

## Effect of Iron Deficiency Anemia on Glycated Hemoglobin (HbA1c) Levels in Normoglycemic Adults

### Author Contributions

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All authors read and approved the final manuscript.

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## Abstract

### Aims & Background:

Iron deficiency anemia (IDA) is the most common nutritional deficiency worldwide and a major contributor to anemia, particularly in developing countries, such as India. Glycated hemoglobin (HbA1c) is widely used for monitoring and diagnosing diabetes mellitus, and is primarily influenced by blood glucose levels. However, emerging evidence suggests that HbA1c levels may also be altered by non-glycemic factors such as iron-deficiency anemia. This study aimed to evaluate HbA1c levels in patients with IDA and analyze their relationship with iron status parameters.

### Materials and Methods:

This cross-sectional study was conducted at the Jaipur National University Institute of Medical Sciences and Research Centre, Jaipur, Rajasthan between 2024 and 2025. A total of 200 subjects aged 18–60 years were included: 100 patients with clinically and biochemically confirmed iron deficiency anemia and 100 age- and sex-matched healthy controls. Hematological parameters were assessed using an automated analyzer. Serum iron, total iron-binding capacity (TIBC), and ferritin levels were estimated using standard biochemical methods. Fasting and postprandial blood glucose levels were measured to confirm normoglycemia. HbA1c levels were estimated using high-performance liquid chromatography (HPLC). Statistical analysis was performed using unpaired Student's *t* test and Pearson's correlation coefficient, with  $p < 0.05$  considered statistically significant.

### Results:

There were no significant differences in age or sex distribution between patients and controls.

Patients with IDA showed significantly lower hemoglobin, hematocrit, MCV, MCH, and MCHC levels, as well as significantly higher RDW ( $p < 0.001$ ). Serum iron and ferritin levels were significantly reduced, whereas TIBC levels were significantly higher in the IDA group than in the controls ( $p < 0.001$ ). Fasting and postprandial blood glucose levels were comparable between the two groups, confirming normoglycemia. The mean HbA1c levels were significantly higher in the IDA group ( $6.55 \pm 0.77\%$ ) than in the controls ( $5.27 \pm 0.83\%$ ) ( $p < 0.001$ ). HbA1c showed a significant negative correlation with hemoglobin, serum iron, and serum ferritin levels and a positive correlation with TIBC.

### **Conclusion:**

Iron deficiency anemia is associated with a significant increase in HbA1c levels independent of glycemic status. Altered iron metabolism appears to influence HbA1c concentrations, potentially leading to falsely elevated values in non-diabetic individuals with IDA.

### **Clinical Significance:**

As HbA1c is increasingly used for the diagnosis and monitoring of diabetes mellitus, iron deficiency anemia should be considered a confounding factor. Assessment and correction of iron status are essential to avoid misinterpretation of HbA1c levels and possible misdiagnosis of diabetes or prediabetes, particularly in populations with a high IDA prevalence.

### **Keywords:**

Iron deficiency anemia; HbA1c; Glycated hemoglobin; Iron status; Serum ferritin; Total iron-binding capacity; Diabetes diagnosis.

## **Introduction**

Iron deficiency is the most common nutritional deficiency worldwide, and continues to be a major public health problem, particularly in developing countries. According to the World Health Organization, iron deficiency is the leading cause of anemia worldwide. Iron deficiency anemia (IDA) and anemia are often used interchangeably, because iron deficiency accounts for nearly 50% of all anemia cases. However, this proportion may vary among different population groups and geographical regions depending on local conditions.<sup>1,2</sup>

Protein glycation is a non-enzymatic, spontaneous biochemical reaction that plays a significant role in the pathogenesis of several clinical disorders. This process is enhanced in the presence of elevated blood glucose concentrations. Among glycated proteins, glycated hemoglobin (HbA1c) is of major clinical importance.<sup>3</sup> HbA1c, also referred to as glycohemoglobin or hemoglobin A1c, is formed by the attachment of glucose to hemoglobin. The term “glycation” is preferred over “glycosylation,” as recommended by the Joint Commission on Biochemical Nomenclature, to describe this non-enzymatic reaction between sugars and proteins.<sup>4</sup>

HbA1c levels are abnormally elevated in patients with poorly controlled diabetes mellitus and correlate positively with long-term glycemic control. According to the American Diabetes Association (ADA) guidelines, HbA1c values below 7% are recommended for optimal glycemic control in diabetic patients, while values above this threshold are associated with an increased risk of microvascular complications such as nephropathy, neuropathy, and retinopathy. More recently,

HbA1c has been incorporated into the diagnostic criteria for diabetes mellitus, with a cutoff value of 6.5%.<sup>9</sup>

Although HbA1c primarily reflects blood glucose concentration, several studies have demonstrated that its levels may be influenced by non-glycemic factors, including iron deficiency anemia. IDA is highly prevalent in India, and increasing evidence suggests that iron deficiency may alter HbA1c levels independently of the glycemic status. However, the available data remains inconsistent, necessitating further evaluation. Therefore, the present study was undertaken to assess HbA1c levels in patients with iron deficiency anemia and to evaluate their association with iron status parameters.

