ORIGINAL ARTICLE

Investigating Iron Deficiency in Non-Anemic Pregnant Women During the Second Trimester

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Abstract- Iron deficiency is the most prevalent nutritional deficiency worldwide ,affecting populations in both developing and developed .despite the absence of anemia, pregnant women may still experience depleted iron stores, which can have significant health implication for both mother and fetus This study aimed to assess the prevalence of iron deficiency among non-anemic pregnant women in their second trimester by analyzing serum ferritin concentrations and identifying related clinical and demographic factors. This cross-sectional study included 246 non-anemic pregnant women at 24-28 weeks' gestation visiting Baqiatallah Hospital in Tehran for routine diabetes screening in 2022. Blood samples were analyzed for complete blood count, ferritin, iron, and total iron binding capacity. Demographic data and supplement use were recorded. Participants were divided into low ferritin (<30 ng/mL) and normal ferritin (≥30 ng/mL) groups. Demographic characteristics, clinical parameters, and laboratory findings were compared between these two groups using Student's t-test, Mann-Whitney U, and Chi-square tests, Mann-Whitney U, and Chi square tests. Of the participants, 64.4% had low ferritin levels despite 94.3% reporting regular iron supplement use. There were no significant differences in demographic characteristics or supplement use between low and normal ferritin groups (P>0.05). Women with low ferritin had significantly lower platelet counts (213.1±43.4 vs 231.0±59.1 thousand/μL, P=0.04) and serum iron levels (97.5±41.9 vs 105.8±34.9 μg/dL, P=0.018) compared to those with normal ferritin. No significant differences were found in hemoglobin, hematocrit, or other red blood cell indices between groups (P>0.05). A high prevalence of iron deficiency was observed among non-anemic pregnant women despite regular supplement use. Current iron supplementation strategies may be inadequate in addressing iron deficiency during pregnancy. Regular monitoring of serum iron profiles is recommended to identify at-risk women and allow for timely interventions.

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Introduction

Iron deficiency (ID) and iron deficiency anemia (IDA) are recognized as significant global health challenges, particularly during pregnancy when the nutritional needs of both the mother and fetus increase dramatically (1,2). Iron deficiency anemia in pregnancy is defined as hemoglobin levels below 11 mg/dL in the first and third trimesters and below 10.5 mg/dL in the second trimester (3). According to the American College of Obstetricians and Gynecologists (ACOG), iron deficiency itself is diagnosed by serum ferritin concentrations below 10 ng/mL, a threshold indicating depleted iron stores even in the absence of anemia (4,5).

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However, evidence suggests that this criterion has only 25% specificity for diagnosing iron deficiency. Consequently, a ferritin level of less than 30 ng/mL, which demonstrates a sensitivity of 98% and specificity of 92%, has been proposed as a more reliable indicator for diagnosing iron deficiency during pregnancy (6).

Pregnant women with iron deficiency anemia face serious health risks, including an increased risk of postpartum hemorrhage, the need for blood transfusions, and heart failure (7). Moreover, both iron deficiency anemia and iron deficiency without anemia are recognized as risk factors for preterm birth, low birth weight, and maternal and fetal mortality before and after delivery (8-10). During pregnancy, ferritin levels typically peak between weeks 12 to 16 but decline as gestation progresses, reaching their lowest point in the third trimester. A study conducted by Ahmad and Al-Sumaie reported that the prevalence of iron deficiency among non-anemic mothers was 14.8% in the first trimester, 49.2% in the second trimester, and 36% in the third trimester (11).

Given that hemoglobin levels also reach their lowest level during the second trimester, anemia screening during this period is strongly recommended in most clinical guidelines (12). Although routine screening for iron stores in non-anemic pregnant women is not widely endorsed by many guidelines, clinical evidence indicates that numerous pregnant women experience symptoms of iron deficiency, including fatigue, general weakness, lethargy, reduced physical capacity, and insomnia, even when their laboratory results do not indicate anemia. Further investigations have shown that these women may indeed suffer from iron deficiency, which can be alleviated with appropriate treatment.

Recent studies have indicated that urban living conditions and exposure to air pollution may lead to polycythemia, potentially explaining why some individuals do not exhibit anemia despite experiencing symptoms (13,14). Treating iron deficiency not only improves symptoms related to low iron levels but also helps establish adequate iron reserves during the third trimester of pregnancy, thereby reducing the risk of developing anemia and mitigating adverse outcomes related to postpartum hemorrhage. Considering this evidence, we have decided to investigate iron stores in non-anemic pregnant women during the second trimester when anemia and diabetes screenings are conducted.

