Determination of Total Oxidative Stress and Total Antioxidant Capacity before and after the Treatment of Iron-Deficiency Anemia

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Background: Oxidative stress is an imbalance between the reactive oxygen species and antioxidant system. In this study, total oxidative stress (TOS) and total antioxidant capacity (TAC) were investigated with a new and practical method in childhood iron-deficiency anemia. **Method:** During the study period 80 children between 6 and 60 months were enrolled; 40 children (study group) had iron-deficiency anemia, and 40 children (control group) were healthy. Complete blood count, serum iron, iron-binding capacity, ferritin levels, TOS, and TAC were evaluated. Children diagnosed iron-deficiency anemia were treated with oral ferric iron. After 2 months of the treatment, blood tests of the study groups were repeated to check the challenge. **Results:** TAC was similar between both groups (1.55 ± 0.26 in control group 1.53 ± 0.19 mmol Trolox Eq./l). Additionally, TOS

was significantly higher in iron-deficiency anemia group before treatment with iron (24.3 ± 18.5 , in controls groups 14.4 ± 7.1 mmol Trolox Eq./l). We have shown that TAC did not change (before treatment 1.55 ± 0.26 , after treatment 1.54 ± 0.26 mmol Trolox Eq./l) although TOS decreased significantly after the treatment of iron-deficiency anemia (before treatment 24.3 ± 18.5 , after treatment 12.4 ± 6.9 mmol Trolox Eq./l). We did not find any correlation between hemoglobin, serum iron, iron-binding capacity, ferritin levels, and TOS or TAC among iron-deficiency anemia patients. **Conclusion:** As a result of this study, oxidative stress increases in children with iron-deficiency anemia and this increase can be returned to normal levels by treatment. J. Clin. Lab. Anal. 27:227–230, 2013.

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Key words: children; iron-deficiency anemia; iron treatment; total oxidative stress; total antioxidant capacity

INTRODUCTION

Iron-deficiency anemia is a major health problem worldwide. During infancy, iron requirements are higher than other age groups due to rapid growth. Iron is an essential element for erythropoiesis, cellular immune responses, and oxidative metabolism (1). Oxidative stress is an imbalanced condition between reactive oxygen species and antioxidant system. This imbalance can cause irreversible damage to the cell compartments. Free radicals show their effects by oxidizing proteins, lipid, carbohydrates, and DNA oxidation on cell membrane and organelles. Free radicals and reactive oxygen species are neutralized by a complex antioxidant system (2). It is declared that patients with iron-deficiency anemia have increased

oxidants and decreased antioxidants levels, and oxidative/antioxidative balance is shifted toward oxidative side in these patients. This is the first study, to our knowledge, to measure the changes of total oxidative stress (TOS) and total antioxidant capacity (TAC) in children with iron-deficiency anemia before and after treatment.

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MATERIALS AND METHODS

Patients and Controls

This study was conducted at Fatih University, School of Medicine, Department of Pediatrics from March 1 to September 1, 2010. Children who periodically attended the pediatric clinic for regular check-ups of their development were included in the present study. All subjects were informed about the study protocol and written consents were obtained from all participants' parents. Ethical approval was obtained from the local ethic committee. Our country has a policy of routine iron treatment on the fourth month of life for term infants and the second month of life for preterm infants.

Forty patients with iron-deficiency anemia and 40 healthy controls were included in the study, whose ages were between 6 and 60 months. Iron-deficiency anemia was defined as hemoglobin (Hb) <10.5 g/dl in children aged 6–24 months, Hb <11 g/dl in children aged 24–60 months, and ferritin levels <10 ng/ml in both groups (3).

Oral ferric iron treatment (Ferro-Sanol[®] syrup, Adeka, Istanbul, Turkey) as a single dose of 5 mg/kg/day was started to the patients with iron-deficiency anemia. Patients were instructed to take medication in the morning on an empty stomach and milk consumption not to exceed 500 ml was given. The families were called 15 days later to check if the treatment is regularly used or not. On follow-up visits at the second month of the treatment, control blood samples were obtained from 26 of 40 patients. Fourteen patients were excluded due to the irregular usage of iron treatment. The controls were only sampled at one point.

Exclusion Criteria

The exclusion criteria were febrile illness within the last 4 weeks and entire study period, chronic disease, and usage of iron or other medication.

