Antioxidant effect of vitamin E in the treatment of nutritional iron deficiency anemia

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ABSTRACT

Oxidant status and antioxidants play important roles in anemias. The present study was conducted to investigate the oxidant-antioxidant status in iron deficiency anemia (IDA), and to evaluate the antioxidant effect of vitamin E in IDA treatment. Ten patients with IDA aged nine months were given only iron treatment, whereas another 10 patients were administered both iron and vitamin E. The complete blood count, plasma malonyldialdehyde (MDA) level, erythrocyte superoxide dismutase level, and the serum vitamin E level, both before and within the treatment phases were examined. The reticulocyte count at the first week of treatment was found lower in the vitamin E-treated group. The mean corpuscular volume (MCV) was found higher in the vitamin E-treated group at the end of therapy. The malonyldialdehyde levels of the group treated with vitamin E were found lower during treatment. These results suggest that iron administration in IDA treatment may stimulate lipid peroxidation, and that vitamin E supplied with iron may reduce the MDA production. The hematological indications of the findings of our study are that the reticulocyte response develops earlier and the microcytosis recovery occurs more rapidly in the vitamin E-administered group in comparison with the group treated with iron only.

Key Words: Iron deficiency anemia, Oxidant status, Antioxidants, Vitamin E, Treatment.

ÖZET

Nütrisyonel demir eksikliği anemisi tedavisinde E vitamininin antioksidan etkisi

Serbest radikal reaksiyonları ve antioksidanlar anemi patogenezinde önemli rol oynar. Bu çalışma demir eksikliği anemisi (DEA) tedavisinde oksidan-antioksidan durumu saptamak ve DEA tedavisinde E vitamininin antioksidan etkisini değerlendirmek amacıyla yapılmıştır. Sadece demir tedavisi verilen dokuz aylık 10 hasta ve demir ile birlikte E vitamini tedavisi verilen 10 hasta çalışmaya alınmıştır. Tam kan sayımı, plazma malonildialdehid (MDA) düzeyi, eritrosit süperoksid dismutaz (ESOD) düzeyi ve serum E vitamini düzeyi tedavi öncesi ve tedavi sırasında ölçülmüştür. E vitamini ile tedavi edilen grupta retikülosit sayısı daha düşük bulunmuştur. Ortalama eritrosit hacmi E vitamini ile tedavi edilen grupta tedavinin sonunda yüksek bulunmuştur. Tedavi boyunca MDA düzeyleri E vitamini ile tedavi edilen grupta daha düşük bulunmuştur. Bu sonuçlar DEA tedavisinde verilen

demirin lipid peroksidasyonunu uyarabileceğini ve demir ile birlikte verilen E vitamininin MDA üretimini azaltabileceğini göstermiştir. Çalışmamızdaki bulguların hematolojik göstergeleri sadece demir verilen gruba göre vitamin E verilen grupta, retikülosit yanıtının daha erken olması ve mikrositozun daha hızlı düzelmesidir.

Anahtar Kelimeler: Demir eksikliği anemisi, Oksidan durum, Antioksidanlar, E Vitamini, Tedavi.

INTRODUCTION

Free radicals, oxidative stress, and antioxidants have become commonly-used terms in modern discussions of disease mechanisms. The degree of lipid peroxidation in organisms can be investigated with malonyldialdehyde (MDA), which is the breakdown product of lipid peroxidation[1-3]. Antioxidants, which act against oxidant damage in a cell, consist of preventive and chain breaking mechanisms. Superoxide dismutase (SOD) is a preventive antioxidant, whereas vitamin E is a chain breaking antioxidant. Vitamin E is also the only lipid soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes. Vitamin E deficiency in erythrocytes causes the formation of lipid peroxidation and hemolysis[4,5].

Numerous investigations have indicated the free radical reactions and preventive roles of antioxidants in the pathogenesis of anemias, especially hemolytic anemias[6-8]. Since the erythrocyte is a cell containing high concentrations of polyunsatured fatty acids, molecular oxygen and ferrous ions, it is highly susceptible to oxidant damage[1,9]. On the other hand, iron is reported to cause the generation of harmful oxygen derivatives and the promotion of lipid peroxidation through the Haber-Weiss and Fenton reactions[10,11].

