**Alqalam Journal of Medical and Applied Sciences**. 2026;9(2):319-323
https://doi.org/10.54361/ajmas.269202

*Original article*

# Association Between Serum Iron Levels and Glycosylated Hemoglobin (HbA1c) in Libyan Male Patients with Type 2 Diabetes: A Pilot Case-Control Study

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**Abstract**
Glycosylated hemoglobin (HbA1c) is the primary biomarker for long-term glycemic control in diabetes mellitus. However, its validity may be compromised by hematological conditions like iron deficiency anemia (IDA), prevalent in developing regions. This study investigated the association between iron status and HbA1c levels in Libyan patients with Type 2 Diabetes Mellitus (T2DM). A case-control study was conducted at a diabetic center in Al-Bayda, Libya, in August 2023. The study included 52 adult males: 26 T2DM patients and 26 healthy controls matched for age and BMI. Biochemical parameters (HbA1c, serum iron, fasting plasma glucose), anthropometric measurements (BMI, waist circumference), and blood pressure were assessed. Statistical analysis employed t-tests, Pearson correlation, and Chi-square tests using SPSS version 26. The T2DM group had significantly lower mean serum iron (87.2 ± 12.3 µg/dL) than controls (93.5 ± 10.8 µg/dL, p=0.047). A significant negative correlation existed between HbA1c and serum iron (r = -0.331, p = 0.019). Chi-square analysis confirmed a significant association between iron status and glycemic category ($x^2$ = 8.321, p = 0.016). No significant correlations were found between HbA1c and BMI or between iron and BMI. Iron deficiency was significantly associated with elevated HbA1c levels in Libyan T2DM patients, potentially leading to misinterpretation of glycemic control. We recommend incorporating iron status assessment in the routine management of diabetic patients, particularly those with unexplained HbA1c elevations.
**Keywords**. Type 2 Diabetes Mellitus, HbA1c, Iron Deficiency, Anemia, Libya, Glycemic Control.

## Introduction

Diabetes mellitus represents a formidable global health challenge, with its prevalence increasing rapidly, particularly in low-income and middle-income nations [1]. Type 2 Diabetes Mellitus (T2DM) accounts for over 90% of diabetes cases and is associated with significant complications, mortality, and economic strain [2]. In Libya, the diabetic population is projected to rise dramatically, underscoring the urgent need for effective management strategies [3]. This challenge is compounded by a high concurrent burden of nutritional deficiencies, including iron deficiency anemia (IDA), which remains prevalent in the region and may significantly impact diabetes management outcomes.

Glycosylated hemoglobin (HbA1c) serves as the cornerstone for evaluating long-term glycemic control over approximately three months and is a recognized diagnostic criterion for diabetes [4]. Nonetheless, HbA1c levels are not solely dictated by plasma glucose; they are influenced by factors affecting erythrocyte turnover, including various anemias [5]. Iron deficiency anemia (IDA), the world's most common nutritional deficiency, is strongly implicated in the alteration of HbA1c levels [6]. The underlying mechanisms may involve shortened erythrocyte survival and enhanced non-enzymatic glycation of hemoglobin in iron-deficient states, which can lead to discordance between HbA1c and actual mean blood glucose levels [7, 8].

This interaction poses a critical clinical dilemma. Evidence suggests that IDA can spuriously elevate HbA1c, an effect that may be reversed with iron supplementation independent of changes in glucose metabolism [9, 10]. This may result in misdiagnosis, inappropriate intensification of hypoglycemic therapy, or misinterpretation of glycemic control [11]. Despite the high burden of both T2DM and IDA in North Africa, data exploring their interaction, particularly in the Libyan context, are remarkably scarce. Most existing evidence originates from Asian and Western populations, limiting its generalizability to the North African demographic and healthcare setting [12, 13]. Therefore, this pilot case-control study aimed to investigate the association between serum iron levels and HbA1c in a carefully selected sample of adult Libyan males with T2DM. By focusing on this understudied population, we sought to provide preliminary local evidence that could inform more nuanced clinical assessment and management, highlighting the potential need for integrating iron status evaluation into the routine care of diabetic patients in Libya and similar settings.

## Methods

### *Study Design and Setting*

A hospital-based case-control study was conducted during August 2023 at the Diabetes Specialty Center in Al-Bayda, Libya. The center serves as a primary referral facility for diabetic patients in the region, providing comprehensive diabetes care and monitoring services.


