Influence of iron deficiency anemia on glycated hemoglobin levels in non-diabetic Saudi women

Iman Abdullah Bindayel

Abstract
Objective: Studies of patients with iron deficiency anemia (IDA) have shown a relationship between high glycated hemoglobin (HbA1c) and low hemoglobin (Hb) concentration. The present study was conducted to determine the influence of IDA on HbA1c in non-diabetic women.

Methods: Fifty-nine Saudi women (20 to 50 years old) were enrolled and categorized into groups according to their circulating hemoglobin concentration: Non-IDA (Hb ≥7.45 mmol/L; n = 38) and IDA (Hb ≤7.44 mmol/L; n = 21). The IDA group was further subdivided according to the severity of the IDA, as follows: mild (Hb 6.83 to 7.44 mmol/L; n = 9) and moderate–severe (Hb <6.83 mol/L; n = 12). HbA1c, Hb, ferritin, fasting blood glucose, and red blood cell (RBC) count were measured in each participant.

Results: HbA1c did not significantly differ between the groups, but the absolute HbA1c level was significantly lower in the mild and moderate–severe anemia groups than the non-anemic group, and was positively associated with Hb, ferritin, and RBC count. In addition, the HbA1c level was inversely associated with the Hb concentration.

Conclusions: HbA1c is significantly associated with parameters related to IDA in non-diabetic Saudi women. Therefore, assessment of IDA-related parameters is recommended prior to making a diagnosis of diabetes.

Keywords
High glycated hemoglobin, anemia, iron deficiency, diabetes mellitus, hemoglobin, serum ferritin, high-performance liquid chromatography

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Introduction
Iron deficiency is a condition that begins with the progressive depletion of iron stores due to a low intake of dietary sources of iron, high iron loss (e.g., through blood loss), or inadequate intestinal iron absorption. Consequently, the synthesis of iron-containing proteins, such as hemoglobin (Hb), is compromised. When a patient's Hb concentration falls below a specified threshold (<7.45 mmol/L for non-pregnant women and <8.07 mmol/L for men), they are described as having iron deficiency anemia (IDA). Anemia is a major public health problem in many parts of the world and affects approximately 30% of the global population. Young, fast-growing children, women of reproductive age, and pregnant women are among the most commonly affected individuals. A study conducted in the Kingdom of Saudi Arabia revealed a high prevalence of anemia in school-age children in various parts of the country, with incidences ranging between 26% and 41.3%. Menstruating girls are twice as likely to be anemic than non-menstruating girls.

Previous epidemiologic studies have linked the IDA-associated depletion of Hb with a high glycated hemoglobin A1c (HbA1c) level; however, the mechanism of this relationship has yet to be elucidated. It is speculated that modification in the Hb backbone, delayed turnover, and the longer lifespan of red blood cells (RBCs) that is associated with IDA predispose the molecule to a higher rate of glycation. HbA1c level is commonly measured for the diagnosis of type 2 diabetes mellitus (DM), which is highly prevalent among Saudi adults (crude prevalence of 23.1% in 2010). The measurement of HbA1c provides a measure of the mean blood glucose concentration over the preceding 2 to 3 months. However, it has been suggested that the HbA1c level does not only reflect glycemia, but also that it is sensitive to alterations in Hb concentration. Therefore, when IDA is not diagnosed, the use of HbA1c testing alone may result in a misdiagnosis of DM. Therefore, it is crucial to assess patients for hematological and IDA-associated risk factors that may influence the outcome of this diagnostic investigation.

The effect of IDA on HbA1c level has been documented in patients with and without DM. However, little is known regarding the relationship between HbA1c and blood indices of iron storage in non-diabetic Arabic women. The public health significance of anemia in Saudi women is considered to be substantial by the World Health Organization. In 2011, it was estimated that 40% of non-pregnant women in Saudi Arabia have a blood hemoglobin concentration of <7.45 mmol/L and 1.4% have a concentration of <4.34 mmol/L.

Therefore, in the present study, I aimed to determine the relationships between HbA1c and circulating measures of iron status in adult Saudi women with and without IDA. Specifically, I aimed to determine whether low Hb is accompanied by high HbA1c in women with IDA and whether the severity of the anemia affects this relationship.

