ($r = -0.545$). Thus, with a decrement of these parameters, there was a significant increase in HbA1c value. Whereas, TIBC showed a positive correlation ($r = 0.524$, $P < .001$), which denotes that with an increase in TIBC, there was a significant increase in HbA1c values (Table 2 and Figure 2).

With each unit of decrease in Hb value, there was 2.61 times (95% CI = 1.65-3.50) and 2.40 times (95% CI = 1.81-3.77) higher risk of having prediabetic and diabetic levels of HbA1C ($P < .001$). Similarly, with decreasing levels of serum iron, serum ferritin, and transferrin saturation, there were 1.87 (95% CI = 1.04-1.91), 1.02 (95% CI = 1.01-1.04), and 1.24 (95% CI = 1.12-1.31) times greater odds of having prediabetic level HbA1c; whereas 1.08 (95% CI = 1.04-1.10), 1.01 (95% CI = 1.01-1.05) and 1.21 (95% CI = 1.12-1.30) times higher chance of getting diabetic levels of HbA1c, respectively ($P < .001$). However, with increasing levels of TIBC, there was 1.02 (95% CI = 1.01-1.02) times and 1.01 (95% CI = 1.01-1.03) times higher risk of having HbA1c value in the prediabetic and diabetic range ($P < .001$) (Table 3).

Table 4 illustrates that decreasing levels of PCV corresponded to 1.30 (95% CI=1.16-1.45) times and 1.21 (95% CI=1.18-1.45) times higher risk of having prediabetic and diabetic range of HbA1c p<0.001). Similarly, decreasing levels of MCV were associated with 1.22 (95% CI=1.11-1.29) times and 1.20 (95% CI=1.13-1.31) times higher risk of having prediabetic and diabetic levels of HbA1c, respectively, (p<0.001). Additionally, each unit decrease in MCH and MCHC was associated with 1.76 (95% CI: 1.41-2.22) and 1.87 (95% CI: 1.41-2.46) times higher odds of prediabetes, and 1.66 (95% CI: 1.32-2.07) and 1.74 (95% CI: 1.30-2.32) times higher odds of diabetic levels of HbA1c, respectively (p<0.001).

Table 1. Distribution of Clinico-Laboratory Parameters Among the 2 Groups

Parameters	Cases (n = 60)	Control (n = 60)	*P*
Age in years, mean (SD)	39.9 (17.52)	37.02 (14.88)	.192*
Gender, n (%)			
Male	19 (31.67)	22 (36.67)	
Female	41 (68.33)	38 (63.33)	.553†
Family history of diabetes, n (%)	4 (6.67)	5 (8.33)	.788†
Adequate exercise, n (%)	32 (53.33)	37 (61.67)	.197†
Comorbidities, n (%)			
Hypertension	16 (26.67)	13 (21.67)	.411†
Ischemic heart disease	7 (11.67)	5 (8.33)	.346†
Others	3 (5.0)	4 (6.67)	.551†
BMI (kg/m^2), median (IQR)	21.4 (19.2-23.3)	21.8 (20.4-22.8)	.754‡
FPG (mg/dL), mean (SD)	87.4 (12.2)	89 (10.8)	.523*
PPG (mg/dL), mean (SD)	118.7 (9.3)	119.3 (9.2)	.812*
HbA1c (%), mean (SD)	6.5 (0.5)	5.13 (0.8)	**<.001***
Hemoglobin (gm/dL), median (IQR)	8.9 (7.9-10.1)	13.7 (13.0-14.3)	**<.001‡**
PCV (%), median (IQR)	29.4 (27.0-33.4)	33.8 (33.4-34.2)	**<.001‡**
MCV (fL), median (IQR)	68.0 (65.0-76.0)	90 (87-92.5)	**<.001‡**
MCH (pg), median (IQR)	23.1 (20.6-26.0)	31.1 (30.2-31.8)	**<.001‡**
MCHC (gm/dL), median (IQR)	29.5 (26.8-31.3)	33.8 (33.4-34.2)	**<.001‡**
Reticulocyte count (%), median (IQR)	1.51 (1.1-2.1)	1.3 (1.2-1.6)	**.047‡**
Serum Iron (µg/dL), median (IQR)	32.8 (19.5-43.0)	86.7 (78.0-98.0)	**<.001‡**
Serum Ferritin (ng/mL), median (IQR)	11.5 (6.6-21.6)	178.5 (110.0-234.1)	**<.001‡**
TIBC (µg/dL), median (IQR)	458 (397.1-520.0)	298 (267.3-328.1)	**<.001‡**
Transferrin saturation (%), median (IQR)	7.2 (4.1-9.8)	28.8 (26.1-32.0)	**<.001‡**

