Turkish Journal of Pediatric Disease
Türkiye Çocuk Hastalıkları Dergisi

Dynamic Thiol-Disulfide Homeostasis in Children with Newly Diagnosed Iron Deficiency Anemia

Yeni Tanı Almış Demir Eksikliği Anemisi Olan Çocuklarda Dinamik Tiyol-Disülfid Homeostazisi

Burcak KURUCU[1], Ali FETTAH[1], Sule YESIL[1], Esra FIRAT OGUZ[2], Ozcan EREL[2,3], Gurses SAHIN[1]

[1]Department of Pediatric Hematology and Oncology, University of Healthy Sciences, Dr Sami Ulus Maternity Child Health and Diseases Training and Research Hospital, Ankara, Turkey
[2]Department of Biochemistry, Ankara City Hospital, Ankara, Turkey
[3]Department of Biochemistry, Faculty of Medicine, Yıldırım Beyazıt University, Ankara, Turkey

ABSTRACT

Objective: Iron is an element, which is found in the structure of antioxidant enzymes and has an important role in the inactivation of reactive oxygen species. Disruption of oxidant-antioxidant balance may be playing a role in the pathogenesis of iron deficiency anemia (IDA). Dynamic thiol-disulfide homeostasis (DTDH) and serum ischemia-modified albumin (IMA) levels are important indicators of pro-oxidant/antioxidant status. In this study, we aimed to evaluate DTDH parameters and serum IMA levels in children with newly diagnosed IDA, who did not receive iron therapy.

Material and Methods: Fifty patients diagnosed with IDA and 33 healthy age- and sex-matched control patients were included in the study. DTDH parameters and IMA levels of the patients and control groups were measured. The same parameters were also compared in patients with Hb<7 g/dl (profound IDA) (n:14/50, 28%) and Hb≥7 g/dl (mild-moderate IDA) (n: 36/50, 72%) in the IDA group. The relationship between DTDH parameters in these groups were investigated.

Results: Native thiol, total thiol, native thiol/total thiol levels, constituting antioxidant capacity indicators, were found to be significantly lower in IDA patients; while oxidant disulfide, disulfide/native thiol, disulfide/total thiol, and IMA levels were found to be statistically higher compared to those in the control group (p<0.050). When DTDH parameters and IMA levels were examined; there was a positive correlation between antioxidant parameters and a negative correlation between oxidative parameters with hemoglobin and ferritin levels (p<0.050). Also, oxidative parameters were found to be much higher in profound IDA group than in the group with Hb>7 g/dl (p<0.050).

Conclusion: In this study, increase in serum disulfide and IMA levels with the decrease in serum native thiol and total thiol levels indicated oxidative stress in IDA patients before treatment, compared to the control group. Evaluation of these indicators in children is important in predicting the toxicity due to IDA.

Key Words: Hemoglobin, Iron deficiency anemia, Ischemia modified albumin, Oxidative damage, Thiol-disulfide

0000-0003-3474-5070 : KURUCU B
0000-0003-4109-2143 : FETTAH A
0000-0002-2328-8612 : YESIL S
0000-0002-8147-5379 : FIRAT OGUZ E
0000-0002-2996-3236 : EREL O
0000-0002-8569-3971 : SAHIN G

Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. The Hospital's Clinical Research Ethics Committee approved the study (numbered 18.03.2021, E-21/03-135).

Contribution of the Authors / Yazarların katkısı*: *KURUCU B: Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***FETTAH A:*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***YESIL S:*** Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***FIRAT OGUZ E:*** Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***EREL O:*** Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***SAHIN G:*** Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar.

How to cite / *Atıf yazım şekli* : Kurucu B, Fettah A, Yesil S, Firat Oguz E, Erel O and Sahin G. Dynamic Thiol-Disulfide Homeostasis in Children with Newly Diagnosed Iron Deficiency Anemia. Turkish J Pediatr Dis 2023;17: 56-61.

