Ferrous Sulfate (Fe^{2+}) Had a Faster Effect Than Did Ferric Polymaltose (Fe^{3+}) on Increased Oxidant Status in Children With Iron-deficiency Anemia

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Objective: The purpose of this study was to compare the total oxidant and antioxidant effect of different oral iron preparations in children with iron-deficiency anemia (IDA).

Methods: A total of 65 children with IDA were randomized to receive 5 mg Fe/kg/d iron (II) sulfate (Fe^{2+} group, n = 33) or iron (III)-hydroxide polymaltose complex (Fe^{3+} group, n = 32); healthy controls (n = 28) were also included in the study. Serum total thiol (–SH), total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), and hematological profile were evaluated at the baseline and on day 8 and day 30 of the therapy.

Results: Serum TOS and OSI levels were significantly higher and total –SH and total antioxidant capacity levels were significantly lower in the study groups at the beginning of therapy than in the controls ($P > 0.001$). In multivariate analysis, after controlling for multiple confounding factors, on days 8 and 30, serum TOS and OSI levels were not different in the Fe^{3+} group, whereas they were significantly reduced in the Fe^{2+} group ($P \leq 0.033$).

Conclusions: Serum total oxidant status was significantly increased in children with IDA, and Fe^{2+} was highly effective in correcting elevated oxidative status.

Key Words: anemia, iron deficiency, ferrous sulfate, ferric polymaltose, antioxidants, oxidants, oxidative stress

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Iron deficiency is the most widespread nutritional deficiency across the world and is common in developing and developed countries alike.[1] The present treatment strategy for iron-deficiency anemia (IDA) involves the oral use of ferrous sulfate (Fe^{2+}) and ferric iron polymaltose complex (Fe^{3+}).[2,3] However, in clinical practice, bivalent iron salts are preferred over ferric iron preparations.[4] Because of its chemical properties—namely, its oxidative potential—iron functions in several biological systems that are crucial to human health.[5] The condition impairs many aspects of health, including the body's antioxidant system; however, the treatment of iron deficiency through oral ingestion of iron supplements may be associated with oxidative damage.[6,7]

Iron shows its deleterious effects by generating reactive oxygen species or hydroxyl radicals and reacting directly with unsaturated fatty acids or preformed lipid hydroperoxides to form alkoxyl and/or peroxyl radicals.[6] Fe^{2+} forms superoxide radicals reacting with oxygen and hydroxyl radicals reacting with hydrogen peroxide.[8] The latter of these radicals is generated by the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + OH^{-}$) and is highly toxic.[7]

In humans, IDA has also been shown to diminish the concentrations and/or activities of antioxidant enzymes such as glutathione peroxidase (GSH-Px),[9–12] catalase (CAT),[11–14] and superoxide dismutase (SOD).[11,14,16] These levels increased after 6 weeks of iron supplementation.[11] It is also reported that iron deficiency without anemia is associated with significant reductions in serum ceruloplasmin and erythrocyte SOD activity, but not serum GSH-Px concentrations, in college-aged females.[17] A recent study also showed that iron deficiency with anemia in adult women is associated with significant reductions in serum total antioxidant capacity (TAC).[18] In our study, the first aim was to determine the oxidative status in children with IDA. The second aim was to investigate the oxidant/antioxidant effects of oral iron supplementation by determining the total oxidant status (TOS) and TAC as well as oxidative stress index and total thiol (–SH) levels in children with IDA.

PATIENTS AND METHODS

A total of 72 consecutive patients who attended the Pediatrics and Pediatric Hematology Outpatient Clinic in Harran University, Turkey, between January 2011 and May 2012 were enrolled in the open-label randomized controlled trial. Informed consent was obtained from each patient's parent. This study was approved by the Ethical Committee of Harran University Medical Faculty. IDA was defined as hemoglobin (Hb) below 10.6 g/dL for children at or below the age of 2 years and below 11 g/dL for children older than 2 years and with a serum ferritin value below 12 ng/mL.[19,20] The children with IDA, aged between 1 and 16 years, using simple randomization with no restrictions or matching, were allocated in the Fe^{2+} (Ferro-Sanol susp. or capsule/Adeka) or Fe^{3+} (Ferrum susp. or coated tablet/Abdi Ibrahim) group. Twenty-eight healthy children, age-matched and sex-matched, were enrolled as controls. Children were excluded if they had used iron preparations in the previous 3 months, had acute infection, had a history of chronic disease or parasites, suffered blood

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loss for any reason, or had occult blood in their stools. There was no control group of children with IDA followed without active treatment as it is viewed as unethical and an infringement of basic human rights.