Materials and Methods

This cross-sectional study was conducted on pregnant women visiting the Baqiatallah Hospital clinic, Tehran affiliated to Baqiatallah University of Medical Sciences for diabetes screening during weeks 24 to 28 of pregnancy in 2022. A census method was used for sample selection, and all eligible women within the specified time frame were included in the study.

The inclusion criteria for the study were an age between 18 and 45 years, singleton pregnancy, and verbal consent to participate in the study. Conversely, the exclusion criteria included bleeding disorders, known hemoglobinopathies, chronic gastrointestinal diseases such as inflammatory bowel disease, bariatric surgery, clinical evidence of active infection, and persistent bleeding in the first and second trimesters.

Ethical committee of baqiyatallah university of medical science approved the study (code: IR.BMSU.BAQ.REC.1401.048). After explaining the study's objectives to eligible pregnant women and obtaining verbal consent, they were referred to the laboratory. At this stage, 5 cc of peripheral blood was drawn from each patient for performing the Glucose Challenge Test (GCT) and Complete Blood Count (CBC), which were mandatory regardless of participation in the study. These tests were performed as part of routine prenatal care. If the CBC test indicated a hemoglobin level above 10.5 mg/dL, serum ferritin, transferrin saturation, and serum iron levels were measured. These steps were taken to identify iron deficiency, which is a common issue during pregnancy. In cases of diagnosed iron deficiency, standard treatment was provided, including iron supplements and nutritional counseling.

All necessary information for the researcher, including demographic characteristics (age, parity, weight, height, and gestational age), history of iron supplement use, and laboratory findings, was recorded in a researcher-designed checklist.

Descriptive statistics for qualitative variables were presented as absolute and relative frequencies, while quantitative variables were expressed as means and standard deviations. To compare two groups, qualitative variables were analyzed using the Chi-Square test, while quantitative variables were compared using the t-test if the data distribution was normal; otherwise, the Mann-Whitney test was utilized. Data analysis was performed using SPSS version 23. A significance level of less than 0.05 was considered statistically significant.

Results

The study included 246 pregnant women, of whom 159 (64.4%) had low ferritin levels and 87 (35.4%) had normal ferritin levels. The majority (94.3%) reported regular iron supplement intake, with 63.4% of those having low ferritin and 36.6% having normal ferritin, but the difference was not statistically significant (P=0.22). Similarly, 85% took multivitamins regularly, with 63.2% of those having low ferritin and 36.8% having normal ferritin, again with no significant difference (P=0.515) (Table 1).

The table 2 compares various characteristics between pregnant women with low and normal ferritin levels. The mean age was 29.4±5.9 years in the low ferritin group and 31.2±6.7 years in the normal group, not significantly different (P=0.056). Gestational age was also similar at 26.04±1.2 weeks and 26.1±1.2 weeks respectively (P=0.479). Gravidity was 2.2±1.0 in both groups (P=0.991). The low ferritin group had slightly more abortions (0.3±0.6 vs 0.2±0.5, P=0.921) and stillbirths (0.03±0.1 vs 0, P=0.095), but these differences were not significant. Live births were the same at 0.9±0.7 and 0.9±0.8 (P=0.685). There was not a significant difference between two groups regarding their BMI status (P=0.729).

Laboratory parameters like RBC, Hb, Hct, and TIBC did not differ significantly between the two ferritin groups. However, platelets were lower in low ferritin (213.1±43.4 vs 231.0±59.1, P=0.04). Ferritin itself was much lower in the low ferritin group at 18.2±7.3 vs 51.8±33.4 ng/mL (P<0.001), as expected. Iron levels were also lower in low ferritin at 97.5±41.9 µg/dL vs 105.8±34.9 µg/dL (P=0.018) (Table 3).

