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ISCHEMIA-MODIFIED ALBUMIN LEVELS AND DYNAMIC THIOL/ DISULFIDE BALANCE IN FEMALE PATIENTS WITH IRON DEFICIENCY ANEMIA

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ABSTRACT

Iron deficiency anemia (IDA) affects about 20-30% of adults worldwide. The present study was aimed to determine the relationship between ischemia-modified albumin (IMA) and dynamic thiol/disulfide balance in female patients with IDA, and to reveal its effects on the development of anemia and the organism. The study included a total of 88 individuals (48 patients/ 40 healthy). IMA levels and disulfide/thiol homeostasis were measured by colorimetric and spectrophotometric methods. Total thiol (TTL) levels were found significantly positively correlated with native thiol (NTL) values ($r = 0.326$, $p<0.05$). A strong positive correlation was found between TTL and disulfide ($r = 0.511$, $p<0.01$). A negative correlation was observed between NTL and disulfide ($r = -0.546$, $p<0.05$) and between TTL and IMA ($r = -0.160$, $p>0.05$); but weak negative correlation was found between NTL and IMA ($r = -0.011$, $p<0.05$) and between IMA and disulfide ($r = -0.035$, $p<0.05$). The progression of this iron deficiency anemia is a risk factor for the occurrence of many diseases. Therefore, it is important to evaluate the parameters together in relation to the prognosis of hypoxic state and oxidant balance caused by anemia.

Keywords: Iron deficiency anemia, ischemia-modified albumin, oxidative stress, thiol/disulfide homeostasis

INTRODUCTION

Iron deficiency affects more than 2 billion people worldwide and iron deficiency anemia (IDA) persists as the main cause of anemia, as approved by the analysis of a large number of reports on disease burden in 187 countries between 1990 and 2010 (McLean *et al.*, 2009). The perusal of literature reveals that IDA has increased in pre-school children and young women (Steven *et al.*, 2013). Iron is the main element for erythropoiesis, oxidative metabolism, and cellular immune response (Pasricha *et al*., 2012). The irregularities between the reactive oxygen species (ROS) and antioxidant system leads to the oxidative stress. These irregularities can be the reason for irreversible damage to the cellular compartments. The free radicals show the effects of oxidizing DNA, proteins, lipids, and carbohydrates in the structure of cell membrane and organelles. Free radicals and ROS are neutralized by antioxidant system (Diaz-Castro *et al.*, 2008). Studies have

shown that oxidative activities increase and antioxidants decrease, thus oxidative/antioxidant balance is directed to the oxidative side in patients with IDA (Akça *et al.*, 2013).

Biochemical markers are utilized to assess the oxidative stress. One of these markers is the thiol/disulfide balance. As main antioxidant, thiols eliminate reactive oxygen species (ROS) in non-enzymatic ways, thiols enter oxidation reactions that form disulfide bonds with oxidant molecules (Elmas *et al*., 2017). Thiol/disulfide homeostasis is necessary for detoxification. The parameters of thiol/disulfide homeostasis contain native and total thiol (Kundi *et al*., 2015; Altıparmak *et al*., 2016). The changes in thiol/disulfide homeostasis are crucial in pathogenesis of many diseases like diabetes mellitus, cardiovascular diseases, kidney diseases, malign diseases, connective tissue disease, Parkinson's disease, multiple sclerosis, AIDS and liver diseases (Ozyazıcı *et al*., 2016). Although thiol/disulfide studies have been reported in some diseases such as pregnancy and menopause, etc. in women, but no thiol/disulfide related research in women suffering from IDA has so far been reported. The present study is the first to evaluate the thiol/disulfide homeostasis in women with IDA.

MATERIALS AND METHODS

Forty-eight female patients, diagnosed with IDA at Hitit University Erol Olçok Training and Research Hospital, (Corum, Turkey) and 40 healthy women had joined in this study. The most important clinical indicators in the diagnosis of IDA for the inclusion of patients in the study were hemoglobin concentration <12 g dL^{-1}, mean red blood cell volume <80 fL, serum iron concentration <45 g dL^{-1}, and serum ferritin concentration <15 ng mL^{-1}). The red cell index includes 3 tests *viz.*, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). MCV, MCH and MCHC values are derived from hemoglobin, packed cell volume (PCV) or hematocrit and red cell count. The values of MCV, MCH and MCHC are derived from the values of hemoglobin, PCV or hematocrit and red cell count. The study exclusion criteria followed were: i) usage of any medication, ii) chronic disease, iii) oral or parenteral iron treatment in the last 3 months, iv) presence of dimorphic anemia, v) history of blood transfusion within 3 months before the study, vi) systemic or local infection, and vii) liver and renal diseases. All the reagents and chemicals used in present study were purchased from Sigma-Aldrich.

