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Serum Oxidative Stress Biomarkers of Iron Deficiency Anemia in the First, Second and Third Trimesters of Pregnancy: A Prospective Cohort Study

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Abstract

Objective: This study assessed the status of dynamic thiol/disulfide balance (Dt/dB) among pregnant women throughout pregnancy in order to examine the possible interaction between iron deficiency anemia (IDA) and oxidative stress (OS).

Methods: The investigation was designed as a prospective cohort study at the Obstetric Care Service of the Haseki Training and Research Hospital (Istanbul). Ninety pregnant women were divided into 2 groups: 45 with IDA and 45 without this condition. They were also placed into first-, second-, and third-trimester groups, with 15 participants in each group. The study gathered sociodemographic information and conducted analyses on blood samples to assess circulating (Dt/dB) status, ferritin, iron, total iron-binding capacity, and other pertinent hematological and biochemical factors.

Results: There were no notable disparities observed in the thiol/disulfide equilibrium between pregnant women with or without IDA. Nevertheless, notable disparities were determined when comparing circulating disulfide values, as well as reduced thiol ratio (RTR) and thiol oxidation-reduction ratio (TORR) in relation to ferritin levels. Women with ferritin levels <20 µg/L exhibited considerably lower circulating disulfide values and significantly higher RTR and TORR values.

Conclusions: Pregnant women with low levels of circulating ferritin show signs of heightened OS, as demonstrated by changes in Dt/dB measures. Tracking ferritin levels offers a more precise evaluation of OS, indicating that ferritin is a crucial indicator for OS in this specific group. It is crucial to conduct additional studies using larger and more similar groups in order to confirm these findings and enhance clinical results for both mothers and their infants.

Keywords: Iron deficiency anemia, oxidative stress, pregnancy, thiol/disulfide balance, ferritin, maternal health

Introduction

Iron deficiency anemia (IDA) is a frequently encountered blood condition, especially in pregnant women, because both the mother and the developing fetus require more iron. Approximately 75% of anemia detected in pregnant women, which is an important public health problem, is iron deficiency anemia.[1,2] Iron deficiency anemia primarily occurs as a result of insufficient consumption of iron-rich food during pregnancy, combined with the body's heightened need for iron during this period. Iron deficiency anemia during pregnancy is linked to several negative consequences, including maternal tiredness, reduced cognitive and physical abilities, and higher chances of complications like preeclampsia, premature birth, and cesarean section.[3]

The metabolism of energy and the transportation of oxygen are both dependent on iron. The oxygen-carrying capacity of erythrocytes is primarily attributed to hemoglobin, a globular protein constituting approximately one-third of their dry mass. This molecule facilitates the efficient transport of oxygen from the pulmonary circulation to peripheral tissues, while concurrently enabling the removal of carbon dioxide for return to the lungs. Iron is a vital component of myoglobin, a substance that delivers oxygen to muscles, as well as several enzymes that play a role in cellular respiration and energy generation.[4,5]

Anemia is defined as "having a hemoglobin level below 11 g/dL or a hematocrit level below 33%" during the first and third trimesters and "a hemoglobin level below 10.5 g/dL or a hematocrit level below 32%" during the second trimester by the World Health Organization (WHO). Nevertheless, the measurement of hemoglobin levels alone may not consistently provide an appropriate assessment of iron status, particularly during the initial phases of insufficiency when the body adapts by utilizing its stored iron

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reserves. Consequently, the measurement of circulating ferritin levels is used to determine the quantity of iron stored in the body. Iron deficiency is defined when blood ferritin levels in pregnant women are below 15 µg/L according to the WHO and below 30 µg/L according to UK guidelines[6,7]

Iron deficiency anemia during pregnancy is a matter of concern not just for the mother but also has substantial implications for the fetus. It can result in reduced birth weight, premature birth, and hindered neonatal development. It is essential to maintain sufficient amounts of iron throughout pregnancy to promote the well-being of mother and fetus dyads.[8]

The term "oxidative stress" (OS) alludes to "an imbalance between the body's antioxidant defenses and the production of reactive oxygen species (ROS)." It is essential in the development of a variety of pregnancy-related issues. ROS are molecules that possess a high degree of reactivity and have the potential to damage biological components, including DNA, proteins, and lipids. Oxidative stress occurs when the body's antioxidant mechanisms are unable to counteract the generation of ROS, which leads to cellular derangement.[9]

Oxidative stress commonly occurs during pregnancy as a result of heightened metabolic demands and the physiological alterations that come with gestation. Increased levels of OS are linked to inadequate fetal and placental development and can contribute to problems such as preeclampsia, gestational diabetes, and intrauterine growth restriction. The aforementioned circumstances can have long-lasting effects upon "the well-being of both the mother and the developing fetus," highlighting the importance of understanding and managing OS throughout pregnancy.[10]