Correspondence Address / Yazışma Adresi:
Burcak KURUCU
Department of Pediatric Hematology and Oncology,
University of Healthy Sciences,
Dr Sami Ulus Maternity Child Health and Diseases Training and Research Hospital, Ankara, Turkey
E-posta: drburcakbilgin@gmail.com

Received / Geliş tarihi : 31.10.2022
Accepted / Kabul tarihi : 20.12.2022
Online published : 12.01.2023
Elektronik yayın tarihi
DOI: 10.12956/tchd.1196564

ÖZ

Amaç: Demir, antioksidan enzimlerin yapısında bulunan ve reaktif oksijen türlerinin inaktivasyonunda önemli rolü olan bir elementtir. Oksidan-antioksidan dengenin bozulması demir eksikliği anemisinin (DEA) patogenezinde rol oynuyor olabilir. Dinamik tiyol-disülfid homeostazisi (DTDH) ve serum iskemi modifiye albümin (IMA) seviyeleri prooksidan/antioksidan durumun önemli göstergeleridir. Bu çalışmada, demir tedavisi almayan, yeni tanı almış demir eksikliği anemisi olan çocuklarda DTDH parametreleri ve serum İMA düzeylerini değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Demir eksikliği tanısı almış 50 hasta ile yaş ve cinsiyet açısından uyumlu 33 sağlıklı kontrol çalışmaya dahil edildi. Hasta ve kontrol gruplarının DTDH parametreleri ve İMA düzeyleri ölçüldü. Aynı parametreler, Hb<7 g/dl (derin DEA) (n:14/50, %28) ve Hb≥7 g/dl (hafif-orta DEA) (n:36/50, %72) olan DEA grubundaki hastalarda da karşılaştırıldı. Bu gruplarda DTDH parametreleri arasındaki ilişki araştırıldı.

Bulgular: Antioksidan kapasite göstergelerini oluşturan nativ tiyol, total tiyol, nativ tiyol/total tiyol seviyeleri DEA hastalarında anlamlı olarak daha düşük bulunurken; oksidan disülfid, disülfid/nativ tiyol, disülfid/total tiyol ve İMA seviyeleri kontrol grubundakilere göre istatistiksel olarak anlamlı daha yüksek bulundu (p<0.050). Dinamik tiyol-disülfid homeostazisi parametreleri ve IMA seviyeleri incelendiğinde; hemoglobin ve ferritin seviyeleri ile antioksidan parametreler arasında pozitif bir korelasyon ve oksidatif parametreler arasında negatif bir korelasyon vardı (p<0.050). Ayrıca derin DEA grubunda oksidatif parametreler, Hb>7 g/dl olan gruba göre çok daha yüksek bulundu (p<0.050).

Sonuç: Bu çalışmada, DEA hastalarında tedavi öncesi kontrol grubuna göre serum nativ tiyol ve total tiyol düzeylerindeki düşüşle birlikte serum disülfid ve IMA düzeylerindeki artış oksidatif strese işaret etti. Çocuklarda bu göstergelerin değerlendirilmesi DEA'ya bağlı toksisiteyi öngermede önemlidir.

Anahtar Sözcükler: Hemoglobin, Demir eksikliği anemisi, İskemi modifiye albümin, Oksidatif hasar, Tiyol-disülfid

INTRODUCTION

Iron deficiency (ID) is the most common nutritional deficiency globally, especially in developing countries. Iron deficiency anemia (IDA) is a condition that develops with a decrease in red blood cell mass or hemoglobin (Hb) amount, depending on ID. This clinical manifestation may occur due to increased iron requirement, malabsorption or chronic blood loss. In children, it is mostly detected during infancy and school-age, when rapid growth occurs, or in adolescence due to menstrual bleeding in girls (1).

Iron is an element that has an important role as catalyst for enzymes, involved in energy production, electron transport chain, inactivation of reactive oxygen species (ROS), deoxyribonucleic acid (DNA), ribonucleic acid (RNA) production and protein synthesis (2). The production of hemoglobin and other iron-containing proteins such as cytochrome, myoglobin, catalase and peroxidase is also affected in ID (3,4). In addition, studies have shown decreases in concentrations and activities of antioxidant enzymes such as glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) in patients with IDA, thus reducing the total antioxidant capacity (TAC) (5,6).

