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Effect of Iron Supplementation on Oxidative Stress and Antioxidant Status in Iron-Deficiency Anemia

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ABSTRACT

This study was designed to measure the effect of iron supplementation on antioxidant status in iron-deficient anemia, including the time for hemoglobin normalization and at the time of filling of iron body stores. The extent of plasma lipid peroxidation was evaluated by measuring the levels of malondialdehyde and glutathione peroxidase (GSH-Px), and the activities of superoxide dismutase (SOD) and catalase in 63 patients with iron-deficiency anemia before and after 6 wk of iron supplementation and at the time when body iron stores are saturated. After 6 wk of iron supplementation, a significant decrease of oxidative stress was observed in the treated subjects relative to controls ($p<0.05$). No significant differences existed between treated patients at 6 wk and at the end of the study. The erythrocyte levels of catalase, SOD, and GSH-Px were significantly lower in treated patients relative to controls ($p<0.05$). These levels increased after 6 wk of supplementation ($p<0.05$) and showed no significant differences with those at the end of the study.

Index Entries: Iron-deficiency anemia; iron replacement; oxidative stress; antioxidants.

INTRODUCTION

Iron deficiency is the most common nutritional deficiency worldwide, with negative effects on work capacity and on motor and mental develop-

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ment of infants, children, and adolescents. Also, maternal iron-deficiency anemia (IDA) is associated with low birth weight and preterm delivery *(1).* Iron deficiency can lead not only to anemia but also to abnormal neurotransmitter function and altered immunologic and inflammatory defenses *(2).* Iron supplementation is a commonly used strategy to meet the increased requirements of risk groups, such as women of childbearing age, especially during pregnancy *(3).* Some factors such as type of contraception in women, blood donation, and minor pathological blood loss (hemorrhoids, gynecological bleeding) considerably increase the difficulty of covering iron needs *(4).*

Oxidative stress has been defined as a loss of balance between free-radical—or reactive oxygen species (ROS)—production and the antioxidant systems, with negative effects on the metabolism of carbohydrates, lipids, and proteins. Oxidative stress has been linked to cardiovascular and infectious diseases, cancer, diabetes, and neurodegenerative pathologies. It was found that IDA might alter the oxidant–antioxidant system by disruption of the antioxidant enzyme system. These alterations can be recovered by iron supplementation *(5,6).* At the present time, however, there is no study about the timing of this recovery and the relationship between recovery and normalization of hemoglobin and saturation of body iron stores.

The present study was designed to measure plasma lipid peroxidation in iron-deficient individuals expressed through serum malondialdehyde levels (MDA) and by the superoxide dismutase (SOD) and catalase (CAT) activities, and glutathione peroxidase levels (GSH-Px). The measurements were performed before treatment with iron supplements, after 6 wk (considered as the time of hemoglobin normalization), and at the end of treatment (time of saturation of body iron stores).

MATERIALS AND METHODS

Subjects

A total of 219 subjects were selected for this study. The subjects were were divided into four study groups as follows:

Group 1 (n = 30): age- and sex-matched healthy controls, selected after clinical, biochemical, and hematological screening
Group 2 (n = 63): patients recently diagnosed with IDA who were not receiving any treatment at the beginning of the study
Group 3 (n = 63): patients with IDA at the sixth week of an iron-replacement program
Group 4 (n = 63): patients with IDA at the end of an iron-replacement treatment

Methods

Serum and hemolyzed red cells were collected for determination of MDA and enzymatic activity, respectively. To obtain the serum, fasting

blood samples were obtained from all subjects without anticoagulant followed by centrifugation at 3000*g* for 10 min. To obtain the red cells, the samples were collected into vacuum tubes containing 1.7 mg/mL K_3EDTA as the anticoagulant. After centrifugation at 3000*g* for 15 min, the plasma and buffy coat were removed. Red blood cells were washed three times in ice-cold isotonic sodium chloride solution and the obtained packed cells were resuspended in the washing solution to give a 50% suspension. The hemoglobin was released from the cells by diluting this suspension 1 : 9 in distilled water.