Iron was given at a dose of 5 mg/kg/d.[20] The duration of the study was 1 month. All patients were seen at the Outpatient Service of Pediatric Hematology Department on days 8 and 30 of the treatment. At each visit, all patients were examined and clinical toxicities and complete data were recorded.

Analytical Methods

Blood samples were collected from a peripheral vein into vacutainers containing ethylenediaminetetraacetic acid and jelled serum tubes. A hemogram was obtained, and reticulocyte, ferritin, iron, and total iron binding capacity levels were determined using commercial kits (Abbott) on the same day. Whole blood count was measured by an automated analyzer (Celldyn 3700; Abbott, IL). Serum samples were separated from the cells by centrifugation at 1500*g* for 10 minutes and were stored at −80°C without preservative until assayed. All analyses were performed at a single laboratory.

Serum thiol (total –SH group) content was measured using dithionitrobenzoic acid.[21] TAC and TOS levels were measured by Erel's methods (Rel Assay Diagnostics, Gaziantep, Turkey), which are automated and colorimetric.[22–24] Erel's TOS method is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and on the measurement of the ferric ion by xylenol orange. The results were expressed in μmol H_2O_2 Eq/L.[25] Erel's TAC method is based on the bleaching of the characteristic color of a more stable 2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants. The results were expressed in mmol Trolox Eq/L.[26] The percentage of TOS level to TAC level was regarded as the OSI.[27] The plasma OSI value was calculated as follows: OSI = [TOS (mmol H_2O_2 Eq/L)][TAC (mmol Trolox Eq/L)] × 100.[23,27]

Statistical Analysis

Homogeneity of variance was tested for all the variables by Levene test. A χ^2 test was used to assess the relationships between categorical independent variables. Differences in the parameters between the Fe^{2+}, Fe^{3+}, and control groups were analyzed by 1-way analysis of variance (ANOVA) with the Tukey honestly significant difference test at the baseline. Differences in hematologic variables between Fe^{2+} and Fe^{3+} groups were analyzed by the Student *t* test. The Pearson correlation was used to identify confounders for the oxidant status variables. The potential confounding factors tested were blood Hb, serum iron, and ferritin levels. Multivariate analysis of covariance (MANCOVA) was applied to test whether the iron treatment affected the oxidant status after controlling for confounding factors. The data were expressed as mean ± SD and differences were considered statistically significant at $P < 0.05$. Statistical analyses were performed using SPSS for Windows Release 11.5 (SPSS Inc., Chicago, IL).

RESULTS

A total of 72 consecutive patients were enrolled in the study, 65 patients were brought on days 8 and 30 of treatment, 11 patients were used improperly of the drugs (5 patients in Fe^{2+} group and 5 patients Fe^{3+} group were excluded the study). The Fe^{2+} group included 33 patients (male/female: 15/18), the Fe^{3+} group included 32 patients (male/female: 15/17), and the control group included 28 patients (male/female: 13/15). The mean age of the Fe^{2+} group was 7.7 ± 5.2 years, that of the Fe^{3+} group was 7.9 ± 5.6 years, and that of the controls was 7.2 ± 5 years. Fifty-four patients were brought on day 30 of the treatment. There was no significant difference between the mean ages and sex distributions of the 3 groups; no statistically significant difference between the 2 study groups according to the side effects; and no patient stopped receiving treatment because of side effects ($P > 0.05$) (Table 1).