Patients and methods

Participants
Female staff and students of King Saud University who were aged between 20 and 50 years were invited to participate in this case–control study. Each had their Hb concentration measured and were then allocated to two groups according to the presence or absence of IDA: an IDA group (n = 21) and a non-IDA group (n = 38). Ethics approval was obtained from the Ethics committee of the College of Applied
Medical Sciences of King Saud University, Riyadh, Saudi Arabia and written informed consent to participate in the study was obtained from all participants. All the participants completed a questionnaire regarding their use of food supplements and medication, history of major surgery, history of active and passive smoking, diet, duration and intensity of physical activity (PA), and duration and severity of menstrual periods. The study was performed between December 2015 and April 2016. Individuals with abnormal renal function, impaired fasting glucose, impaired glucose tolerance, hypothyroidism, or a type of anemia other than IDA were excluded, as were pregnant and lactating women and those who had been taking iron or multi-nutrient supplements during the preceding 3 months. Hb variants were not assessed in this study because the participants had no history of hemoglobinopathies. Blood glucose concentration was also measured randomly to exclude individuals who had DM or pre-DM.

**Anthropometrics**

Body mass (kg) was measured using an electronic scale and height (m) was measured using a stadiometer. Body mass index (BMI) was calculated using these two measures. Individuals with a BMI of <18.5 kg/m² were excluded from the study.

**Blood collection and measurements**

The participants made two visits to the clinic at the College of Applied Medical Science at King Saud University. At the first visit, they were screened for IDA by measuring their Hb concentration using a hemoglobinometer (*HemoCue*, AB Leo Diagnostics, Helsingborg, Sweden), signed the consent form, completed the questionnaire, and had their body mass, height, and fasting blood glucose (FBG) measured. At their second visit, the fasted participants had 10 to 15 mL of blood drawn, which was placed into two tubes (an EDTA and a serum tube) that were labeled with the participants’ assigned codes and refrigerated at 4°C for ≤4 hours. The derived serum samples were aliquoted into sterile 1-mL Eppendorf tubes, and both blood and serum samples were sent to the Hematology and Biochemistry Department of King Khalid University Hospital for analysis. FBG was measured using an Accu-Chek glucometer (Bio-Dynamics, Boehringer Mannheim, Indianapolis, IN, USA), with a reference range of <6.1 mmol/L. The normal range for Hb concentration was 7.45 to 9.93 mmol/L in women, and the cut-off value for anemia was <7.44 mmol/L. The HbA1c level (normal range 4% to 6%) of whole blood was analyzed using high-performance liquid chromatography (Bio-Rad D10 analyzer, Hercules, CA, USA). The intra-assay and inter-assay coefficients of variation (n = 10) were 0.56% and 0.60%, respectively. The absolute HbA1c concentrations were calculated from the measured HbA1c levels using the following formula:

\[
\text{Absolute HbA1c (mmol/L)} = \frac{\text{HbA1c} \% \times \text{Hb (mmol/L)}}{100}
\]

The total blood count was analyzed by SFRI diagnostics (SFRI, Berganton, France). Finally, serum ferritin was analyzed using an electrochemiluminescence immunoassay on a Modular E-170 Immunology Analyzer (Roche Diagnostics, Mannheim, Germany).

**Definition of anemia**

For the purpose of this study, non-IDA participants were defined as having a Hb concentration of ≥7.45 mmol/L. Participants with an Hb concentration ≤7.44 mmol/L, a mean corpuscular volume (MCV) <80
fL, a mean corpuscular hemoglobin (MCH) <1.61 fmol/cell, a mean corpuscular hemoglobin concentration (MCHC) <19.9 mmol/L, and/or a serum ferritin concentration <33.7 pmol/L were considered to have IDA and included in the IDA group. Participants in the IDA group were further subdivided according to the severity of their anemia, as follows: mild (Hb 6.83 to 7.44 mmol/L) and moderate–severe (Hb < 6.83 mol/L).15

