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Oxidative Status in Iron-Deficiency Anemia

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Oxidative stress is an imbalance between free radicals and antioxidant molecules that can play an important role in the pathogenesis of iron-deficiency anemia (IDA). The aim of this study was to investigate oxidative status in patients with IDA and alteration of oxidative status after iron treatment. Thirty-three female patients with IDA and 25 healthy controls were included in this study. Oxidant and total antioxidant capacity were determined using free oxygen radicals test and free oxygen radicals defence (Form CR 3000, Callegari, Parma, Italy). Catalase activity was measured by spectrophotometer using a commercially available kit (Bioxytech Catalase-520, OxisResearch, Portland, OR). Oxidant activity in patients with IDA was significantly higher than controls ($P < 0.05$), while total antioxidant and catalase activity were significantly lower ($P < 0.05$). After treatment, oxidant, antioxidant, and catalase activity reached the levels of the control group, and no significant differences were observed among groups ($P > 0.05$). In conclusion, our data indicate that blood reactive oxygen species was lower and total antioxidant and catalase activity were higher after rather than before treatment in patients with IDA. The results of our study support the higher oxidative stress hypothesis in IDA; however, due to the limited number of cases included, more studies may be required to confirm the results. J. Clin. Lab. Anal. 23:319–323, 2009.

Key words: iron-deficiency anemia; oxidative stress; reactive oxygen species; antioxidant; catalase

INTRODUCTION

Iron-deficiency anemia (IDA) is the most common nutritional deficiency worldwide (1), affecting 500–600 million people (2). Reduction in serum iron concentration causes insufficient hemoglobin synthesis with subsequent reduction in erythrocyte proliferation. Furthermore, reduced red cell survival may be present in patients with IDA. Iron deficiency also affects the production of other proteins containing Fe^{2+}, such as cytochromes, myoglobin, catalase, and peroxidase (3). Therefore, impaired catalase activity might be anticipated in iron deficiency. Catalase is thought to represent the first line of defense in the rapid detoxification of high concentrations of H_2O_2 (4). Red cells from iron-deficient subjects lyse more readily than normal cells on exposure to H_2O_2 in vitro (4), suggesting some defect in the mechanism for protection of the iron-deficient red cell against oxidant damage.

Oxidative stress is a condition characterized by a disturbance in the pro-oxidant–antioxidant balance in

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favor of pro-oxidation. The term "oxidative stress" is used to describe a number of chemical reactions involved in the production of free radicals known as reactive oxygen species (ROS), such as hydroxyl radicals, superoxide radicals, hydrogen peroxide, and singlet oxygen that are potentially able to induce cellular injury (5,6). Disturbance of the pro-oxidant/antioxidant balance is also considered to be a causative factor underlying oxidative damage to cellular molecules such as lipids, proteins, and DNA (7,8). Through oxidation free radicals cause damage to these molecules, disturbing their normal function, and may therefore contribute to a variety of diseases (8).

Increased oxidative stress has been reported in patients with IDA. Studies have shown that oxidants were increased and antioxidants were decreased and as a result, oxidative/antioxidative balance shifted toward the oxidative side in patients with IDA. Thus, increased oxidative stress may contribute to the pathogenesis of patients with IDA (4,9–11). The literature offers limited data on oxidative stress and antioxidant defense in patients with IDA. Additionally, alterations in oxidative/antioxidative balance using measurement of free oxygen radicals, catalase, and total antioxidant status by iron treatment in patients with IDA have not been studied.

The aim of this study was to investigate oxidative status in patients with IDA and alteration of oxidative status after iron treatment.

MATERIALS AND METHODS

Experimental Design

This study was conducted in the Department of Laboratory Medicine, National Health Insurance Corporation Ilsan Hospital, Goyang-si, Korea. Thirty-three female patients with IDA who had not undergone any previous treatment for IDA and 25 healthy controls were chosen for this study. All subjects were informed about the study protocol and written consent was obtained from all participants. The group of patients received oral iron (160 mg bid ferrous sulfate; Ferroba-U®, Bukwang, Seoul, Korea). Oral iron treatment period was 4 months; at the end that period, the subjects having hemoglobin concentration below 12 g/dL continued with the same treatment for another 2 months (12). After 4–6 months treatment, hemoglobin concentration of a total of 21 patients was above 12 g/dL. A total of 12 patients dropped out at the end of the study as they did not visit the hospital.