## Materials And Methods

### Study Design, Setting, and Period

This cross-sectional study was conducted at Jaipur National University Institute for Medical Sciences and Research Center. Study participants were recruited from patients attending the outpatient department (OPD) and those admitted to inpatient wards. The study was conducted between 2024–2025.

### Sample Size and Study Groups

A total of **200 subjects** were included in the study, comprising:

- I. **Cases (n = 100):** Clinically and biochemically confirmed cases of iron deficiency anemia (IDA).
- II. **Controls (n = 100):** healthy individuals with normal hematological and biochemical parameters matched for age and sex.

### Inclusion Criteria

- a. **Cases:** Individuals with laboratory-confirmed iron deficiency anemia.
- b. **Controls:** Apparently healthy individuals with normal hematological indices and iron profile.

### Exclusion Criteria

Subjects with any of the following conditions were excluded:

1. Impaired glucose tolerance
2. Diabetes mellitus
3. Hemoglobinopathies
4. Hemolytic anemia
5. Chronic renal disease
6. Chronic alcoholism
7. Pregnancy
8. History of blood transfusion within the preceding six months

### Sample Collection

After written informed consent was obtained, venous blood samples were collected under aseptic conditions by trained personnel.

1. **2 mL fasting venous blood** was collected in fluoride bulbs for estimation of fasting plasma glucose (BSL-F).
2. **2-hour postprandial venous blood** was collected in fluoride bulbs for postprandial plasma glucose estimation (BSL-PP).
3. **2 mL venous blood** was collected in EDTA bulbs for complete blood count (CBC) and glycated hemoglobin (HbA1c) estimation.
4. **2 mL venous blood** was collected in plain bulbs for estimation of serum iron, total iron-binding capacity (TIBC), and serum ferritin.

Serum and plasma were separated by centrifugation at **3000 rpm for 10 minutes**, and all samples were analyzed within **two hours** of collection.

### Laboratory Investigations

The following parameters were evaluated:

#### Hematological Parameters

Hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) were analyzed using a **HORIBA H550 automated hematology analyzer** based on the electrical impedance principle.

#### Biochemical Parameters

- I. **Serum iron and TIBC:** Ferrozin method
- II. **Serum ferritin:** Enzyme-linked immunosorbent assay (ELISA)
- III. **Blood glucose (fasting and postprandial):** Glucose oxidase–peroxidase (GOD-POD) method
- IV. **HbA1c:** Ion-exchange high-performance liquid chromatography (HPLC)

Biochemical analyses were performed using fully automated analyzers (**Randox RX Daytona and RX Imola**) under standard laboratory conditions.

#### Diagnostic Criteria for Iron Deficiency Anemia

Iron deficiency anemia was diagnosed based on the following criteria:

1. Reduced hemoglobin concentration
  - a) <13 g/dL in males
  - b) <12 g/dL in females
2. Reduced MCV, MCH, and MCHC
3. Increased RDW
4. Decreased serum iron and serum ferritin levels
5. Increased total iron-binding capacity (TIBC)
6. Peripheral blood smear showing microcytic hypochromic anemia

## Statistical Analysis

Data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) software (version 26.0 (IBM Corp., Armonk, NY, USA)). Continuous variables were expressed as mean  $\pm$  standard deviation (SD). Comparisons between cases and controls were performed using unpaired Student's t-test. Pearson's correlation coefficient (r) was used to assess the association between HbA1c and hematological and iron profile parameters. Statistical significance was set at  $p < 0.05$ , and  $p < 0.001$  was considered highly significant.

## Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee of Jaipur National University Institute for Medical Sciences and Research Centre, Jaipur (**IEC Approval No.: JNUIMSRC/IEC/2024/117; dated 01 June 2024**). Written informed consent was obtained from all participants prior to enrollment. The study was conducted in accordance with the **Declaration of Helsinki (2013, revised)** and the **Indian Council of Medical Research (ICMR) National Ethical Guidelines for Biomedical and Health Research Involving Human Participants** and complied with ICH-GCP and local regulatory requirements.

## Facilities and Quality Control

All laboratory investigations were performed using standardized protocols and calibrated instruments. To ensure accuracy and reliability of the results, sample collection, processing, and analysis were performed under strict aseptic conditions by trained and experienced laboratory personnel.

## Results

A total of 200 subjects were included in the study, comprising 100 patients with iron deficiency anemia (IDA) and 100 age- and sex-matched healthy controls.

The mean age of subjects in the IDA group was  $39.23 \pm 9.57$  years, while that of the control group was  $37.83 \pm 9.08$  years. The difference in the mean age between the two groups was not statistically significant ([Table 1](#)).

Both groups were comparable in terms of sex distribution. Each group consisted of 20 males (20%) and 80 females (80%), indicating appropriate sex matching between cases and controls ([Table 2](#); [Figure 1](#)).

