Türk Klinik Biyokimya Derg 2024; 22(1): 1-10

Araştırma Makalesi / *Research Article*

https://doi.org/10.56615/tkbd.2024.01

Oxidative Stress Markers in Reproductive-Age Women with Iron Deficiency Anemia

Demir Eksikliği Anemisi Olan Üreme Çağındaki Kadınlarda Oksidatif Stres Belirteçleri

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Received / Başvuru Tarihi: 06 Aralık 2023 *Accepted* / Kabul Tarihi: 11 Ocak 2024

ABSTRACT

Aim: During iron deficiency anemia (IDA), increased oxidative stress is accompanied by reduction in antioxidant defense systems and increased lipid peroxidation. We aimed to assess oxidative balance and Deoxyribonucleic Acid (DNA) damage in reproductive-aged women with IDA.

Material and Methods: This study included 30 women who had clinically confirmed diagnoses of IDA at the Internal Medicine Polyclinics. Also, we had 30 healthy women compatible with age and body mass index in the control group. Serum levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were quantified via the ELISA technique, while levels of total oxidant status (TOS) and total antioxidant status (TAS) were determined through a spectrophotometrically.

Results: Patients with IDA exhibited lower TAS (p=0.001), but higher TOS, 8-OHdG and OSI levels than the controls (p<0.001). TOS had relatively better diagnostic performance with 73.3% sensitivity and 90.0% specificity at a cut-off value 23.7. TOS levels were found to be correlated better with hemoglobin, hematocrit, ferritin, erythrocyte indices, iron, and transferrin saturation.

Conclusion: In reproductive-age women with IDA, we showed reduced antioxidant status, and the balance shifted to the oxidative side, including DNA damage.

Key Words: Iron Deficiency Anemia; Oxidative Stress; 8-Hydroxy-2-Deoxyguanosine.

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Etik onay: Fırat Üniversitesi Tıp Fakültesi, Etik Kurulu 27.07.2023 tarihi ve 2023/10- 26 sayılı kurul kararı

ÖZET

Amaç: Demir eksikliği anemisi (DEA) sırasında, artan oksidatif strese, antioksidan savunma sistemlerinde azalma ve lipid peroksidasyonunda artış eşlik eder. DEA olan üreme çağındaki kadınlarda, oksidatif dengeyi ve Deoksiribonükleik Asit (DNA) hasarını değerlendirmeyi amaçladık.

Materyal ve Metod: Dahiliye Polikliniğinde DEA tanısı klinik olarak doğrulanan 30 kadın çalışmaya alındı. Ayrıca, yaş ve vücut kitle indeksi uyumlu 30 sağlıklı kadın kontrol grubumuzdaydı. Serum 8-hidroksi-2'-deoksiguanozin (8-OHdG) seviyeleri ELISA tekniği ile ölçülürken, toplam oksidan durum (TOS) ve toplam antioksidan durum (TAS) seviyeleri spektrofotometrik olarak belirlendi.

Bulgular: DEA'lı hastalarda kontrollere göre daha düşük TAS (p=0,001), ancak daha yüksek TOS, 8-OHdG ve OSI düzeyleri görüldü (p<0,001). TOS, 23,7 kesme değerinde %73,3 duyarlılık ve %90,0 özgüllük ile nispeten daha iyi tanısal performansa sahipti. TOS düzeylerinin hemoglobin, hematokrit, ferritin, eritrosit indeksleri, demir, transferrin satürasyonu ile daha iyi korele olduğu görüldü.

Sonuç: Üreme çağındaki DEA'lı kadınlarda antioksidan durumunun azaldığını ve dengenin, DNA hasarı da dahil olmak üzere oksidatif tarafa kaydığını gösterdik.

Anahtar Sözcükler: Demir Eksikliği Anemisi; Oksidatif Stres; 8-Hidroksi-2-Deoksiguanozin.