Values in bold indicate statistical significance.
P-value was obtained by using *Independent *t*-test, †Chi-square test, and ‡Mann–Whitney *U*-test.BMI, body mass index; FPG, fasting plasma glucose; IQR, interquartile range; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; PPG, postprandial plasma glucose; TIBC, total iron binding capacity.

Discussion

Anemia remains a concealed global pandemic, with IDA being the most prevalent nutritional deficiency, constituting over 50% of all anemia cases worldwide.[1] Diabetes, on the other hand, is rapidly

Table 2. Correlation Matrix Showing the Correlation of HbA1c with Hemoglobin, Iron Profile Parameters, and Red Blood Cell (RBC) Indices

	HbA1c	
Parameters	**Correlation coefficient (r)¥**	***P***
Hemoglobin (%)	−0.638	**<.001**
Serum iron (mcg/dl)	−0.609	**<.001**
Serum ferritin (ng/ml)	−0.522	**<.001**
TIBC (mcg/dl)	0.524	**<.001**
Transferrin saturation (%)	−0.587	**<.001**
PCV (%)	−0.592	**<.001**
Reticulocyte count (%)	0.216	**.031**
MCV (fl)	−0.570	**<.001**
MCH (pg)	−0.637	**<.001**
MCHC (gm/dl)	−0.545	**<.001**

Values in bold indicate statistical significance.
¥Spearman Rho correlation coefficient was calculated as data was not normally distributed (Kolmogorov–Smirnov test *P* value < .05).MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PCV, packed cell volume; TIBC, total iron binding capacity.

evolving into a global health emergency, with over two-thirds of its burden being contributed by LMICs.[18] Over the past decade, the diagnosis and severity assessment of prediabetes and diabetes have increasingly involved the measurement of blood glycated proteins and HbA1c, which are key indicators of glycemic status over the preceding 3 months. These indicators offer the advantages of not requiring fasting, demonstrating low inter-user variability, and correlates well with the risk of long-term diabetes complications.[4] However, emerging studies suggest that HbA1c estimation can be influenced by factors that alter RBC lifespan and Hb structure. Therefore, the reliability of HbA1c values should be carefully considered during the diabetes diagnosis and treatment process (Table 3).

The current study found that patients with IDA have higher HbA1c levels as compared to non-anemic patients and the levels of HbA1c have a strong negative correlation with Hb, serum iron, serum ferritin, and transferrin saturation (Table 4).

In our study, most individuals with IDA were female (68.33%), which is consistent with the findings of other Indian studies.[19,20]

The current study revealed no significant difference in glycemic profiles based on FPG and PPG values between the 2 groups, consistent with findings from similar studies.[11-13] However, the level of HbA1c was notably higher in patients with IDA compared to controls (6.5 ± 0.5 vs. 5.13 ± 0.8, $P < .001$). This observation closely mirrors findings from studies conducted by other researchers.[11,21-24] Studies have also shown a significant reduction in HbA1c levels following iron replacement therapy (IRT) in these patients.[25] Though the exact mechanisms remain unclear, IDA-induced alteration in the quaternary structure of Hb leads to more rapid glycation of the globin chain and reduction in RBC turnover, which is accompanied by an increase in the glycation rate of Hb and decreased hemoglobinization results in

