



## Influence of Iron Deficiency on HbA1c Levels in non- Diabetic Pregnant women.

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### ABSTRACT

Glycosylated hemoglobin (HbA1c) is the form of hemoglobin that is used widely to identify the average blood glucose levels of a person over the past three months and can correlate to complications of high blood glucose. This study aimed to determine the effect of iron deficiency anemia (IDA) on glycated hemoglobin (HbA1C) in non-diabetic pregnant women. This study was conducted on 86 pregnant women in the last trimester of pregnancy, 66 pregnant women had IDA and were non-diabetic, and 20 pregnant women were non-anemic as control, their ages were between 20 - 37 years, fasting blood sugar (FBS), glycated hemoglobin (HbA1C), hemoglobin (Hb), and the serum iron was determined on all blood sample. The mean HbA1c level was significantly lower in pregnant with IDA ( $4.42 \pm 0.89$ .) than in pregnant that have not IDA ( $5.35 \pm 0.38$ ),  $P < 0.05$ . Mild anemia was (56.1%), moderate anemia was (37.9%), and severe anemia was (6.06%), The mean HbA1C level in cases of mild anemia was slightly higher than in non-anemic pregnant, ( $5.42 \pm 0.69$ .,  $5.4 \pm 0.38$ ), with no significant differences, while in the cases of moderate anemia, the mean of HbA1C level was lower than non-anemic pregnant  $4.6 \pm 0.85$ , with significant differences at  $P < 0.05$ . the mean HbA1C level in cases of severe anemia was lower than its level in non-anemic pregnant ( $3.45 \pm 0.29$ ) with significant differences at ( $P < 0.05$ .), there was a significant decrease in the mean value of HbA1c in pregnant with IDA compared to that pregnant non-anemia, the presence of anemia in pregnant affects the HbA1C level.

**Keywords** Hb A1c · Iron deficiency anemia · Diabetes mellitus.

تأثير فقر الدم بعوز الحديد على مستوى الهيموجلوبين السكري لدى النساء الحوامل غير المصابات بالسكري

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### المخلص

الهيموجلوبين السكري هو شكل الهيموجلوبين المستخدم على نطاق واسع لتحديد متوسط مستويات الجلوكوز في الدم لدى الشخص خلال الأشهر الثلاثة الماضية ويمكن أن يرتبط أيضًا بمضاعفات ارتفاع نسبة السكر في الدم (داء السكري). هدفت هذه الدراسة لمعرفة تأثير فقر الدم بعوز الحديد على الهيموجلوبين السكري عند النساء الحوامل الغير مصابات بالسكري. أجريت هذه الدراسة على 86 امرأة في الأشهر الثلاثة الأخيرة من



الحمل، 66 نساء حوامل يعانين من فقر الدم الناجم عن نقص الحديد وغير المصابات بالسكري، و 20 امرأة حامل غير مصابة بفقر الدم كعينة تحكم، تراوحت أعمارهن بين 20 و 37 عامًا. تم قياس سكر الصيام في الدم ومستوى الهيموجلوبين السكري وحديد المصل في جميع العينات. كان متوسط مستوى الهيموجلوبين السكري أقل بشكل ملحوظ لدى الحوامل المصابات بفقر الدم نقص الحديد ( $4.42 \pm 0.89$ )، مقارنة بالحوامل الاتي يعانين من فقر الدم ( $0.38 \pm 5.35$ )، بوجود فروق معنوية عند مستوى دلالة اقل من 0.05، كان نسبة فقر الدم الخفيف (56.1%)، و فقر الدم المتوسط (37.9%)، وفقر الدم الشديد (6.06%)، متوسط الهيموجلوبين السكري في حالات فقر الدم الخفيف أعلى قليلاً منه في الحوامل غير المصابات بفقر الدم، ( $0.69 \pm 5.42$ )، مع عدم وجود فروق معنوية، متوسط مستوى الهيموجلوبين السكري أقل منه في الحوامل غير المصابات بفقر الدم ( $0.85 \pm 4.6$ ) بوجود فروق معنوية. كان متوسط مستوى الهيموجلوبين السكري في حالات فقر الدم الشديد أقل من مستواه في الحوامل غير المصابات بفقر الدم، ( $0.29 \pm 3.45$ ) عند مستوى دلالة اقل من 0.05. من هذه الدراسة لوحظ وجود انخفاضاً معنوياً في متوسط قيمة الهيموجلوبين السكري لدى الحوامل المصابات بفقر الدم الناجم عن نقص الحديد مقارنة بالحوامل غير المصابات بفقر الدم، وبالتالي فإن وجود فقر الدم لدى الحوامل يؤثر على مستوى الهيموجلوبين السكري.