INTRODUCTION

Iron deficiency anemia (IDA) is a chronic medical and public health issue accompanying decreased serum ferritin and transferrin levels (1). Unless the etiological factors causing IDA are resolved, finding a solution with iron replacement alone is very difficult. Many health problems due to IDA have been reported in underdeveloped and developing countries, especially women of reproductive age. Besides inadequate nutrition status, an increase in duration and amount of menstrual bleeding, bleeding due to obstetric problems, or postpartum pathologies are the leading causes of IDA in those women (2). The changes in oxidative stress balance markers and Deoxyribonucleic Acid (DNA) damage indicators in the circulation of women diagnosed with IDA during reproductive age are unknown exactly.

Oxidative stress is the disruption of the balance between the production, accumulation, and neutralization of reactive oxygen species (ROS) (3, 4). Deficiencies in various trace elements, particularly ionic iron, can lead to oxidative stress, which in turn can cause DNA damage by increasing ROS levels (4). Increased fluorescent heme degradation products were demonstrated in a mouse model fed an iron-deficient diet (5). Increased susceptibility to pro-oxidant effects, decreased antioxidant defense mechanisms, and increased peroxidation of lipids all contribute to increased oxidative stress during anemia. In iron deficiency, programmed death mechanisms such as stiffness in the erythrocyte membrane, inability to respond appropriately to shape change, and increased cytosolic calcium can also cause oxidative stress (6). Moreover, it was shown that microcytic erythrocytes were more susceptible to oxidants, with increased membrane stiffness and hemolysis (7).

Measuring individual oxidative stress indicators in circulation and tissues is difficult and time-consuming. We can have preliminary information about oxidative stress due to IDA through the measurement of total antioxidant status (TAS) and total oxidant status (TOS), which represent a balance between antioxidant and oxidant molecules (8, 9). By proportioning these two molecules, the oxidative stress index (OSI) is obtained. The increase or decrease in OSI determines whether oxidative damage will develop (10). 8-hydroxy-2′-deoxyguanosine (8-OHdG) serves as an indicator of DNA damage caused by oxidative stress initiated by ROS. When ROS, like the hydroxyl radical, interact with cellular components such as membranes, proteins, and nucleic acids, they induce modifications in both nuclear

and mitochondrial DNA, leading to the formation of 8-OHdG (3). Therefore, DNA damage caused by oxidative stress can be estimated by measuring the rise in the concentrations of 8-OHdG in biological fluids or tissues (3, 4).

Although we have observed studies on DNA damage and oxidative stress in IDA in the general population, we think our study could have clinical importance as it analyzed the effects of IDA on oxidative stress in women of reproductive age with these parameters for the first time. This study aimed to assess oxidative balance and oxidative stress-induced DNA damage in reproductive-aged women with IDA.

MATERIALS AND METHODS

This research was conducted under the Rules of the Declaration of Helsinki, with the approval of the Fırat University Non-Interventional Research Ethics Committee (Number: 2023/10-26 and Date: 27.07.2023). A total of 60 women in the reproductive period were included in the study; 30 women were clinically confirmed diagnosed with IDA at the Internal Medicine Polyclinic from August to October 2023. Also, we had 30 healthy women compatible with body mass index (BMI) and age in the control group. Every participant involved in the study received information about it and provided their consent.

Individuals with blood hemoglobin (HGB) levels≤12 g/dL (11) and ferritin≤15 μg/L (12) were included in the patient group. Patients with acute or chronic infection, erythrocyte or whole blood transfusion in the last three months, a history of anemia due to other causes, vitamin B12<150 pg/mL (13), and folate<3 ng/mL levels (14), use of steroids or immunosuppressive drugs, systemic inflammatory diseases, diabetes mellitus, thyroid dysfunction, cardiovascular diseases, malignancy, hematological diseases such as thalassemia and multiple myeloma, below 18 years or pregnant were excluded this study.

Serum 8-OHdG levels were measured by ELISA using the Human 8-OHdG (Sunred Biotechnology Company, Cat No: 201-12-1437, Shanghai, China). The absorbances were read at a wavelength of 450 nanometers via the Bio-Tek ELX800 (BioTek Instruments, USA) device. In the kit insert, it was stated that intra-assay CV<10% and inter-assay CV<12%. The measurement range of 8-OHdG was 1-100 ng/mL, the sensitivity was 0.558 ng/mL.