It is thought that oxidative stress increases with the elevation in pro-oxidant levels or decrease in antioxidant enzyme capacities in IDA. Due to oxidative stress, the loss of balance between free radicals or ROS production and the antioxidant system leads to deterioration in molecular and cellular functions (7,8). Thiols, a group of organic sulfur compounds, occupy an important place in the antioxidant system and protect the organism against the harmful effects of ROS by coordinating the antioxidant defense (9). Oxygen radicals, ROS and thiol groups are oxidized to form reversible disulfide bonds. The disulfide bonds are reduced back to thiol groups, ensuring dynamic thiol-disulfide homeostasis (DTDH). Dynamic thiol-disulfide homeostasis is one of the most important indicators of the oxidant/antioxidant status in the body. Thiol-disulfide balance measurements are used to evaluate native thiol (-SH), disulfide (-SS), total thiol [(-SH)+(-SS)] levels and dynamic thiol-disulfide (-SH/-SS) homeostasis (10). Ischemia-modified albumin (IMA) is an altered form of albumin, characterized by decreased cobalt-binding affinity, mainly occurring in ischemic conditions and due to free radical damage. It has been shown that there is an increase in IMA levels in cases of oxidative damage (11).

Evaluation of thiol-disulfide homeostasis and IMA levels in IDA-related oxidative damage in children, can predict future toxic effects and indicates the importance of early diagnosis and appropriate treatment. Therefore, in our study, we aimed to evaluate thiol-disulfide homeostasis and serum IMA levels in patients with newly diagnosed IDA, who had not received iron therapy.

MATERIALS and METHODS

The study was conducted between June 2020 and January 2021 at Dr. Sami Ulus Maternity Child Health and Diseases Training and Research Hospital, Department of Pediatric Hematology and Oncology, Ankara, Turkey. Fifty patients, diagnosed with IDA and 33 healthy age- and sex-matched control group patients were included. The patients and control group did not have any acute or chronic diseases or medication use during the evaluation. The diagnosis of IDA (with Hb value below -2SD according to age group and ferritin below 12 ng/ml) was made by evaluating the patients' complete blood count and iron parameters. In addition, patients in the IDA group were divided according to their hemoglobin levels as Hb<7g/dl (profound IDA) (n:14/50, 28%) and Hb≥7 g/dl (mild-moderate IDA) (n:36/50, 72%). Dynamic thiol-disulfide homeostasis

parameters and IMA levels of the patients and control group were measured.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The Hospital's Clinical Research Ethics Committee approved the study (numbered 18.03.2021, E-21/03-135). Informed consent was obtained from all participants and their parents.

Peripheral venous blood samples were taken to measure complete blood count, ferritin, DTDH parameters and IMA levels. To measure DTDH parameters and IMA levels, additional 3 ml samples were taken from blood samples, taken from children with IDA and the control group, during their routine controls.

Collected blood samples were centrifuged at 3600 rpm for 10 minutes in the biochemistry laboratory and stored at -80°C. After completion of sample collection, all of them were thawed simultaneously and studied on a Roche Hitachi Cobas c501 automatic analyzer, usinga new spectrophotometric method defined by Erel and Neşelioğlu (10). Accordingly, disulfide bonds (SS) were reduced to free thiol groups (SH) with sodium borohydride. Total thiol (SH + SS) amount was measured using 5,5'-dithiobis-(2 nitrobenzoic) acid. Half the difference between total thiol and native thiol provided the dynamic disulfide amount. After measuring these levels, the percentage ratios of disulfide/native thiol (SS/SH), disulfide/total thiol (SS/SH+SS), and native thiol/total thiol (SH/SH+SS) were calculated. IMA was measured by a colorimetric method developed by Bar-Or et al. (12), based on the measurement of unbound cobalt after incubation with patient serum, and the results were reported in absorbance units (ABSU). Serum ferritin level was measured by immunoassay on Advia Centaur XPT analyzer (Siemens IRL08191533).

Statistical Analysis

Statistical analyses were performed using the SPSS software version 20. Frequency and percentage values were calculated with the Kolmogorov-Smirnov test for categorical variables; mean±standard deviation (SD), median, minimum and maximum values were calculated for continuous variables. The significance of the difference between groups was evaluated using Student's t-test or Mann-Whitney U test. Nominal variables were compared using Pearson Chi-Square or Fisher's exact probability test. While investigating the associations between non-normally distributed and/or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test. $p<0.050$ values were considered statistically significant.