The equilibrium between thiol and disulfide compounds is a crucial indicator of OS. Thiols, which are organic molecules that contain sulfhydryl groups, have a crucial function in preserving cellular redox equilibrium. Thiols have the ability to undergo oxidation and create disulfides. Intracellular disulfide bonds serve as a reversible redox couple, undergoing reduction to free thiols. This dynamic equilibrium reflects the prevailing cellular redox state with fidelity. Many diseases, such as cardiovascular diseases, diabetes, neurological disorders, and cancer are associated with oxidative stress.[11,12]

Examining the correlation between IDA and OS during pregnancy can offer a useful understanding of the fundamental processes and possible treatment targets. Prior research has demonstrated that iron deficiency anemia might worsen OS by augmenting the generation of ROS and compromising antioxidant mechanisms. This can result in additional cellular harm and worsen the negative consequences linked to anemia.[13]

We intend to determine the interaction between IDA and circulating OS biomarkers (OSBs) in pregnant women. This will be achieved by conducting dynamic thiol/disulfide balance (Dt/dB) measurements during all trimesters. This study aims to reveal the interaction between IDA and OS during pregnancy by comparing the levels of OSBs in pregnant women with and without IDA. Gaining insight into this correlation can provide valuable information for medical practice and direct treatments aimed at enhancing the health outcomes of both the mother and the fetus.

Methods

A prospective cohort study implemented in this investigation was conducted at the Obstetric Care Service at the Haseki Training and Research Hospital (İstanbul), taking place from October 2022 to December 2022. The research followed the standards specified in the Declaration of Helsinki, assuring ethical compliance throughout the investigation. All subjects were required to provide informed written permission before participating in the study. The hospital's Ethics Committee provided ethical approval for the study (Approval no: 104/22, Date: September 29, 2022).

Participants

The study included 2 main cohorts of pregnant women: those who were diagnosed with IDA and those who did not have anemia. The study included a range of gestational ages from 8 to 41 weeks. Anemia was diagnosed by assessing hemoglobin concentrations using the criteria established by the WHO. Anemia was diagnosed in the first and last trimesters when "hemoglobin levels were below 11 g/dL" and in the midtrimester when "its levels were below 10.5 g/dL." Furthermore, the diagnostic analysis incorporated measurements of circulating iron, red cell distribution width (RDW), and ferritin levels. A total of 45 pregnant women were selected for each group, with 15 participants assigned to each trimester of pregnancy.

Criteria for inclusion: women between the ages of 18 and 42 who are currently expecting a baby; gestational age ranges from 8 to 41 weeks; voluntary participation; and the ability to give informed consent.

Criteria for exclusion: multiple pregnancies; conceiving a child within 1 year of giving birth to a previous child; adhering to a vegetarian diet; placental abnormalities affecting fetal well-being; receiving a blood transfusion or undergoing parenteral iron therapy within 6 months prior to becoming pregnant; tobacco use or alcohol consumption; prolonged and intense physical exertion; and chronic inflammation or other diseases linked to OS.

The sociodemographic data obtained from the participants encompassed their age, economic condition, and education level. The assessment of economic position was conducted by a survey that utilized national income ranges, while the classification of participants according to education level was performed as "before high school" and "high school and above."

Collection and Preparation of Blood Samples

Each subject had 4 mL of venous blood drawn between 8 and 9 in the morning. This was done to maintain uniformity in terms of the participants' fasting state and the natural rhythm of iron metabolism. The blood samples were obtained in tubes with ethylene diamine tetra-acetic acid as an anticoagulant. Subsequently, the blood specimens were centrifuged at 4°C for 10 minutes at a force of 3000 rpm to separate the serum from the blood cells. The serum samples were divided into smaller portions and stored at −80°C until laboratory tests were performed.

Laboratory Tests

Measurement of the Dynamic Balance between Thiol and Disulfide Compounds

The evaluation of the Dt/dB was conducted using an automated spectrophotometric technique developed by Rel Assay Diagnostics (Gaziantep, Türkiye). This technique entails the conversion of reducible disulfide bonds into unbound functional thiol groups, which are then measured as native thiol (NT) levels. Total cellular thiols (TT), encompassing both free thiols and those engaged in disulfide bonds, were assessed by a DTNB (5,5'-dithiobis-(2-nitrob enzoic) acid) assay. Disulfide levels were subsequently calculated as half the difference between TT and the complementary native thiol (NT) measurement. To evaluate the cellular redox state, we employed a battery of thiol-disulfide exchange ratio measurements. The oxidized thiol level (OTR) reflects the proportion of disulfides relative to total thiols (TT), while the reduced thiol ratio

(RTR) indicates the free thiol content compared to TT. Additionally, the thiol-oxidation reduction ratio (TORR) quantifies the relative abundance of disulfides to free thiols.

Further Biochemical and Hematological Evaluations

Standard laboratory techniques were used to evaluate levels of circulating iron, total iron-binding capacity (TIBC), transferrin, ferritin, and C-reactive protein (CRP). An automated hematology analyzer (Sysmex, Kobe, Japan) was used to assess the complete blood count parameters, such as hemoglobin, hematocrit, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), RDW, platelet, and leukocyte counts.