TAS and TOS levels were measured spectrophotometrically via an AU680 device (Beckman Coulter Inc., Brea, CA, USA) using a Rel Assay kit (Mega Medicine Industry & Trade Co., Gaziantep, Turkey). The formula was utilized to calculate OSI: [(TOS, μmol H2O2 Eq/L) / (TAS, mmol Trolox Eq/L)*100]. TAS levels were transformed into units of μmol Trolox Equivalents per liter when determining the OSI values.

Complete blood count (CBC) was analyzed via ABX Pentra DX 120 (Horiba Medical, Montpellier, France). Serum iron and iron binding capacity were determined via an AU680 device (Beckman Coulter Inc., Brea, CA, USA). Ferritin levels were determined via Maglumi 4000 Plus (Snibe Diagnostics, Shenzhen, China). The following formula was used for determining transferrin saturation (TS): [serum iron/serum total iron binding capacity (TIBC)] *100.

Statistical Analysis

To evaluate the normal distribution of continuous data, the Shapiro-Willk test was employed. Continuous data were displayed as either mean with standard deviation or median with the 25th and 75th percentiles. The Mann-Whitney-U or Student's t-test was employed to compare continuous variables. The relationships between parameters were evaluated using Spearman correlation analyses. Separate logistic regression analysis was undertaken to account for multi-collinearity between the oxidative stress indicators. The diagnostic performance of the oxidative stress

parameters was evaluated through the Receiver Operating Characteristic (ROC). Statistical analyses were conducted using IBM SPSS v. 26.0 (IBM Corp., Armonk, NY, US), GraphPad Prism 8.0 (GraphPad Software, San Diego, California, US), and MedCalc v. 22.009 (MedCalc Software Ltd, Ostend, Belgium). A significance level (p-value) of less than 0.05 was used for consideration.

RESULTS

No statistical differences were observed between the control group and patients with IDA with respect to BMI, age, red blood cell (RBC), leukocyte count, and mean platelet volume (MPV) levels. TIBC, platelet count, and RBC distribution width coefficient of variation (RDW-CV) levels were found to be higher; iron, TS, ferritin, HGB, hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) levels were found to be lower in IDA. TAS levels in IDA patients were lower than those in the controls, while TOS, 8-OHdG, and OSI were significantly higher in IDA patients (Table 1 and Figure 1).

TOS performed the highest area under the curve (AUC) value with 0.871 (95% confidence interval [CI]=0.759–0.944) at a cut-off value of 23.7, p<0.001. OSI performed the AUC value of 0.831 (95% CI=0.712–0.915) at a cut-off value of 2.44, p<0.001. TAS performed the AUC value of 0.750 (95% CI=0.621–0.853) at a cut-off value of 0.89, p<0.001. 8-OHdG performed the AUC value of 0.829 (95% CI=0.710–0.914) at a cut-off value of 5.52, p<0.001 (Table 2 and Figure 2).

Table 1. Comparison of demographic and laboratory findings between the controls and iron deficiency anemia