Collection of blood samples

Blood samples were taken valid for biochemical parameters required for the diagnosis of anemia. For thiol/disulfide and ischemia-modified albumin (IMA) parameters, blood samples were centrifuged at 4000 rpm for 10 min to obtain serum. The control samples were also subjected to centrifugation and serum separation. The separated serum samples were stored at - 80°C until use. The informed consent of study group was taken and included in the study. The study was approved by the Research Ethics Committee of the Institution vide their approval No.32/2019.

Analysis of demographic data

Complete blood counts from the samples (hemoglobin, MCV, MCH, MCHC) were determined by measuring the blood samples taken into K3-EDTA tube with Beckman Coulter device. Iron, ferritin and TIBC levels in the samples were determined in Mindray Biochemistry autoanalyzer.

Thiol-disulfide measurement

After obtaining serum samples of all the patients and control subjects, native thiol (NTL), total thiol (TTL), disulfide, native thiol/total thiol, disulfide/native thiol and disulfide/total thiol ratios were performed by a fully automated method developed by Erel and Neselioglu (2014).

Ischemia-modified albumin levels

IMA levels were determined by albumin cobalt binding test, a rapid colorimetric method developed by Bar-Or *et al.* (2000). The method is based on the binding ability of reduced cobalt ions (Co^{2+}) of HSA due to ischemia. Briefly, a known amount of exogenous Co ($CoCl_2$) was added to serum samples. Albumin, which is altered because of ischemic processes, binds to the Co(II) to a far lesser extent and the excess (unbound) amount of Co^{2+} forms a coloured complex with dithiothreitol, which is measured spectrophotometrically at 480 nm (Bar-Or *et al.*, 2000) using Biochrom Libra S70 spectrophotometer (Harvard Biosciences, Holliston, MA, USA) with a temperature-controlled cuvette holder.

Statistical analysis

Statistical analysis was performed using the SPSS 22.0 software. Data has been presented as mean ± SD and standard error mean (SEM). Normality distribution was determined through Shapiro-Wilk test (Peat and Bartın, 2015). In cases where these assumptions were not met and n<30, non-parametric Mann Whitney-U test were used for comparison of differences between means. Spearman's rho correlation coefficient was used to determine the association among all parameters (Magnello, 2009). In addition, critical significance level was set at 0.05.

RESULTS AND DISCUSSION

Iron deficiency is one of the most common nutritional problems in the world. Women of reproductive age are at particularly high risk of iron deficiency (Coad and Pedley, 2014). The present study was aimed to determine the relationship between ischemia-modified albumin (IMA) and dynamic thiol/disulfide balance in women patients with IDA, and to reveal its effects on the development of anemia and the organism.

In present study, the mean age of IDA patient female group was 37.21 yr while that of control group was 34.17 yr. The volunteers in patient group and control group were assessed together in relation to their age and gender (p>0.05). The demographic characteristics of two groups have been summarized in Table 1. Hemoglobin levels were 9.89 g dL^{-1} in IDA female group and 13.33 µg dL^{-1} in control group. MCV levels were 70.92 L in IDA female group and 82.61 L in control group. MCH were 22.13 pg in IDA patient female group and 26.61 pg in control group. MCHC level in IDA patient female group was 29.27 g L^{-1} and in control group 31.30 g L^{-1}. TIBC levels were 445.62 µg dL^{-1} in IDA female group and 385.21 µg dL^{-1} in control group. Serum iron levels were

Table 1: Demographic information of the patients and control groups

Parameters	Patients		Control		U	P
	Mean ± S.D.	SEM	Mean ± S.D.	SEM		
Age (yr)	37.21 ± 1.49	1.49	34.17 ± 9.60	2.28	297.5	0.228**
Hemoglobin (g dL^{-1})	9.89 ± 0.29	0.29	13.33 ± 2.19	0.16	12.0	0.000*
MCV (L)	70.92 ± 1.80	1.80	82.61 ± 9.89	0.65	71.5	0.000*
MCH (pg)	22.13 ± 0.72	0.72	26.61 ± 4.85	0.97	124.5	0.000*
MCHC (g L^{-1})	29.27 ± 0.57	0.57	31.30 ± 5.00	1.38	126.0	0.000*
Serum Fe (µg dL^{-1})	20.78 ± 1.60	1.60	75.65 ± 35.60	7.11	25.0	0.000*
TIBC (µg dL^{-1})	445.62 ± 9.19	9.19	385.21 ± 63.71	13.07	169.0	0.001*
Transferrin saturation (%)	4.58 ± 0.42	0.42	27.43 ± 23.86	6.82	29.0	0.000*
Ferritin (ng dL^{-1})	6.52 ± 2.10	2.10	46.33 ± 68.82	21.32	70.0	0.000*

S.D. = Standard deviation, SEM = Standard error of mean, **p>0.05, *p<0.05; U = Mann-Whitney U test data, P = Critical significance level as statistically, MCV = Mean cell volume, MCH = Mean cell hemoglobin, MCHC = MCH concentration; and TIBC = Total iron binding capacity

20.78 µg dL^{-1} in IDA patient female group, and 75.65 µg dL^{-1} in control group. There was also a statistically significant difference between serum Fe, transferrin, transferrin saturation, and ferritin in the patient and control groups. (Table 1).