Statistical Analysis

To determine the sample size of the study, a calculation by Power 3.1 software (www.psychologie.hhu.de) was used to achieve a power value of 0.80 and a *P* value of .05 by using an effect size value of 0.53. A total of 45 participants in each study group was considered sufficient.

The clinical and laboratory data were analyzed with the Statistical Package for Social Sciences version 26.0 software (IBM Corp.; Armonk, NY, USA). The Shapiro–Wilk test was chosen to assess the normality statuses of numeric variables. Variables that did not pass the normality test were depicted as medians (minimum and maximum). Conversely, variables that conformed to a normal distribution were represented as the average value with its standard deviation. Statistical comparisons were conducted using the *t*-test or ANOVA after normality was confirmed, while the Mann–Whitney *U* or Kruskal–Wallis ANOVA test was employed after normality was denied. The categorical variables were expressed as count with percentage. A significance level of alpha less than 0.05 was adopted to assess statistical significance.

Results

Initially, a cohort of 108 pregnant women underwent screening to determine their eligibility for this study. In terms of inclusion and exclusion criteria, 18 people were excluded. The reasons for their withdrawal were incorrect blood samples, chronic inflammation, or personal requests to withdraw. Out of the total of 90 pregnant women, half (n = 45) were anemic and the other half were non-anemic. The groups were separated into 3 trimester categories, with 15 individuals in each category.

Assessment of Descriptive Statistics

Demographic and Social Characteristics

Analysis of demographic characteristics (age, gravidity, parity, type of birth, socioeconomic status, and education) revealed no statistically significant differences ($P > .05$) between the study groups across trimesters (Table 1).

Comparison of Laboratory Parameters between Groups

Hematological Parameters

Notable disparities were noted in various hematological parameters between the groups with anemia and those without anemia. The anemic group consistently had lower hemoglobin levels in all trimesters (first trimester: 9.7 (8.1-10.3) g/dL, second trimester: 10

Table 1. Pregnant Women with and without Iron Deficiency Anemia; Comparison of Age, Gravidity, Parity, Type of Birth, Economic Status, and Educational Status

	Iron Defiency Anemia						
	Present (n = 45)			Absent (n = 45)			
	First Trimester (n = 15) (%)	Second Trimester (n = 15) (%)	Third Trimester (n = 15) (%)	First Trimester (n = 15) (%)	Second Trimester (n = 15) (%)	Third Trimester (n = 15) (%)	*P*
Maternal age	27.9 ± 5.2	28.7 ± 5.9	25.5 ± 4.6	29.1±.9	28.8 ± 5.8	28.1 ± 5.6	.477[a]
Economic status							
Lower	7 (46.7)	7 (46.7)	8 (53.3)	7 (46.7)	6 (40.0)	8 (53.3)	.337[a]
Lower middle	6 (40.0)	5 (33.3)	6 (40.0)	4 (26.7)	2 (13.3)	4(26.7)	
Middle	2 (13.3)	3 (20.0)	2 (13.3)	4 (26.7)	4 (26.7)	2 (13.3)	
Upper middle	0	0	1 (6.7)	0	3 (20.0)	1 (6.7)	
Education level							
Before high school	10 (66.7)	9 (60.0)	9 (60.0)	12 (80.0)	7 (46.7)	7 (46.7)	.416[a]
Upper high school	5 (33.3)	6 (40.0)	6 (40.0)	3 (20.0)	8 (53.3)	8 (53.3)	
Gravida	3.0 (2.0-4.0)	4.0 (1.0-12.0)	2.5 (1.0-4.0)	2.2 (1.0-4.0)	3.0 (1.0-5.0)	3.0 (1.0-7.0)	.297[b]
Parite	1.6 (1.0-3.0)	2 (1.0-5.0)	1.6 (1.0-3.0)	1.5 (1.0-3.0)	1.3 (1.0-2.0)	1.9 (1.0-4.0)	.610[b]
Mode of birth							
Spontaneous vaginal:	5 (62.5)	4 (40.0)	6 (60.0)	9 (64.3)	5 (38.5)	5 (50.0)	.700[a]
Caesarian section:	3 (37.5)	6 (60.0)	4 (40.0)	5 (35.7)	8 (61.5)	5 (50.0)	

[a]One Way ANOVA test was used (values given as mean or number (%)), [b]Kruskal–Wallis test was used (values given as median (min-max)).

(7.2-10.4) g/dL, third trimester: 9.9 (7.4-10.8) g/dL) compared to the non-anemic group (first trimester: 12.2 (11.2-13.5) g/dL, second trimester: 11.4 (11.0-12.8) g/dL, third trimester: 11.3 (11.0-12.4) g/dL) ($P < .05$). The anemic group had significantly lower hematocrit levels compared to the non-anemic group in all trimesters (first trimester: 29 (26.1-30.6)% vs. 36.8 (32.8-38.6)%, second trimester: 30.3 (25.0-30.8)% vs. 34 (31.9-37.8)%, third trimester: 30 (24.3-32.6)% vs. 33 (31.5-36.4)%) ($P < .05$) (Table 2).