The levels of MDA in serum were determined by the fluorimetric method of Wasowicz et al. *(7)* and the results were expressed as nanomoles per milliliter of MDA. In the hemolysates, the enzymatic activities of SOD, GSH-Px, and CAT were measured following established procedures and the results were expressed as units per gram of Hb for SOD and GSH-Px and as k per gram of Hb for CAT, where k is the rate constant for CAT activity. The erythrocyte Cu,Zn-SOD activity was assayed by the spectrophotometric indirect inhibition technique of Misra and Fridovich based on the ability of SOD to inhibit the auto-oxidation of adrenaline to adrenochrome at pH>7 *(8).* The CAT activity in erythrocytes was measured by the method of Aebi using hydrogen peroxide as a substrate *(9).* This method is based on the decomposition of hydrogen peroxide, which is indicated by a decrease in absorbance at 240 nm. The GSH-Px activity was measured by the coupled method of Pagila and Valentine using *t*-butyl hydroperoxide as a substrate *(10).*

Statistical Analysis

The statistical analysis was performed using Kruskal–Wallis and Mann–Whitney tests. Mean values and standard deviations (SDs) were calculated for every variable in each group and were compared between different groups; $p<0.05$ was selected as statistically significant.

RESULTS

The iron-deficient patients showed MDA levels that were significantly higher than in the controls ($p<0.05$). These levels significantly decreased both at the sixth week and at the end of the treatment, relative to the pretreatment values ($p<0.05$). These was no significant difference in thiobarbituric acid-reactive substances between those at the 6th week and at the end of the treatment (*see* Fig. 1).

The erythrocyte SOD, CAT activities, and GPx levels were significantly lower in IDA patients relative to controls ($p<0.05$). These values were significantly increased after 6 wk of supplementation ($p<0.05$) but remained without significant changes until the end of treatment (*see* Figs. 2–4).

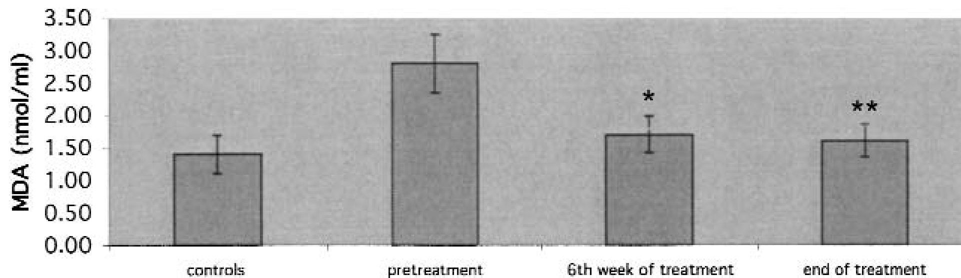

Fig. 1. Plasma MDA levels (nmol/mL) in IDA patients and controls before supplementation, at the sixth week, and at end of treatment. Values are represented as mean ± SD. *$p<0.05$ versus control subjects. **$p<0.05$ versus pretreatment in IDA patients.

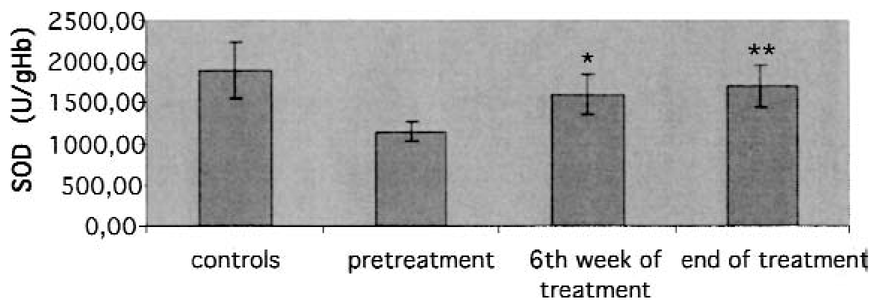

Fig. 2. Erythrocyte SOD activity (U/g Hb) in IDA patients and controls before, after 6 wk, and at the end of treatment. Values are represented as mean ± SD. *$p<0.05$ versus control subjects. **$p<0.05$ versus pretreatment in IDA patients.