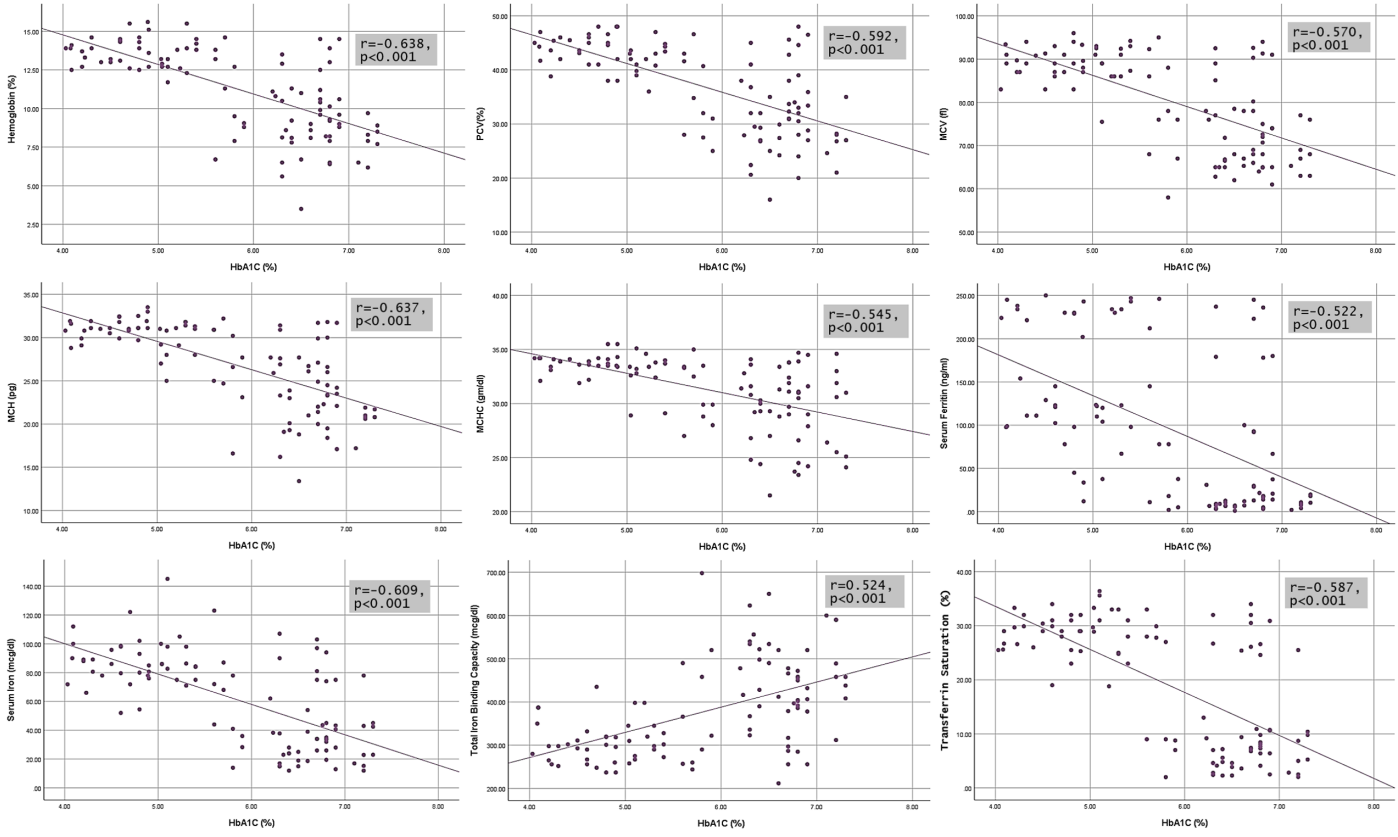

Figure 2. Scatter plots displaying the correlation of HbA1c with hemoglobin, iron profile parameters, and various RBC indices (*r* = Spearman's correlation coefficient). PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table 3. Multinomial Logistic Regression Showing Association of HbA1c with Hemoglobin and Iron Profile Parameters