The hematological parameters revealed significant alterations in the IDA group. Mean hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were significantly lower in IDA patients compared to controls ( $p < 0.001$ ). In contrast, red cell distribution width (RDW) was significantly higher in the IDA group ( $p < 0.001$ ) ([Table 3](#); [Figure 3](#)).

Iron profile parameters showed a highly significant reduction in mean serum iron and serum ferritin levels in the IDA group compared with the controls ( $p < 0.001$ ). Total iron-binding capacity (TIBC) was significantly elevated in patients with IDA ( $p < 0.001$ ) ([Table 4](#); [Figure 4](#)).

Evaluation of glycemic status demonstrated no statistically significant difference in fasting blood sugar (BSL-F) and postprandial blood sugar (BSL-PP) levels between the two groups ( $p > 0.05$ ), confirming normoglycemia in both cases and controls (Table 5; Figure 5).

Despite normal plasma glucose levels, mean HbA1c levels were significantly higher in the IDA group ( $6.55 \pm 0.77\%$ ) than in the controls ( $5.27 \pm 0.83\%$ ), and the difference was highly statistically significant ( $p < 0.001$ ) (Table 6).

Pearson's correlation analysis within the IDA group revealed a significant negative correlation between HbA1c and hemoglobin ( $r = -0.34$ ,  $p = 0.03$ ) (Figure 6), serum iron ( $r = -0.30$ ,  $p = 0.04$ ), and serum ferritin ( $r = -0.33$ ,  $p = 0.03$ ) (Figure 8). A positive but statistically non-significant correlation was observed between TIBC and HbA1c levels ( $r = 0.19$ ,  $p = 0.15$ ) (Figure 7).

## Discussion

Glycated hemoglobin (HbA1c) is widely accepted as a reliable marker of long-term glycemic control and endorsed as a diagnostic criterion for diabetes mellitus by major international bodies.<sup>1,2</sup> However, accumulating evidence indicates that HbA1c levels can be influenced by factors unrelated to plasma glucose concentration, particularly conditions affecting erythrocyte lifespan, hemoglobin structure, and erythropoiesis, such as iron-deficiency anemia (IDA). Iron deficiency anemia continues to be a major public health concern in developing countries including India, making the interpretation of HbA1c levels in this population clinically significant.<sup>7</sup>

The present study evaluated HbA1c levels in individuals with iron deficiency anemia and compared them with those in age- and sex-matched healthy controls. Both groups were demographically comparable, minimizing confounding variables. Laboratory confirmation of IDA was established using hematological and iron profile parameters. As expected, patients with IDA demonstrated significantly reduced hemoglobin, hematocrit, MCV, MCH, and MCHC with increased RDW. Additionally, serum iron and ferritin levels significantly decreased, whereas TIBC levels significantly increased, confirming iron deficiency.<sup>7-9</sup>

Normoglycemia was confirmed in both groups using fasting and post-prandial plasma glucose measurements, excluding hyperglycemia as a confounding factor. However, the HbA1c levels were significantly higher in the IDA group than in the control group. These findings support the growing evidence that iron-deficiency anemia may result in falsely elevated HbA1c values, independent of the glycemic status.<sup>3,5,10</sup>

Recent systematic reviews and population-based studies have corroborated these findings, demonstrating higher HbA1c levels in iron-deficient individuals, with significant reductions following iron supplementation.<sup>3,4,10-12</sup> The proposed mechanisms include prolonged erythrocyte lifespan, altered hemoglobin quaternary structure, and increased susceptibility of beta-globin chains to non-enzymatic glycation.<sup>13,14</sup> Reduced erythropoiesis in iron deficiency leads to an increased mean age of circulating red blood cells, thereby increasing cumulative glycation and HbA1c concentration.<sup>14</sup>

Correlation analysis in the present study revealed a significant negative correlation between HbA1c and hemoglobin, serum iron, and serum ferritin levels, and a positive correlation between HbA1c and TIBC. Similar associations have been reported in recent studies in India and other populations, thus reinforcing the relationship between iron status and HbA1c levels.<sup>5,11,15</sup>

The clinical implications of these findings are substantial. Recent consensus statements and expert reviews caution that reliance on HbA1c alone for diagnosing diabetes or prediabetes in individuals with anemia may lead to misclassification, particularly when HbA1c values are near diagnostic thresholds.<sup>2,4,16</sup> The American Diabetes Association and IFCC recommend that conditions affecting red cell turnover be considered when interpreting HbA1c results.<sup>1,2</sup>

In conclusion, the present study demonstrated that iron deficiency anemia is associated with significantly elevated HbA1c levels, despite normal blood glucose concentrations. Given the high prevalence of IDA in India and the widespread use of HbA1c for diagnosing diabetes, assessment of iron status should be considered an essential adjunct to HbA1c interpretation. Larger prospective studies are warranted to further define correction strategies and improve the diagnostic accuracy in anemic populations.