**الكلمات الدالة:** الهيموجلوبين السكري · فقر الدم الناجم عن نقص الحديد · مرض السكري.

### Introduction:

Glycosylated hemoglobin (HbA1c) is a widely used biomarker to estimate an individual's average blood glucose level over the previous three months. This measure is also associated with possible complications resulting from blood sugar levels, especially in the context of diabetes mellitus. International guidelines support its use to evaluate the general management and control of this disease [1,2]. The HbA1c value is used by the American Diabetes Association (ADA) and the World Health Organization (WHO) to diagnose diabetes [3, 4]. An HbA1c of less than 5.7% is normal; a level of 5.7% to 6.4% indicates prediabetes; and a level of more than 6.5% indicates diabetes. [5]. HbA1c levels are influenced by various physiological factors that can have a significant impact on their measurement. In particular, hemoglobin abnormalities, such as those seen in patients with hemoglobinopathies (which may be genetic or chemical), fetal hemoglobin (HbF) and methemoglobin, can cause changes in HbA1c levels due to changes in the lifespan of red blood cells. In addition, altered erythropoiesis can lead to elevated HbA1c levels, usually associated with vitamin B12, folate and iron deficiency, and decreased erythropoiesis due to renal failure or malignancy. Conversely, false HbA1c values can occur when patients receive treatments such as erythropoietin, iron supplements, or vitamin B12 injections; this is also observed in cases of reticulocytosis and chronic liver disease. [6]. Abnormal glycation - Increased HbA1c value observed in alcoholism, chronic renal failure, decreased intraerythrocyte pH and decreased HbA1c value due to the ingestion of aspirin, vitamins C and E, certain hemoglobinopathies, increased intraerythrocyte pH. Destruction of red blood cells - Increase in HbA1c in cases where the life of red blood cells is increased (splenectomy, vitamin B12 or folate deficiency). [7] Low HbA1c levels in cases of reduced red blood cell life (hemoglobinopathy, splenomegaly, glucose-6-phosphate dehydrogenase



deficiency, sickle cell anemia, rheumatoid arthritis or medications such as antiretrovirals, ribavirin, and dapson). Analysis - Increased HbA1c in hyperbilirubinemia, carbamylated hemoglobin, alcoholism, high-dose aspirin, chronic opioid use, variable HbA1c values in hemoglobinopathy, and decreased HbA1c in hypertriglyceridemia. [8,9]. HbA1c levels have been found to decrease during the second trimester of a normal, non-diabetic pregnancy and increase during the third trimester [10]. Coban and colleagues reported that HbA1c levels could be artificially increased in patients with iron deficiency anemia [11]. In contrast, glycated albumin (GA) remains unchanged in the presence of iron deficiency. In particular, women with IDA have a slightly higher HbA1c level in premenopausal women than in those without such deficiencies [12]. It has also been proven that HbA1c tends to increase at the end of pregnancy [13]. Anemia is an important public health problem worldwide, affecting about 30% of the population, especially children in rapid growth phases, as well as women of reproductive age and pregnant women. [14,15]. Anemia is defined as a condition in which the level of hemoglobin (Hb) in the body is lower than normal, resulting in a decrease in the oxygen-carrying capacity of the red blood cells in the tissues [16]. It affects all age groups, but pregnant women and children are more vulnerable. Stevens *et al.*, [17] reported that the global prevalence of anemia in non-pregnant women, pregnant women, and children is 29%, 38%, and 43%, respectively. Studies indicate that the prevalence of anemia among pregnant women in Tripoli was 31.6%, with iron deficiency anemia accounting for 13% of cases [18]. In Sabrata, iron deficiency was found to be responsible for 50% of anemia cases among pregnant women [19]. According to WHO guidelines, anemia in pregnancy is defined as a hemoglobin level < 11 g/dl in the first trimester and less than 10.5 g/dl in the second and third trimester [20]. The prevalence of anemia is an important health indicator. A previous study found a relationship between IDA and HbA1c levels and tried to explain the difference in HbA1c levels in IDA using differences in hemoglobin structure and HbA1c levels in old and young red blood cells [21]. Research on the impact of IDA on HbA1c levels is conflicting; however, some studies show an increase in HbA1c, while others show a significant decrease in HbA1c in patients with IDA compared to control groups [22, 23]. This study aimed to determine the effect of iron deficiency anemia on glycated hemoglobin in non-diabetic pregnant women.