The patients in the Fe^{2+} group had significantly higher iron, ferritin, and Hb levels on days 8 and 30 of the

TABLE 1. Comparison of Demographic, Hematologic, and Oxidative/Antioxidative Parameters in the 2 Study Groups and Controls

	Fe^{2+} (n = 33)	Fe^{3+} (n = 32)	Control (n = 28)	*P*†
Age (y)	7.7 ± 5.2	7.9 ± 5.6	7.2 ± 5	0.792
Height (cm)	112 ± 34	117 ± 36	113 ± 32	0.604
Body weight (kg)	23.7 ± 17	23.2 ± 14	22.6 ± 13	0.516
Gender (M/F)	15/18	15/17	13/15	0.215‡
Inconsistencies in treatment (n)	5	5	NA	0.965‡
Abdominal pain (n)	4	2	NA	0.351‡
Nausea (n)	3	2	NA	0.515‡
Hb (g/dL)	8 ± 1.5	8.1 ± 1.8	13.1 ± 1.2	< 0.001*
Ferritin (ng/mL)	7.8 ± 6.9	7.5 ± 10.2	92.5 ± 17.8	< 0.001*
Iron (μg/dL)	18.5 ± 7.3	17.4 ± 6.5	89.6 ± 12.3	< 0.001*
TIBC (μg/dL)	443 ± 78	449 ± 58	309 ± 137	0.031*
Reticulocyte ($\times 10^3/mm^3$)	17 ± 11	22 ± 13	42 ± 27	< 0.001*
Total –SH group (mmol/L)	0.51 ± 0.07	0.51 ± 0.08	0.61 ± 0.06	< 0.001*
TAC (mmol Trolox Eq/L)	0.80 ± 0.17	0.79 ± 0.18	0.95 ± 0.13	0.001*
TOS (μmol H_2O_2 Eq/L)	20.9 ± 7.8	21.2 ± 7.3	13.1 ± 2.7	< 0.001*
OSI (arbitrary unit)	2.9 ± 1.2	2.9 ± 1.5	1.4 ± 0.3	< 0.001*

Data are given as mean ± SD.
*$P > 0.05$ versus control.
†One-way analysis of variance test.
‡χ^2 test.
Hb indicates hemoglobin; TIBC, total iron-binding capacity.

TABLE 2. Comparison of Hematologic Parameters of the Fe^{2+} and Fe^{3+} Groups at the Day 8 and 30 Evaluations

	Day 8			Day 30		
	Fe^{2+} (n = 28)	Fe^{3+} (n = 27)	*P*†	Fe^{2+} (n = 28)	Fe^{3+} (n = 27)	*P*†
Hb (g/dL)	9.5 ± 1.3	8.5 ± 2.2	0.036	11.4 ± 1.4	9.6 ± 1.8	< 0.001
Ferritin (ng/mL)	24.9 ± 15.8	12.8 ± 11.6	0.006	29.4 ± 22.4	18.7 ± 20.1	0.047
Iron (μg/dL)	37.5 ± 19.6	25.8 ± 9.8	0.006	45.9 ± 22.4	28.4 ± 14.8	0.001
TIBC (μg/dL)	370 ± 74	429 ± 47	< 0.001	326 ± 49	395 ± 63	< 0.001
Retic ($\times 10^3/mm^3$)	134 ± 37	102 ± 30	0.006	90 ± 54	80 ± 27	0.026

Data are given as mean ± SD.
†Student *t* test.
Hb indicates hemoglobin; Retic, reticulocyte; TIBC, total iron-binding capacity.

treatment, although there was no difference between the 2 study groups at the beginning of the study. On day 30 of the study, the Fe^{2+} group had Hb levels of 11.4 ± 1.4 g/dL and ferritin levels of 29.4 ± 22.4 ng/mL, whereas the Fe^{3+} group had Hb levels of 9.6 ± 1.8 g/dL and ferritin levels of 18.7 ± 20.1 ng/mL ($P < 0.05$) (Table 2).

There was no statistically significant correlation between Hb, ferritin, iron, and total –SH, TAC, TOS, and OSI levels in the Fe^{2+} and Fe^{3+} groups at day 8 or day 30 evaluations ($P > 0.05$). In the multivariate analysis using blood Hb, serum iron, and ferritin levels as covariates, interactions were not marked with antioxidant/oxidant markers at day 8 (MANCOVA; $P > 0.05$) or day 30 evaluations (MANCOVA; $P > 0.05$). There were no differences in total –SH and TAC levels in the study groups on day 8 (MANCOVA; $P = 0.134$ and $P = 0.085$, respectively) (Table 3), whereas TOS (Fig. 1) and OSI levels were lower in the Fe^{2+} group than they were in the Fe^{3+} group (MANCOVA; $P = 0.04$ and $P = 0.033$, respectively). Moreover, multivariate analysis showed that total –SH, TOS, and OSI levels were significantly different in the Fe^{2+} group at day 30 evaluations (MANCOVA; $P = 0.008$, 0.011, and 0.010, respectively).

