Original Article

Frequency of Gestational Diabetes Mellitus in Non-Anaemic Women with and Without Use of Iron Supplements

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Abstract

Objective: To determine the frequency of gestational diabetes mellitus (GDM) in non-anaemic women with and without iron supplementation.

Methods: This comparative cross-sectional study was conducted at the Department of Obstetrics and Gynecology, Holy Family Hospital, Rawalpindi, from January to June 2025. A total of 110 non-anaemic pregnant women (20–24 weeks' gestation) were enrolled: 55 using iron supplements and 55 not using them. Haemoglobin (Hb) and serum ferritin levels were measured. All participants were screened using a 75 g oral glucose tolerance test (OGTT) at 28 weeks of gestation to assess GDM. Data were analysed using chi-square tests and multivariable logistic regression; adjusted odds ratios (aOR) with 95% confidence intervals (CI) were calculated ($p \leq 0.05$).

Results: GDM occurred in 28 (25.5%) participants and was more frequent in supplement users than in non-users (36.4% vs. 14.5%; $p = 0.01$). The mean serum ferritin level was higher in users (85.4 ± 17.8 ng/mL) than in non-users (51.1 ± 14.9 ng/mL). In the unadjusted analysis, iron supplementation was associated with higher odds of GDM (OR 3.36, 95% CI: 1.33–8.49). After adjusting for confounders, iron supplementation remained significantly associated with GDM (aOR = 3.12, 95% CI: 1.21–8.03; $p = 0.018$). Ferritin levels >100 ng/mL were also associated with an increased risk of GDM (aOR = 3.70, 95% CI: 1.13–12.11; $p = 0.030$). No differences were observed in terms of parity, education, or diet.

Conclusion: Iron supplementation in non-anaemic pregnant women is associated with an increased risk of GDM, particularly at high ferritin levels. Careful assessment and individualised supplementation are recommended.

Keywords: Gestational diabetes mellitus, Iron supplementation, Pregnancy, Non-anaemic women, Maternal health.

Introduction

Iron, an essential trace element, is involved in critical biological processes, including gene expression control, enzyme metabolism, mitochondrial energy generation, and hormone synthesis.[1] It is a fundamental constituent of haemoglobin and myoglobin, both vital for oxygen delivery to tissues and muscles.[2] Pregnancy significantly increases the physiological demand for iron due to maternal plasma volume expansion, foetal erythropoiesis, and placental development, necessitating nearly double the iron intake compared to that in non-pregnant women.[3]

Although iron supplementation is widely recommended in antenatal care to prevent iron deficiency anaemia, recent evidence has indicated a more complex relationship between iron status and gestational diabetes mellitus (GDM). Several studies have identified a potential association between elevated iron levels and increased GDM risk, particularly in non-anaemic women. This association may be mediated by iron-induced oxidative stress and impaired insulin signalling pathways.[4,5] Increased ferritin levels in non-anaemic individuals receiving supplementation can promote free radical formation, contributing to oxidative damage of pancreatic β-cells and compromised glucose regulation. A study conducted in Pakistan observed that elevated maternal serum ferritin levels may be associated with an increased risk of GDM, prompting reconsideration of universal iron supplementation policies in this context.[6]

Contributions:
MK MS HN SA SY - Conception, Design
KI MS HN SA - Acquisition, Analysis, Interpretation
MK MS HN SA - Drafting
KI SY - Critical Review

All authors approved the final version to be published & agreed to be accountable for all aspects of the work.

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Gestational diabetes mellitus (GDM) presents a substantial challenge to both maternal and foetal health. Women diagnosed with GDM are at a notably elevated risk of adverse pregnancy outcomes, including caesarean delivery, preterm birth, and macrosomia. A retrospective analysis revealed that singleton pregnancies had a 1.5-fold higher chance of caesarean delivery and a 30% greater risk of macrosomia.[7] The pathophysiology involves maternal hyperglycaemia disrupting endocrine pathways, particularly adipokine regulation, thereby altering foetal growth trajectories and metabolic profiles, which may predispose offspring to long-term cardiometabolic diseases.[8] These complex interactions highlight the importance of timely diagnosis of GDM and systematic perinatal care approaches. The incidence of gestational diabetes mellitus (GDM) has increased significantly over the years. Current evidence shows that approximately 14.7% of pregnancies are affected, according to the diagnostic standards set by the International Association of Diabetes and Pregnancy Study Groups (IADPSG).[9] Moreover, the International Diabetes Federation estimates that 16.7% of live births worldwide involve hyperglycaemia during pregnancy, most of which are due to GDM.[10] In Pakistan, the prevalence of GDM appears particularly high; however, available studies are limited to specific regions and may not represent the entire national population. A recent study estimated a pooled national rate of 16.7% (95% confidence interval (CI): 13.1–21.1%), showing notable variation across provinces of Pakistan, ranging from 11.4% in Punjab to 35.8% in Balochistan.[11] Despite this significant burden, the absence of standardised screening protocols has resulted in many cases remaining undiagnosed, emphasising the urgent need for standardised national screening protocols.[9]