Table 1. Comparison of iron and multivitamin supplement intake among pregnant women with low and normal ferritin levels

Variable		Total (%)	Low ferritin (%)	Normal ferritin (%)	*P*
Total		246	159 (64.4%)	87 (35.4%)	
Iron supplement intake	**Regular intake**	232 (94.3)	147 (63.4%)	85 (36.6%)	0.22
	Irregular intake	13 (5.3)	11 (84.6%)	2 (15.4%)	
	No intake	1 (0.4)	1 (100%)	0 (0%)	
Multivitamin intake	**Regular intake**	209 (85)	132 (63.2)	77 (36.8)	0.515
	Irregular intake	15 (6.1)	11 (73.3)	4 (26.7)	
	No intake	22 (8.9)	16 (72.7)	6 (27.3)	

Table 2. Comparison of demographic and clinical characteristics between pregnant women with low and normal ferritin levels

Characteristic	Low ferritin (Mean±SD)	Normal ferritin (Mean±SD)	*P*
Age (years)	29.4 ± 5.9	31.2 ± 6.7	0.056
Gestational age (weeks)	26.04 ± 1.2	26.1 ± 1.2	0.479
Gravidity	2.2 ± 1.0	2.2 ± 1.0	0.991
Number of abortions	0.3 ± 0.6	0.2 ± 0.5	0.921
Number of live births	0.9 ± 0.7	0.9 ± 0.8	0.685
Number of stillbirths	0.03 ± 0.1	0 ± 0	0.095
BMI (kg/m^2)	30.58 ± 4.71	30.36 ± 4.68	0.729

Table 3. Comparison of laboratory parameters between pregnant women with low and normal ferritin levels

Laboratory parameter	Low ferritin (Mean±SD)	Normal ferritin (Mean±SD)	*P*
WBC (cells/µL)	9274.5 ± 1781.4	9640.21 ± 699.6	0.147
RBC (million cells/µL)	4.3 ± 0.3	4.0 ± 0.3	0.837
Hemoglobin (g/dL)	11.8 ± 0.8	11.9 ± 0.8	0.155
Hematocrit (%)	35.6 ± 2.4	36 ± 2.5	0.318
MCV (fL)	87.4 ± 5.1	88.1 ± 5.2	0.330
Platelets (thousands/µL)	213.1 ± 43.4	231.0 ± 59.1	0.04
Ferritin (ng/mL)	18.2 ± 7.3	51.8 ± 33.4	<0.001
Iron (µg/dL)	97.5 ± 41.9	105.8 ± 34.9	0.018
TIBC (µg/dL)	415.3 ± 51.2	406.6 ± 55.1	0.373

Discussion

The present study revealed that a substantial proportion of participants had low ferritin levels, despite the majority reporting regular iron supplement intake. Interestingly, the use of iron supplements and multivitamins did not significantly differ between women with low and normal ferritin levels. Furthermore, demographic and obstetric characteristics such as age, gestational age, gravidity, and BMI were homogenous between the two groups. While most hematological parameters were similar, women with low ferritin levels exhibited significantly lower platelet counts and serum iron levels compared to those with normal ferritin levels.

Currently, American and European protocols do not recommend routine iron supplementation for all pregnant women. Instead, they advise s screening for anemia using complete blood count (CBC), and initiating iron-contain supplement only if anemia is detected. These guidelines also do not recommend routine screening using serum iron studies (such as ferritin, transferrin saturation or serum iron). This is largely due to concern about cost-effectiveness, variability in test interpretation, and a lack of strong evidence that early detection of isolated iron deficiency without anemia significantly improves maternal and fetal (1,15). There is also no global consensus on how to prescribe iron supplements during pregnancy. Some guidelines recommend universal supplementation throughout pregnancy as preventive measure, especially in population with high anemia prevalence. Others advocate for targeted approach, reserving supplementation for women as high risk (e.g., those with multiple pregnancies, low dietary intake, or a history of iron deficiency). A few protocols suggest individualized supplementation based on ferritin level, though this practice remains controversial due to variability in laboratory standards and the influence of inflammation on ferritin (16).

In our study, among 246 pregnant women, 85% regularly used multivitamin supplements and 94.3% used iron-containing tablets. This rate is higher than that reported in conducted study by Vandevijvere *et al.,* (17) in Belgium, where about two-thirds of pregnant women used iron supplements. This difference may be due to variations in protocols used for prescribing iron supplements during pregnancy. In the study by Vandevijvere *et al*., (17), although two-thirds of pregnant women were taking iron supplements, around 40% still had serum ferritin levels below 15 micrograms. Similarly, our findings revealed that 64.4% of non-anemic patients had ferritin levels below 30 micrograms, despite 94.3% of the pregnant women regularly using iron supplements. This deficiency indicates a significant depletion of iron stores in pregnant women, suggesting that even consistent use of iron supplements has not adequately met the increased iron requirements during pregnancy.