Parameter	Control Group (n=30)	IDA Group (n=30)	p
Age (years)	26.9±4.79	27.1±4.61	0.827*
Body Mass Index (kg/m^2)	24.2 (22.9 to 24.7)	23.4 (22.4 to 24.2)	0.085**
8-OHdG (ng/ml)	4.71±1.67	6.91±1.88	**<0.001***
TAS (mmol Trolox Eq/L)	1.04 (0.95 to 1.32)	0.87 (0.72 to 0.99)	**0.001****
TOS (μmol H_2O_2 Eq/ L)	21.9 (19.2 to 23.2)	24.1 (23.5 to 25.8)	**<0.001****
OSI (arbitrary unit)	1.94±0.59	2.95±0.96	**<0.001***
Iron (μg/dL)	61.0 (47.0 to 84.0)	23.0 (19.0 to 28.0)	**<0.001****
TIBC (μg/dL)	379 (343 to 413)	450 (423 to 464)	**<0.001****
Transferrin Saturation (%)	15.9 (13.0 to 25.3)	4.79 (4.32 to 6.52)	**<0.001****
Ferritin (µg/L)	48.5 (29.2 to 65.5)	5.14 (4.04 to 6.93)	**<0.001****
Leukocyte count (10^9/L)	6.69±1.41	7.26±1.38	0.120*
Platelet count (10^9/L)	257 (239 to 305)	344 (234 to 356)	**0.034****
MPV (fL)	9.48±0.95	9.33±0.83	0.518*
Red blood cell count (10^9/L)	4.84±0.32	4.83±0.43	0.954*
Hemoglobin (g/dL)	13.2 (12.8 to 14.1)	10.1 (9.20 to 10.6)	**<0.001****
Hematocrit (%)	39.4 (38.6 to 42.7)	31.9 (30.4 to 33.6)	**<0.001****
MCV (fL)	84.5±2.97	67.4±6.78	**<0.001***
MCH (pg)	28.0±1.23	21.1±2.91	**<0.001***
MCHC (g/dL)	33.3±1.05	30.9±1.58	**<0.001***
RDW-CV (%)	13.4±0.49	18.6±2.25	**<0.001***

*Student t-test, **Mann-Whitney U test. TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative stress index, 8-OHdG: 8-hydroxy-2-deoxyguanosine, TIBC: total iron-binding capacity, MPV: mean platelet volume, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW-CV: red blood cell distribution width coefficient of variation.

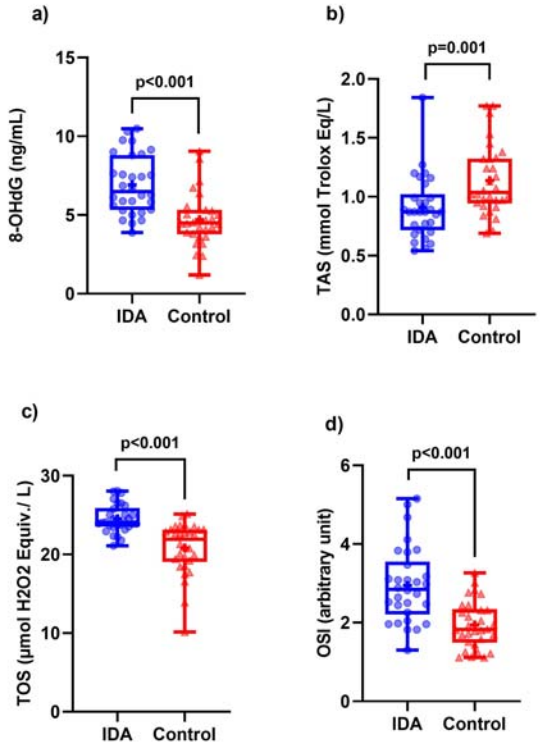

Figure 1. Comparison of oxidative stress indicators between the controls and iron deficiency anemia

In Spearman correlation, TOS, 8-OHdG, and OSI were observed to have a negative correlation with HGB, ferritin, HCT, MCH, MCHC, MCV, iron, and TS; positively correlated with RDW-CV, TIBC. On the contrary, TAS levels exhibited a positive correlation with HGB, ferritin, HCT, MCH, MCHC, MCV, iron, and TS, inversely correlated with TIBC (Figure 3). However, it was observed that platelet and MPV values did not correlate with oxidative stress markers. When the relationship between hematological parameters and platelets was evaluated, platelet levels were found to be negatively related to MCV (r=-0.415, p=0.001), TS (r=-0.414, p=0.001), iron (r=-0.366, p=0.004), HGB (r=-0.383, p=0.003), ferritin (r=- 0.405, p=0.001).

In the logistic regression analysis, it was observed that 8-OHdG [odds ratio (95% CI)=2.235 (1.436-3.479), p<0.001], TAS [odds ratio (95% CI)=0.041 (0.004-0.428), p=0.008], TOS [odds ratio (95% CI)=2.508 (1.463-4.298), p=0.001], OSI [odds ratio (95% CI)=5.978 (2.182-16.38), p=0.001] were independently linked to IDA after adjusted with age and BMI (Table 3).