## Materials and methods:

### Patient and Methods

**Patients:** This study was conducted on 86 samples from pregnant women in the last trimester of pregnancy, 66 from pregnant women who suffer from iron deficiency anemia and not diabetic, 20 samples from pregnant women who do not suffer from anemia, their ages are between (20 -37 years).

### Laboratory Methods

Venous blood samples were collected from all pregnant women participating in the study after overnight fasting (8-12 hours). They were processed for the determination of by Spectrophotometer 4040, Fasting blood sugar was measured by used a calorimetric method using, commercial Kit (Biocon) based on glucose oxidase method using a glucose analyzer (BECKMAN, USA); glycated hemoglobin (HbA1C) percentage was determined according to a boronate affinity chromatography method



using NYCO Card reader II; hemoglobin level was measured using Sysmex KX21N hematology analyzer.

### Statistical Analyses

The data was entered into the program Microsoft Office Excel 2013, Data were statistically presented as means and standard deviations (SD). Paired t-test was used by the statistical program MINTAB version 16, to compare between two groups to find out whether there were significant differences and to evaluate the relationship between HbA1c levels and different variables, a P value  $\leq 0.05$  was regarded as statistically significant, Correlation between the different variables was tested by using Pearson moment correlation coefficient.

### Results:

Table No. (1) shows the comparison between the two groups., The Mean of FBS was similar between the two groups., results of the present study showed that there is a significant decrease ( $p < 0.05$ ) of the levels of Hb, HbA1C, and Iron in the pregnant with IDA compared to Non- anemic pregnant, ( $12.5 \pm 5.0$ ,  $8.7 \pm 1.5$ ), ( $5.4 \pm 0.38$ ,  $4.42 \pm 0.89$ ), ( $61.9 \pm 15$ ,  $16 \pm 9.95$ ) respectively.

**Table (1) Comparison of Hb, FBS, HbA1c and Iron levels in pregnant with IDA and non-anemic pregnant**

Parameter	IDA –pregnant (66) Mean $\pm$ SD	Non-anemic pregnant (20) Mean $\pm$ SD	P value
FBS (mg/dl)	83.7 $\pm$ 14.97	85.2 $\pm$ 0.11	0.38
Hb (g/dl)	8.7 $\pm$ 1.5	12.5 $\pm$ .50	0.000*
HbA1C (%)	4.42 $\pm$ 0.89	5.4 $\pm$ 0.38	0.000*
Iron ( $\mu$ g/dl)	16 $\pm$ 9.95	61.9 $\pm$ 15	0.000*

**SD: standard deviations, \* Significant.**

The cases of anemia were divided according to the severity of the anemia into mild anemia was 37 (56.1%), moderate anemia was 25 (37.9%), and severe anemia was 4 (6.06%), figure (4), The mean HbA1C level in mild anemia was slightly higher than in non-anemic pregnant, ( $5.42 \pm 0.69\%$ ,  $5.4 \pm 0.38\%$ ), with no significant differences, while in the cases of moderate anemia, the mean of HbA1C level was lower than non-anemic pregnant  $4.6 \pm 0.85\%$ , with significant differences at  $P < 0.05$ . Also, the mean HbA1C level in severe anemia was lower than its level in non-anemic pregnant,  $3.45 \pm 0.29\%$  with significant differences at  $P < 0.05$ . Table 2.