In iron deficiency anemia (IDA), previous studies suggest that decreased red cell survival and red cell membrane stiffness are secondary to an increased susceptibility of red cells to oxidant stress[12,13]. It is also reported that if erythrocytes are incubated with an oxidant substance, there occur increased levels of MDA and decreased levels of catalase, glutathione peroxidase, and vitamin E accur[14]. Also an increased level of red cell SOD is reported in IDA, a finding suggesting a compensatory increase in this enzyme secondary to an increased oxidant stress[15]. Several authors like Acharya et al., however, showed that there is no evidence of an increased susceptibility of red cell to lipid peroxidation in iron deficiency[16].

Oxidant evidences suggest that iron treatment could induce lipid peroxidation similar to thalassemias in IDA[9-11]. Treatment and prophylactic strategies of IDA have been researched in previous studies. The role of excess iron in causing intestinal oxidative stress has drawn attention to other approaches of iron supplementation. Prophylactic administration of iron, along with antioxidants like vitamins E and C, or with foods rich in these vitamins may be a strategy[17].

The present study was conducted to investigate the oxidant-antioxidant status in IDA, and to evaluate both the oxidant effect of iron and the antioxidant effect of vitamin E in IDA treatment.

MATERIALS and METHODS

The present study was conducted at Gazi University School of Medicine, Department of Pediatrics. Totally 20 infants aged 9 months (13 males + 7 females), who were diagnosed to have IDA during their well-baby examinations were examined. Informed consent was provided by all parents. The research project was approved by the Ethics Committee of the School of Medicine.

The diagnosis criteria of IDA involved hemoglobin values below 10.5 g/dL, hematoc-

rit values below 33%, erythrocyte count below 3.7 x 10^{12}/L, MCV values below 70 fl, and mean corpuscular hemoglobin (MCH) values below 23 pg.

The patients were randomly divided into two groups. Group one consisted of 10 patients who were given only iron treatment (ferroglycine sulfate, per oral drops, 4 mg/kg/d, for 3 months). Group 2 consisted of 10 patients administered both iron (ferroglycine sulfate, per oral drops, 4 mg/kg/d, for 3 months) and vitamin E (100 mg/d, per oral drops, for 1 month). Ten healthy non-anemic subjects aged 9 months (6 males + 4 females) were studied as controls.

The patients and controls were followed in well-baby examinations. They had no history of anemia in their prenatal, natal or neonatal periods. Fifteen patients in the IDA group were receiving cow's milk. In the control group, seven subjects were still breast-fed, and three subjects were receiving cow's milk.

The complete blood count, peripheral blood analysis, reticulocyte count, serum ferritin levels, serum iron and iron binding capacity levels, serum vitamin E levels, erythrocyte superoxide dismutase (ESOD) values and plasma MDA levels were obtained from patients and healthy subjects before the treatment.

The patients underwent clinical and laboratory evaluations within the first week, first month, and the third month of treatment. The following blood analyses were performed during treatment: complete blood count, peripheral blood analysis, reticulocyte count, ESOD, and plasma MDA. At the end of treatment, the serum ferritin and vitamin E levels were also analyzed.

The complete blood count was analyzed by Beckman & Coulter MaxM. The reticulocyte count was determined by the standard manual method. Serum ferritin levels (spectroferritin kit, RAMCO Laboratory, Inc., Houston, Texas, USA) were determined by the enzyme immunoassay method. The serum iron and iron binding capacity were measured by a spectrophotometric assay. Plasma MDA levels were determined by thiobarbituric acid reaction substance (TBARS) methods, and ESOD levels were determined using the method described by Winterbourn[18,19]. Serum vitamin E levels were analyzed spectrophotometrically using the method described using Rindi[20].

Statistical Analysis

Data were analyzed by SPSS 10.0 for Windows. Values are given in Tables as mean ± standard deviation.