		HbA1c Level		
Parameter	**Statistics**	**Normal (≤5.6)**	**Prediabetic (5.7-6.4)**	**Diabetic (≥6.5)**
↓ Hemoglobin (%)	Median (IQR)¥	13.7 (12.7-14.3)	9.4 (8.1-11.3)	9.0 (8.1-10.6)
	OR (95% CI)	1	2.61 (1.65-3.50)	2.40 (1.81-3.77)
	P	_	**<.001**	**<.001**
↓ Serum iron (µg/dL)	Median (IQR)¥	85.9 (76-98)	32.1 (20-65)	34 (23-45)
	OR (95% CI)	1	1.87 (1.04-1.91)	1.08 (1.04-1.10)
	P	_	**<.001**	**<.001**
↓ Serum ferritin (ng/mL)	Median (IQR)¥	123.4 (98.8-230)	9.7 (6.6-57.9)	18 (7.5-66.8)
	OR (95% CI)	1	1.02 (1.01-1.04)	1.01 (1.01-1.05)
	P	_	**<.001**	**<.001**
↑ TIBC (µg/dL)	Median (IQR)¥	298 (267-332)	443 (329.5-528)	432 (379-478)
	OR (95% CI)	1	1.02 (1.01-1.02)	1.01 (1.01-1.03)
	P	_	**<.001**	**<.001**
↓ Transferrin saturation (%)	Median (IQR)¥	29 (25.6-32)	7.1 (4.4-19.9)	8.1 (5.0-10.9)
	OR (95% CI)	1	1.24 (1.12-1.31)	1.21 (1.12-1.30)
	P	_	**<.001**	**<.001**

Values in bold indicate statistical significance.
The symbol (↑) denotes a per unit increase, while (↓) denotes a per unit decrease.
¥Data was not normally distributed (Kolmogorov–Smirnov test *P* value < .05).IQR, interquartile range; OR, odds ratio; TIBC, total iron-binding capacity.

older circulating erythrocytes. These are the most accepted hypotheses for the falsely elevated HbA1c concentrations observed in IDA patients.[26,27] Conversely, a few studies have reported lower HbA1c levels in patients with IDA,[14,15] and some have shown no change to a significant rise in HbA1c levels after IRT.[28] The heterogeneity of these results may stem from various factors, including the minimal impact of the hemolytic component on both mature and immature erythrocytes in IDA, resulting in a normal erythrocyte lifespan. Additionally, differences in the doses, composition, and duration of IRT, as well as variations in the methods used to estimate HbA1c, could also contribute. To avoid misdiagnosis or missed diagnosis, the ADA recommends performing the HbA1c test using a method certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized to the Diabetes Control and Complications Trial (DCCT) reference assays. The current study used the latex agglutination method, which is one of the NGSP-certified methods and standardized to DCCT references.[29] However, a study by Rai KB et al,[30] where different biochemical techniques such as colorimetric assays, ion-exchange chromatography, and affinity chromatography were utilized for measuring HbA1c, found no significant difference.[30]

Table 4. Multinomial Logistic Regression Showing the Association of HbA1c with Red Blood Cell (RBC) Indices

		HbA1c Level		
Parameter	**Statistics**	**Normal (≤5.6)**	**Prediabetic (5.7-6.4)**	**Diabetic (≥6.5)**
↓ PCV (%)	Median (IQR)¥	43.4 (41-45.4)	31.5 (27.3-37.4)	30.9 (27-35)
	OR (CI)	1	1.30 (1.16-1.45)	1.21 (1.18-1.45)
	P	_	**<.001**	**<.001**
↑ Reticulocyte count (%)	Median (IQR)¥	1.3 (1.1-1.5)	1.4 (1.0-2.1)	1.5 (1.2-2.0)
	OR (CI)	1	2.74 (0.81-9.29)	4.32 (1.50-12.49)
	P	_	.105	**.007**
↓ MCV (fl)	Median (IQR)¥	89 (87-92.4)	76 (65.8-81.6)	69 (65.3-78)
	OR (CI)	1	1.20 (1.11-1.29)	1.22 (1.13-1.31)
	P	_	**<.001**	**<.001**
↓ MCH (pg)	Median (IQR)¥	31 (29.9-31.8)	25.3 (21.6-27.7)	22.3 (20.8-26.6)
	OR (CI)	1	1.76 (1.32-2.07)	1.66 (1.41-2.20)
	P	_	**<.001**	**<.001**
↓ MCHC (gm/dl)	Median (IQR)¥	33.6 (33.1-34.1)	30.2 (29-32.7)	30.6 (26.6-31.9)
	OR (CI)	1	1.87 (1.30-2.32)	1.74 (1.41-2.46)
	P	_	**<.001**	**<.001**

Values in bold indicate statistical significance.
The symbol (↑) denotes a per unit increase, while (↓) denotes a per unit decrease.
¥Data was not normally distributed (Kolmogorov–Smirnov test *P* value < .05).IQR, interquartile range; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; OR, odds ratio; PCV, packed cell volume.