RESULTS

Fifty patients diagnosed with IDA and 33 healthy controls were included in the study. Twenty one (42%) of the patients in the study group and 18 (54%) of the control group were female.

Table I: Comparison of the demographical characteristics and hematological parameters of children with IDA and control group.

	IDA* (n=50)	Controls (n=33)	p
Gender: Female/Male	21 / 29	18 / 15	0.262
Age (year)	3.7±2.6	3.7±1.2	0.969
Hemoglobin (Hb) (g/dL)	8.95±1.84	12.57±0.85	<0.001
Hematocrit (Hct) (%)	29.63±4.17	41.81±2.15	<0.001
Mean Corpuscular Volume (MCV) (fL)	62.87±7.43	83.81±1.60	<0.001
Red blood cell (RBC) ($x10^{12}$/L)	4.21±0.19	4.90±0.13	<0.001
(Red Cell Distribution Width (RDW) (%)	17.54±1.54	12.12±1.08	<0.001
Platelet count ($\times10^3$/µL)	375.80±84.77	265.87±54.69	<0.001
Ferritin (ng/mL)	4.59±3.15	30.90±12.07	<0.001

***IDA:** Iron deficiency anemia*

Table II: Comparison of DTDH parameters and IMA levels of children with IDA and control group

	IDA* (n=50)	Control group (n=33)	p
Native thiol (µmol/L)	430.07±63.02	476.58±38.36	<0.001
Total thiol (µmol/L)	451.23±59.80	489.41±42.31	<0.001
Disulphide (µmol/L)	11.74±6.0	6.10±4.30	<0.001
Disulphide/native thiol†	2.81±1.97	1.33±1.01	<0.001
Disulphide/total thiol†	2.61±1.65	1.27±0.94	<0.001
Native thiol/total thiol†	95.11±3.36	97.44±1.89	<0.001
Ischemia modified albumin (IMA) (ABSU)	0.79±0.29	0.57±0.20	<0.001

**IDA: Iron deficiency anemia, †:(%)*

Table III: Comparison of DTDH parameters and IMA levels of children with profound IDA (Hb below 7 g/dL) and Mild-moderate IDA (Hb above 7 g/dL) groups.

	Profound IDA* group (n=14/50, %28)	Mild-moderate IDA* group (n=36/50, %72)	p
Native thiol (µmol/L)	426.53±57.34	431.44±65.81	0.503
Total thiol (µmol/L)	448.58±57.46	452.26±61.45	0.574
Disulphide (µmol/L)	15.17±4.95	10.41±5.85	0.004
Disulphide/native thiol†	3.27±1.34	2.63±2.16	0.036
Disulphide/total thiol†	3.08±1.21	2.43±1.77	0.034
Native thiol/total thiol†	95.03±2.93	95.14±3.55	0.635
Ischemia modified albumin (IMA) (ABSU)	0.79±0.20	0.80±0.32	0.738

**IDA: Iron deficiency anemia, †: (%)*

While the mean age of the patients was 3.7±2.6 years in the study group, it was 3.7±1.2 years in the control group. There was no significant difference between the two groups regarding

Table IV: Correlation analyses between hemoglobin, ferritin and platelet values with DTDH parameters and IMA levels.

	Native thiol	Total thiol	Disulphide	Disulphide/ native thiol	Disulphide/ total thiol	Native thiol/ total thiol	IMA*
Hemoglobin							
r	0.329	0.297	-0.261	-0.260	-0.271	0.256	-0.248
p	0.002	0.006	0.017	0.018	0.013	0.019	0.024
Ferritin							
r	0.282	0.247	-0.320	-0.241	-0.255	0.240	-0.301
p	0.010	0.024	0.003	0.028	0.020	0.029	0.006
Platelet							
r	-0.257	-0.191	0.269	0.285	0.284	-0.274	0.209
p	0.019	0.084	0.014	0.009	0.009	0.012	0.058

IMA: *Ischemia modified albumin*

gender distribution and age (p >0.050). The study group had significantly lower Hb, hematocrit (Hct), mean corpuscular volume (MCV), red blood cell (RBC) counts, ferritin levels and higher red cell distribution width (RDW) and platelet levels compared to the control group. Demographic characteristics and hematological parameters of patients and the control group are presented in Table I.