All laboratory parameters between IDA groups and between the control group and each IDA group were analyzed using the Mann-whitney U test. This test was used to analyze values not abtained parametrically.

All laboratory parameters were evaluated by chi-square test regarding feeding strategies in all infants, including the patients and control infants.

RESULTS

Laboratory Evaluations Before the Treatment

Tables 1 and 2 show the laboratory parameters of all patient and control groups. Before the treatment, some hematological indices with the exception of red cell distribution width (RDW) were lower in all patients with IDA as compared to the control group. Serum ferritin, serum iron, and serum vitamin E levels were lower in patient groups than in the control group. RDW and serum iron binding capacity were significantly higher in the patient groups. The reticulocyte count, ESOD, and plasma MDA levels were found similar between the IDA groups and controls.

All patient and control infats were, evaluated regarding feeding strategies. It was found that the infants who were fed with cow's milk demonstrated lower vitamin E levels compared to the infants who were fed with breast milk ($p< 0.05$).

Table 1. Hematological parameters of patients and controls

	Hb (g/dL)	Htc (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	RBC (10^{12}/L)	RDW (%)	Reticulocyte count (%)
Group 1	8.44 ± 0.91	28.13 ± 2.77	59.17 ± 6.46	18.52 ± 3.03	30.76 ± 1.68	4.53 ± 0.32	17.63 ± 1.58	0.4 ± 0.21
Group 2	8.66 ± 0.56	28.77 ± 2.17	61.41 ± 3.15	20.61 ± 2.4	31.52 ± 0.99	4.42 ± 0.3	16.09 ± 1.8	0.32 ± 0.14
P*	p> 0.05	p> 0.05	p> 0.05	p> 0.05	p> 0.05	p> 0.05	p> 0.05	p> 0.05
Control	11.84 ± 0.42	35.12 ± 1.74	76.51 ± 2.53	24.6 ± 1.34	32.94 ± 0.53	4.86 ± 0.32	14.32 ± 1.24	0.28 ± 0.14
P**	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p> 0.05
P***	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p< 0.05	p> 0.05

Hb: Haemoglobin, Htc: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RBC: Red blood cell count, RDW: Red cell distribution width.

Values given as mean ± standard deviation.

* Group 1 versus Group 2

** Group 1 versus control group.

*** Group 2 versus control group.

Table 2. Laboratory parameters of patients and controls

	Ferritin (ng/mL)	SI (mg/dL)	SIBC (µg/dL)	ESOD (U/g Hb)	MDA (nmol)	Vitamin E (mg/dL)
Group 1	7.47 ± 4.06	51 ± 11.8	41 ± 91.23	1494.01 ± 29.44	2.08 ± 0.71	0.54 ± 0.24
Group 2	7.05 ± 3.3	60.5 ± 9.85	361.1 ± 23.32	1829.31 ± 571.8	2.24 ± 0.58	0.57 ± 0.21
Control	36.03 ± 5.9	68.8 ± 5.08	305.9 ± 14.04	1632.53 ± 517.1	2.24 ± 0.7	0.74 ± 0.12
P*	**p< 0.05**	**p< 0.05**	**p< 0.05**	p> 0.05	p> 0.05	**p< 0.05**
P**	**p< 0.05**	**p< 0.05**	**p< 0.05**	p> 0.05	p> 0.05	**p< 0.05**

SI: Serum iron, SIBC: Serum iron binding capacity, ESOD: Erythrocyte superoxide dismutase, MDA: Malonyldialdehyde.

Values given as mean ± standard deviation.

* Group 1 versus control group.

** Group 2 versus control group.

Laboratory Evaluations at Treatment Phases

Hemoglobin and MCV values and the reticulocyte count from IDA groups during the treatment phase are shown in Table 3. Evaluation of each of the IDA groups during the first week of treatment revealed that the reticulocyte count values were lower in the vitamin E-treated group in comparison to the other patient group. This finding was statistically significant (p< 0.05).