Study Criteria

Description criteria for IDA are microcytic hypochromic erythrocytes; with a mean corpuscular volume < 80 fL, hemoglobin concentration < 12 g/dL, serum iron concentration < 45 g/dL, and serum ferritin concentration < 15 ng/ml. Exclusion criteria include acute bleeding, history of blood transfusion within 6 months before the study, existence of diabetes mellitus, coronary artery disease, rheumatoid arthritis, malignancy, systemic or local infection, hypertension, liver diseases, renal dysfunction, usage of supplemental vitamins, alcohol abuse, and smoking (11,12).

Blood Samples

Five milliliter of blood were extracted by venous puncture from all experimental subjects for the determination of catalase activity. The blood was allowed to clot and serum was extracted and stored at 70°C until use. Two milliliter of heparinized venous blood samples were used from all experimental subjects for the determination of ROS and total antioxidant activity.

Determination of Blood Levels of Oxidants, Antioxidants, and Catalase

Blood levels of oxidants were determined using the free oxygen radicals test (FORT) (Callegari, Parma, Italy); the radical species produced by the reaction that are directly proportional to the quantity of lipid peroxides present in the sample interact with an additive (phenylenediamine derivative) that forms a radical molecule evaluable by spectrophotometer at 505 nm (Form CR 3000, Callegari). The results are expressed as FORT units where 1 FORT unit corresponds to 0.26 mg/L of H_2O_2 (13). Antioxidant capacity was evaluated by Trolox equivalent antioxidant capacity assay using free oxygen radicals defence (Callegari). In the presence of an acidic buffer (pH = 5.2) and a suitable oxidant ($FeCl_3$), the chromogen (which contains 4-amino-*N*,*N*-diethylaniline sulphate) forms a stable and colored radical cation photometrically detectable at 505 nm (Form CR 3000, Callegari). Antioxidant compounds in the sample reduce the radical cation of the chromogen quenching the color and producing a decoloration of the solution, which is proportional to their concentration. The absorbance values obtained for the samples are compared with a standard curve obtained using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a permeable cell derivative of vitamin E commonly employed as an antioxidant. The results are expressed as mmol/L of Trolox. Catalase activity was measured by spectrophotometer using a commercially available kit (Bioxytech Catalase-520, OxisResearch, Portland, OR).

Statistical Analysis

Data are expressed as mean $\pm$ standard deviation (SD) and were analyzed using SPSS for Windows, Release

12.0 (SPSS Inc., Chicago, IL). Comparisons of parameters were performed with Mann–Whitney *U*-test and correlation analyses were performed using Pearson's correlation test. Before and after treatment changes were compared by Wilcoxon signed ranks test. Results were considered significant when $P<0.05$.

RESULTS

Oxidant activity of patients with IDA was significantly higher than controls ($P<0.05$) while total antioxidant and catalase activity were significantly lower ($P<0.05$) (Fig. 1). After treatment, oxidant, antioxidant, and catalase activity reached the levels of the control group, and no significant differences were observed among groups ($P>0.05$). Although statistically not significant ($P>0.05$), hemoglobin level, total antioxidant activity, catalase activity, and serum iron level were negatively correlated with oxidant activity in patients ($r=-0.222$, -0.146, -0.236, and -0.205, respectively) (Table 1). Total antioxidant activity was positively correlated with catalase activity in patients ($r=0.621$, $P<0.05$).

DISCUSSION

In this study, the major findings are as follows: (1) High ROS activity was significantly decreased after treatment and (2) low antioxidant and catalase activity were significantly increased after treatment. To the best of our knowledge, these findings have not been reported previously.