Antioxidant capacity indicators of native thiol, total thiol, native thiol/total thiol levels were significantly lower in IDA patients than in controls. In contrast, oxidant disulfide, disulfide/native thiol, disulfide/total thiol and IMA levels were found to be statistically significantly higher (p<0.050) in IDA patients (Table II). In addition, when oxidative stress markers were evaluated in patients with profound anemia with Hb below 7 g/dL and patients with IDA with Hb above 7 g/dL; disulfide, disulfide/native thiol, disulfide/total thiol levels were found to be statistically significantly higher in patients with profound anemia, but no significant difference was found in terms of IMA levels (p=0.004; p=0.036; p=0.034; p=0.738, respectively) (Table III).

There was a weak negative correlation between disulfide, disulfide/native thiol, disulfide/total thiol and IMA levels with serum hemoglobin levels. However, there was a moderate-weak positive correlation between native thiol and weak positive correlation between total thiol, native thiol/total thiol with serum hemoglobin levels. Also, these relationships were statistically significant (p<0.050). When correlation analyses were performed between ferritin levels and these parameters; a moderate-weak negative correlation between disulfide and IMA, and weak negative correlation between disulfide/native thiol, disulfide/total thiol were found. Also, a weak positive correlation between native thiol, total thiol, and native thiol/total thiol were found. This relationship was statistically significant, as well (p<0.050). Moreover, there was a weak negative correlation between native thiol, total thiol, native thiol/total thiol, and a weak positive correlation between disulfide, disulfide/native thiol, disulfide/total thiol, and IMA levels with platelet count. Among them, native thiol, native thiol/total thiol, disulfide, disulfide/native thiol, disulfide/total thiol correlations were found to be statistically significant (p<0.050) (Table IV).

DISCUSSION

Iron is an element that is essential for human life. It is found in the structure of Hb, which provides oxygen transport in the body, participates in the structure of enzyme systems in some tissues and ensures the complementation of iron-related functions (1).

Iron deficiency anemia, which is very common in the world and especially in developing countries, occurs with the decrease in the amount of Hb as a result of ID. Since ID is a systemic disease that affects all bodily functions, many complications such as growth retardation, neurocognitive deficiencies, impaired immune system and learning disabilities can occur. Especially in childhood, oxidative damage in IDA may contribute to deterioration in neurocognitive functions (13,14). Therefore, understanding the pathogenesis, early detection and prevention of IDA are important in terms of child health (15,16).

In oxidative stress caused by increased free radicals and ROS, the oxidant-antioxidant balance is disrupted in favor of oxidation. Membrane changes and damage to erythrocytes due to ROS-induced oxidative stress, have been demonstrated (17). Several studies have reported that the concentrations and activities of antioxidant enzymes such as glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) decreased and oxidant stress increased in IDA (18, 19). Aycicek et al. (20), have found that total oxidant status (TOS) and oxidative stress index (OSI) were higher in patients with IDA, while total thiol (–SH) and total antioxidant capacity (TAC) levels were lower than in the control group. Similarly, Akça et al. (21), have reported that oxidative stress parameters increased in children with IDA. In addition, Akarsu et al. (22), have stated that in IDA, the total antioxidant capacity measurements were low.

Dynamic thiol-disulfide homeostasis is one of the indicators, playing an important role in intracellular signal transduction, apoptosis, and many antioxidant and enzyme activities (9). Topal et al. (23), have reported that disulfide, disulfide/native thiol, and disulfide/total thiol levels were significantly higher in the IDA group. Consistent with previous reports, in our study, we found that DTDH parameters of native thiol, total thiol, native thiol/total thiol levels showing antioxidant capacity

decreased and oxidative stress indicators (disulfide, disulfide/native thiol, disulfide/total thiol, and IMA levels) increased in IDA patients compared to in control group. Also, we showed in our study that the increase in oxidant parameters due to oxidative damage could be higher especially in profound anemia where the Hb value is below 7 g/dL.