The anemic group had a significant decrease in both the MCV and MCHC. The MCV values were significantly decreased in anemic participants compared to those without anemia in all trimesters (first trimester: 74.2 (68.6-87.5) fL vs 87 (82-94.1) fL, second trimester: 81.3 (55.5-89.1) fL vs 84.3 (76.8-93.3) fL, third trimester: 80.2 (68.3-89.9) fL vs 87.4 (70-100) fL) ($P < .05$). The MCHC values were significantly lower in participants with anemia compared to those without anemia in all trimesters (first trimester: 33 (23.2-34.6) g/dL vs 34.1 (32.9-34.9) g/dL, second trimester: 33 (28.5-34.6) g/dL vs 33.6 (25.7-34.7) g/dL, third trimester: 32.4 (30.3-33.1) g/dL vs 34 (31.5-34.9) g/dL) ($P < .05$) (Table 2).

In Table 2, it was observed that the RDW values of pregnant women in the first trimester who had anemia were significantly higher compared to those without anemia ($P = .023$).

Evaluation of hematological parameters (leukocytes, platelets, transferrin) and inflammatory markers (CRP) in all pregnant women, regardless of anemia status, revealed no significant differences between groups across trimesters ($P > .05$) (Table 2).

Study of Iron Metabolism and Biochemical Parameters

In all trimesters, the anemic group had significantly lower circulating iron levels compared to the non-anemic group ($P < .05$). The iron levels for the anemic group were as follows: first trimester: 47 (44-47) µg/dL, second trimester: 33 (18-92) µg/dL, and third trimester: 33 (23-97) µg/dL. On the other hand, the non-anemic group had higher iron levels: first trimester: 140 (18-920) µg/dL, second trimester: 160 (23-700) µg/dL, and third trimester: 70 (26-680) µg/dL. During different trimesters of pregnancy, the levels of ferritin were found to be significantly lower in women with anemia compared to women without anemia. In the first trimester, the levels were 7.5 (5-32.9) µg/L for women with anemia and 16.1 (6.0-64.7) µg/L for women without anemia. In the second trimester, the levels were 7.5 (3.7-63.4) µg/L for women with anemia and 21.4 (7.3-44.2) µg/L for women without anemia. In the third trimester, the levels were 8.6 (2.7-24) µg/L for women with anemia and 15.0 (6.9-98) µg/L for women without anemia. The observed differences achieved statistical significance ($P < .05$) (Table 2).

The TIBC levels were found to be significantly higher in the group of participants with anemia compared to those without anemia during all trimesters of pregnancy ($P < .05$). In the first trimester, the TIBC levels ranged from 403 to 6000 µg/dL in the anemia group, while they ranged from 326 to 2800 µg/dL in the non-anemia group. Similarly, in the second trimester, the TIBC levels were higher in the anemia group (388 to 6490 µg/dL) compared to the non-anemia group (313 to 4610 µg/dL). Finally, in the

Table 2. Comparison of Laboratory Parameters between Groups

	Iron Deficiency Anemia						
	Present (n = 45)			Absent (n = 45)			
	First Trimester (n = 15)	Second Trimester (n = 15)	Third Trimester (n = 15)	First Trimester (n = 15)	Second Trimester (n = 15)	Third Trimester (n = 15)	*P*
WBC (×10^9/L)	8.2 ± 1.91	9.15 ± 1.99	9.98 ± 2.28	8.09 ± 1.79	8.06 ± 2.63	7.56 ± 2.26	.143[a]
HCT (%)	29 (26.1-30.6)	30.3 (25.0-30.8)	30 (24.3-32.6)	36.8 (32.8-38.6)	34 (31.9-37.8)	33 (31.5-36.4)	.001[b]
HB (g/dL)	9.7 (8.1-10.3)	10 (7.2-10.4)	9.9 (7.4-10.8)	12.2 (11.2-13.5)	11.4 (11.0-12.8)	11.3 (11.0-12.4)	.001[b]
MCV (fL)	74,2 (68.6-87.5)	81,3 (55.5-89.1)	80,2 (68.3-89.9)	87 (82-94.1)	84.3 (76.8-93.3)	87.4 (70-100)	.001[b]
MCHC (g/dL)	33 (23.2-34.6)	33 (28.5-34.6)	32.4 (30.3-33.1)	34.1 (32.9-34.9)	33.6 (25.7-34.7)	34 (31.5-34.9)	.003[b]
PLT (×10^9/L)	261 ± 50.3	246 ± 61.8	270 ± 79.7	234 ± 48.9	238 ± 68.3	241 ± 62.5	.678[a]
RDW (%)	16.4 (12.7-19.4)	14.7 (12.5-24.3)	15.1 (13.8-17.4)	13.4 (12.1-14.9)	14.1 (12.5-23.8)	13.2 (12-23)	.023[b]
Ferritin (µg/L)	7.5 (5-32.9)	7,5 (3.7-63.4)	8,6 (2.7-24)	16,1 (6.0-64.7)	21.4 (7.3-44.2)	15.0 (6.9-98)	.004[b]
Iron (µg/dL)	47 (44-47)	33 (18-92)	33 (23-97)	140 (18-920)	160 (23-700)	70 (26-680)	.002[b]
TIBC (µg/dL)	3910 (403-6000)	4740 (388-6490)	589 (443-6970)	391 (326-2800)	419 (313-4610)	454 (330-5830)	.001[b]
Transferrin (%)	3.4 (0.15-4.95)	3.8 (0.14-5.04)	3.9 (0.16-5.67)	2.7 (0.23-3.99)	3.3 (0.12-4.03)	3.4 (0.14-4.60)	.629[b]
Total bilirubin (mg/dL)	0.11 (0.01-0.86)	0,21 (0.07-0.58)	0.18 (0.08-0.63)	0.24 (0.11-0.86)	0.16 (0.08-0.36)	0.23 (0.07-0.64)	.473[b]
Direct bilirubin (mg/dL)	0.09 (0.04-0.34)	0.12 (0.05-0.31)	0.14 (0.04-0.36)	0.12 (0.06-0.35)	0.08 (0.03-0.13)	0.13 (0.07-0.26)	.035[b]
Indirect bilirubin (mg/dL)	0.24 (0.01-4.45)	0.2 (0.05-4.42)	0.23 (0.04-5.29)	0.14 (0.02-3.22)	0.09 (0.03-3.31)	0.40 (0.10-3.52)	.527[b]
CRP (mg/L)	3.9 (0.3-23.5)	4.3 (0.7-38)	5.0 (1.1-29.3)	2.9 (0.80-9.7)	5.7 (0.9-23)	4.1 (1.2-29.5)	.591[b]