This study aimed to explore the association between iron supplementation and GDM risk in non-anaemic pregnant women in the study population. Given the widespread use of antenatal iron supplementation and emerging evidence linking iron status with dysregulated glucose metabolism, this study aimed to evaluate whether iron intake among non-anaemic women contributes to a higher incidence of GDM. The findings are expected to support evidence-informed maternal nutrition strategies that balance the prevention of anaemia with the minimisation of potential metabolic risks.

Materials And Methods

This comparative cross-sectional study was conducted at the Department of Obstetrics and Gynaecology, Unit II, Holy Family Hospital, Rawalpindi, Pakistan, over six months from 1 January to 30 June 2025. Ethical approval was obtained from the Ethical Review Committee of Rawalpindi Medical University (Letter Ref. No. 196/IREF/RMU/2025, dated 17 June 2025). Informed consent was obtained from all participants before inclusion. This study was conducted in accordance with the STROBE guidelines for Observational Studies in Epidemiology (STROBE) guidelines.

A total of 120 pregnant women with singleton pregnancies between 20 and 24 weeks' gestation were screened for eligibility. During follow-up, six women were excluded due to anaemia (haemoglobin <11 g/dL; n = 6), and four women were lost to follow-up (n = 4). The remaining 110 participants were enrolled and categorised into two groups: 55 women using iron supplements and 55 women not using supplements. Participants with incomplete data or those who were lost to follow-up were excluded from the final analysis. No imputation methods were applied, and only complete cases were included in the statistical analyses.

Based on previous literature indicating a 15% prevalence of GDM among non-anaemic women, with an expected increase to 35% in iron supplement users, a sample size of 55 participants per group (total n = 110) was calculated using the WHO sample size calculator with a 95% confidence level and 80% power. The inclusion criteria comprised pregnant women with singleton pregnancies between 20 and 24 weeks of gestation and haemoglobin levels ≥11 g/dL. The exclusion criteria were a diagnosis or family history of type 1 or type 2 diabetes mellitus, anaemia, chronic renal or hepatic disease, tuberculosis, autoimmune disorders, malignancy, haemoglobinopathies, inflammatory bowel disease, or obesity (BMI >30 kg/m²).

Participants were recruited from the outpatient department and obstetric wards and subsequently categorised into two groups based on their iron supplementation status. Patients who used iron supplements for at least 4 weeks during the 12–20 weeks of the gestation period were categorised as iron supplement users. Demographic and baseline clinical information, including patient name, age, gestational age, gravidity, parity, BMI, educational attainment, socioeconomic status, family history of diabetes, dietary habits, and any history of infectious or inflammatory conditions, were documented using a structured pro forma.

Blood samples were collected between 20 and 24 weeks of gestation to assess the complete blood count (CBC) and serum ferritin levels, with iron status stratified into 15–50, 51–100, and 101–150 ng/mL. Serum ferritin and OGTT analyses were performed in a blinded manner with respect to the participants' iron supplementation status, ensuring an unbiased assessment of the primary outcomes. Haemoglobin levels were also analysed in relation to GDM status to assess any potential association between maternal haemoglobin (Hb%) and the development of gestational diabetes mellitus.

At 28 weeks' gestation, all participants underwent a 75 g oral glucose tolerance test (OGTT) to screen for gestational diabetes mellitus (GDM), with results interpreted according to the internationally accepted IADPSG diagnostic criteria. All participants continued to receive routine antenatal care and were monitored during their routine antenatal visits.

Data were recorded and analysed using IBM SPSS Statistics version 25.0. Quantitative variables were expressed as mean ± standard deviation and compared using independent t-tests, while categorical variables were expressed as frequencies and percentages and compared using chi-square tests. Multivariable logistic regression analysis was performed to assess the association between iron supplementation and gestational diabetes mellitus (GDM), while controlling for potential confounders. The variables included in the regression model were age, BMI, gravidity, parity, education, dietary patterns, socioeconomic status, and serum ferritin levels. Adjusted odds ratios (aOR) with 95% confidence intervals (CI) and exact p-values are reported. Statistical significance was set at $p \leq 0.05$. This approach ensured a reliable assessment of the independent association between iron supplementation, haemoglobin, iron stores, and GDM risk in non-anaemic pregnant women. Efforts were made to minimise potential sources of bias. Selection bias was reduced by applying strict inclusion and exclusion criteria to the study population. Measurement bias was minimised through blinded laboratory assessment. Multivariable logistic regression analysis was performed to control for potential confounders.