DISCUSSION

The current treatment of IDA is successfully performed orally with either Fe^{2+} or Fe^{3+} preparations. However, data assessing together the effect on oxidant and antioxidant systems of these different ionic forms is limited. To the best of our knowledge, all of the published studies related to the oxidative/antioxidative effects of oral iron treatment are about GSH-Px,[9–12] CAT,[11–15] and SOD[11,14,16]; this is the first report showing an association between decreased serum TOS and oxidative stress index in children who were treated orally with Fe^{2+}.

Free iron may lead to the production of free oxygen radicals and consequently increase the risk of developing potentially serious organ damage.[7,8,28] It was reported that Fe^{2+} forms hydroxyl and superoxide radicals and causes oxidant stress.[28] Slivka et al[7] stated that oral iron causes in vivo formation of hydroxyl iron in the gastrointestinal tract and that ferrous iron in ionic form can be easily oxidized by molecular oxygen. Meral et al[29] reported that lipid peroxidation increased in children with thalassemia major. Combining these reports with our results, iron excess or deficiency causes oxidant effect in the body.

For antioxidant enzymes such as GSH-Px, CAT, and SOD, the concentrations and/or activities have been shown to diminish in IDA. Yoo et al[30] reported that total antioxidant activity was higher after rather than before treatment in patients with IDA. Moreover, Kavakli et al[31] found that erythrocyte malondialdehyde (MDA), SOD, CAT, and GSH-Px levels showed minimal differences between children treated with ferric or ferrous iron in antioxidant system activities. However, they compared the 2 different oral iron preparations and did not have a control group; the results of the patients obtained during the treatment were compared to their baseline values, and each patient was assessed with his/her own control in both the groups.

Kurtoglu et al[11] measured serum MDA, erythrocyte SOD, CAT activities, and GSH-Px levels in adult IDA patients at 3 stages: before, at 6 weeks, and at the end of the treatment. The indicators of increased serum MDA levels were significantly higher, whereas the antioxidant enzymes were significantly lower in IDA patients compared with the controls. Erythrocyte antioxidant enzyme values were significantly increased after 6 weeks of supplementation but remained without significant changes until the time of

TABLE 3. Comparison of Serum Oxidative/Antioxidative Parameters of the Fe^{2+} and Fe^{3+} Groups at the Day 8 and 30 Evaluations

	Day 8			Day 30		
	Fe^{2+} (n = 28)	Fe^{3+} (n = 27)	*P**	Fe^{2+} (n = 28)	Fe^{3+} (n = 27)	*P**
Total –SH group (mmol/L)	0.49 ± 0.08	0.53 ± 0.08	0.134	0.54 ± 0.066	0.48 ± 0.043	0.008
TAC (mmol Trolox Eq/L)	0.79 ± 0.2	0.8 ± 0.17	0.849	0.82 ± 0.23	0.81 ± 0.17	0.961
TOS (μmol H_2O_2 Eq/L)	16.6 ± 4.8	22.2 ± 8.2	0.004	15.9 ± 4	18.4 ± 4.2	0.011
OSI (arbitrary unit)	2.11 ± 0.6	2.86 ± 1.5	0.033	1.85 ± 0.6	2.92 ± 1.5	0.010

Data are given as mean ± SD.
*MANCOVA test.
MANCOVA indicates multivariate analysis of covariance; OSI, oxidative stress index; TAC, total antioxidant capacity; TOS, total oxidant capacity.