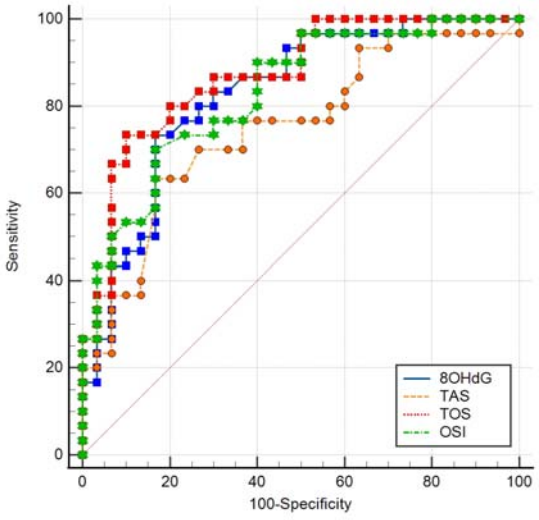

Figure 2. Receiver operating curve analysis of oxidative stress indicators for iron deficiency anemia

Table 2. Receiver operating curve analysis of oxidative stress indicators for iron deficiency anemia

Parameter	AUC	95 CI%	Cut-off	Sensitivity	Specificity	P
TOS (μmol H_2O_2 Eq/ L)	0.871	0.759 to 0.944	>23.7	73.3%	90.0%	**<0.001**
OSI (arbitrary unit)	0.831	0.712 to 0.915	>2.44	70.0%	83.3%	**<0.001**
8-OHdG (ng/ml)	0.829	0.710 to 0.914	>5.52	73.3%	83.3%	**<0.001**
TAS (mmol Trolox Eq/L)	0.750	0.621 to 0.853	≤0.89	63.3%	83.3%	**<0.001**

TOS: total oxidant status, OSI: oxidative stress index, 8-OHdG: 8-hydroxy-2-deoxyguanosine, TAS: total antioxidant status

Table 3. Factors independently associated with iron deficiency anemia, multiple logistic regression analysis.

Unadjusted Univariate Regression				*Adjusted Multivariate Regression*			
Parameter	**OR**	**95% CI**	**P**	**Parameter**	**OR***	**95% CI**	**P**
8-OHdG (ng/ml)	2.071	1.377-3.115	**<0.001**	**8-OHdG (ng/ml)**	2.235	1.436-3.479	**<0.001**
TAS (mmol Trolox Eq/L)	0.041	0.004-0.405	**0.006**	**TAS (mmol Trolox Eq/L)**	0.041	0.004-0.428	**0.008**
TOS (μmol H_2O_2 Eq/ L)	2.356	1.435-3.868	**0.001**	**TOS (μmol H_2O_2 Eq/L)**	2.508	1.463-4.298	**0.001**
OSI (arbitrary unit)	6.257	2.281-17.16	**<0.001**	**OSI (arbitrary unit)**	5.978	2.182-16.38	**0.001**

*Adjusted: age, BMI. 8-OHdG: 8- hydroxy-2-deoxyguanosine, TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative stress index.