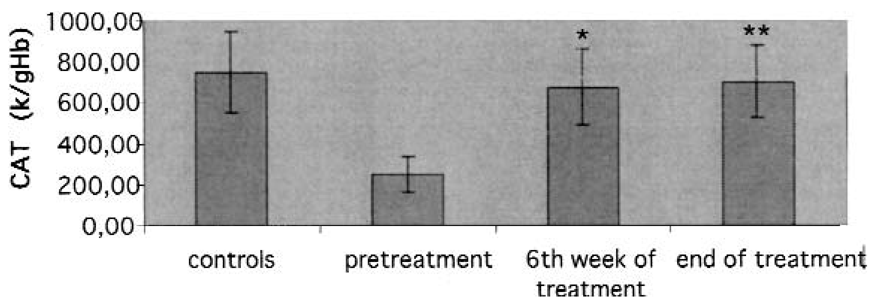

Fig. 3. Erythrocyte catalase activity (k/g Hb) in IDA patients and controls before, at the sixth week, and at the end of treatment. Values are represented as mean ± SD. *$p<0.05$ versus control subjects. **$p<0.05$ versus pretreatment in IDA patients.

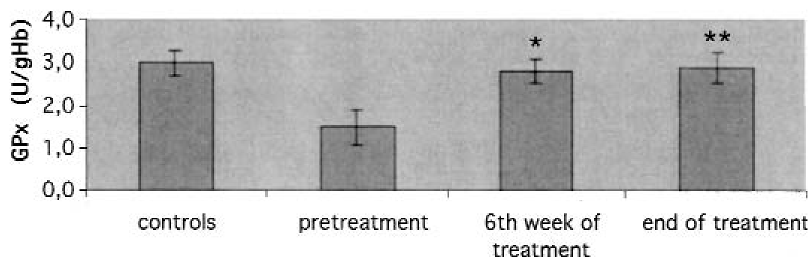

Fig. 4. Erythrocyte GSH-Px activity (U/g Hb) in IDA patients and controls before, at the sixth week, and at the end of treatment. Values are represented as mean ± SD. *$p<0.05$ versus control subjects. **$p<0.05$ versus pretreatment in IDA patients.

DISCUSSION

Oxidative damage to microcytic red blood cells has been related to the generation of free radicals *(11)*. Increased lipid peroxidation has been linked to iron-deficiency anemia, but the topic is still controversial *(12,13)*. Kumerova et al. found that the antioxidant defenses were decreased and lipid peroxidation was increased in IDA patients *(14)*. Moriarty et al. showed that the GSH-Px activity decreased secondary to IDA in rats *(15)*. Tekin et al. found that there was no significant difference in the SOD and CAT activities in IDA patients relative to controls *(5)*. Meral et al. stated that IDA does not lead to lipid peroxidation, reporting a normal antioxidant enzyme activity *(16)*.

In this study, we measured the oxidant and antioxidant status in IDA patients at three stages: before, at 6 wk, and at the end of the treatment. The indicators of increased oxidative status were significantly higher, whereas the antioxidant enzymes were significantly lower in IDA patients compared to controls.

In IDA patients, iron supplementation is standard treatment to restore the body iron stores. Although hemoglobin levels increase within 1–2 mo, the treatment must be continued until the body iron stores are up to capacity *(17)*.

Although it is well known that iron-replacement treatment decreases antioxidant stress by regulating enzyme levels, data about the timing of this recovery are lacking. In a recent study of Isler et al., iron supplementation was used to recover the antioxidant defense system *(6)*. Also, Bartal et al. showed that although IDA cells are more susceptible to oxidation, they maintain good capacity for recovery *(18)*. These studies only compare the pretreatment and posttreatment values and not at different stages of the treatment.

Our results provide evidence of decreased oxidative stress after 6 wk of iron supplementation. This condition remains without significant changes until the end of the study, suggesting that the oxidant–antioxidant status is regulated at the same time as the normalization of hemoglobin.

Although we observed a decrease in lipid peroxidation expressed as lower MDA levels in both groups of IDA patients receiving iron supplements, this decrease was not significant. The MDA test is very sensitive but has poor specificity. Certainly, the most direct approach for the assessment of lipid peroxidation is the direct quantification of hydroperoxides, which, in practice, is very difficult because of their labile nature. Consequently, detection of lipid peroxidation relies largely on indirect methods (i.e., analyses of secondary or end products such as malondialdehyde) *(19).*

Our results show that oxidative stress was lower both at the time of hemoglobin normalization and at the time of saturation of iron body stores. The lack of significant differences in oxidant status among the groups at these stages of treatment suggests that the patients recover following the normalization of hemoglobin, before normalization of ferritin.