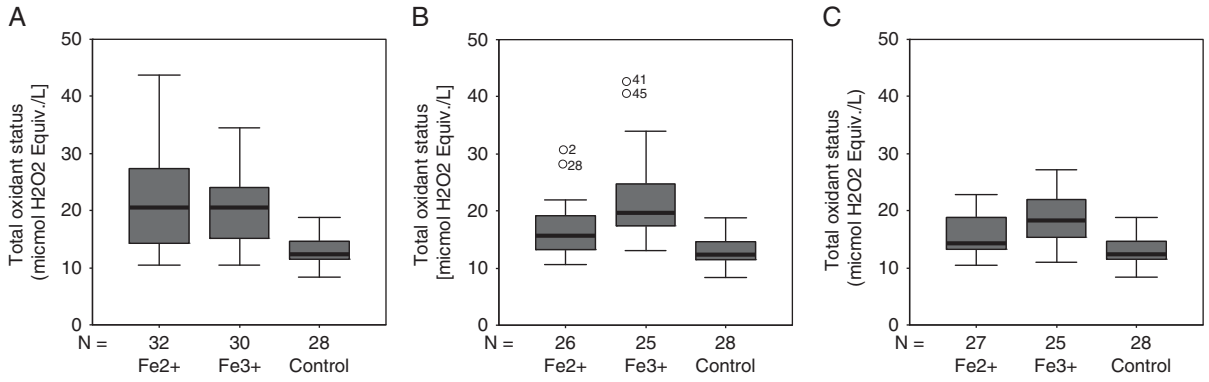

FIGURE 1. Boxplot graph of plasma total oxidant status levels at the baseline (A), on day 8 (B), and on day 30 (C) of iron treatment in Fe^{2+} group, Fe^{3+} group, and controls.

saturation of body iron stores. However, in that study, they compared distinctive groups at different stages of the treatment. Isler et al[32] reported that oral iron treatment improved adult IDA and repaired the antioxidant defense system by increasing the SOD activity and maintaining GSH-Px activity at the normal level.

In our study, before the treatment, serum TOS and OSI levels were significantly higher, total –SH and TAC levels were lower in anemic patients than in the control group ($P < 0.001$). One month later, these parameters significantly improved in the Fe^{2+} group and but did not reach the values found in the control group.

Ferric iron is a powerful oxidizing agent. The most critical point here is the risk that Fe^{2+}, which provides a faster and more efficient iron treatment because of better absorption, might cause toxic oxidant reactions.[31] The clinical and laboratory results that we obtained in our study do not support the reports stating that iron treatment causes toxic oxidant effects in children with IDA. In this study, no meaningfully high increase in oxidative status was observed in children in the Fe^{2+} group compared with those in the Fe^{3+} group.

Gropper et al[6] reported that oxidative damage was assessed in the plasma of college-aged females with adequate iron status and with nonanemic iron deficiency before and after 8 weeks of iron supplementation, and no significant differences in plasma lipid hydroperoxide or protein carbonyl concentrations were found between groups or within groups at the end of the study period. Neither nonanemic iron deficiency nor its treatment with oral iron supplements is associated with oxidative damage in the plasma of college-aged females.

Aslan et al[18,33] reported that serum TAC was significantly reduced in adult women with iron deficiency and anemia. They also found a positive correlation between TAC and Hb levels but did not have the results of the patients obtained during the treatment. In our study, TAC total –SH levels were significantly reduced in both the Fe^{2+} and Fe^{3+} groups compared with the controls. However, there was no difference in serum TAC between the Fe^{2+} and Fe^{3+} groups at the baseline or day 8 or 30 evaluations.

Our results show that oxidative stress was higher at the time of Hb normalization in the Fe^{2+} group than in controls. The lack of significant differences in antioxidant status on days 8 and 30 of the treatment suggests that the patients recover after the normalization of Hb and before normalization of antioxidant status. The limitation of this research were the short duration of follow-up and was not a double-blind trial. In general, if researchers describe a trial as double-blind, readers can assume that they have avoided bias.[34] The comparison of the drugs is most readily accepted if the results are from randomized controlled trials.[35] Open-label studies are frequently incorporated in the design of randomized controlled trials. More research is needed to identify and measure the total oxidant and antioxidant effect of different iron preparations in children with IDA.

In conclusion, serum TOS was significantly increased in children with IDA, and Fe^{2+} was highly effective in correcting elevated oxidative status.

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