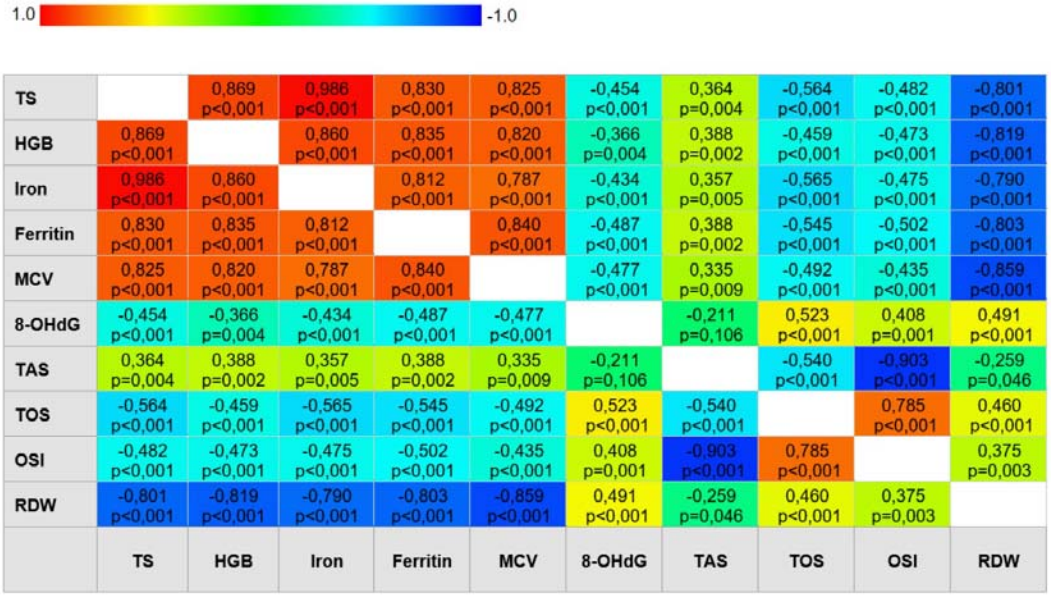

Figure 3. Correlations between oxidative stress indicators and the other variables in all groups.

DISCUSSION

Patients with IDA exhibited lower TAS levels in comparison to the controls. Conversely, TOS, 8-OHdG, and OSI were higher in IDA patients. Moreover, regression analysis showed that these oxidative stress markers had independent associations with the presence of IDA when adjusted according to age and BMI values. As a result, we showed that antioxidant status decreased, and the balance shifted to the oxidative side, including oxidative stress-induced DNA damage, in reproductive-age women with IDA.

Iron, the most prevalent trace element in the human body, plays a crucial role in regulating oxidative balance. Both iron excess and deficiency could lead to deterioration in mitochondrial functions. During iron excess, the production of ROS increases as electron transport in the oxidative respiratory chain is impaired. Certain toxic substances, such as ROS, cause iron-dependent cell death, known as ferroptosis (15-17). Iron plays a crucial role in various biological pathways, including DNA metabolism and mitochondrial function, in addition to its function in oxygen transport as part of hemoglobin. Heme synthesis is

particularly linked to mitochondrial function, highlighting the importance of proper iron functionality in this process (17). Mitochondria play critical roles in the hematopoietic system, from the differentiation and commitment of hematopoietic stem cells to the functioning and survival of mature blood cells (18). Mitochondrial dysfunction has been associated with a range of hematopoietic disorders, including bone marrow failure syndromes (19). Iron restriction has been found to suppress mitochondrial activity in erythroid precursor cells, highlighting the connection between mitochondrial function and erythropoiesis (20). Iron deficiency can contribute to ineffective erythropoiesis, probably due to mitochondrial dysfunction with abnormal transport and incorporation of iron into heme (21). It is not possible to replace iron with food or supplements in patients diagnosed with IDA. Therefore, exogenous iron medication is required in IDA. When the literature data are reviewed, the data on oxidative stress changes observed after iron supplementation is contradictory. In a study on this subject, prophylactic iron supplementation in non-anemic pregnant led to an elevation in oxidative stress and inflammation. Nevertheless, iron supplementation in pregnant individuals with anemia was found to reduce inflammation without affecting oxidative stress (22). Administering excess exogenous iron and causing an overfilling of stores could trigger oxidative damage (23). However, we did not evaluate whether the oxidative status of the patient group changed after exogenous iron supplementation. While TAS decreased in the IDA group, TOS and OSI increased, supporting the existence of oxidative damage due to low iron levels, in our study.