### Conclusion

The present study demonstrated that iron deficiency anemia is associated with significantly elevated HbA1c levels despite normal fasting and post-prandial blood glucose levels.<sup>1-3</sup> HbA1c showed a significant negative correlation with hemoglobin, serum iron, and serum ferritin, and a positive correlation with total iron-binding capacity, indicating a clear influence of iron status on HbA1c values independent of glycemia. Given the high prevalence of iron deficiency anemia in the Indian population and the widespread use of HbA1c as a diagnostic and monitoring tool for diabetes mellitus, the assessment of iron status should be considered when interpreting HbA1c results to avoid misclassification of glycemic status.<sup>1,2,7</sup> Further large-scale prospective studies are warranted to validate these findings and to establish appropriate correction strategies for HbA1c interpretation in anemic individuals.<sup>2,8</sup>

### Clinical Significance

Iron deficiency anemia can lead to spuriously elevated HbA1c levels in normoglycemic individuals, potentially resulting in misclassification as diabetes or prediabetes when HbA1c values are close to the diagnostic cut-off points.<sup>1,3</sup> In populations with a high prevalence of anemia, such as in India, reliance on HbA1c alone for the diagnosis or monitoring of diabetes may therefore be misleading. The assessment of iron status should be considered before interpreting HbA1c results, and the correction of iron deficiency may improve the accuracy of HbA1c-based diagnosis and management.<sup>1,2,7</sup>

**Table I. Distribution of age in cases and controls**

Group	n	Age (years) (Mean ± SD)	p value
Case (IDA)	100	39.23 ± 9.57	>0.05

Group	n	Age (years) (Mean ± SD)	p value
Control	100	37.83 ± 9.08	

Values are expressed as mean ± standard deviation (SD). Statistical analysis was performed using unpaired Student's t-test.  $p < 0.05$  was considered statistically significant. IDA: Iron deficiency anemia.

Table II. Sex distribution of subjects in case and control groups

Sex	Case (n=100)	Control (n=100)
Male	20	20
Female	80	80

Data are presented as number (percentage). The groups were matched for sex distribution. No statistically significant difference was observed (Chi-square test;  $p > 0.05$ ).

Table III. Comparison of hematological parameters between case and control groups

Parameter	Case (Mean ± SD)	Control (Mean ± SD)	p value
Hb (g/dL)	8.9 ± 1.5	13.83 ± 1.04	0.0001*
HCT (%)	26.64 ± 5.2	40.97 ± 3.4	0.0001*
MCV (fL)	70.03 ± 5.1	91.44 ± 3.87	0.0001*

Parameter	Case (Mean $\pm$ SD)	Control (Mean $\pm$ SD)	p value
MCH (pg)	23.51 $\pm$ 2.6	30.88 $\pm$ 1.59	0.0001*
MCHC (g/dL)	28.03 $\pm$ 1.74	33.86 $\pm$ 1.86	0.0001*
RDW (%)	19.14 $\pm$ 3.92	13.42 $\pm$ 0.64	0.0001*

\*Values are expressed as mean  $\pm$  SD. Statistical comparison was done using unpaired Student's t-test.  $p < 0.05$  was considered statistically significant. Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red cell distribution width.

**Table IV. Comparison of iron profile parameters between case and control groups**

Parameter	Case (Mean $\pm$ SD)	Control (Mean $\pm$ SD)	p value
Serum Iron ( $\mu$ g/dL)	31.9 $\pm$ 10.3	98.98 $\pm$ 21.25	0.0001*
TIBC ( $\mu$ g/dL)	399 $\pm$ 27.2	302.1 $\pm$ 33.47	0.0001*
Serum Ferritin (ng/mL)	6.17 $\pm$ 3.34	62.96 $\pm$ 18.33	0.0001*

\*Values are expressed as mean  $\pm$  SD. Comparison between groups was performed using unpaired Student's t-test.  $p < 0.05$  was considered statistically significant. TIBC: Total iron binding capacity.

**Table V. Comparison of fasting and postprandial blood sugar levels between case and control groups**

Parameter	Case (Mean $\pm$ SD)	Control (Mean $\pm$ SD)	p value
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Parameter	Case (Mean ± SD)	Control (Mean ± SD)	p value
Fasting blood sugar (mg/dL)	88.9 ± 9.03	84.70 ± 7.6	>0.05
Postprandial blood sugar (mg/dL)	120.00 ± 10.53	124.00 ± 9.08	>0.05

Values are expressed as mean ± SD. Statistical analysis was done using unpaired Student's t-test.  $p < 0.05$  was considered statistically significant. No significant difference was observed between groups.

**Table VI. Comparison of HbA1c levels between case and control groups**

Parameter	Case (Mean ± SD)	Control (Mean ± SD)	p value
HbA1c (%)	6.55 ± 0.77	5.27 ± 0.83	0.0001*

\*Values are expressed as mean ± SD. Comparison between groups was performed using unpaired Student's t-test.  $p < 0.05$  was considered statistically significant.