IMA is caused by chemical changes in albumin, which, in turn, is caused by oxidative free radicals during ischemia. IMA is one of the earliest markers of ischemia (24). Therefore, elevated IMA levels have been associated with increased oxidative stress in IDA, which can be considered a chronic hypoxic state. Bilgili et al. (25), in their study on adults, have reported high IMA levels in the patient group with IDA. Topal et al. (23), have reported relatively elevated IMA levels in children with IDA compared to in healthy controls. Similar to the literature, in our study, we found higher levels of IMA indicating oxidative damage in the IDA group compared to in control group. Also, there was a positive correlation between antioxidant parameters with hemoglobin and ferritin levels in the current study, while oxidative parameters showed a negative correlation with both hemoglobin and ferritin levels.

Causes of reactive thrombocytosis in children include infections, malignancies, splenectomy, acute blood loss, and iron deficiency anemia. Although the mechanisms are not fully known, it is thought that the similar amino acid sequence of thrombopoietin and erythropoietin may explain reactive thrombocytosis in children with iron deficiency anemia (26-28). Durmuş et al. (29), have reported that oxidative stress increased in patients with essential thrombocythemia, but there is no study showing the oxidant-antioxidant balance in reactive thrombocytosis due to iron deficiency anemia in children. In our study, we found a positive correlation with disulfide, disulfide/native thiol, disulfide/total thiol, possibly due to the increase in platelet count and oxidative stress.

In literature, there is limited data on DTDH and IMA levels in children with IDA. This study has some limitations, such as having a small sample group and avoidance of evaluation of antioxidant enzyme levels. However, our study found statistically significantly lower DTDH parameters, showing antioxidant capacity and higher DTDH parameters and IMA levels, showing oxidative damage in patients with IDA compared to healthy controls. Evaluation of thiol-disulfide homeostasis in oxidative damage due to IDA in children, is important in predicting the toxic effects, to which the patient will be exposed to in the subsequent period. Otherwise, the relationship between thrombosis and neurocognitive functions and oxidative stress in patients with IDA needs to be investigated. Therefore, further studies are needed to elucidate this situation. In addition, these results will guide hematologists in the management of children diagnosed with IDA.

REFERENCES

1. Armitage AE, Moretti D. The importance of iron status for young children in low- and middle-income countries: a narrative review. Pharmaceuticals (Basel) 2019;12:59.
2. Gao J, Zhou Q, Wu D, Chen L. Mitochondrial iron metabolism and its role in diseases. Clin Chim Acta 2021;513:6-12.
3. Sharma R, Nachane H. Oxidative stress in iron deficiency anemia-a gender-based analysis-a pilot study. J Evolution Med Dent Sci 2020;9:3739-42.
4. Kontoghiorghes GJ, Kontoghiorghe CN. Iron and chelation in biochemistry and medicine: new approaches to controlling iron metabolism and treating related diseases. Cells 2020;9: 1456.
5. Hamed HM, Motawie AA, Abd Al-Aziz AM, El-Saeed GSM, El Wasseif M, Mourad AA, et al. Low Dose Iron Therapy in Children with Iron Deficiency: DNA Damage and Oxidant Stress Markers. Indian J Hematol Blood Transfus 2021;37:287-94.
6. Sharif Usman S, Dahiru M, Abdullahi B, Abdullahi SB, Maigari UM, Ibrahim Uba A. Status of malondialdehyde, catalase and superoxide dismutase levels/activities in schoolchildren with iron deficiency and iron-deficiency anemia of Kashere and its environs in Gombe State, Nigeria. Heliyon 2019;5:e02214.
7. Mehram EB, Ahmed SA, Elhassaneen YA. Role of Oxidant/Antioxidant Status in the Pathogenesis of Iron-Deficiency Anemia: Case Study on Children of Qalyubiyya and Minoufiya Governorates, Egypt. Bulletin of the National Nutrition Institute of the Arab Republic of Egypt 2020;55:39-71.
8. Guven S, Meral I, Kurdoglu M, Demir H. The Nutritional Habits and Relationship Between The Antioxidant Activity and Iron Deficiency Anemia During Pregnancy. East J Med 2022;27:317-24.
9. Ulrich K, Jakob U. The role of thiols in antioxidant systems. Free Radic Biol Med 2019;140:14-27.
10. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326-32.
11. Özcan O, Erdal H, İlhan G, Demir D, Gürpınar AB, Neşelioğlu S, et al. Plasma Ischemia-Modified Albumin Levels and Dynamic Thiol/Disulfide Balance in Sickle Cell Disease: A Case-Control Study. Turk J Haematol 2018;35:265-70.
12. Bar-Or D, Lau E, Winkler JV. A Novel assay for cobaltalbumin binding and its potential as a marker for myocardial ischemia-a preliminary report. J Emerg Med 2000;19:311-15.
13. East P, Doom JR, Blanco E, Burrows R, Lozoff B, Gahagan S. Iron deficiency in infancy and neurocognitive and educational outcomes in young adulthood. Dev Psychol 2021;57: 962-75.
14. Zaky EA, Mahmoud ZK, Abd El Wahab MM, Kamel SK. The effect of Iron Deficiency Anemia on Intelligence Quotient in children. MJMR 2021;32:66-8.
15. Sundararajan S, Rabe H. Prevention of iron deficiency anemia in infants and toddlers. Pediatr Res 2021;89:63-73.
16. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann N Y Acad Sci 2019;1450:15-31.
17. 1Jakubczyk K, Dec K, Kałduńska J, Kawczuga D, Kochman J, Janda K. Reactive oxygen species-sources, functions, oxidative damage. Pol Merkur Lekarski 2020:22;48:124-7.
18. Dilber B, Akbulut UE, Serin HM, Alver A, Menteşe A, Kolaylı CC, et al. Plasma and Erytrocyte Oxidative Stress Markers in Children with Frequent Breath-Holding Spells. Klin Padiatr 2021;233:173-80.