There was limited data on how IDA affects oxidative stress in studies conducted on patients of different age groups and genders. It was reported that the oxidative balance was negatively affected, especially in childhood IDA (7, 24). In line with this, it was shown that IDA was among the causes of attention deficit in school age, and many studies covered the association between oxidative stress and IDA in children. The elevation of OSI and TOS levels, coupled with the reduction in TAS levels among children with IDA, demonstrated an alteration in the oxidant-antioxidant balance, favoring oxidative stress (24). In children with IDA, TOS levels increased, and this elevation could be normalized through iron replacement (7). In another study, an increase in the disulfide/thiol ratio and a decrease in ferroxidase activity were observed. Moreover, a negative correlation was found between disulfide and HGB, iron, and ferritin levels, and a positive relationship was found between ferroxidase activity and these variables (25). While lipid peroxidation increased dramatically in a hydroperoxide-superoxide-dependent manner in children with IDA, on the contrary, enzymatic antioxidant capacity (catalase and superoxide dismutase) was significantly reduced in the patient groups (26). In some other studies, several oxidative stress markers were elevated in pediatric patients with IDA (7, 24, 27, 28).

Since mitochondria cannot perform their ROS scavenging function in the presence of excessive ROS, first lipid peroxidation and then DNA damage occurs. The possible mechanism of the increase in 8-OHdG levels in the IDA group could be nuclear and mitochondrial DNA destruction due to increased ROS (3, 4, 29). To our knowledge, there are limited studies regarding the 8-OHdG parameter in patients with IDA of both genders. Nevertheless, serum 8-OHdG levels were found to be higher in IDA groups than in the controls (29), in a study supporting our study. Another study showed a significant association between DNA damage in lymphocytes and the presence of IDA. HGB levels were also inversely associated with lymphocyte DNA damage (30). In both iron deficiency and IDA, increased DNA damage was shown in patients compared to controls (31, 32). It

has been stated that using a low-dose iron treatment might be adequate to enhance antioxidant status and reduce DNA damage (32). In females with IDA, HGB levels were positively associated with TAS and negatively associated with DNA damage (31). We observed an inverse correlation between 8-OHdG levels and parameters such as HGB, RBC indices, ferritin, iron, and TS.

In women with IDA in the reproductive period similar to our patient group, antioxidant markers, such as native thiol and total thiol levels, were observed to be reduced. Conversely, higher ischemia-modified albumin values were found to indicate disturbed oxidative balance in favor of hypoxia in the organism (6). In a study conducted on women over 40 years of age, TOS and OSI values of the IDA group were higher than the non-IDA group, and TAS and myeloperoxidase levels were lower (33). In another study, women with IDA had higher total peroxide and OSI values and lower TAS levels than in the controls. In addition, HGB levels showed a positive relationship with TAS levels and an inverse relationship with total peroxide, and OSI levels (34). According to another study conducted on women, arylesterase and paraoxonase were lower in those with IDA than in the controls, while lipid hydroperoxide levels were higher. Moreover, HGB, ferritin, and MCV showed a positive link to paraoxonase and arylesterase, and a negative link to lipid hydroperoxide (35). By forming a chelate with free iron when oxidative stress is elevated, ferritin protects against oxidative damage (7). In support of these findings, our study found correlated oxidative stress indicators with parameters such as HGB, ferritin, TS, and RDW, which indicate the severity of iron deficiency.

Obesity was related to impaired iron metabolism (36). As a strength of this study, we had no difference between the groups with regard to age and BMI. Limitations include the inclusion of only female gender, a single-center and cross-sectional study design, and the fact that the effect of iron treatment response to oxidative stress was not examined. Soluble transferrin receptor, reticulocyte, and reticulocyte hemoglobin content values could not be studied due to technical reasons. We also did not evaluate 8-OHdG levels in tissues such as leukocytes or urine.

CONCLUSION

We showed an independent relationship between IDA and oxidative stress-induced DNA damage. Elevated 8-OHdg levels in women in the reproductive period diagnosed with IDA could indicate a vicious cycle regarding prognosis, including mitochondrial dysfunction and impaired heme synthesis. Since TOS levels correlated better with erythrocyte indices and iron status, it can be used as a prognostic marker for the severity of IDA. We believe that it could be useful to monitor serum TOS and 8-OHdg levels with iron replacement. Prospective cohort studies are needed to confirm the improvement in oxidative damage with appropriate iron treatment by changes in these markers.

Ethical Approval: Approval was obtained from the ethics committee of our institution with the decision numbered 2023/10-26 and dated July 27, 2023.

Funding: The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest: None

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