Erel and Neselioglu (2014) developed a novel automated colorimetric method for measuring hemostasis in dynamic thiol-disulfide. Thiol and disulfide levels were previously measured as low molecular weight thiol compounds. Erel and Neselioglu (2014) demonstrated hemostasis in dynamic thiol-disulfide. This novel method can determine whether thiol metabolism contributes to the pathogenesis of various diseases. Dynamic thiol/disulfide homeostasis is a plasma oxidative stress biomarker that shows the importance of oxidant/antioxidant balance in antioxidant protection and provides valuable information about various normal or abnormal biochemical processes. Thiol-disulfide pair plasma levels are reliable and valuable biomarkers for assessing biological redox status (Prakash *et al.*, 2004; Kolagal *et al.*, 2009; Erel and Neselioglu, 2014). As a result, a research gap was identified: whether plasma biomarkers identified for "dynamic thiol/disulfide homeostasis" differ in iron-deficiency anemia samples compared to healthy women, supports the data on oxidative hypothesis in iron-deficiency anemia in women. This case-control study investigated the relationship between iron deficiency anemia and serum oxidative stress markers. Total thiol levels were also found significantly different in iron-deficiency anemia group. Total thiol concentrations in patients were significantly lower as compared to their control (*$p<0.05$). But no significant differences were observed in native thiol, disulfide level, and IMA Abs levels (**$p>0.05$) in between the patients and control group (Table 2; Fig. 1 and 2). The disturbed dynamic thiol/disulfide balance in women with iron deficiency anemia is the study's most striking finding. The current findings suggest a plausible link between increased iron deficiency and decreased antioxidant capacity, as well as iron deficiency anemia. The findings of impaired thiol-disulfide homeostasis were supported. The discovery of a disruption in dynamic thiol/disulfide homeostasis in defined iron deficiency anemia group reflected a shift in oxidant-antioxidant balance to the oxidative stress side, with no expected compensatory increase in antioxidant response to oxidative stress.

Albumin is an essential component of antioxidant systems. Its structure changes during oxidative stress, resulting in a decrease in metal binding capacity. It is then converted into a new molecule known as ischemia-modified albumin (IMA) (Grzebyk and Piwowar, 2013). The formation of reactive oxygen species (ROS) and free radicals alters the N-terminal region of albumin, causing an increase in IMA concentration (Bar–Or *et al*., 2000). Chronic hypoxia is the major cause of the production of IMA. IDA is also considered a hypoxic state. Although elevated IMA levels have been studied in other types of anemia associated with chronic diseases such as chronic kidney disease or b-thalassemia major, data on IMA changes in IDA are limited (Cichota *et al*., 2008; Awadallah *et al*., 2012; Grzebyk and Piwowar, 2013). Bilgili *et al.* (2017) found that an IDA group had higher IMA levels than a control group of adults. We also found that IMA levels were lower in women with IDA than in healthy controls, although the difference was not

Table 2: Ischemia-modified albumin levels and dynamic thiol/disulfide balance in control and patient groups

Treatments		Total thiol (µmol L^{-1})	Native thiol (µmol L^{-1})	Disulfide (µmol L^{-1})	Ischemia modified albumin (ABSU)
Control	Mean ± S.D.	707.50 ± 119.23	399.50 ± 158.89	136.75 ± 63.36	0.68 ± 0.11
	SEM	28.10	37.45	14.93	0.02
Patients	Mean ± S.D.	613.00 ± 125.80	378.00 ± 135.66	113.50 ± 76.09	0.59 ± 0.15
	SEM	25.16	27.13	15.22	0.031
	U	109.00	204.00	171.00	172.00
	P	0.004*	0.604**	0.184**	0.191**

S.D. = Standard deviation, SEM = Standard error of mean, U = Mann-Whitney U test data, P = Critical significance level as statistically, **$p>0.05$, *$p<0.05$