**Statistical analysis**

Data are reported as means ± standard deviations. The datasets were tested for normality using the Kolmogorov–Smirnov test (p > 0.05 for all). Following testing, datasets were compared using the analysis of variance (ANOVA), followed by Tukey’s post-hoc test, and a p-value of <0.05 was considered to represent statistical significance. Statistical analyses were performed using SPSS 25.0 (IBM Inc. Armonk, NY, USA). Analyses of the responses to the lifestyle questionnaire by the three Hb groups were conducted using Fisher’s exact test (<5 cell count) or the chi-square test (>5 cell count) and corrected using Bonferroni’s correction for multiple comparisons; a p-value of <0.016 was considered to represent statistical significance. The relationships between the severity of anemia (severe, moderate, mild, or absent) with the outputs of the health lifestyle, and diet questionnaire were analyzed using Spearman’s correlations. Pearson’s correlation was used to determine the relationship between HbA1c and the measured blood parameters. A p-value of <0.05 was considered to represent statistical significance in these analyses.

**Results**

Fifty-nine participants were enrolled, among whom the prevalence of IDA was 36% and 20% had an Hb concentration <6.83 mmol/L. Table 1 shows the characteristics of participants, according to the severity of their anemia. Participants with IDA had a mean HbA1c of 5.4 ± 0.4%.

| Table 1. Characteristics of the participants, according to the severity of anemia (n = 59). |
|---------------------------------------------------|-----------------|-----------------|-----------------|----------------|
| N (%)                                             | No anemia       | Mild anemia     | Moderate–severe anemia | p-value       |
| N (%)                                             | 38 (64)         | 9 (16)          | 12 (20)           |               |
| Age (years)                                       | 21.0 ± 2.0      | 21.0 ± 1.0      | 24.0 ± 8.0        | NS            |
| BMI (kg/m²)                                       | 24.0 ± 4.1      | 22.0 ± 2.8      | 24.2 ± 4.1        | NS            |
| FBG (mmol/L)                                      | 4.5 ± 0.4       | 4.7 ± 0.6       | 4.6 ± 0.4         | NS            |
| HbA1c (%)                                         | 5.15 ± 0.36     | 5.39 ± 0.38     | 5.40 ± 0.35       | 0.052         |
| Absolute HbA1c (mmol/L)                           | 0.430 ± 0.038*† | 0.384 ± 0.025*  | 0.335 ± 0.025     | <0.001        |
| Hb (mmol/L)                                       | 8.19 ± 0.43*†   | 7.14 ± 0.19*    | 6.21 ± 0.50       | <0.001        |
| Ferritin (pmol/L)                                 | 8.43 ± 6.16*†   | 3.51 ± 3.15*    | 0.97 ± 0.40       | <0.001        |
| RBC count (10⁶/μL)                                | 4.7 ± 0.4       | 4.6 ± 0.4       | 4.7 ± 0.3         | NS            |
| MCV (fl)                                          | 83.8 ± 16.3*†   | 79.1 ± 5.3*     | 69.9 ± 5.3        | 0.012         |
| MCH (mmol/L)                                      | 1.76 ± 0.12*    | 1.54 ± 0.14*    | 1.33 ± 0.11       | <0.001        |
| MCHC (mmol/L)                                     | 20.2 ± 0.6*     | 19.4 ± 0.5*     | 19.1 ± 0.4        | <0.001        |

Results are shown as means ± standard deviations for continuous variables. *Denotes significance versus moderate–severe anemia by ANOVA followed by Tukey’s post-hoc test, p < 0.05; †Denotes significance versus mild anemia using ANOVA followed by Tukey’s post-hoc test, p < 0.05.

BMI, body mass index; Hb, hemoglobin; HbA1c, glycated hemoglobin A1c; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; FBG, fasting blood glucose.
which was not significantly higher than that of the non-IDA group (5.15±0.36%). In addition, the HbA1c level value did not differ according to the severity of the anemia (mild 5.39±0.38% and moderate–severe 5.40±0.35%, p > 0.05; Table 1). However, the absolute HbA1c concentrations significantly differed according to the Hb distribution (p < 0.05; Table 1; Figure 1), and the mean FBG did not differ between the mild and moderate–severe IDA groups and the non-IDA group.