Blood Sample Collection

Venous blood samples were obtained for measurement of complete blood count, serum iron, and total iron-binding capacity, ferritin level, TOS, and TAC from all children participating in the study. Blood samples for iron tests were taken early in the morning because serum iron levels vary during the day (4). Complete blood count, serum iron, serum total iron-binding capacity, and ferritin levels were studied on the same day. Blood was centrifuged at 4,000 rpm for 5 min for the separation of plasma. Plasma samples were stored at -20°C until analysis of TOS and TAC.

Measurement of Parameters

The samples were studied by the biochemistry expert who was unaware of groups in biochemistry laboratory. Complete blood tests were measured with an automatic flow cytometric method (Beckmann-Coulter Hmx), serum iron levels were measured with colorimetric method (Roche-Cobas Integra 800), serum iron-binding capacity was determined by direct measurement method by ferrozine (Roche-Cobas Integra), and ferritin was measured by using the immunoturbidimetry (Roche E170). TOS and TAC were measured spectrophotometrically, developed by Erel (5). All measurements were repeated at the end of 2 months of treatment.

Statistical Analysis

Statistical analysis was performed using SPSS 13.0 version for windows (SPSS, Inc., Chicago, IL). Data were expressed as mean $\pm$ standard deviation (SD). Differences were regarded as significant at $P < 0.05$. In determination of data variation of "Kolmogorov Smirnov" normalization test compared to data of chi-square test, evaluation of continuous variables with normal variation parametric test (independent Student's *t*-test) for variables with abnormal variation nonparametric test (Mann-Whitney *U* test) was used. In the comparison of TAC and TOS before and after the treatment, Student's *t*-test was used for dependent groups. Correlation analyses were performed using Pearson's correlation test.

RESULTS

Demographic characteristics and laboratory results of iron-deficiency anemia and control groups are shown in Table 1.

TAC was similar in both groups, but TOS was significantly higher in iron-deficiency anemia group. We have shown that TAC did not change although TOS decreased significantly after the treatment of iron-deficiency anemia. TOS and TAC were similar in control group and after the treatment of iron-deficiency anemia (Table 2).

No correlation between hemoglobin, serum iron, iron-binding capacity, ferritin levels, and TOS and TAC was found in iron-deficiency anemia.

DISCUSSION

In the present study, we evaluated the TOS which thought to arise from iron-deficiency anemia. Our findings indicated that TOS increased, but after treatment with iron supplementation, the levels were shown to achieve to the normal levels. These data suggested that iron-deficiency anemia obviously causes oxidative stress.

TABLE 1. Demographic and Laboratory Results in Iron-Deficiency Anemia Patients and Controls

	Iron-deficiency anemia		Control (n = 40)	P	
	BT (n = 40)	AT (n = 26)		BT-AT	AT control
Age (months)	21.7 ± 14.0		26.0 ± 15.2		0.192
Sex					
Female	15		19		0.498
Male	25		21		
Hb (g/dl)	10.1 ± 0.8	11.7 ± 0.8	12.5 ± 0.7	<0.001	<0.001
Hct (%)	31.4 ± 2.6	35.0 ± 2.6	37.0 ± 2.0	<0.001	<0.001
MCV (fl)	69.2 ± 7.6	74.9 ± 5.7	78.5 ± 4.0	<0.001	<0.001
Iron (μg/dl)	34.3 ± 17.9	49.2 ± 29.2	74.4 ± 38.8	0.077	<0.001
IBC(μg/dl)	400.0 ± 76.5	359.0 ± 74.0	324.0 ± 77.7	0.039	<0.001
TSI (%)	9.2 ± 6.6	14.0 ± 8.0	24.5 ± 15.7	0.075	<0.001
Ferritin (ng/ml)	7.4	17.3	20.8	<0.001	<0.001

Hb, hemoglobin; Hct, hemotocrit; MCV, mean corpuscular volume; IBC, iron-binding capacity; TSI, transferrin saturation index; BT, before treatment; AT, after treatment.

Thus, after the treatment with iron, this oxidative stress turns to normal.