**Table (2) Comparison of Hb, FBS, HbA1c, and Iron levels in patients and controls (according to severity of anemia)**

Parameter	Pregnant with IDA			Non-anemic pregnant	P value
	Mild Mean $\pm$ SD	Moderate mean $\pm$ SD	Sever Mean $\pm$ SD		
FBS (mg/dl)	88.7 $\pm$ 14.9	83 $\pm$ 13	89 $\pm$ 18.9	85.2 $\pm$ 0.11	0.38
Hb (g/dl)	10.7 $\pm$ 0.4	8.5 $\pm$ 0.87	6.3 $\pm$ 0.4	12.5 $\pm$ .50	0.000*
HbA1C (%)	5.4 $\pm$ 0.69	4.6 $\pm$ 0.85	3.5 $\pm$ 0.46	5.4 $\pm$ 0.38	0.000*
Iron ( $\mu$ g/dl )	40 $\pm$ 0.4	33 $\pm$ 20.8	10.4 $\pm$ 3.8	61.9 $\pm$ 15	0.000*

SD: standard deviations, \* Significant

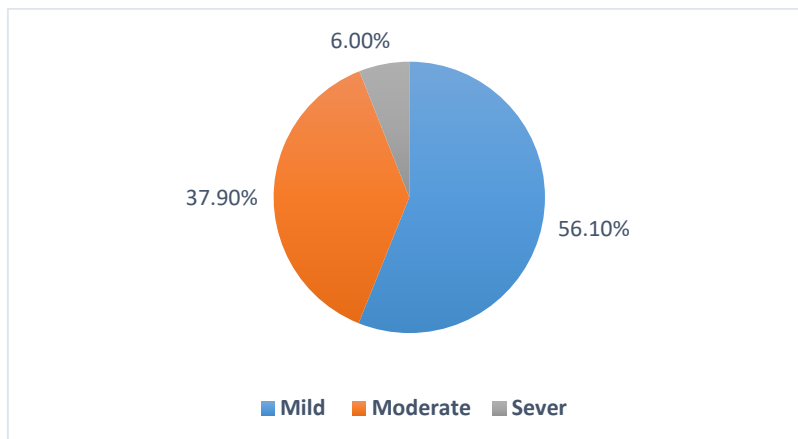


Figure (1): Distribution of anemia cases as regards severity of anemia

The Pearson correlation test showed a positive correlation between HbA1c levels and Hb levels was  $r=-0.614$  ( $p=0.000$ ); in anemic pregnancy, also Pearson's correlation between HbA1c levels and serum iron level was  $r=-0.401$  ( $p=0.000$ ); in anemic pregnancy.

### Discussion

iron deficiency anemia (IDA) are prevalent form of nutritional deficiency. Globally, 50% of anemia is attributed to iron deficiency. Reduced iron stores have been linked to increased glycation of hemoglobin A1C (HbA1c) [24-25]. Most previous studies were conducted on pregnant and non-pregnant women who did not have diabetes, although iron deficiency anemia is very common in pregnancy, to our best knowledge, there has been limited research investigating HbA1c levels in non-diabetic anemic pregnant women; therefore, this study was conducted on non-diabetic pregnant (a group with IDA and group without IDA) to determine the effect of iron deficiency anemia on HbA1C. In the present study, the results showed mean HbA1c level in Pregnant with IDA (4.619 %) was significantly lower than the non-anemic pregnant (5.446%) at ( $P < 0.05$ ). Although HbA1c levels were slightly lower, they were still in the normal reference range, these results are consistent with a study by Seher *et al*, conducted their study on 131 patients who had IDA, which showed their results that mean HbA1c level was significantly lower in the group with IDA (5.4%) than in the healthy control group (5.9%;  $p < 0.05$ ). [26]. In a study by Sinha *et al.*, [9]. involving 50 patients, the mean baseline HbA1c level was significantly lower in anemic patients (4.6%) compared to the control group (5.5%,  $P < 0.01$ ). Similarly, Sankar *et al*. also reported significantly lower baseline HbA1c levels in anemic patients compared to the control group [28]. Also similar to a study conducted by Nalini *et al*, on 120 patients who had IDA, the mean HbA1c level in IDA patients (4.619 %) was significantly lower than the control group (5.446 ± 0.281%). Their results showed A significant increase (5.816 %) was observed in the mean HbA1c of the anemia group after treatment [27]. In a research conducted by Bindayel in Saudi Arabia, a group of non-diabetic women was studied, including those with and without IDA. Within the IDA group, which consisted of 21 women, the mean HbA1c level in IDA patients was significantly lower than in the