CONCLUSION

The level of oxidative stress indicated by the serum levels of MDA and the SOD, CAT, and GSH-Px status in IDA patients decreases after the sixth week of iron supplementation. This decrease remains until the iron body stores are fulfilled. Oral iron supplementation is recommended for the recovery of the impaired antioxidant defense system in iron deficiency anemia.

REFERENCES

1. A. C. Looker, M. E. Cogswell, and E.W. Gunter, Iron deficiency, United States, 1999–2000. *MMWR*, **51,** 897–899 (2001).
2. E. M. Ross, Evaluation and treatment of iron deficiency in adults, *Nutr. Clin. Care* **5,** 220–224 (2002).
3. R. Yip, Iron supplementation: country level experiences and lessons learned, *J. Nutr.* **132,** 859–861 (2002).
4. S. Hercberg, P. Preziosi, and P. Galan, Iron deficiency in Europe, *Public Health Nutr.* **4,** 537–545 (2001).
5. D. Tekin, S. Yavuzer, M. Tekin, et al., Possible effects of antioxidants status on increased platelet aggregation in childhood iron-deficiency anemia, *Pediatr. Int.* **43,** 74–77 (2001).
6. M. Isler, N. Delibas, M. Guclu, et al., Superoxide dismutase and glutathione peroxidase in erythrocytes of patients with iron deficiency anemia: effects of different treatment modalities, *Croat. Med. J.* **43,** 16–19 (2002).
7. W. Wasowicz, J. Neve, and A. Peretz, Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage, *Clin. Chem.* **39,** 2533–2536 (1993).
8. H. P. Misra and I. Fridovich, The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase, *J. Biol. Chem.* **247,** 3170–3175 (1973).
9. H. E. Aebi, Catalase of enzymatic analysis, Volume III *Enzymes. I. Oxidoreductases, Transferases,* in H. U. Bergmeyer, ed., VCH, Weinheim, pp. 273–285 (1987).

10. D. E. Paglia and W. N. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, *J. Lab. Clin. Med.* **70,** 158–169 (1967).
11. J. L. Vives Corrons, A. Miguel-Garcia, M. A. Pujades, et al., Increased susceptibility of microcytic red blood cells to in vitro oxidative stress, *Eur. J. Haematol.* **55,** 327–331 (1995).
12. A. A. Golovin and V. D. Konvai, Lipid peroxidation in patients with iron deficiency anemia complicated by frequent acute respiratory distress, *Klin. Med.* **69,** 73–75 (1991).
13. J. Acharya, N. A. Punchard, J. A. Taylor, et al., Red cell lipid peroxidation and antioxidant enzymes in iron deficiency, *Eur. J. Haematol.* **4,** 287–291 (1991).
14. A. Komerova, A. Lece, A. Skesters, et al., Anemia nad antioxidant defence of the red blood cells, *Mater. Med. Pol.* **30,** 12–15 (1998).
15. P. M. Moriarty, M. F. Picciano, J. L. Beard, et al., Classical selenium-dependent glutathione peroxidase expression is decreased secondary to iron deficiency in rats, *J. Nutr.* **125,** 293–301 (1995).
16. A. Meral, P. Tuncer, E. Surmen-Gur, et al., Lipid peroxidation and antioxidant status in beta-thalassemia, *Pediatr. Hematol. Oncol.* **17,** 687–693 (2000).
17. G. M. Brittenham, Disorders of iron metabolism: iron deficiency and iron overload, in *Hematology: Basic Principles and Practice,* R. Hoffman, E. J. Benz, Jr., S. J. Shattil, et al., eds., Churchill Livingstone, Philadelphia, p. 416 (2000).
18. M. Bartal, D. Mazor, A. Dvilansky, et al., Iron deficiency anemia: recovery from in vitro oxidative stress, *Acta Hamatol.* **90,** 94–98 (1993).
19. M. E. Inal, G. Kanbak, and E. Sunal, Antioxidant enzyme activities and malondialdehyde levels related to aging, *Clin. Chim. Acta* **305,** 75–80 (2001).