During the first month of treatment, the erythrocyte indices were found similar between the two IDA groups.

At the end of treatment, the MCV value in the vitamin E-treated group was similar to that in the control group. At that time, however, the MCV value in the vitamin E-treated group was found higher compared to the value in the group treated with iron only. This difference between the patient groups was also found statistically significant (p< 0.05).

Table 4 shows the serum ferritin and vitamin E levels of the iron deficiency groups. The serum ferritin and vitamin E levels were not different at the basal and third-month evaluations.

As shown in Table 5 and Figure 1, the plasma MDA values during the treatment phase were significantly higher in the group treated only with iron compared to the group treated with both vitamin E and iron. This finding, however, revealed a statistical significance only in the third month of treatment (p< 0.05). At the first week of treatment, the ESOD levels were higher in the group treated with vitamin E than in the group treated with iron only (p< 0.05) (Figure 2).

DISCUSSION

Decreased erythrocyte survival, which is secondary to an increased susceptibility to oxidant damage was reported in IDA. There is an increased level of ESOD in this anemia, suggesting a compensatory increase in this

Table 3. Hemoglobin, mean corpuscular volume, and reticulocyte count in patients during treatment

	Before treatment			First week			First month			Third month		
	Hb (g/dL)	MCV (fl)	Ret. Count (%)	Hb (g/dL)	MCV (fl)	Ret. Count (%)	Hb (g/dL)	MCV (fl)	Ret. Count (%)	Hb (g/dL)	MCV (fl)	Ret. Count (%)
Group 1	8.44 ± 0.91	59.17 ± 6.46	0.4 ± 0.21	9.02 ± 1	62.25 ± 5.95	2.22 ± 2.1	11 ± 0.5	68.7 ± 3.12	0.32 ± 0.19	11.87 ± 0.41	73.90 ± 1.97	0.24 ± 0.08
Group 2	8.66 ± 0.56	61.41 ± 3.15	0.32 ± 0.14	9.41 ± 0.64	65.97 ± 5.82	1.58 ± 0.95	11.42 ± 0.54	69.48 ± 4.88	0.21 ± 0.07	12.09 ± 0.55	76.65 ± 1.91	0.28 ± 0.14
Control	11.84 ± 0.42	76.51 ± 2.53	0.28 ± 0.14	11.84 ± 0.42	76.51 ± 2.53	0.28 ± 0.14	11.84 ± 0.42	76.51 ± 2.53	0.28 ± 0.14	11.84 ± 0.42	76.51 ± 2.53	0.28 ± 0.14
P*	p> 0.05	p> 0.05	p> 0.05	p> 0.05	p> 0.05	p< 0.05	p> 0.05	p> 0.05	p> 0.05	p> 0.05	p< 0.01	p> 0.05
P**	p< 0.05	p< 0.05	p> 0.05	p< 0.05	p< 0.05	p< 0.05	p> 0.05	p< 0.05	p> 0.05	p> 0.05	p< 0.05	p> 0.05
P***	p< 0.05	p< 0.05	p> 0.05	p< 0.05	p< 0.05	p< 0.05	p> 0.05	p< 0.05	p> 0.05	p> 0.05	p> 0.05	p> 0.05

Hb: Haemoglobin, MCV: Mean corpuscular volume, Ret. count: Reticulocyte count.
Values given as mean ± standard deviation.
*Group 1 versus Group 2, ** Group 1 versus control group, *** Group 2 versus control group.

Table 4. Serum ferritin and serum vitamin E levels in patients

	Before treatment		Third month	
	Ferritin (ng/mL)	Vitamin E (mg/dL)	Ferritin (ng/mL)	Vitamin E (mg/dL)
Group 1	7.47 ± 4.06	0.54 ± 0.24	34.62 ± 8.67	0.67 ± 0.26
Group 2	7.05 ± 3.3	0.57 ± 0.21	39.14 ± 8.74	0.67 ± 0.16
P*	p> 0.05	p> 0.05	p> 0.05	p> 0.05

Values given as mean ± standard deviation.
* Group 1 versus Group 2.