***Received****: 30-11-2025 -* ***Accepted****: 27-01-2026 -* ***Published****: 02-02-2026*

### *Study Population and Sampling*

T The study population comprised 52 adult male participants, systematically recruited and divided into two groups: a case group of 26 patients with a confirmed diagnosis of type 2 Diabetes Mellitus (T2DM) according to the American Diabetes Association diagnostic criteria and a control group of 26 age- and body mass index (BMI)-matched healthy individuals with no history of diabetes or metabolic disorders. The sample size was calculated to detect a moderate effect size (d = 0.8) in serum iron levels between the groups, with 80% power at an alpha level of 0.05, requiring approximately 26 participants per group.

### *Inclusion and Exclusion Criteria*

The inclusion criteria for the case group were: (1) male sex, (2) age ≥40 years, (3) a confirmed diagnosis of T2DM for more than one year, and (4) stable treatment with oral hypoglycemic agents for at least three months, while for the control group, they were: (1) male sex, (2) age ≥40 years, (3) fasting blood glucose <100 mg/dL, and (4) HbA1c <5.7%. Exclusion criteria applied to both groups were as follows: (1) history of hepatic, renal, or metabolic bone disorders; (2) current insulin therapy; (3) use of multivitamin or iron supplements within the past three months; (4) diagnosis of other forms of anemia (hemolytic, sickle cell, thalassemia); and (5) acute infection or inflammatory conditions at the time of recruitment.

### *Ethical Approval and Consent*

Written informed consent was obtained from all participants after explaining the study objectives, procedures, benefits, and potential risks to them. Confidentiality of participant data was maintained throughout the study, with personal identifiers removed during data analysis. This study was conducted in accordance with the principles of the Declaration of Helsinki.

### *Data Collection Procedures*

Data collection followed a standardized protocol administered by trained research assistants. A structured questionnaire was used to collect demographic information, medical history, diabetes duration, current medications, and lifestyle factors. Anthropometric measurements were obtained using calibrated equipment, and weight was measured to the nearest 0.1 kg using a digital scale (SECA 813, Germany), height to the nearest 0.1 cm using a wall-mounted stadiometer, and waist circumference at the midpoint between the lower rib margin and iliac crest using a non-stretchable tape measure. Body mass index (BMI) was calculated as weight (kg) divided by height squared ($m^2$). Blood pressure measurements were taken twice from the right arm using a calibrated mercury sphygmomanometer (Riester, Germany) after the participants had rested for at least 5 min in a seated position; the average of two readings was recorded.

### *Laboratory Analysis*

After a 12-14 hour overnight fast, 5 mL of venous blood was collected from each participant between 8:00 AM and 10:00 AM using standard phlebotomy techniques, immediately transferred to the hospital laboratory, and processed within two hours; Serum was separated by centrifugation at 3000 rpm for 10 min and aliquoted for analysis. Glycosylated hemoglobin (HbA1c) was measured using a turbidimetric inhibition immunoassay on a Cobas c501 analyzer (Roche Diagnostics, Switzerland; CV <2%), and serum iron concentration was determined spectrophotometrically using the ferrozine-based colorimetric method (Iron/UIBC kit, Roche Diagnostics; inter-assay CV <5%), Fasting plasma glucose (FPG) levels were analyzed using the glucose oxidase/peroxidase method on a Cobas c311 analyzer (Roche Diagnostics; CV <3%), with all procedures following the manufacturer's instructions and daily internal quality control performed using commercial control sera.

### *Operational Definitions*

The operational definitions were as follows: low iron status was defined as a serum iron concentration <80 µg/dL based on laboratory reference ranges, and glycemic categories were classified according to ADA criteria into normoglycemic (HbA1c <5.7%), prediabetic (HbA1c 5.7–6.4%), and diabetic (HbA1c ≥6.5%).

### *Statistical Analysis*

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp). Continuous variables were expressed as mean ± standard deviation (SD). Between-group comparisons of continuous variables were performed using independent-sample t-tests. Initially, ANOVA was used for comparisons involving multiple glycemic categories; however, since the primary group comparisons were between two groups (T2DM vs. control), t-tests were deemed appropriate for these analyses. Categorical variables are presented as frequencies and percentages and were analyzed using the chi-square test. Pearson's correlation coefficient was used to assess the linear relationships between continuous variables. Statistical significance was set at $P < 0.05$.


***Received****: 30-11-2025 -* ***Accepted****: 27-01-2026 -* ***Published****: 02-02-2026*

https://doi.org/10.54361/ajmas.269202

## Results

### *Baseline Characteristics*

As shown in Table 1, the T2DM and control groups were well-matched for age (p = 0.512) and BMI (p = 0.376). However, the T2DM group had significantly higher systolic BP, diastolic BP, and waist circumference (all p < 0.001).