[a]One Way ANOVA test was used (values given as mean), [b]Kruskal–Wallis test was used (values given as median (min-max)). CRP, C-reactive protein; HB, hemoglobin; HCT, hematocrit; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, platelet count; RDW, red distribution width; TIBC, total ıron binding capacity; WBC, white blood cell.

third trimester, the anemia group had TIBC levels ranging from 443 to 6970 µg/dL, while the non-anemia group had levels ranging from 330 to 5830 µg/dL. However, there were no significant differences observed in the levels of total bilirubin and indirect bilirubin among the groups ($P > .05$) (Table 2).

Comparative Analysis of the Dt/dB among Different Groups

General Comparison

When analyzing the total Dt/dB parameters, the anemic and non-anemic groups had no meaningful difference ($P > .05$). The average levels of native thiol (NT) were similar between participants with anemia and those without anemia in all trimesters (first trimester: 244 (176.8-439.7) µmol/L vs. 248.7 (184.8-372.2) µmol/L, second trimester: 270 (196.6-383) µmol/L vs. 280.4 (208.8-420.4) µmol/L, third trimester: 276.4 (212-472) µmol/L vs. 237.5 (203.9-415.3) µmol/L) ($P > .05$). The levels of TT were similar between the study groups in all trimesters (first trimester: 321 ± 83.4 µmol/L vs. 346 ± 68.3 µmol/L; second trimester: 353 ± 75.4 µmol/L vs. 377±89.7 µmol/L; and third trimester: 375 ± 92.5 µmol/L vs. 341 ± 74.4 µmol/L). No statistically significant differences were detected between the groups ($P > .05$). Likewise, no significant difference was found in terms of disulfide, RTR, OTR, or TORR in the same trimester comparison in the group with and without anemia ($P > .05$) (Table 3).

We compared the average Dt/dB parameters of pregnant women with and without IDA within the groups, taking into account all trimesters. There were no notable variations observed among women in different trimesters within the same group when considering the average values of Dt/dB parameters ($P > .05$) (Table 4).

Comparison Based on Ferritin Concentrations

A considerable disparity was seen in the thiol/disulfide equilibrium when comparing subjects according to their ferritin levels. Participants who had ferritin levels below 20 µg/L had significantly lower circulating disulfide values compared to participants with high ferritin levels (41.6 ± 29.5 vs 51.7 ± 8.16.2; $P = .043$) (Table 5). Furthermore, circulating RTR and TORR levels exhibited

Table 3. Comparison of Dynamic Thiol-Disulfide Balance between Groups

	Absent (n = 45)			Present (n = 45)			
	First Trimester (n = 15)	Second Trimester (n = 15)	Third Trimester (n = 15)	First Trimester (n = 15)	Second Trimester (n = 15)	Third Trimester (n = 15)	*P*
TTL (µmol/L)	346 ± 68.3	377 ± 89.7	341 ± 74.4	321 ± 83.4	353 ± 75.4	375 ± 92.5	.406[a]
NTL (µmol/L)	248.7 (184.8-372.2)	280.4 (208.8-420.4)	237.5 (203.9-415.3)	244 (176.8-439.7)	270 (196.6-383)	276.4 (212-472)	.133[b]
Disulfide (µmol/L)	44.6 ± 16.5	46.6 ± 20.3	40.5 ± 16.9	40.8 ± 13.9	39.3 ± 31.4	41.3 ± 17.5	.871[a]
RTR (%)	74.6 ± 6.3	75.6 ± 7.6	76.9 ± 8.0	75.1 ± 5.1	78.4 ± 8.9	75.6 ± 12.0	.836[a]
OTR (%)	12.6 ± 3.16	12.1 ± 3.83	11.4 ± 4.04	12.4 ± 2.57	11.5 ± 4.36	12.2 ± 6.0	.958[a]
TORR (%)	565.4 (333.7-981.9)	579.4 (405.9-2667.0)	584.2 (355.6-5053.2)	640.4 (367.8-1002.4)	660.6 (316.2-2566.5)	726.5 (133.7-3111.4)	.971[b]