19. Aslaner H, Dündar MA, Arslan A, Karakükcü Ç, Altuner Torun Y. Reduced antioxidant capacity in children with iron deficiency and iron-deficiency anemia. Minerva Pediatr (Torino) 2021. Epub ahead of print.
20. Aycicek A, Koc A, Oymak Y, Selek S, Kaya C, Guzel B. Ferrous sulfate (Fe2+) had a faster effect than did ferric polymaltose (Fe3+) on increased oxidant status in children with iron-deficiency anemia. J Pediatr Hematol Oncol 2014;36:57-61.
21. Akca H, Polat A, Koca C. Determination of total oxidative stress and total antioxidant capacity before and after the treatment of iron-deficiency anemia. J Clin Lab Anal 2013; 27:227-30.
22. Akarsu S, Demir H, Selek S, Oguzoncul F. Iron deficiency anemia and levels of oxidative stress induced by treatment modality. Pediatr Int 2013;55:289-95.
23. Topal I, Mertoglu C, Sürücü Kara I, Gok G, Erel O. Thiol-Disulfide Homeostasis, Serum Ferroxidase Activity, and Serum Ischemia Modified Albumin Levels in Childhood Iron Deficiency Anemia. Fetal Pediatr Pathol 2019;38:484-9.
24. Shevtsova A, Gordiienko I, Tkachenko V, Ushakova G. Ischemia-Modified Albumin: Origins and Clinical Implications. Dis Markers 2021;2021:9945424.
25. Bilgili S, Bozkaya G, Tütüncüler FK, Akşit M, Yavuz M. Investigation of ischemia modified albumin levels in iron deficiency anemia. Turk J Biochem 2017;42:259-64.
26. Fahim FM, Helal SR, Fathalla E, Saeed AN, Galal SM. Platelets abnormalities in children with iron deficiency anemia. J Curr Med Res Pract 2022;7:22-8.
27. Han DD, Ma C, Zhao L, Gao YJ, Su YH. Relationship between Iron Metabolism and Platelet Parameters in Patients with Iron Deficiency Anemia. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2018;26:1738-41.
28. Chalise S, Acharya N, Pradhan SB. Correlation between Iron Parameters and Platelet Parameters in Iron Deficiency Anemia. JIOM Nepal 2019;41:35-8.
29. Durmus A, Mentese A, Yılmaz M, Sumer A, Akalın I, Topal C. Increased oxidative stress in patients with essential thrombocythemia. Eur Rev Med Pharmacol Sci 2013;17:2860-66.