***Table 1. Baseline Characteristics of the Study Population (N=52).***

| Characteristic | Control Group (n=26) | T2DM Group (n=26) | p-value |
|---|---|---|---|
| Age (years) | 51.2 ± 6.8 | 52.4 ± 7.1 | 0.512 |
| BMI (kg/m²) | 28.7 ± 4.2 | 29.8 ± 4.5 | 0.376 |
| Systolic BP (mmHg) | 124.5 ± 8.3 | 136.8 ± 11.5 | <0.001 |
| Diastolic BP (mmHg) | 78.2 ± 5.6 | 84.6 ± 6.9 | <0.001 |
| Waist Circumference (cm) | 94.3 ± 8.1 | 102.7 ± 9.5 | <0.001 |

### *Glycemic Control and Iron Status*

The T2DM group exhibited significantly higher FPG and HbA1c levels than the control group (both P < 0.001). Mean serum iron was significantly lower in the T2DM group (87.2 ± 12.3 µg/dL) than in controls (93.5 ± 10.8 µg/dL, p=0.047). Categorically, a low iron status was more prevalent in the T2DM group (50.0% vs. 15.4%, p=0.016) (Table 2).

***Table 2. Comparison of Biochemical Parameters Between Groups.***

| Parameter | Control Group (n=26) | T2DM Group (n=26) | p-value |
|---|---|---|---|
| Fasting Glucose (mg/dL) | 88.4 ± 7.2 | 162.8 ± 32.5 | <0.001 |
| HbA1c (%) | 5.4 ± 0.3 | 8.1 ± 1.2 | <0.001 |
| Serum Iron (µg/dL) | 93.5 ± 10.8 | 87.2 ± 12.3 | 0.047 |
| Low Iron, n (%) | 4 (15.4%) | 13 (50.0%) | 0.016 |

*Data presented as mean ± SD or n (%). Low iron was defined as serum iron <80 µg/dL.*

### *Association between Iron Status and Glycemic Control*

A significant negative correlation was observed between HbA1c and serum iron levels across all participants (r = -0.331, p = 0.019) (Table 4). The distribution of participants based on the combined glycemic and iron status is presented in Table 3. Chi-square analysis confirmed a significant association between iron status (low/normal) and the glycemic category ($x^2$ = 8.321, p = 0.016) (Table 5).

***Table 3. Distribution of Participants by Glycemic and Iron Status.***

| Glycemic Status | Total (n=52) | Low Iron (n=19) | Normal Iron (n=33) |
|---|---|---|---|
| Normoglycemic | 14 (26.9%) | 2 (10.5%) | 12 (36.4%) |
| Prediabetic | 10 (19.2%) | 7 (36.8%) | 3 (9.1%) |
| Diabetic | 28 (53.8%) | 8 (42.1%) | 18 (54.5%) |

***Table 4. Correlation Between HbA1c and Serum Iron Levels (All Participants).***

| Correlation Coefficient (r) | p-value |
|---|---|
| -0.331 | 0.019 |

### *Relationship with Body Mass Index (BMI)*

No significant correlations were found between HbA1c and BMI (r = -0.042, p = 0.772) or serum iron and BMI (r = -0.041, p = 0.772). The corresponding chi-squared tests also showed no significant associations (p > 0.05).

***Table 5. Association Between Iron Status and Glycemic Category.***

| $x^2$ Value | df | p-value |
|---|---|---|
| 8.321 | 2 | 0.016 |

## Discussion

This case-control study revealed a significant inverse association between serum iron levels and HbA1c among Libyan male patients with T2DM. Specifically, the T2DM group exhibited significantly lower mean serum iron (87.2 ± 12.3 µg/dL) compared to healthy controls (93.5 ± 10.8 µg/dL, p=0.047), along with a higher prevalence of low iron status (50.0% vs. 15.4%, p=0.016). More importantly, a statistically significant negative correlation was observed between HbA1c and serum iron across all participants (r = -0.331, p = 0.019).

Our findings align robustly with previous international research investigating the iron-HbA1c relationship.