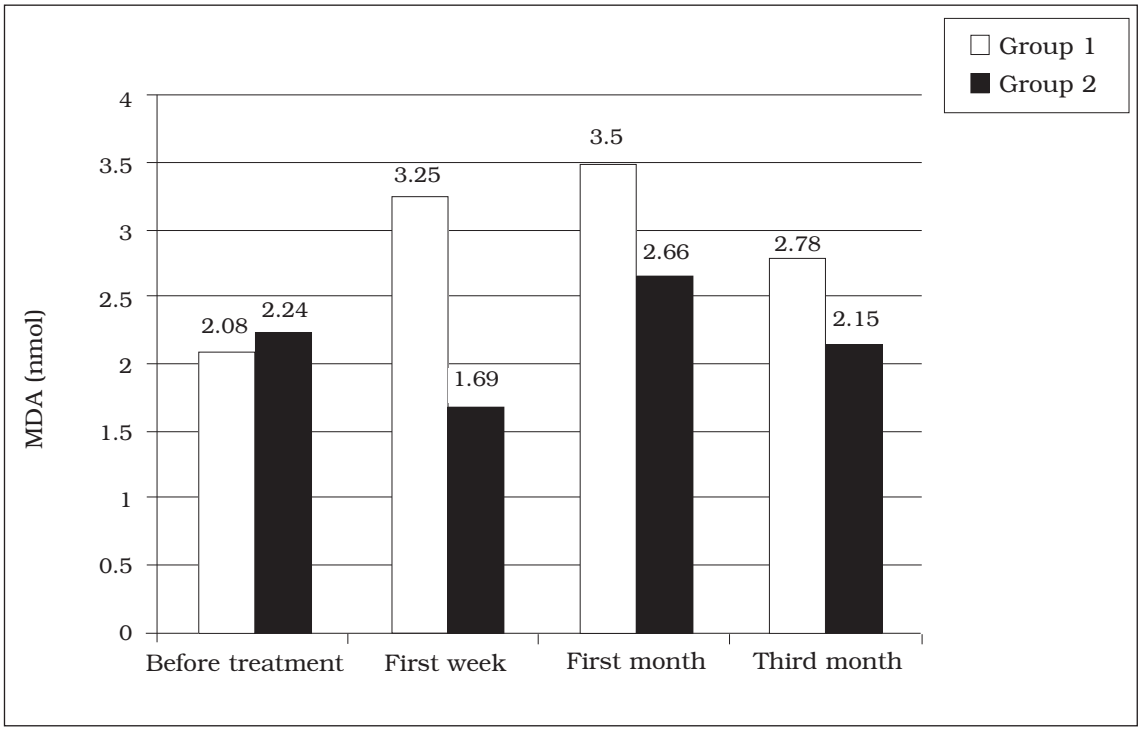

Figure 1. Plasma malonyldialdehyde levels in patients at treatment phases.

enzyme secondary to an increased oxidant stress[15]. On the contrary, several studies have indicated no increased sensitivity to peroxidative damage and no differences in antioxidant enzyme activities in IDA[16,21]. In a pediatric age group, no differences were observed in SOD and catalase activities between an IDA group and controls[22]. The present study shows that in patients with IDA, the basal levels of plasma MDA and ESOD were similar to in levels healthy children. These findings are similar to those of previous studies wherein iron-deficiency dependent oxidative damage was established.

Various biochemical changes such as reduced gastric acid secretion, impaired intestinal absorption of fat, vitamin A and xylose, and decreased serum vitamin E levels were reported in iron deficiency[23,24]. Mino et al. reported that vitamin E deficiency was seen more frequently the infants receiving cow's milk than in breast-fed infants[25]. In the

Table 5. Plasma malonyldialdehyde and erythrocyte superoxide dismutase activities in patients