The current study observed a negative, moderate, and statistically significant correlation of HbA1c with Hb, serum iron, serum ferritin, transferrin saturation, PCV, MCV, MCH, and MCHC, consistent with findings from other studies.[31,32] Ferritin serves as the storage form of iron, and lower serum ferritin concentrations indicate delays in RBC turnover, leading to increased Hb glycation and potentially higher HbA1c levels in these patients. However, a few studies have sporadically reported a positive correlation of HbA1c with different RBC indices and iron profile parameters.[15,33] These correlations between red cell indices and HbA1c underscore the role of erythrocyte morphology and lifespan in elevating HbA1c levels.

In our study, for each unit decrease in Hb value, the likelihood of obtaining HbA1c values in prediabetic and diabetic ranges increased by 2.4 times (95% CI = 1.65-3.50) and 2.61 times (95% CI = 1.81-3.77), respectively. This observation is consistent with the findings of Rajagopal et al[34] and Bhargava, S. et al,[35] who also noted that HbA1c levels tend to rise proportionally as the severity of anemia increases. However, neither of these studies addressed the clinician's need for a specific cutoff value of Hb below which HbA1c interpretation is significantly affected. Although generalizing a cutoff value can be challenging, establishing one could provide valuable guidance for clinicians, especially in regions with high anemia prevalence, such as India. Nevertheless, further large-scale, long-term studies with homogeneous patient groups are necessary to draw definitive conclusions.

This study not only highlighted the impact of IDA on HbA1c levels in non-diabetic individuals but also explored the correlation of HbA1c with anemia severity, RBC indices, and iron profile parameters. Few studies from eastern India have examined these relationships, and to the best of our knowledge, this is the first study from West Bengal to do so. All biochemical analyses were performed on freshly collected blood samples using globally accepted standard methods. However, the study has a few limitations. As a single-center study with small sample size and control subjects drawn from hospital attendees, its findings may not be widely generalizable. Additionally, follow-up data after IRT were not evaluated, which could have provided new insights. Moreover, whether the severity of IDA increases the risk of diabetes mellitus and its complications remains unexplored. Therefore, there is a pressing need for large-scale, long-term studies with robust methodologies to establish the relationship between IDA, HbA1c, and the effects of IRT.

In conclusion, the current study statistically demonstrates that the presence of IDA elevates the HbA1c level towards the prediabetic or diabetic range in euglycemic individuals. Clinicians should not rely solely on HbA1c measurements to diagnose prediabetes or diabetes in individuals with IDA. Instead, they should conduct a comprehensive assessment and consider and treat the IDA before planning any further glycemic control strategies to minimize the risk of drug-related adverse events.

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: This study was approved by the Institutional Ethics Committee of Medical College, Kolkata (approval number: MCK/KOL/IEC/NON-SPON/508/12/2019; date December 21, 2019).

Informed Consent: Written informed consent was obtained from the participants who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – N.D., I.D., A.G.; Design – B.K., A.G., S.R.; Supervision – I.D.; Materials – A.G., S.R.; Data Collection and/or Processing – N.D., B.K.; Analysis and/or Interpretation – A.G., S.R.; Literature Search – B.K., N.D., A.G.; Writing – N.D., B.K., A.G.; Critical Review – I.G., S.R.

Acknowledgments: The authors would like to thank all the participants for their willingness to participate in this study. They are also grateful to Dr. Ripan Saha (M.D. Community Medicine), Department of Health and Family Welfare, Swasthya Bhawan, Kolkata, India for his potential inputs in statistical analysis.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: This study received no funding.