[a]One Way ANOVA test was used (values given as mean), [b]Kruskal–Wallis test was used (values given as median (min-max)). NTL, native thiol level; OTR, oxidized thiol level; RTR, reduced thiol ratio; TORR, thiol oxidation-reduction ratio; TTL, total thiol level.

Table 4. Comparison of Dynamic Thiol-Disulfide Balance between Trimesters

Iron Deficiency Anemia								
	Absent (n = 45)				Present (n = 45)			
	First Trimester (n = 15)	Second Trimester (n = 15)	Third Trimester (n = 15)	*P*	First Trimester (n = 15)	Second Trimester (n = 15)	Third Trimester (n = 15)	*P*
TTL (µmol/L)	346 ± 68.3	377 ± 89.7	341 ± 74.4	.406[a]	321 ± 83.4	353 ± 75.4	375 ± 92.5	.065[a]
NTL (µmol/L)	248.7 (184.8-372.2)	280.4 (208.8-420.4)	237.5 (203.9-415.3)	.317[b]	244 (176.8-439.7)	270 (196.6-383)	276.4 (212-472.2)	.081[b]
Disulfide (µmol/L)	44.6 ± 16.5	46.6 ± 20.3	40.5 ± 16.9	.644[a]	40.8 ± 13.9	39.3 ± 31.4	41.3 ± 17.5	.948[a]
RTR (%)	74.6 ± 6.3	75.6 ± 7.6	76.9 ± 8.0	.698[a]	75.1 ± 5.1	78.4 ± 8.9	75.6 ± 12.0	.571[a]
OTR (%)	12.6 ± 3.16	12.1 ± 3.83	11.4 ± 4.04	.650[a]	12.4 ± 2.57	11.5 ± 4.36	12.2 ± 6.0	.864[a]
TORR (%)	565.4 (333.7-981.9)	579.4 (405.9-2667.0)	584,2 (355.6-5053.2)	.508[b]	640,4 (367.8-1002.4)	660.6 (316.2-2566.5)	726.5 (133.7-3111.4)	.447[b]

[a]One Way ANOVA test was used (values given as mean), [b]Kruskal–Wallis test was used (values given as median (min-max)). NTL, native thiol level; OTR, oxidized thiol level; RTR, reduced thiol ratio TORR, thiol oxidation-reduction ratio; TTL, total thiol level.

Table 5. Comparison of Dynamic Thiol-Disulfide Balance with Serum Ferritin Levels

	Ferritin <20 µg/L (n = 64)	Ferritin ≥20 µg/L (n = 26)	*P*
TTL (µmol/L)	347.4 ± 97.7	385 ± 75.2	.052[a]
NTL (µmol/L)	247.9 (176.8-472.2)	274.1 (203.9-420.4)	.164[b]
Disulfide (µmol/L)	41.6 ± 29.5	51.7 ± 16.2	.043[a]
RTR (%)	77.1 ± 8.8	73.4 ± 5.7	.024[a]
OTR (%)	11.6 ± 4.3	13.2 ± 2.9	.051[a]
TORR (%)	650.5 (133.7-5053)	554.1 (333.7-977.3)	.043[b]

[a]Independent sample *t*-test was used (values given as mean), [b]Mann–Whitney *U* test was used (values given as median (min-max)). NTL, native thiol level; OTR, oxidized thiol level; RTR, reduced thiol ratio TORR, thiol oxidation-reduction ratio; TTL, total thiol level.

a statistically significant elevation in the low ferritin group compared to the high ferritin group ($P < .05$). Detailed analysis of thiol-disulfide exchange ratios revealed a significant elevation ($P < .05$) in the low ferritin group compared to the high ferritin group. Specifically, the low ferritin group exhibited a mean RTR of 77.1 ± 8.8 and a median TORR of 650.5 (interquartile range: 133.7-5053), while the high ferritin group had a mean RTR of 73.4 ± 5.7 and a median TORR of 554.1 (interquartile range: 333.7-977.3) (Table 5).

Consistent with these findings, serum iron levels and hemoglobin values were also lower in the low ferritin group compared to the high ferritin group. The median serum iron level in the low ferritin group was 42 µg/dL (range: 18-80), whereas the high ferritin group had a median of 81 µg/dL (range: 23-92). Similarly, the median hemoglobin level in the low ferritin group was 10.1 g/dL (range: 7.2-12.7), compared to a median of 11.4 g/dL (range: 9.8-13.5) in the high ferritin group.