As expected, the Hb, ferritin, MCV, MCH, and MCHC were significantly lower in the IDA group (p-values: < 0.001, < 0.001, 0.012, < 0.001, and < 0.001, respectively), and the differences were more pronounced in the moderate–severe group (p < 0.05 for each). Table 2 shows the outputs of the health, lifestyle, and diet questionnaire, according to the severity of anemia. There was a significant difference in the prevalence of passive smoking (42% in the moderate–severe group versus 0% in the mild group and 13.2% in the non-IDA group, p < 0.01). All the participants in the moderate–severe group stated that they drank either coffee, tea, or carbonated drinks at mealtimes, and this habit was significantly more prevalent in the moderate–severe group than in the mild or non-IDA groups (p < 0.01). The majority of the participants in all the groups reported that they experienced menstrual periods of 4 to 7 days in length, and in the non-IDA group, fewer participants reported that they experienced menstrual periods of >7 days’ duration (p = 0.007). The severity of menstruation differed among the groups (Table 2, p = 0.002). The remaining variables did not significantly differ among the groups.

There was a significant inverse relationship between HbA1c and Hb concentration (Pearson correlation, R = −0.348; p < 0.01) and between HbA1c and other parameters relevant to the assessment of anemia: MCHC (R = −0.337; p < 0.01) and ferritin (R = −0.350; p < 0.01) (Figure 2). There were significant positive relationships between the absolute HbA1c concentration and serum ferritin, Hb, MCV, MCH, and MCHC (R = 0.293, p < 0.05; R = 0.820, p < 0.001; R = 0.619, p < 0.0001; R = 0.722, p < 0.0001; and R = 0.535, p < 0.0001, respectively). (Figure 3).

**Discussion**

In the present study, I aimed to determine the relationship between IDA and HbA1c level in adult Saudi women who did not have DM. Despite not identifying a statistically significant difference in HbA1c between the non-IDA and IDA groups or between the two groups with differing...
Table 2. Differences in the health, lifestyle, and diet questionnaire output according to the degree of anemia [n (%); percentage yes].

|                          | No anemia | Mild anemia | Moderate severe anemia | p-value |
|--------------------------|-----------|-------------|------------------------|---------|
| n                        | 38        | 9           | 12                     |         |
| Supplement use (n, %)    | 5 (13.2)  | 0 (0)       | 2 (16.7)               | NS      |
| Medication use (n, %)    | 2 (5.3)   | 0 (0)       | 0 (0)                  | NS      |
| Exposure to smokers (n, %) | 3 (7.9)  | 0 (0)       | 5 (41.7)               | 0.008   |
| Major Surgery (n%)       | 4 (10.5)  | 2 (22.2)    | 1 (8.3)                | NS      |
| Physical activity (n, %) |           |             | 0.008                  |         |
| 1–2 times/week           | 19 (50.0) | 4 (44.4)    | 6 (50.0)               |         |
| 3–4 times/week           | 18 (47.4) | 5 (55.6)    | 6 (50.0)               |         |
| 4–7 times/week           | 1 (2.6)   | 0 (0)       | 0 (0)                  |         |
| Duration of physical activity (n/%) |   |             | 0.008                  |         |
| <30 minutes              | 15 (39.5) | 3 (33.3)    | 6 (50.0)               |         |
| 30–60 minutes            | 19 (50.0) | 4 (44.4)    | 5 (41.7)               |         |
| >60 minutes              | 4 (10.5)  | 2 (22.2)    | 1 (8.3)                |         |
| Dieting (n, %)           | 11 (28.9) | 1 (11.1)    | 4 (33.3)               | NS      |
| Consumption of coffee or tea with meals (n, %) | 26 (68.4) | 3 (33.3) | 12 (100.0) | 0.001 |
| Consumption of carbonated drinks with meals (n, %) | 21 (55.3) | 3 (33.3) | 12 (100.0) | 0.001 |
| Duration of menstruation (n, %) |   |             | 0.007                  |         |
| 1–3 days                 | 1 (2.6)   | 0 (0)       | 1 (8.3)                |         |
| 4–7 days                 | 34 (89.5) | 5 (55.6)    | 7 (58.3)               |         |
| >7 days                  | 3 (7.9)   | 4 (44.4)    | 4 (33.3)               |         |
| Severity of menstruation (n, %) |   |             | 0.002                  |         |
| Low                      | 2 (5.3)   | 0 (0)       | 0 (0)                  |         |
| Medium                   | 36 (94.7) | 6 (66.7)    | 8 (66.7)               |         |
| High                     | 0 (0)     | 3 (33.3)    | 4 (33.3)               |         |

Categorical variables are expressed as numbers and percentages. Fisher’s exact test (2-sided) was used to compare the groups (cell count <5).