Results

A total of 110 pregnant women were included and analysed in this study, with 55 in the iron supplement group and 55 in the non-supplement group. The demographic and baseline clinical characteristics of the study participants are presented in Table 1. The mean age of the participants was 27.35 ± 4.09 years, with the majority being 26–30 years old. The mean gestational age at recruitment was 21.78 ± 1.14 weeks, and the mean body mass index (BMI) was 26.25 ± 2.84 kg/m². Most participants were multigravida (63.6%), and 80% belonged to the middle socioeconomic class. The educational status and dietary intake were comparable between the groups. There were no statistically significant differences between iron supplement users and non-users in terms of age, gestational age, parity, socioeconomic status, education level, dietary intake, or haemoglobin level (all $p > 0.05$); however, BMI was significantly higher in the iron supplement group than in the non-user group ($p = 0.021$) (Table 1).

The mean haemoglobin level at enrolment was 12.34 ± 0.86 g/dL. The mean serum ferritin level was 68.25 ± 21.19 ng/mL. Participants in the iron supplement group had higher mean serum ferritin levels (85.4 ± 17.8 ng/mL) than those in the non-user group (51.1 ± 14.9 ng/mL) (Table 1).

Table 1: Demographic and Clinical Characteristics of Study Participants (N = 110)

Variable	Total (n=110)	Iron Users (n=55)	Non-Users (n=55)	Mean Difference (95% CI)	P-value
Age (years)	27.35 ± 4.09	27.8 ± 3.9	26.9 ± 4.3	0.9 (-0.6–2.4)	0.23
Gestational Age (weeks)	21.78 ± 1.14	21.6 ± 1.2	21.9 ± 1.1	-0.3 (-0.7–0.1)	0.12
BMI (kg/m²)	26.25 ± 2.84	26.8 ± 2.9	25.6 ± 2.7	1.2 (0.2–2.2)	0.021
Multigravida (%)	70 (63.6%)	36 (65.5%)	34 (61.8%)	—	0.67
Middle Socioeconomic Class	88 (80%)	44 (80%)	44 (80%)	—	1.0
Education ≥ Secondary	67 (60.9%)	33 (60%)	34 (61.8%)	—	0.83
Average/Good Dietary Intake	96 (87.3%)	49 (89.1%)	47 (85.4%)	—	0.57
Haemoglobin (g/dL)	12.34 ± 0.86	12.42 ± 0.84	12.26 ± 0.89	0.16 (-0.12–0.44)	0.26
Serum Ferritin (ng/mL)	68.25 ± 21.19	85.4 ± 17.8	51.1 ± 14.9	34.3 (28.2–40.4)	<0.001

Continuous variables were compared using independent t-tests, and categorical variables were compared using chi-square tests. Statistical p-value $p \leq 0.05$.

Among the 110 participants, 28 (25.5%) were diagnosed with gestational diabetes mellitus (GDM) based on an oral glucose tolerance test (OGTT) performed at 28 weeks of gestation. The incidence of GDM was significantly higher in the iron supplement group, where 20 out of 55 women (36.4%) developed GDM, compared with 8 out of 55 women (14.5%) in the non-supplement group ($p = 0.01$), indicating a strong association between iron supplementation and GDM development (Table 2).

Chi-square p-value = 0.01 for unadjusted comparison, statistically significant. The odds ratios presented in (Table 2) represent the unadjusted (crude) analysis.

Interpretation: Women taking iron supplements had 3.36 times higher odds of developing GDM than non-users, which was statistically significant ($p = 0.01$).

Table 2: Incidence of GDM and Unadjusted Logistic Regression Analysis

Group	Total	Developed GDM	Did Not Develop GDM	% with GDM	Crude OR	95% CI	p- value
Iron Supplement Users	55	20	35	36.4%	3.36	1.33 – 8.49	0.01
Non-Users	55	8	47	14.5%	Reference	-	-
Total	**110**	**28**	**82**	**25.5%**	**-**	**-**	**-**

Multivariable logistic regression analysis was performed to assess the association between iron supplementation and gestational diabetes mellitus. After adjusting for potential confounders, iron supplementation was significantly associated with an increased risk of GDM (aOR = 3.12, 95% CI: 1.21–8.03, p = 0.018) (Table 5).
The mean haemoglobin levels were analysed in relation to GDM status. Participants who developed GDM had slightly higher mean haemoglobin (12.51 ± 0.82 g/dL) than those who did not develop GDM (12.28 ± 0.88 g/dL); however, this difference was not statistically significant (p = 0.21) (Table 3).