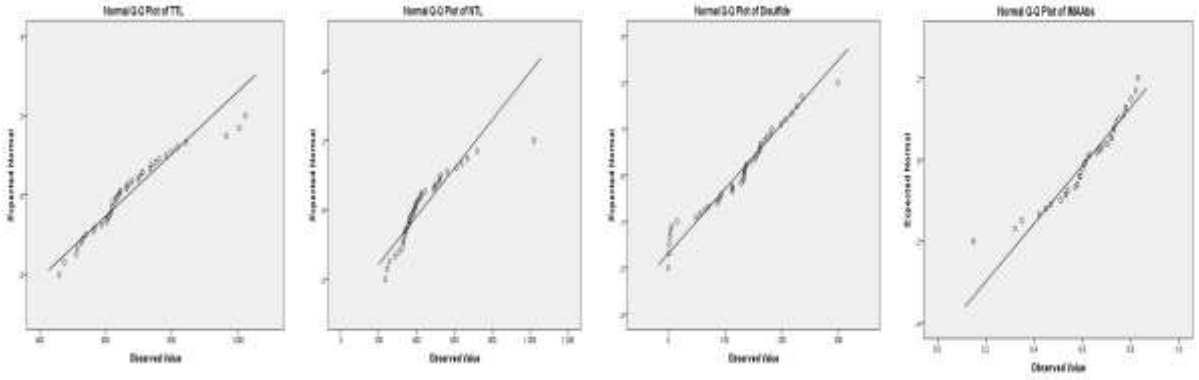

Fig. 1: The symmetric scatter plot of total thiol, disulfide, native thiol and ischemia-modified albumin in iron deficiency anemia

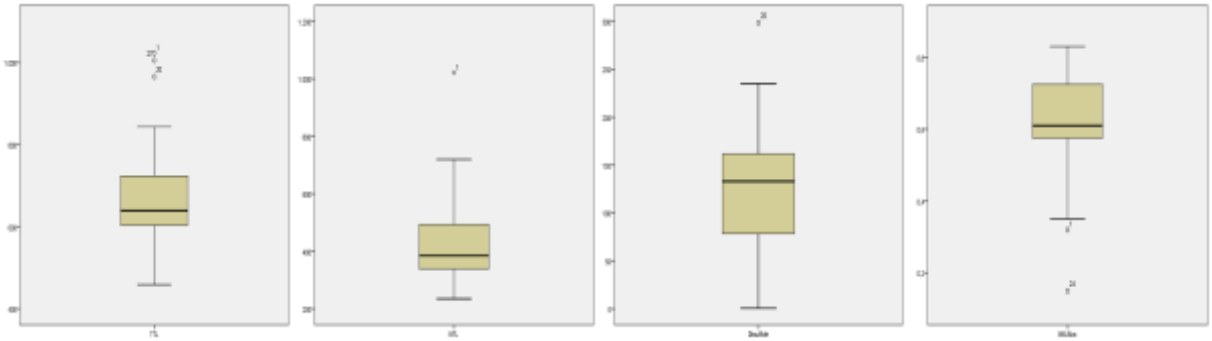

Fig. 2: The box and whisker symmetric scatter of total thiol, disulfide, native thiol and ischemia-modified albumin in iron deficiency anemia

Table 3: Correlation coefficient (r) between ischemia-modified albumin levels and dynamic thiol/disulfide balance in control and patient groups

Correlation coefficient (r)	Total thiol (μmol L^{-1})	Native thiol (μmol L^{-1})	Disulfide (μmol L^{-1})	Ischemia modified albumin (ABSU)
Total thiol		0.326*	0.511**	-0.160
Native thiol	0.326*		0.326*	-0.011
Disulfide	0.511**	-0.546**		-0.035
Ischemia-modified albumin	-0.160	-0.011	-0.035	

statistically significant. This could be related to the duration of IDA in the women. The IMA levels could be higher in patients who had IDA for a longer period. Large-scale studies on the changes of IMA in patients with IDA at different stages of the disease are needed.

The total thiol levels were significantly positive correlated with native thiol values ($r = 0.326$, $p = 0.033$, $p<0.05$) and strong positively correlated with disulfide levels ($r = 0.511$, $p = 0.000$, $p<0.01$). Also, negative correlation was noticed between native thiol and disulfide ($r = 0.546$, $P = 0.000$, $p<0.05$) and between total thiol and IMA ($r = 0.160$, $P = 0.304$, $p>0.05$); however, weak negative correlation was observed between native thiol and IMA ($r = 0.011$, $P = 0.945$, $p<0.05$) and between IMA and disulfide ($r = 0.035$, $P = 0.825$, $p<0.05$) (Table 3).

Conclusion: The current study is the first in the literature to show that the thiol-disulfide balance is disturbed in women with iron deficiency anemia. prognosis. As a result, we discovered both thiol/disulfide homeostasis parameters and IMA levels increase in women with IDA. Iron

deficiency anemia reduces the antioxidant capacity of erythrocytes and causes oxidative stress. Many tissues are negatively impacted by oxidative stress. Based on these findings, we propose that antioxidants can be given to patients with IDA to help them correct their IDA and reduce oxidative stress. Larger prospective studies evaluating the activities of other plasma enzymes involved in oxidative stress are needed.

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