The lack of a significant relationship between ferritin deficiency and supplement use in our study raises important questions about the effectiveness of current iron supplement prescription methods. Factors such as supplement consumption patterns, dosage and formulation, and individual differences in iron metabolism may play a role in this observation (18,19). The absence of a significant difference in hemoglobin levels between women with and without ferritin deficiency suggests that hemoglobin alone may be an inadequate indicator, and a combined screening approach should be considered. suggests that iron stores, as indicated by ferritin levels, may not be the primary determinant of hemoglobin concentration in non-anemic pregnant women. This finding contradicts previous study that reported a strong association between iron deficiency and anemia during pregnancy (20). Several factors may explain this unexpected result. First, the body may employ various compensatory mechanisms to maintain adequate hematopoiesis even in the presence of reduced iron stores, particularly in non-anemic individuals (21). These mechanisms could include increased iron absorption, mobilization of iron from stores, and more efficient utilization of available iron for erythropoiesis. Additionally, the timing of ferritin assessment may influence the relationship between ferritin levels and hemoglobin concentration, as this relationship may vary depending on the stage of pregnancy and duration of iron deficiency (22). Furthermore, our study found no significant association between ferritin deficiency and changes in mean corpuscular volume (MCV), red blood cell (RBC) count, white blood cell (WBC)count, or hematocrit during pregnancy. These findings challenge the traditional understanding of iron's central role in hematopoiesis and suggest that additional or compensatory regulatory mechanisms may influence hematologic parameters during gestation (23). The absence of a correlation between ferritin deficiency and MCV is particularly noteworthy as microcytosis is commonly regarded a s a hallmark indicator of iron deficiency anemia this discrepancy implies that relying solely on hemoglobin and red cell indices may be insufficient to detect early iron deficiency, especially in pregnant women, where physiological changes may obscure classical sign of iron depletion. These results highlight the potential need for a more comprehensive screening approach, such as combining hemoglobin measurement with ferritin levels or other iron biomarkers, to more accurately identify subclinical iron deficiency before anemia develops and to guide timely intervention (24).

These findings challenge the conventional understanding of iron's role in hematopoiesis during pregnancy and indicate that other regulatory mechanisms may be at work. Further research is needed to elucidate these compensatory mechanisms and develop new strategies for managing iron status during pregnancy. Finally, longitudinal studies extending into the postpartum period could be beneficial in understanding the long-term implications of ferritin deficiency and its potential impact on maternal and neonatal health outcomes.

This study has several limitations that should be considered when interpreting the results. Firstly, the cross-sectional design limits our ability to establish causal relationships and provides only a snapshot of the population at a specific point in time. Secondly, conducting the study at a single hospital in Tehran may restrict the generalizability of our findings to other populations or regions. Additionally, the lack of pre-pregnancy data makes it challenging to determine the true impact of pregnancy on ferritin levels. Finally, the study may not have accounted for all possible confounding factors, such as dietary habits or other health conditions.

Our study underscores the complexity of iron homeostasis during pregnancy and raises concern about the effectiveness of current iron supplementation strategies. Despite widespread use of iron supplement among participants, a substantial portion of pregnant women continue to exhibit low ferritin level .this suggests that supplementation alone may not be sufficient and point to several contributing factors ,including gastrointestinal malabsorption, poor adherence to supplementation regimens, or the use of suboptimal or poorly absorbed iron formulations .the lack of correlation between ferritin level and traditional hematological parameters such as e hemoglobin and MCV further complicate early detection. These findings highlight a potential gap in exiting screening practice and suggest that reliance on standard hematologic indices may fail to identify iron deficiency before anemia develops. Given this insight, there is a compelling need for routine monitoring of serum iron status using serum biomarkers such as ferritin and transferrin saturation particularly in high-risk populations. Early identification of iron depletion could enable timely and targeted intervention, potentially improving maternal and fetal outcomes. Future research should focus on developing more sophisticated methods for assessing iron status, understanding the underlying mechanisms of iron regulation, and evaluating the long-term implications of maternal iron status on health outcomes.

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