control group. A significant increase was observed in the mean HbA1c of the anemia group after treatment. [29]. Similarly, McGill et al. (2017) suggested that IDA may falsely lower HbA1c measurements due to reduced exposure of hemoglobin to glucose during glycation. This mechanistic insight supports the observation of lower HbA1c levels in the present study. However, most of the literature focuses on mixed populations or individuals with diabetes, making the current findings in non-diabetic pregnant women particularly valuable. (30). In the current study, the mean HbA1C level in severe anemia was significantly low when compared to non-anemic pregnant at  $P < 0.05$ ., HbA1c levels were observed to decrease with severity anemia (4.42%, 4.6%, and 3.5%, respectively;  $p < 0.05$ ). There are few studies available that demonstrate the relationship between HbA1c and the severity of anemia. A study conducted by Seher *et al.*, reported that when hemoglobin levels decreased, a statistically insignificant decrease in the level of glycated hemoglobin was observed between the groups suffering from mild anemia, moderate anemia, severe anemia, and groups without anemia. [26]. In a recent study conducted by D'Sa Janice *et al*, 50 non-diabetic female patients with decreased levels of Hb, MCV, and MCHC were examined. The researchers found a positive correlation between HbA1c and serum ferritin, Hb, MCV, MCH, and MCHC ( $P < 0.05$ ). There was a significant decrease in the mean HbA1c in those with severe anemia compared to those with moderate anemia, and a significant decrease in the mean HbA1c among those with severe anemia compared to those with moderate anemia. This trend was consistent when comparing women with severe anemia to those with moderate anemia. These findings suggest that HbA1c levels decrease as the severity of (IDA) increases in non-diabetic women. [31]. A study done by Bindayel, divided the patients based on the severity of anemia: mild and moderate-severe. the mean HbA1C level in cases of mild anemia was slightly higher than. non-anemic pregnant, with no significant differences, while in the cases of moderate anemia, the mean HbA1C level was lower than non-anemic pregnant with significant differences at  $P < 0.05$ . [29]. On the other hand, the results of a study conducted by Omar *et al.*, on 154 non-diabetic pregnant women, showed no significant difference in the level of HbA1c between women with IDA and women with no IDA. Likewise, there was no significant difference in the median (interquartile) of HbA1c levels in anemic women compared with non-anemic women [32]. In contrast to the results of this study, a study by Basheer included 40 non-diabetic women has IDA and compared to 46 healthy women. The HbA1c levels in patients with IDA are elevated compared to the control group. [33].

The discrepancies between the findings of this study and previous researches can be attributed to differences in participant characteristics, physiological effects of anemia during pregnancy, measurement methods, geographical and nutritional factors, and study design. This study focused on non-diabetic pregnant women, whose altered red blood cell turnover and hemoglobin metabolism during pregnancy may influence HbA1c levels differently from other populations. Hemodilution and iron deficiency during pregnancy also play a role, with conflicting results in prior studies regarding their impact on HbA1c levels. [34]. Variations in measurement techniques, such as HPLC versus immunoassays, and differences in dietary and environmental factors across regions further contribute to these discrepancies. [35,36]. Additionally, this study's focused sample highlights specific dynamics not apparent in larger, more



diverse studies, while controlling for diabetes to isolate the effects of iron deficiency anemia [37].

#### Conclusion:

In the current study, there was a significant decrease in the mean value of HbA1c in pregnant with iron deficiency anemia compared to those pregnant without iron deficiency anemia, Therefore, the presence of anemia in pregnant affects the HbA1C level.

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