	Before treatment		First week		First month		Third month	
	MDA (nmol)	ESOD (U/g Hb)	MDA (nmol)	ESOD (U/g Hb)	MDA (nmol)	ESOD (U/g Hb)	MDA (nmol)	ESOD (U/g Hb)
Group 1	2.08 ± 0.71	1494.01 ± 429.4	3.25 ± 0.91	987.47 ± 475	3.5 ± 1.54	874.74 ± 318.09	2.78 ± 0.59	1650.87 ± 735 2
Group 2	2.24 ± 0.58	1829.31 ± 571.8	1.69 ± 0.55	1829.16 ± 786.76	2.66 ± 0.93	951.78 ± 208.69	2.15 ± 0.44	1339.7 ± 381.24
P*	p> 0.05	p> 0.05	**p< 0.001**	**p< 0.05**	p> 0.05	p> 0.05	**p< 0.05**	p> 0.05

MDA: Malonyldialdehyde, ESOD: Erythrocyte superoxide dismutase.
Values given as mean ± standard deviation.
* Group 1 versus Group 2.

present study, the infants fed with cow's milk showed decreased vitamin E levels. Vitamin E deficiency can be secondary to decreased absorption of vitamin E, especially in infants fed with cow's milk.

The role of excess iron in causing intestinal oxidative stress has drawn attention to other approaches of iron supplementation. Prophylactic administration of iron along with antioxidants like vitamins E and C, or with foods rich in these vitamins is one such strategy[17]. Mejia et al. showed that vitamin A supplementation produced significant elevations in the serum levels of blood hemoglobin, hematocrit, erythrocytes, serum iron, and percent transferring saturation in children with IDA[26]. Taniguchi et al. found that in IDA, food-iron may be efficiently absorbed as iron with vitamin C[27]. Zlotkin et al. also showed that the use of ferrous sulfate drops or ferrous fumarate sprinkles plus ascorbic acid resulted in a similar rate of successful treatment of anemia without side effects[28]. In an animal study, Srigiridhar et al. showed that supplementation of α-tocopherol alone or in combination with ascorbic acid protects the gastrointestinal tract of iron-deficient rats against iron-mediated oxidative damage during iron repletion[29]. Treatment of iron deficiency with ferrous fumarate deteriorated plasma antioxidant status and increased specific clinical symptoms in patients with active Crohn disease[30]. The present study provides a treatment approach involving the use of both ferroglycine sulfate and vitamin E for treating IDA. Our findings reflect the variations in hematological parameters emerging depending on the antioxidant affect of vitamin E administered in IDA.

We determined in the present study lower reticulocyte count values in the vitamin E-treated group in comparison to the other patient group. On the other hand, any technical inadequacies arising from the aforementiored manual method might lead to relatively lower reticulocyte counts in control infants. At the end of treatment, the present