It is generally accepted that oxidative stress is elevated due to the increase in oxidant levels and/or decrease in antioxidant enzyme capacities in iron-deficiency anemia (6). Iron deficiency affects also the production of other proteins and enzymes containing iron, such as cytochromes, myoglobin, catalase, and peroxidase. There are various studies about the protective effects of these enzymes against oxidative damage (7, 8).

Furthermore, in literature, microcytic red blood cells were shown to be more sensitive to oxidants, involving increased membrane rigidity, decreased deformability, and hemolysis due to oxidative damage to erythrocytes (9, 10). In vitro studies showed that erythrocytes of patients with iron deficiency were broken down more easily than normal cells when exposed to hydrogen peroxide. Therefore, these results were thought that protective mechanisms of erythrocytes against oxidative damage in iron deficient states were disordered (11). On the other hand, excess iron accumulation was determined to cause DNA damage and had cytotoxic effects because of hydroxyl radicals with Fenton and Haber–Weiss reactions (12). Ferritin is protective against oxidative stress by making chelation with free iron during increased oxidative stress (13).

It was determined that pro-oxidant/antioxidant imbalance can cause oxidative damage on cellular molecules. Additionally, oxidative stress was related to cardiovascular, infectious diseases, cancer, diabetes mellitus, and neurodegenerative pathologies (14, 15). In different childhood diseases, many biochemical tests measuring the oxidative status, TOS and TAC were shown to be practical, inexpensive, and reliable (16–18). However, limited studies exist using these tests both before and after the treatment of iron-deficiency anemia (6). Moreover, there are limited and controversial data about oxidative stress and antioxidant capacity in patients with iron-deficiency anemia. Some studies supported that erythrocytes have increased sensitivity against oxidant agents and reactive oxygen species could not be detoxified due to reduction of antioxidant enzyme (6, 19), as well as other studies showed that oxidant–antioxidant systems has not changed in iron deficiency (20–22).

Isler et al. (7) reported that superoxide dismutase activity was significantly lower in iron-deficiency anemia and increased after treatment. It has been demonstrated that glutathione peroxidase activity was similar among iron-deficiency anemia and control groups. Coghetto et al. (23), Acharya et al. (24) and Jansson et al. (8) studies have shown increased activity of antioxidant

TABLE 2. TAC and TOS in Patients with Iron Deficiency Anemia and Controls

	Iron deficiency anemia		Control	P		
	BT	AT		BT-AT	BT-C	AT-C
TAC (mmol Trolox Eq./l)	1.55 ± 0.26	1.54 ± 0.26	1.54 ± 0.20	0.393	0.856	0.939
TOS (mmol Trolox Eq./l)	24.3 ± 18.5	12.4 ± 6.9	14.4 ± 7.1	0.001	0.002	0.252

TAC, total antioxidant capacity; TOS, total oxidative stress; BT, before treatment; AT, after treatment; C, control.

enzymes such as catalase and superoxide dismutase in iron-deficiency anemia. The authors explained this situation to compensate for increased oxidative stress. In our study, there was no significant difference in TAC between iron-deficiency anemia and control groups and among the results of before and after treatment in iron-deficiency anemia.

Kurtoglu et al. (25) reported that oxidant enzyme levels, such as malonyldialdehyde, were increased, antioxidant enzymes, such as glutathione peroxidase, were decreased in iron-deficiency anemia group and oxidative stress has been shown to decrease noticeably after iron treatment. Yoo et al. (19) reported that TAC levels were lower in iron-deficiency anemia and the comparison between the TAC levels of these women after treatment with that of controls were similar. Similarly to the literature, in our study, TOS was high in iron-deficiency anemia and decreased after iron treatment. This study supports the hypothesis of the presence of increased oxidative stress in iron-deficiency anemia.

Correlation analysis showed that TAC had positive correlation with catalase and hemoglobin, and negative correlation with DNA damage and TOS (6). We did not find any correlation between hemoglobin, serum iron, iron-binding capacity, ferritin levels, and TOS or TAC in iron-deficiency anemia.

In the literature there is little and controversial information about oxidative stress and antioxidant capacity in patients with iron-deficiency anemia. As a result of this study, oxidative stress increases in children with iron-deficiency anemia and this increase returns to normal levels by treatment. Our study is important because it shows the changes of TOS and TAC after treatment in childhood iron-deficiency anemia.

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