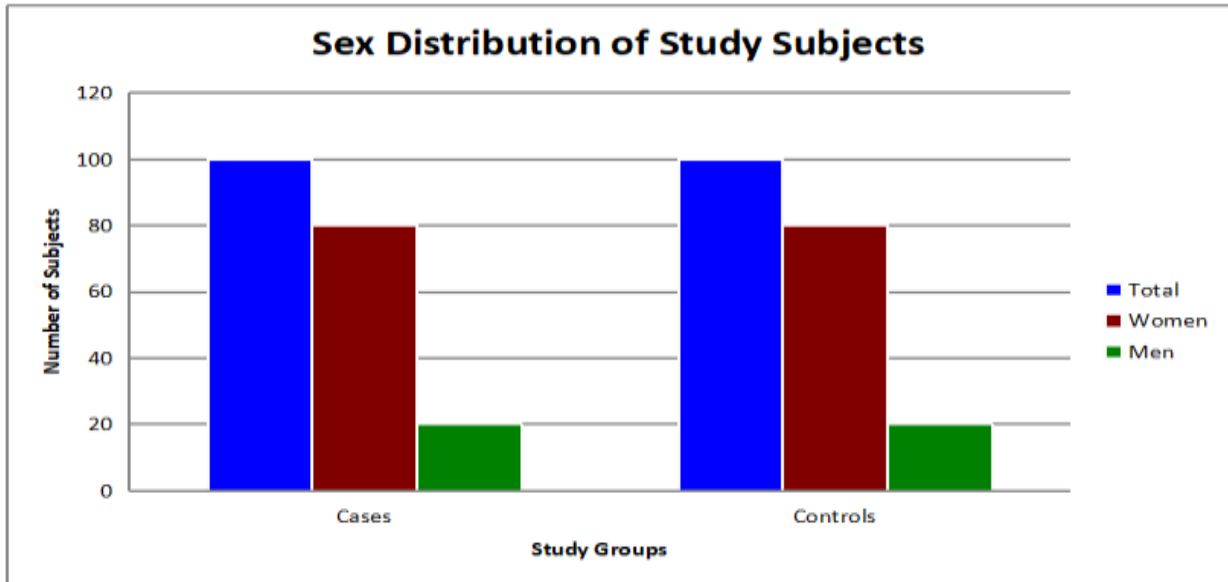
**Table VII. Pearson's correlation between hematological/iron parameters and HbA1c in IDA group**

Sl. No.	Parameter	Pearson's correlation coefficient (r)	p value
1	Hemoglobin	-0.34	0.03*
2	Serum Iron	-0.30	0.04*
3	TIBC	0.19	0.15
4	Serum Ferritin	-0.33	0.03*

\*Correlation analysis was performed using Pearson's correlation test.  $p < 0.05$  was considered statistically significant. A negative correlation indicates that HbA1c levels increased as the respective parameter decreased.

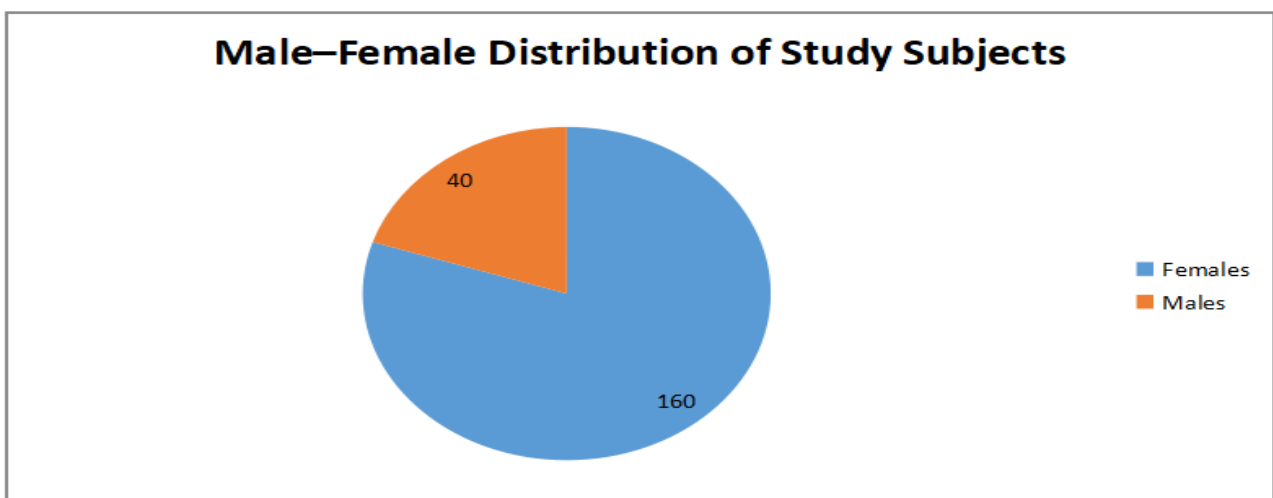
**Figure 1. Sex distribution of study subjects in case and control groups.**

Bar diagram showing comparable distribution of male and female participants between iron deficiency anemia (IDA) cases and healthy controls. No statistically significant difference was observed between groups ( $p > 0.05$ ).