Figure 2. Scatter plots of HbA1c (%) versus hematologic parameters related to anemia. Pearson’s correlation. n = 59.
Hb, hemoglobin; HbA1c, glycated hemoglobin A1c; MCHC, mean corpuscular hemoglobin concentration.
severities of anemia, the effect of IDA on absolute HbA1c concentration, which is not affected by hemodilution, was more pronounced: the absolute HbA1c concentration was significantly lower in the mild and moderate–severe anemia groups than in the non-anemic group. In addition, lower absolute HbA1c concentration was associated with lower circulating indices of iron storage and RBC parameters. Lower serum ferritin concentration is linked to delays in RBC turnover, which increases the susceptibility of Hb molecules to glycation, resulting in a higher HbA1c. In the present study, I found inverse associations between HbA1c and Hb, ferritin, and MCHC.

The relationship identified in the present study is consistent with those identified in previous epidemiologic and clinical studies, in which high HbA1c levels were found in patients with IDA, whereas the absolute HbA1c concentration was low. However, some previous studies have generated conflicting results. It has been suggested that methodological variations, heterogeneity of the participants, and differences in study design may explain these differences in the magnitude and direction of the outcomes.

Because of the effect of hyperglycemia on HbA1c, the present study was carried in female participants who did not have DM to ensure the accuracy of the HbA1c data. Women who are iron deficient are at risk of an inaccurate classification of diabetes when HbA1c measurements are used. Therefore, it is necessary to assess all the relevant hematologic indices and IDA risk factors to avoid misdiagnoses and the resulting adverse effects on patient health.

Figure 3. Scatter plots of absolute HbA1c versus hematologic parameters related to anemia. Pearson’s correlation. n = 59. Hb, hemoglobin; HbA1c, glycated hemoglobin A1c; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.
Analysis of risk factors for IDA in the present study revealed significant associations of the length and severity of menstruation, exposure to second-hand smoke, and the consumption of caffeinated drinks with meals with IDA. As expected, women in the IDA groups tended to have heavier and longer menstrual periods than those in the control group. Excessive blood loss directly affects hematologic parameters and leads to anemia. In addition, the higher exposure to secondhand smoke reported by participants with moderate–severe anemia may have reduced their vitamin C levels, which could compromise iron absorption. Furthermore, women in the moderate–severe anemia group reported a significantly higher consumption of caffeinated drinks and tea with meals than those in the mild anemia group. Such beverages inhibit iron absorption because they contain polyphenols and caffeine, which can also reduce the circulating vitamin C concentration.

Previous clinical studies have investigated the effect of iron supplementation for the treatment of IDA on HbA1c level. However, these studies generated conflicting results. A few studies showed that this treatment reduced HbA1c, but another found no effect on this parameter.

There are several strengths to the present study. I recruited women because they are predisposed to IDA. However, women over the age of 50 were excluded because of the potential confounding effect of menopause, and diabetic participants were excluded because of their abnormal insulin–glucose metabolism. Both of these factors affect HbA1c level; therefore, the exclusion of individuals from these groups ensured greater accuracy of the study findings.

I must also acknowledge some limitations. The small sample size may have reduced the power of the study to detect differences between the IDA and non-IDA groups. Furthermore, the present findings cannot be generalized to other populations, and may only be applicable to women who do not have DM. Further larger studies that include men and perhaps children are needed to confirm whether IDA is linked to the apparent progression of DM in the Saudi population because of its influence on the glycation of hemoglobin A1c.

In summary, the absolute HbA1c concentration was found to be affected by the circulating Hb concentration in adult Saudi women, and the magnitude of this effect depended on the severity of the anemia. However, whether the severity of IDA increases the risk of DM and its complications remains to be determined. It is recommended that clinicians perform hematologic measurements relevant to IDA when HbA1c is used for the diagnosis of DM. Interventional studies with larger sample sizes are necessary to determine whether the correction of IDA normalizes HbA1c.

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The author declares that there is no conflict of interest.

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**ORCID iD**
Iman Abdullah Bindayel  
https://orcid.org/0000-0001-5452-115X
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