References

1. GBD. Prevalence, years lived with disability, and trends in anaemia burden by severity and cause, 1990-2021: findings from the Global Burden of Disease Study 2021. *Lancet Haematol*;10(9):e713-e734. [CrossRef] [published correction appears in *Lancet Haematol*. 2023;10(10):e796] [published correction appears in *Lancet Haematol*. 2024;11(1):e10. (https://doi.org/10.1016/S2352-3026(23)00373-3)]
2. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and mineral nutrition information system; 2011. Geneva, World Health Organization. https://apps.who.int/iris/bitstream/handle/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf. Last assessed-22 May 2024 (World Health Organization, NMH/NHD/MNM/11; 1).
3. Charles CV, Maddock J. Iron deficiency anemia: a public health problem of global proportions. *Public Health – Methodol. Environ Syst*;109. http://cdn.intechopen.com/pdfs/37291/InTechIron_deficiency_anemia_a_public_health_problem_of_global_proportions.pdf(2012), Last assessed-22 May 2024.
4. American Diabetes Association Professional Practice Committee. 2. Diagnosis and classification of diabetes: standards of care in Diabetes-2024. *Diabetes Care*. 2024;47(suppl 1):S20-S42. [CrossRef]
5. English E, Idris I, Smith G, Dhatariya K, Kilpatrick ES, John WG. The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review. *Diabetologia*. 2015;58(7):1409-1421. [CrossRef]
6. Church D, Simmons D. More evidence of the problems of using HbA1c for diagnosing diabetes? The known knowns, the known unknowns and the unknown unknowns. *J Intern Med*. 2014;276(2):171-173. [CrossRef]
7. Koga M, Saito H, Mukai M, Matsumoto S, Kasayama S. Influence of iron metabolism indices on glycated haemoglobin but not glycated albumin levels in premenopausal women. *Acta Diabetol*. 2010;47(suppl 1):65-69. [CrossRef]
8. Cavagnolli G, Pimentel AL, Freitas PA, Gross JL, Camargo JL. Effect of ethnicity on HbA1c levels in individuals without diabetes: systematic review and meta-analysis. *PLOS ONE*. 2017;12(2):e0171315. [CrossRef]
9. El-Agouza I, Abu Shahla A, Sirdah M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. *Clin Lab Haematol*. 2002;24(5):285-289. [CrossRef]
10. Tarim O, Küçükerdoğan A, Günay U, Eralp O, Ercan I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. *Pediatr Int*. 1999;41(4):357-362. [CrossRef]
11. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in non-diabetic patients. *Acta Haematol*. 2004;112(3):126-128. [CrossRef]
12. Hardikar PS, Joshi SM, Bhat DS, et al. Spuriously high prevalence of prediabetes diagnosed by HbA(1c) in young indians partly explained by hematological factors and iron deficiency anemia. *Diabetes Care*. 2012;35(4):797-802. [CrossRef]
13. Kim C, Bullard KM, Herman WH, Beckles GL. Association between iron deficiency and A1C Levels among adults without diabetes in the National