**Figure 2. Frequency distribution of sex among iron deficiency anemia (IDA) cases.**

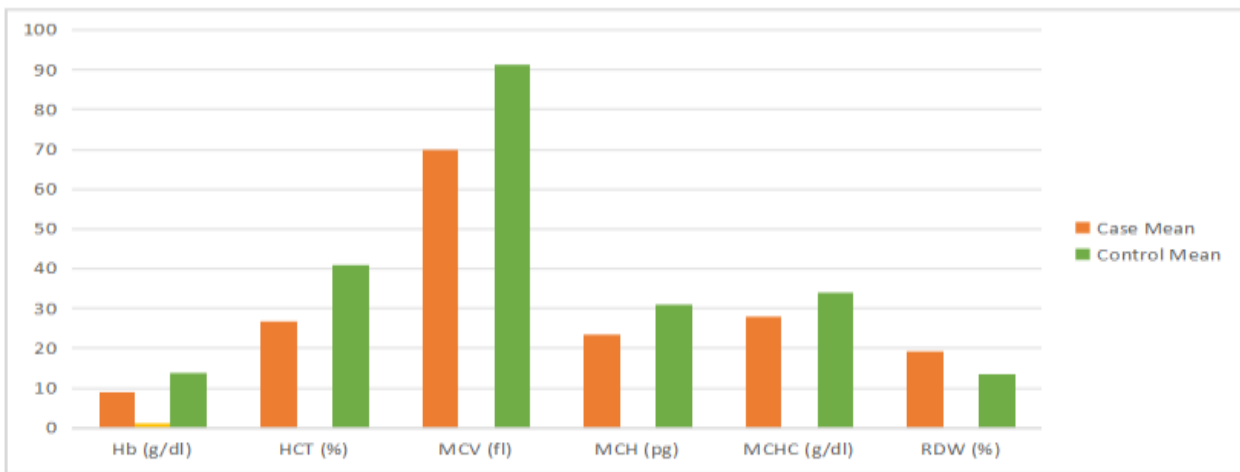
Pie chart illustrating predominance of female subjects among IDA cases. Females constituted the majority of affected individuals.



**Figure 3. Comparison of hematological parameters between case and control groups.**

Bar diagram demonstrating significantly lower mean hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in IDA cases compared to controls. Red cell distribution width (RDW) was significantly higher in the IDA group ( $p < 0.001$ ).

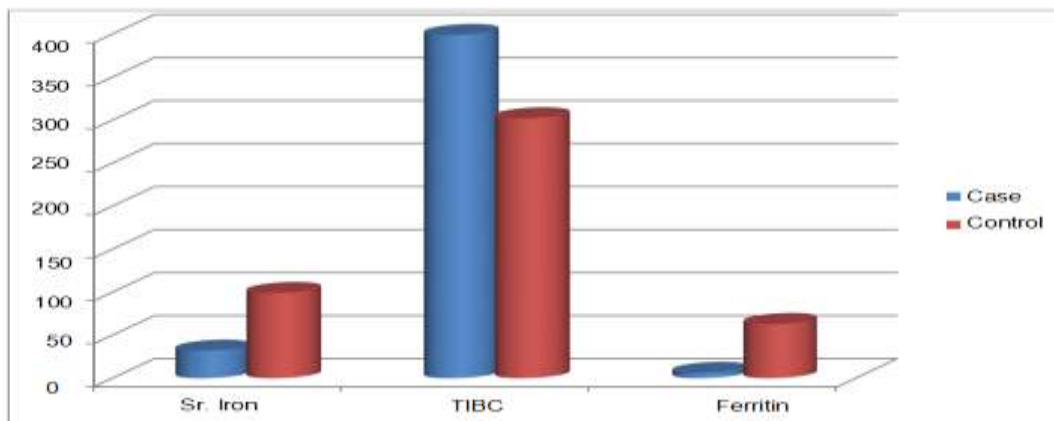
Abbreviations: Hb – hemoglobin; Hct – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; RDW – red cell distribution width.



**Figure 4. Comparison of serum iron profile parameters between case and control groups.**

Bar diagram showing significantly reduced serum iron and serum ferritin levels and significantly elevated total iron-binding capacity (TIBC) in IDA cases compared to controls ( $p < 0.001$ ).