The organism has enzymatic, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase, and nonenzymatic (e.g., Vitamin C and Vitamin E) antioxidant mechanism that work as scavenger for harmful oxidant activity (11). Because antioxidant defenses are not completely efficient, increased ROS formation in the body is likely to increase damage. The term "oxidative stress" is often used to refer to this effect (5).

ROS such as hydrogen peroxide have been shown to change membrane properties of erythrocytes. Decreased cell deformability, increased echinocyte formation, increased membrane rigidity, and lipid peroxidation occurred after hydrogen peroxide exposure (14). Erythrocytes are equipped with a highly effective antioxidant defense system. In comparison to other cell types, they possess highly active antioxidant enzymes, such as SOD, GSH-Px and catalase (15).

In spite of experimental and clinical evidence for ROS and its important role in IDA, there has been little research on changes in oxidative status after treatment (11,12,16–18). Clinical studies have focused on the

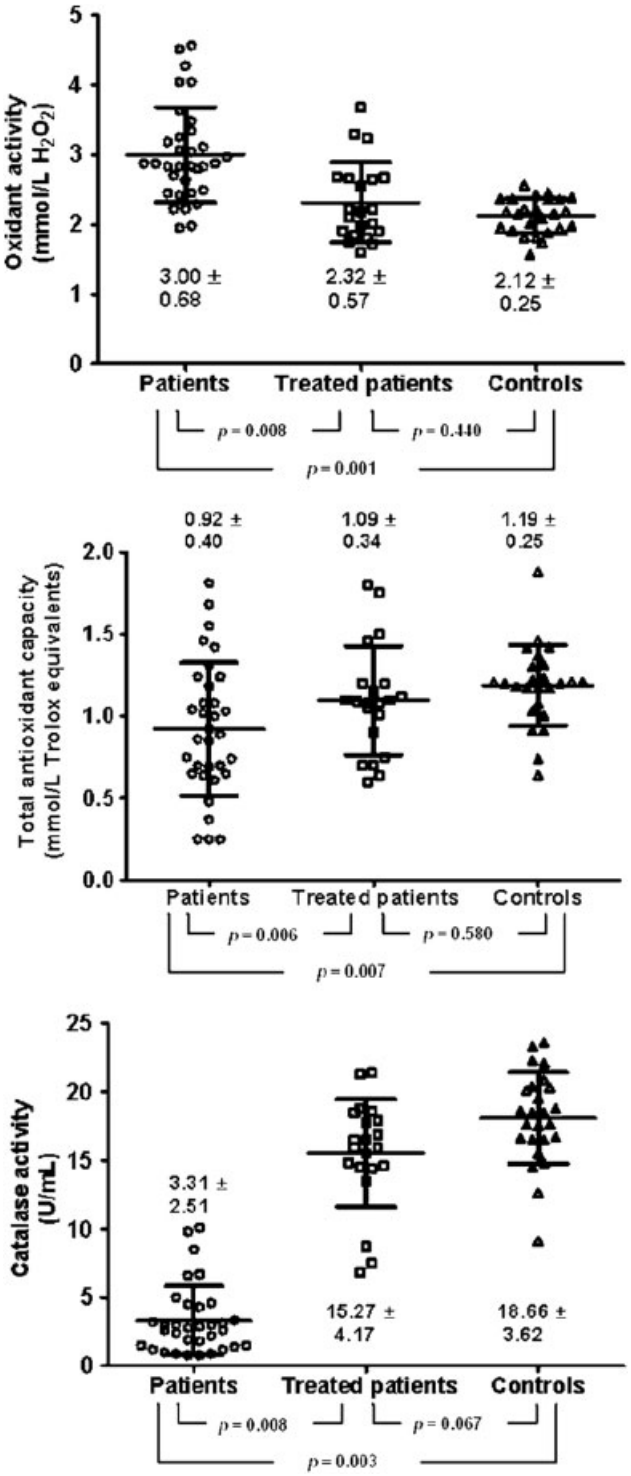

Fig. 1. Result of oxidant activity, total antioxidant capacity, and catalase activity in IDA patients ($n=33$), treated patients ($n=21$), and normal controls ($n=25$). Data were presented as mean ± SD. The Mann–Whitney *U*-test was used to investigate significant differences between the groups. The Wilcoxon signed ranks test was used to investigate significant differences between the patient's groups before and after the treatment.