Discussion

This study sought to elucidate the interplay between IDA and circulating OSBs in pregnant women, with a particular focus on evaluating the dynamic thiol/disulfide redox balance across the gestational trimesters. The current results suggest that there is no meaningful difference in thiol/disulfide equilibrium between pregnant women with and without IDA. Nevertheless, notable discrepancies were noted when comparing circulating disulfide values, RTR, and TORR levels in relation to ferritin concentrations. These findings provide important insights into the intricate relationship between iron metabolism and OS during pregnancy.

The research's findings suggest that there were no significant differences in the Dt/dB among pregnant women with or without IDA. This indicates that the existence of anemia, as determined by hemoglobin and hematocrit levels, may not be an adequate indicator of the OS status in this particular group of people. Nevertheless, upon analyzing ferritin levels, which provide a more precise indication of the body's iron reserves, we noticed notable disparities in OSBs. Pregnant women with ferritin levels below 20 µg/L displayed significantly lower circulating disulfide levels and, conversely, higher circulating RTR and TORR levels compared to those with ferritin exceeding 20 µg/L. Notably, the median hemoglobin values in both groups fell within the WHO's acceptable range for anemia in pregnancy. However, it's important to acknowledge that individual hemoglobin values within each group could potentially fall below or surpass the anemia threshold.

In addition, iron plays a role in the production of ROS through the Fenton reaction. This reaction involves the interaction between ferrous iron (Fe2+) and hydrogen peroxide (H_2O_2), resulting in the formation of highly reactive and damaging hydroxyl radicals (•OH). Iron deficiency anemia decreases the body's ability to produce ROS due to decreased iron levels, which can unexpectedly decrease oxidative damage in specific tissues. Low ferritin levels suggest a depletion of iron stores, which might weaken the body's antioxidant defense mechanisms, leading to an increase in OS.[14]

Studies suggest elevated OS during pregnancy.[15] Ischemia-modified albumin (IMA) has emerged as a potential marker of OS, particularly in ischemic conditions.[16] To further investigate this relationship, a 2020 study compared the thiol/disulfide redox balance, which indicates cellular oxidative status, with IMA levels in 215 healthy pregnant women and 74 healthy non-pregnant controls. The study showed that Dt/dB tended to be shifted toward OS in pregnant women, and serum IMA values were higher in pregnant women.[17] In a study conducted by Zelanzniewick et al[18] in 2015, 8-hydroxy-2'-deoxyguanosine and 8-iso-ProstaglandinF2α levels were found to be higher in first trimester pregnant women compared to non-pregnant women. Another study examining malondialdehyde (MDA) and total antioxidant capacity (TCA) levels in placental tissues in all 3 trimesters provides additional evidence for increased oxidative stress levels during pregnancy.[19] These studies provide evidence that pregnancy can lead to OS. In our study, we specifically focused on pregnant women to ensure that the impact of pregnancy-related OS does not interfere with our research findings.

In addition, it has been shown that OS is a consequence of IDA. Disrupting the resistance of erythrocytes to OS can result in structural and functional damage associated with iron deficiency anemia.[20] Studies have shown that there may be disruptions in the Dt/dB in people with IDA. A study was conducted to measure Dt/dB in 39 women diagnosed with IDA and 39 women without anemia. When both groups were compared, it was discovered that patients with IDA had lower levels of TT and native thiols, and higher levels of IMA.[21] The fact that no significant difference was found between the groups with and without iron deficiency in our study may be due to the fact that both groups consisted of pregnant women. In the study conducted by Bozkaya et al[22], thiol levels (NT, TT) were found to be lower in pregnant women with IDA compared to healthy pregnant women. The fact that the study was conducted on patients with more severe anemia and did not cover all 3 trimesters may have produced different results. In a separate study conducted in 2013, a group of 40 children diagnosed with IDA and another group of 40 children without anemia, ranging in age from 6 to 60 months, were analyzed. The findings showed a significant rise in overall OS among individuals with anemia, while the total antioxidant capacity remained similar in both groups.[23] In a study conducted by Bilgili et al[24], a group of 140 female patients with IDA was compared to a control group of 84 healthy women without anemia. The levels of IMA were found to be significantly elevated in the group with IDA. According to previous studies, low hemoglobin levels, which are characteristic of IDA, have been found to cause hypoxia, a condition that is known to increase IMA levels. The connection between IDA and OS has been further reinforced by Nagababu et al[25], who showed that increased OS affects erythrocytes in mice with iron-deficient anemia. The findings of these studies provide evidence for the heightened OS observed in individuals with IDA. In contrast to other studies, our study found