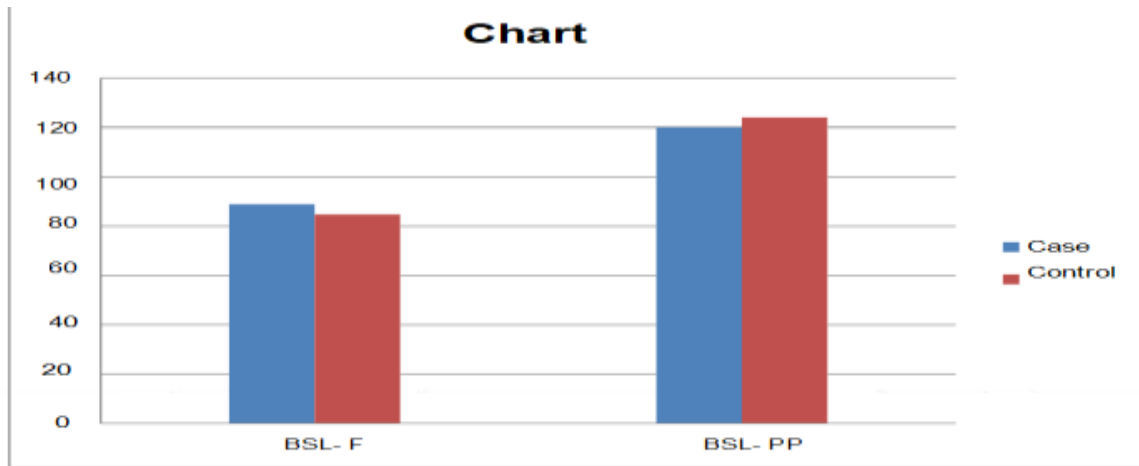
Abbreviations: TIBC – total iron-binding capacity.



**Figure 5. Comparison of fasting and postprandial blood glucose levels between case and control groups.**

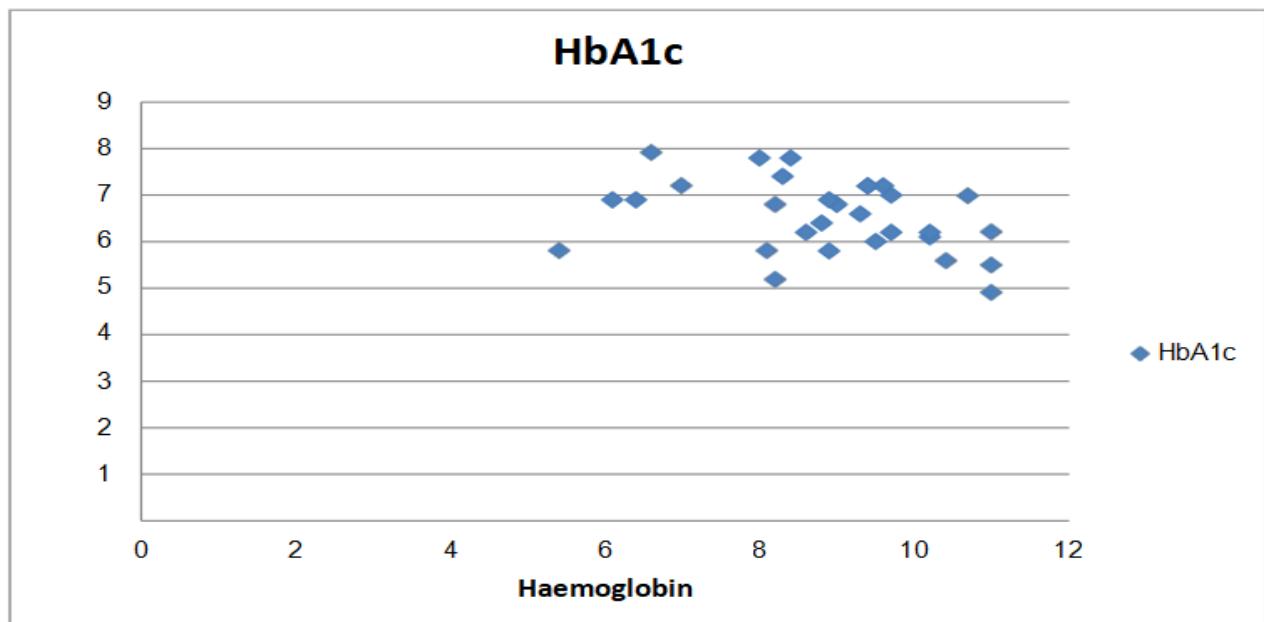
Bar diagram illustrating fasting blood sugar (BSL-F) and postprandial blood sugar (BSL-PP) levels in both groups. No statistically significant difference was observed ( $p > 0.05$ ), confirming normoglycemia.

Abbreviations: BSL-F – fasting blood sugar; BSL-PP – postprandial blood sugar.