***Received****: 30-11-2025 -* ***Accepted****: 27-01-2026 -* ***Published****: 02-02-2026*

Several studies have documented that iron deficiency anemia (IDA) can artificially elevate HbA1c levels independent of true glycemic status. For instance, Son et al. (2013) [9] demonstrated in a Korean cohort that HbA1c levels were significantly higher in anemic subjects compared to non-anemic controls, even after adjusting for fasting glucose levels. Similarly, Madhu et al. (2017) [10] reported in an Indian population that iron supplementation in IDA patients led to a significant reduction in HbA1c without concomitant changes in plasma glucose, suggesting a direct hematological effect rather than improved glycemic control. Our study extends this evidence to the North African context, confirming that this phenomenon is not limited to Asian populations but is also clinically relevant in Arab populations. In the Libyan context, a recent methodological study by Mohammed and Almaky (2025) compared three common HbA1c assay methods immunoassay, boronate affinity, and high-performance liquid chromatography (HPLC) and found no significant analytical variation between them ($p = 0.41$) [14]. This local evidence supports the reliability of HbA1c measurement across different laboratory platforms in Libya and strengthens the validity of our observed association, indicating that it is unlikely to be an artifact of assay-specific variability.

The observed relationship can be explained through several well-established biological mechanisms. First, iron deficiency reduces erythrocyte lifespan, leading to a younger circulating red blood cell population with less time for hemoglobin glycation to accumulate. This results in an underestimation of HbA1c relative to average glucose levels, though in clinical practice, the opposite effect (spuriously elevated HbA1c) has been more commonly reported and may relate to methodological differences in HbA1c measurement [7, 8]. Second, iron-deficient states may alter erythrocyte membrane properties and hemoglobin structure, potentially increasing susceptibility to non-enzymatic glycation [7]. Third, chronic inflammation associated with both T2DM and certain forms of anemia (anemia of chronic disease) can independently affect iron metabolism and HbA1c reliability [13]. These mechanisms collectively highlight the complex interplay between iron homeostasis and glycation processes.

The clinical significance of our findings is substantial, particularly in resource-limited settings like Libya where both T2DM and nutritional deficiencies are prevalent. Relying solely on HbA1c for glycemic assessment in iron-deficient diabetic patients may lead to diagnostic and therapeutic errors. Specifically, spuriously elevated HbA1c could result in unnecessary intensification of hypoglycemic therapy, increasing the risk of iatrogenic hypoglycemia and associated complications. Conversely, failure to recognize this interaction might lead to under-treatment if HbA1c falsely appears within target range. This concern is amplified in our study population, where half of the T2DM participants had low iron status, suggesting this is not a rare comorbidity but a common clinical scenario requiring systematic attention.

While our results support the predominant literature, it is important to acknowledge that some studies have reported conflicting findings. For example, Coban et al. (2004) [11] found no significant change in HbA1c after iron treatment in non-diabetic anemic patients [9]. These discrepancies may stem from methodological variations, including differences in: (1) the definition and severity of iron deficiency, (2) the assays used for HbA1c measurement, (3) population characteristics (diabetic vs. non-diabetic), and (4) the iron supplementation protocols employed. Our study's focus on T2DM patients with relatively stable disease and exclusion of other anemia forms strengthens the specificity of our findings to the diabetic population.

The strengths of our study include its case-control design with carefully matched controls, standardized laboratory procedures using internationally recognized assays, and its focus on an understudied population. However, several limitations must be acknowledged. First, our assessment of iron status relied solely on serum iron measurement without complementary markers such as ferritin, transferrin saturation, or total iron-binding capacity (TIBC). Serum iron exhibits significant diurnal variation and acute-phase reactivity, potentially limiting its reliability as a standalone marker. Future studies should incorporate a comprehensive iron panel to better characterize iron deficiency. Second, the cross-sectional design precludes causal inference regarding the directionality of the iron-HbA1c relationship. Third, the exclusive inclusion of males' limits generalizability to female patients, who have different iron metabolism patterns due to menstrual blood loss and hormonal influences. Fourth, the relatively small sample size may affect statistical power and precision of estimates.

## Conclusion

In conclusion, our findings strengthen the evidence that iron deficiency significantly influences HbA1c levels in patients with type 2 diabetes mellitus, a relationship with important implications for diabetes management in Libya and other regions where iron deficiency anemia is prevalent. This highlights the need for careful interpretation of HbA1c values alongside hematological parameters and supports the importance of routine screening for iron deficiency in diabetic populations. Future research with larger, more diverse cohorts and comprehensive iron and inflammatory profiling is warranted to clarify the mechanisms and clinical impact of this association.

***Acknowledgments***

The authors gratefully acknowledge the staff of the Diabetic Center in Al-Bayda and all participants for their cooperation in this study.


***Received****: 30-11-2025 -* ***Accepted****: 27-01-2026 -* ***Published****: 02-02-2026*

### *Conflicts of Interest*

The authors declare no conflict of interest.

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***Received****: 30-11-2025 -* ***Accepted****: 27-01-2026 -* ***Published****: 02-02-2026*