TABLE 1. Correlations (*r*) Between Iron and Oxidant Parameters in Patients with IDA (*P*-value)

	ROS (mmol/L H_2O_2)	TAC (mmol/L Trolox equivalents)	Catalase (U/mL)	Hemoglobin (g/dL)	Iron (g/dL)	Ferritin (ng/mL)
TAC	−0.146	*				
(mmol/L Trolox equivalents)	(0.418)					
Catalase	−0.236	0.621	*			
(U/mL)	(0.224)	(<0.05)				
Hemoglobin	−0.222	−0.141	−0.152	*		
(g/dL)	(0.214)	(0.433)	(0.452)			
Iron	−0.205	0.081	0.139	0.644	*	
(g/dL)	(0.252)	(0.654)	(0.543)	(<0.001)		
Ferritin	0.275	−0.084	−0.095	0.268	−0.007	*
(ng/mL)	(0.122)	(0.642)	(0.683)	(0.131)	(0.971)	

ROS, reactive oxygen species; TAC, total antioxidant capacity.

measurement of stable markers of oxidative status in patients with IDA. In this study, we studied the direct automated measurement of ROS, total antioxidant activity, and catalase in patients with IDA before and after treatment.

In the literature (9,14,19), microcytic red blood cells had higher susceptibility to oxidant and high malonyldialdehyde production. Similarly, our results indicated that blood ROS concentrations were higher in patients with IDA than controls. We also found that levels of ROS were lower after treatment than before. Although statistically not significant, ROS was associated with hemoglobin and serum iron level. This result implies that IDA causes an increase in blood ROS formation and after correction of anemia, these ROS decrease.

In several studies, patients with IDA showed decreased antioxidant capacity (4,10–12), but few opposing results have been reported (16,18). They suggested that increased antioxidant capacity was a compensatory factor for increased oxidant stress (16,18). In this study, total antioxidant capacity in patients with IDA was lower than controls. Decreased SOD and GSH-Px activity are generally accepted. Hodgson et al. (20) reported that ROS, especially hydrogen peroxide, inactivate SOD. GSH-Px is a selenium-dependent enzyme, and selenium concentration was significantly lower in patients with IDA (21,22). Catalase is one of the proteins containing Fe^{2+} and decreased catalase activity is expected, but only one study has been reported (4). In this study, catalase activity in patients with IDA was lower than controls. In addition, we found that total antioxidant capacity and catalase activity were higher after treatment than before.

Recent studies have shown the association between chronic kidney disease (CKD) and oxidative stress, which increases patients' risk for cumulative injury to multiple organs (23–25). Anemia is a common feature of CKD and seems to be a main cause of oxidative stress; correction of anemia represents an effective approach to reduce oxidative stress (23). The vast majority of CKD patients seem to be iron-deficient, because of multiple forms of interference with all phases of iron metabolism, so regular supplements of intravenous iron and erythropoiesis-stimulating agents are standard therapies for treatment of anemia in patients with CKD (24). There is increasing evidence that correction of anemia with erythropoiesis-stimulating agents could protect from oxidative stress in patients with CKD (23–25). However, iron can cause hydroxyl radical production via the Fenton and Haber–Weiss reactions and promote lipid peroxidation, which can promote cytotoxicity and tissue injury (26). After parenteral iron administration, absorption may last for at least four weeks; hence free radical formation may be prolonged (12). Iron deficiency is a common cause of resistance to erythropoiesis-stimulating agents, and the overall risk-benefit ratio favors use of intravenous iron to treat iron deficiency in patients with CKD (23,24). In this study, we excluded patients with renal dysfunction and patients with use of parenteral iron.

In conclusion, our data indicate that blood ROS was lower and total antioxidant and catalase activity were higher after rather than before treatment in patients with IDA. The results of our study support the higher oxidative stress hypothesis in IDA. Because of the limited number of cases included in this study, more research may be required to confirm the results.

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