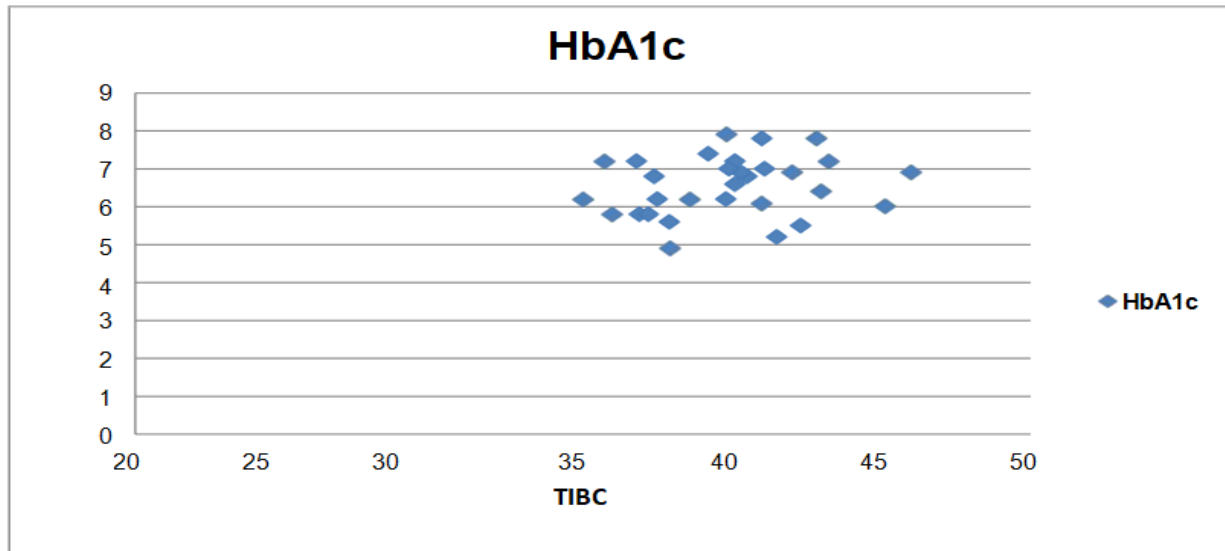
**Figure 6. Correlation between hemoglobin and HbA1c levels in IDA cases.**

Scatter plot showing a statistically significant negative correlation between hemoglobin and glycated hemoglobin (HbA1c) levels in IDA patients ( $r = -0.34$ ,  $p < 0.05$ ).



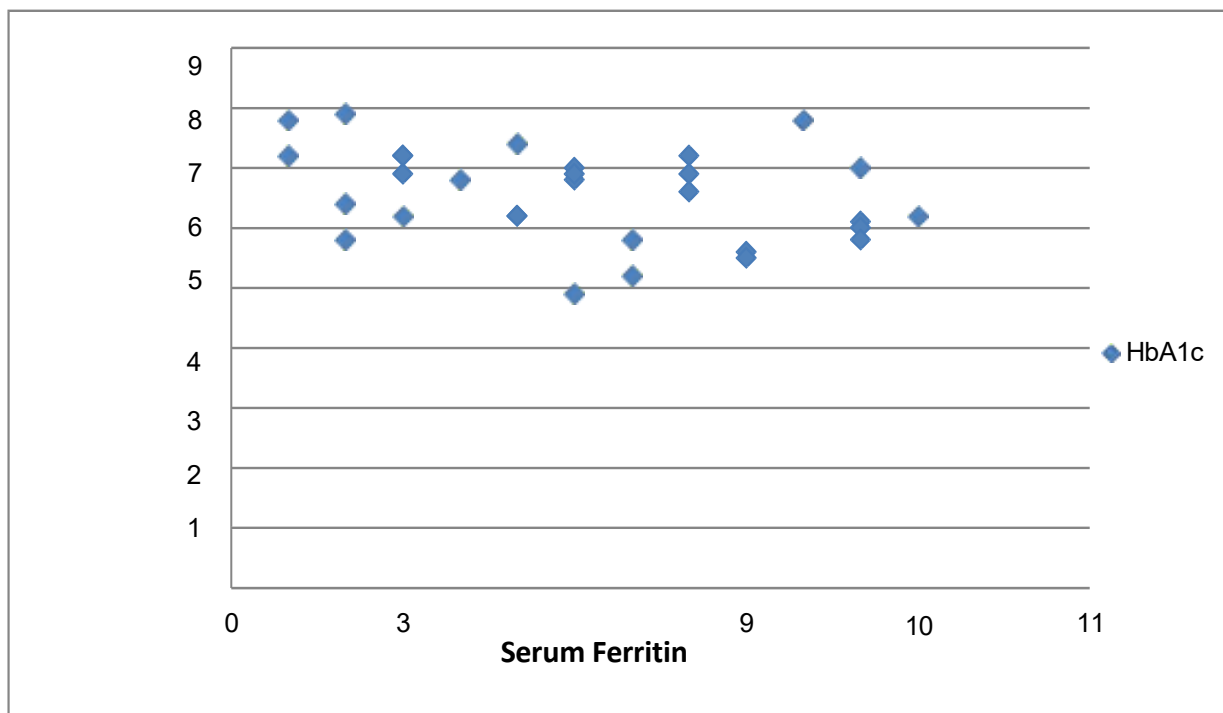
**Figure 7. Correlation between TIBC and HbA1c levels in IDA cases.**

Scatter plot demonstrating a positive, though statistically non-significant, correlation between total iron-binding capacity (TIBC) and HbA1c levels ( $r = 0.19$ ,  $p > 0.05$ ).



**Figure 8. Correlation between serum ferritin and HbA1c levels in IDA cases.**

Scatter plot illustrating a statistically significant negative correlation between serum ferritin and HbA1c levels in IDA patients ( $r = -0.33$ ,  $p < 0.05$ ).



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