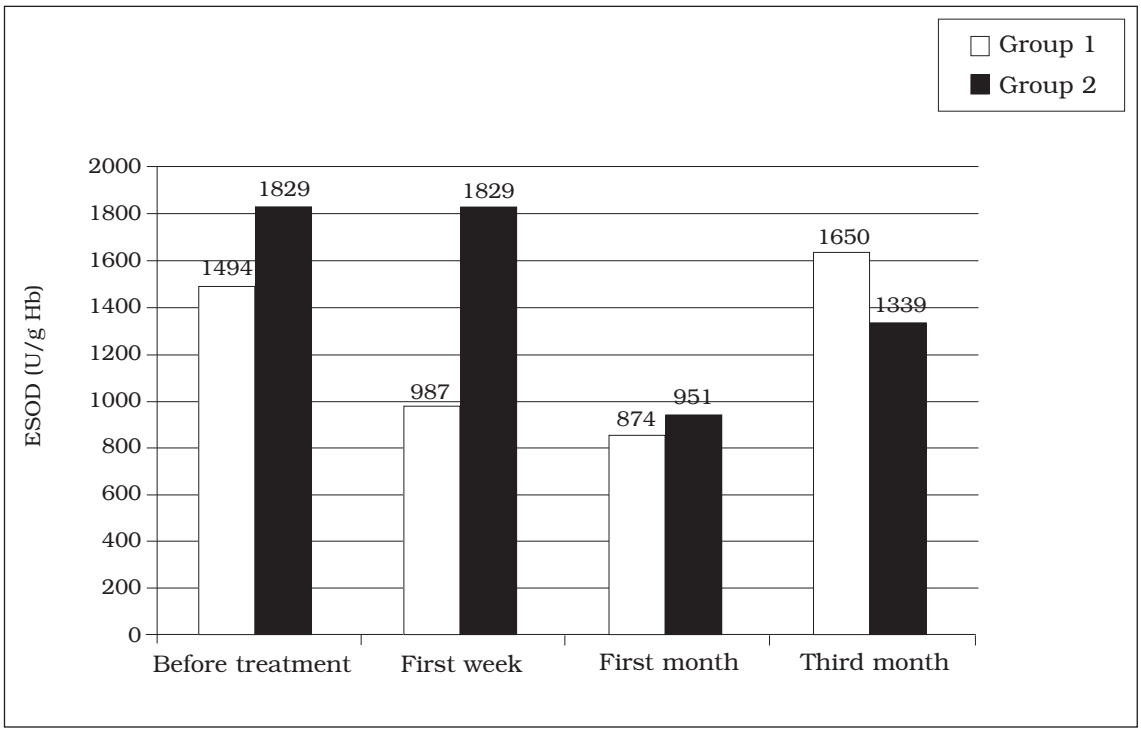

Figure 2. Erythrocyte superoxide dismutase activities in patients at treatment phases.

findings of the group treated with vitamin E revealed higher MCV values than in the group treated with iron only. Microcytosis, an indicator of erythropoetic activity in IDA treatment, is known as the last recovering parameter. In the group treated with vitamin E, our findings regarding the high MCV values and early reticulocyte crisis revealed that the erythrocyte membrane was protected against the lipid peroxidation by vitamin E.

In a previous study, Kurtoğlu et al. showed that the level of oxidative stress in IDA patients decreases after the sixth week of iron supplementation, and they suggested that oral iron supplementation is recommended for the recovery of the impaired antioxidant defense system in IDA[31]. Kavaklı et al. showed that there were minimal differences between children treated with ferric or ferrous iron in antioxidant system activities and the status of oxidizable substrates[32]. The present study was conducted to investigate the oxidant-antioxidant status in IDA and in the treatment thereof. Our findings show that increased MDA levels coexist both with impaired ESOD and vitamin E activity at the first month. These results can be explained with several mechanisms. The most likely explanation is the direct oxidant damage caused by iron. Accordingly, the patients might not be protected from the oxidant effect of iron, as a result of having vitamin E deficiency. On the other hand, it is known that iron supplement represents lower ESOD activities. Since iron, copper, and zinc have similar physicochemical properties, interactions can occur among them, which can adversely affect the metabolism and absorption of these metals. Similarly, these interactions explain the decreased ESOD levels in the IDA treatment[33,34]. The decreased MDA levels observed during treatment may be explained with the preventive effect of vitamin E against increased oxidant damage caused by iron. Those factors except vitamin E may

possibly be involved in the decrease in MDA. Increased vitamin E levels at the end of treatment, which were observed in all patients with IDA, may be related to an improvement in nutrition subsequent to the commencement of anemia treatment and to the recovery of absorption irregularities in the intestinal mucosa.

In conclusion, an increase in lipid peroxidation may be observed in IDA treatment, similar to iron overload such as thalassemias. An important effect of vitamin E supplementation is the significant decreases in MDA levels. This yields a preventive mechanism of vitamin E against the oxidative effect of iron. The hematological significances of these results are both the rapid recovery of microcytosis and the earlier formation of reticulocyte responses. Therefore, it has been concluded that vitamin E supplementation to iron treatment provides earlier recovery in microcytosis at the end of treatment, without any effect on hemoglobin values in children with IDA, especially those fed with cow's milk. It is recommended that comparative studies be conducted on this subject, especially for determining the dosage and duration of vitamin E supplementation to iron treatment in IDA.

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