Table 3: Haemoglobin Levels in Relation to GDM Status

Group	Mean Haemoglobin (g/dL)	p-value
Developed GDM	12.51 ± 0.82	0.21
Did not develop GDM	12.28 ± 0.88	-

An independent t-test is used for comparison of continuous variables.
Further stratified analysis demonstrated that the risk of GDM increased with increasing serum ferritin level. Participants with serum ferritin levels between 101 and 150 ng/mL had the highest incidence of GDM, supporting the hypothesis that increased iron stores may contribute to glucose intolerance during pregnancy (Table 4).

Table 4: Association Between Serum Ferritin Levels and Incidence of GDM

Serum Ferritin Range	Participants (n)	Developed GDM (n)	% with GDM
15–50 ng/mL	41	7	17.1%
51–100 ng/mL	49	12	24.5%
101–150 ng/mL	20	9	45.0%

Chi-square value = 8.74, p-value = 0.013 → Statistically significant
Odds Ratio (high vs. Low Ferritin) = 3.9 [95% confidence interval (CI): 1.2 – 12.3]
The stratified analysis of BMI, ferritin levels, iron supplementation, and other potential risk factors related to GDM is shown in Table 5.

Table 5: Risk Factors Associated with GDM (Multivariable Logistic Regression)

Variable	Category	Total (n)	Developed GDM (n)	% with GDM	aOR	95% CI	p-value
BMI (kg/m²)	< 25	42	6	14.3%	Ref		
	≥ 25	68	22	32.4%	2.45	1.01 – 5.94	0.047*
Serum Ferritin (ng/mL)	≤ 100	90	19	21.1%	Ref		
	>100	20	9	45.0%	3.70	1.13 – 12.11	0.030*
Iron Supplementation	Non-Users	55	8	14.5%	Ref	-	
	Users	55	20	36.4%	3.12	1.21 – 8.03	0.018*
Parity	Primigravida	40	9	22.5%	Ref	-	
	Multigravida	70	19	27.1%	1.20	0.48 – 3.01	0.69
Education Level	< Secondary	43	12	27.9%	Ref	-	
	≥ Secondary	67	16	23.9%	0.88	0.37 – 2.07	0.77
Dietary Intake	Poor	14	4	28.6%	Ref	-	
	Average/Good	96	24	25.0%	0.92	0.28 – 3.03	0.90

**Indicates statistical significance (p < 0.05). Categorical variables were analysed using the chi-square test. Outcome variable: Gestational Diabetes Mellitus (GDM); aOR, adjusted odds ratio; CI, confidence interval; model adjusted for all variables listed.*

Stratified analysis demonstrated that a higher BMI (≥25 kg/m²), elevated serum ferritin levels (>100 ng/mL), and iron supplementation were significantly associated with an increased risk of GDM (p < 0.05). Participants with BMI ≥25

kg/m² had a higher incidence of GDM (32.4%) than those with a lower BMI (14.3%). Similarly, women with serum ferritin levels >100 ng/mL showed a markedly higher frequency of GDM (45.0%) than those with lower ferritin levels (21.1%).
The association between iron supplementation and GDM remained statistically significant across the stratified analyses, indicating that iron supplementation and elevated iron stores are associated with an increased risk of gestational diabetes mellitus.