no significant difference in terms of OS among anemic patients when pregnant women were evaluated individually. A study conducted in 2016 examined 35 children between the ages of 5 and 15 who had normal hemoglobin levels but low ferritin levels (<12 ng/mL). The findings revealed that the case group, with low ferritin levels but no anemia, had significantly higher OS compared to the normal control group with normal ferritin levels. This suggests that low ferritin levels alone can lead to OS.[26] Our study yields comparable findings to this study, suggesting that the use of hemoglobin and hematocrit values alone may not lead to a conclusive diagnosis of OS. Nevertheless, a more significant outcome can be achieved when taking ferritin values into account. This study further supports our perspective that ferritin is a more suitable indicator when it comes to predicting OS. There are few studies examining oxidative stress in cases of low iron stores without anemia. In the study by McAnulty et al[27], serum selenium and glutathione peroxidase levels of patients with ferritin ≤20 µg/L without anemia were found to be similar to the control group. Similarly, no significant difference was found in plasma lipid hydroperoxide and protein carbonyl levels in the study by Gropper et al[28] in which two non-anemic groups defined the same limit for ferritin were compared. Studying different parameters in different groups may have caused contradictory results. Our study was designed in pregnant women, unlike other studies. While the increase in disulfide and TORR indicates the oxidative environment, the increase in TT and NT indicates the antioxidant environment.[29,30] In our study, the decrease in disulfide in the ferritin <20 µg/L group reflects the antioxidant environment, suggesting a compensatory mechanism against the oxidative environment.

Anemia during pregnancy is risky in terms of factors that increase mortality, such as sepsis, preeclampsia, eclampsia, heart failure, increased risk of cesarean section and bleeding requiring a blood transfusion, and hemorrhagic shock.[31,32] There are also studies that maternal anemia affects cognitive functions both during pregnancy and after birth and increases the risk of depression.[33] Bozkaya et al[22] stated that iron deficiency anemia can cause cardiac pathologies due to increased oxidative stress.

Considering the negative fetomaternal effects of iron deficiency anemia and oxidative stress, it is important to pay due attention to the prevention and treatment of anemia. Studies have found that prophylactic iron administration increases oxidative stress but reduces oxidative stress when used for the treatment of anemia.[34] In a study examining fetomaternal complications and oxidative stress in 28-32 weeks pregnant women, it was recommended that treatment be started as early as possible in order to prevent negative effects.[35]

The initial approach to treatment involves the use of oral iron preparations, which are available in two forms: ferric and ferrous. To quickly address moderate to severe anemia, intravenous (IV) iron formulations are more commonly recommended.[36] Compared to bivalent oral iron preparations, trivalent oral iron forms, which are thought to have fewer side effects, appear to offer advantages in terms of patient compliance.[37] Some studies suggest that parenteral iron treatments, such as iron sucrose, should be administered alongside antioxidant agents to mitigate their negative effects on oxidative stress.[38] The oxidative stress caused by iron that fails to bind to transferrin during electron exchange in daily oral administration also indicates the need for research into alternative iron treatment methods.[39] Studies have also explored the relationship between effective iron absorption and increased hepcidin levels across various administration schedules. It is evident that further research is required to assess the efficacy of daily versus weekly iron supplementation.[37,40]

Our findings showed that ferritin levels, as opposed to hemoglobin or iron levels, serve as more dependable OSBs. Monitoring ferritin levels in expectant mothers may provide a more accurate assessment of their iron status and help identify those at higher risk of experiencing adverse effects due to iron deficiency anemia.

Implementing more focused interventions, such as administering iron supplements or antioxidant therapy, could help reduce the impact of OS and improve the health outcomes of mother and fetus dyads. We believe that future studies are needed that include various treatment methods and a wider population.

Limitations and Strengths

Our research, while carefully conducted with a methodology that included a cohort design and thorough assessment of OSBs, does have its limitations that need to be acknowledged. One important point to consider is the number of samples in our study particularly when analyzing subgroups based on trimesters. This means that our findings may not be broadly applicable to the population. Further studies are necessary to validate our results and explore how iron deficiency anemia affects survival in populations. Factors that could impact OS levels, such as diet, exercise, and environmental exposures, were not examined in this study. There is a need for studies that standardize these parameters. In addition, our study did not conduct a longitudinal study in which participants were followed throughout their pregnancies. Future studies conducting a longitudinal study following mothers throughout pregnancy may reveal dynamic changes in Dt/dB and provide a more comprehensive understanding of the interaction between iron status and oxidative stress. To gain an understanding of the connection between IDA and OS during pregnancy, future research should take these factors into account.

Conclusions

In conclusion, our research emphasizes the relationship between anemia and OS in women, highlighting the significance of ferritin levels as an OSB. While conventional methods of diagnosing anemia may not fully capture stress levels, focusing on ferritin levels can provide a precise evaluation and guide targeted interventions to mitigate complications associated with OS.

Further investigation is needed to delve into this correlation by overcoming the constraints of existing research and discovering avenues and therapeutic choices to enhance the well-being of mother and fetus dyads. Understanding the details of the link between iron metabolism and OS can enable us to formulate approaches to assist in the care of expectant mothers and their infants.

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Republic of Türkiye Ministry of Health, Haseki Training and Research Hospital (Approval no: 104/22, Date: September 29, 2022).

Informed Consent: Written informed consent was obtained from women who participated in this study.

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