Health and Nutrition Examination Survey, 1999-2006. *Diabetes Care*. 2010;33(4):780-785. [CrossRef]
14. Çetinkaya Altuntaş S, Evran M, Gürkan E, Sert M, Tetiker T. HbA1c level decreases in iron deficiency anemia. *Wien Klin Wochenschr*. 2021;133(3-4):102-106. [CrossRef]
15. Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. *Ann Lab Med*. 2012;32(1):17-22. [CrossRef]
16. Basheer A. Effect of Iron Deficiency anemia on HbA1c Levels in non-diabetic females in Brack Alshatti. *Alq J Med App Sci*. 2023;6(2):476-481. [CrossRef]
17. Ganz T. Iron deficiency and overload. In: Kaushansky K, Lichtman MA, Prchal JT, et al., eds. *Williams Hematology, 9ème*. McGraw-Hill Education; New York; 2015*Last Assessed*; 2024).
18. Magliano DJ, Boyko EJ. IDF Diabetes Atlas. 10th ed scientific committee. *IDF Diabetes Atlas*. 10th ed; 2021. Brussels: International Diabetes Federation. https://www.ncbi.nlm.nih.gov/books/NBK581934/ (*Last Assessed*; 2024).
19. Givens DI, Anitha S, Giromini C. Anaemia in India and its prevalence and multifactorial aetiology: A narrative review. *Nutrients*. 2024;16(11):1673. [CrossRef]
20. Natekar P, Deshmukh C, Limaye D, Ramanathan V, Pawar A. A micro review of a nutritional public health challenge: iron deficiency anemia in India. *Clin Epidemiol Glob Health*. 2022;14:100992. [CrossRef]
21. Alzahrani BA, Salamatullah HK, AIsharm FS, et al. The effect of different types of anemia on HbA1c levels in non-diabetics. *BMC Endocr Disord*. 2023;23(1):24. [CrossRef]
22. Bansal RK, Yadav YR, Kulkarni HS, et al. Effect of iron deficiency anemia on HbA1c in non-diabetics. *Jour Diab Endo Assoc Nepal*. 2020;4(1):10-16. [CrossRef]
23. Kalasker, Vishal, Sudhamadhuri MV. Kodliwadmath and harish S. Bhat. Effect of iron deficiency anemia on glycosylated hemoglobin levels in non diabetic Indian adults. *Int J Med Health Sci*. 2014;3:40-43.
24. Shanthi B, Revathy C, Manjula Devi AJ, Subhashree. Effect of iron deficiency on glycation of haemoglobin in nondiabetics. *J Clin Diagn Res*. 2013;7(1):15-17. [CrossRef]
25. AlQarni AM, Alghamdi AA, Aljubran HJ, Bamalan OA, Abuzaid AH, AlYahya MA. The effect of iron replacement therapy on HbA1c levels in diabetic and nondiabetic patients: A systematic review and meta-analysis. *J Clin Med*. 2023;12(23):7287. [CrossRef]
26. Brooks AP, Metcalfe J, Day JL, Edwards MS. Iron deficiency and glycosylated haemoglobin A. *Lancet*. 1980;2(8186):141. [CrossRef]
27. Radin MS. Pitfalls in hemoglobin A1c measurement: when results may be misleading. *J Gen Intern Med*. 2014;29(2):388-394. [CrossRef]
28. van Heyningen C, Dalton RG. Glycosylated haemoglobin in iron-deficiency anaemia. *Lancet*. 1985;1(8433):874. [CrossRef]
29. List of National Glycohemoglobin Standardization Program (NGSP) Certified Methods (updated 07/24). https://ngsp.org/docs/methods.pdf Accessed29 July 2024.
30. Rai KB, Pattabiraman TN. Glycosylated haemoglobin levels in iron deficiency anaemia. *Indian J Med Res*. 1986;83:234-236.
31. Madhu SV, Raj A, Gupta S, Giri S, Rusia U. Effect of iron deficiency anemia and iron supplementation on HbA1c levels - implications for diagnosis of prediabetes and diabetes mellitus in Asian indians. *Clin Chim Acta*. 2017;468:225-229. [CrossRef]
32. Silva JF, Pimentel AL, Camargo JL. Effect of iron deficiency anaemia on HbA1c levels is dependent on the degree of anaemia. *Clin Biochem*. 2016;49(1-2):117-120. [CrossRef]
33. Kumar D, Rasheed T, Zuberi BF, Sadaf R, Ali FS. Correlation of HbA1c with serum iron & transferrin saturation in non-diabetic patients with iron deficiency. *Pak J Med Sci*. 2023;39(4):956-960. [CrossRef]
34. Rajagopal L, Ganapathy S, Arunachalam S, Raja V, Ramraj B. Does iron deficiency anaemia and its severity influence HbA1c level in Non diabetics? An analysis of 150 cases. *J Clin Diagn Res*. 2017;11(2):EC13-EC15. [CrossRef]
35. Bhargava S, Mahato K, Manocha A, et al. Interpreting HbA1c in presence of deficiency anemias. *Indian J Clin Biochem*. 2021;36(3):360-364. [CrossRef]