Discussion

Iron supplementation remains an integral part of routine antenatal care to prevent iron deficiency anaemia, especially in low- and middle-income countries such as Pakistan. However, its role in glucose metabolism in non-anaemic individuals warrants further investigation. Several international studies have suggested a positive correlation between elevated iron stores and glucose intolerance. Zhang et al. and Rawal et al. reported similar trends, noting that excess iron may lead to oxidative stress, adversely affecting pancreatic β-cell function and insulin sensitivity, ultimately increasing the risk of gestational diabetes mellitus (GDM).[4,5,13]
A large international study conducted in Shanghai was among the first to explore the association between longitudinal changes in maternal serum ferritin levels and GDM risk. The study demonstrated that women with persistently elevated ferritin concentrations throughout pregnancy had a significantly higher risk of developing GDM,[12] suggesting that maternal iron stores and their dynamic changes during pregnancy may influence glucose metabolism.
Consistent with these findings, our study observed a direct correlation between serum ferritin levels and GDM incidence. Participants with serum ferritin >100 ng/mL had a 45% incidence of GDM, compared to 17.1% in those with ferritin 15–50 ng/mL (aOR = 3.70, 95% CI: 1.13–12.11; P = 0.030, Table 4). Similarly, iron supplement users had higher odds of developing GDM (aOR = 3.12, 95% CI: 1.21–8.03; p = 0.018; Table 5). These findings indicate that elevated iron stores, even in the absence of anaemia, are associated with disrupted glucose homeostasis and an increased risk of GDM.[14,15] Similar observations were noted in a local study by Siddiqui et al., who reported that higher maternal ferritin levels were significantly associated with GDM in Pakistani women.[6] Additional recent cohort and meta-analysis studies have also reported that high serum ferritin levels are associated with an increased risk of GDM.[16,17]
Interestingly, mean haemoglobin levels were slightly higher among participants who developed GDM (12.51 ± 0.82 g/dL) than among those who did not (12.28 ± 0.88 g/dL); however, this difference was not statistically significant (p = 0.21). This indicates that haemoglobin alone may not be a reliable predictor of GDM in non-anaemic women. In contrast, serum ferritin levels, which more accurately reflect early iron stores, were significantly associated with the risk of GDM. These results support previous evidence indicating that elevated iron stores, rather than haemoglobin concentrations, play a more critical role in the pathogenesis of glucose intolerance during pregnancy. This emphasises the importance of assessing iron status using ferritin levels rather than relying solely on haemoglobin levels to guide iron supplementation in non-anaemic pregnant women.
Contrary to studies that found no significant difference between supplement users and non-users, our findings highlight the importance of stratifying participants based on iron status and controlling for confounding factors such as anaemia, obesity, and chronic illness. In our study, the exclusion of women with anaemia allowed for a clearer understanding of the isolated effects of supplemental iron in patients with normal iron levels.
BMI was found to be a significant effect modifier in the stratified analysis. Women with a higher BMI (≥25 kg/m²) were more likely to develop GDM (aOR = 2.45, 95% CI: 1.01–5.94; P = 0.047), especially when the serum ferritin level exceeded 100 ng/mL (Table 5). This synergistic effect of obesity and elevated iron stores on GDM risk has been reported in previous studies, suggesting that iron may exacerbate pre-existing metabolic susceptibility.[5] No significant differences were found in GDM incidence when stratified by educational status, parity, or dietary intake (Table 5). These findings align with prior studies indicating that metabolic risks associated with iron overload may be more biochemically driven rather than dependent on social or reproductive history.[3,4,17]
The main strength of this study lies in its focused design, targeting non-anaemic women and accounting for multiple effect modifiers, such as BMI, dietary habits, and education status. The diagnostic criteria for GDM are internationally accepted, enhancing the reliability of this study. This study did not require complex or invasive investigations and addressed a serious health problem affecting many pregnant women. However, this study has several limitations. First, the sample size was relatively small, and the study was conducted in a single tertiary care hospital, which may affect the generalisability of the results. Furthermore, there was limited biochemical evaluation to explain the underlying mechanisms of this association.

In conclusion, the results of this study highlight the importance of further evaluating iron supplementation practices in non-anaemic pregnant women. Although iron is essential for maternal and foetal health, excessive supplementation in women with adequate iron stores may contribute to metabolic complications, including gestational diabetes mellitus. Therefore, it is recommended that iron supplementation be guided by maternal iron status, as indicated by serum ferritin levels, and that the routine practice of providing iron supplements to all pregnant women, irrespective of their iron status, should be reconsidered.

Conclusions

This study found that iron supplementation in non-anaemic pregnant women was associated with an increased risk of developing gestational diabetes mellitus (GDM), particularly in those with high serum ferritin levels and elevated BMI. Although iron is essential during pregnancy to prevent anaemia and its complications, its use in nonanemic women with adequate iron stores appears to have adverse metabolic consequences. This study emphasises the need to review iron supplementation practices in non-anaemic populations based on serum ferritin levels, particularly in populations at a higher risk of GDM. Future studies should consider measuring ferritin levels later in pregnancy after discontinuing iron supplementation, as some evidence suggests that initially elevated ferritin levels may decrease in the later stages, potentially reducing the risk of GDM. Additionally, large-scale studies are recommended to establish evidence-based guidelines for iron supplementation in non-anaemic pregnant women.

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