The Effect of Iron Deficiency Anemia and Different Treatment Methods on DNA Damage: 8-hydroxy-2-deoxyguanosine Level

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Abstract

Ionic iron causes damages at the cellular level by forming free radicals. Reactive oxygen species lead to the formation of oxidative base damages in DNA. Among these forms the most common one and the one which has the best known mutagenity is 8-hydroxy-2′-deoxyguanosine (8-OHdG). We aimed to determine iron deficiency anemia (IDA) and its different forms of treatments; probable oxidative damage on DNA by looking at the level of 8-OHdG. The patients were divided into 4 subgroups: Oral treatment (p.o.) group; Intramuscular treatment (i.m.) group; Intravenous treatment (i.v.) group; Healthy control group. Blood and urine samples were taken from all patients totally 4 times. 8-OHdG levels detected in blood and urine samples were compared with the control group. IDA and the treatment of it affect the level of 8-OHdG. p.o. therapy should be the top priority on children.

Introduction

Iron deficiency anemia (IDA) is a frequently encountered health problem all over the world.[1] It is associated with mortality and hospitalization. It is accepted that reactive oxygen species (ROS) production is altered during IDA.[2,3] Iron deficiency also affects the production of iron-containing proteins such as cytochrome, myoglobin, catalase (CAT), and peroxidase. In IDA, antioxidant defense system deteriorates, cellular immunity, and myeloperoxidase activities decrease.[4,5] It has been stated that IDA increases oxidative stress and its treatment increases antioxidant capacity.[6,7] Iron is a transition-metal ion, and it can introduce free radical formation, which leads to formation of various lesions in DNA, proteins, and lipids.[4] Therefore, the treatment of IDA is very important.

In high concentrations, ROS can damage cellular structures such as nucleic acid, lipid, and protein. Hydroxyl radicals damage all components of DNA molecules; both purine and pyrimidine bases and even deoxyribose structure. Phospholipid residues of polyunsaturated fatty acids are very sensitive to oxidation. Once peroxidation occurs, they can spread and convert 100s of fatty acids into lipid hydroperoxides. Thus the loss of fatty acids causes membrane damage. Since they are diffusable, they can also react with the nitrogen bonds of DNA. Because of these properties, they are mutagenic, genotoxic and carcinogenic. The oxidative damage to DNA are an important factor in carcinogenesis. Despite extensive repair oxidatively modified DNA is abundant in human tissues, in particular in tumors, that is, in terms of 1 to 200 modified nucleosides per 10^5 intact nucleosides. The damaged nucleosides accumulate with age in both nuclear and mitochondrial DNA. The products of repair of these lesions are excreted into the urine in amounts corresponding

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Table 1. Demographic Characteristics of the Cases.

	IDA Group	Group I (p.o.)	Group II (i.m.)	Group III (i.v.)	Group IV (control)
Age (mean $\pm$ SD)	10.6 $\pm$ 5.62	10 $\pm$ 5.76	10.5 $\pm$ 4.60	11.4 $\pm$ 5.45	9.7 $\pm$ 4.93
(min-max.)	(2-17)	(2-17)	(3-17)	(2-17)	(2-17)
Gender n (%)					
Male	31 (51.6)	10 (50)	10 (50)	11 (55)	11 (55)
Female	29 (48.4)	10 (50)	10 (50)	9 (45)	9 (45)

Abbreviations: n, number of patients; mean, arithmetic mean; SD, Standard deviation.

to a damage rate of up to 10^4 modifications in each cell every day. The most abundant of these lesions, 8-hydroxy-2'-deoxyguanosine (8-OHdG), is also the most mutagenic. These biomarker reflect the rate of damage and the balance between the damage and repair rate, respectively. 8-OHdG is an important biomarker for the evaluation of oxidative DNA damage.[8,9]

Nearly 100 oxidative DNA base damage have been identified. The most commonly encountered and well known mutagenicity is associated with 8-OHdG. The 8-OHdG is a mutagen formed in DNA by endogenous or exogenous ROS produced during normal oxidative metabolism. Guanine is the base that has the lowest ionization potential among DNA components and is most susceptible to oxidation.[5,10,11]

In our study, the oxidative DNA damage that may be caused by IDA and its different forms of treatment (oral [p.o.], intramusculer [i.m.], intravenous [i.v.]) was tried to be determined by examining the level of 8-OHdG. In this way, it was aimed to determine the effects of IDA itself and its different treatment modalities in repeated doses on human body.

Methods

Participants and Design

The total number of patients studied was 80 and 60 of them had IDA and the rest were healthy control group (2-17 years). The control group consisted of children who applied to the outpatient clinic of pediatrics for any reason (for vaccination, and control etc.) (Table 1). Patients with chronic or past infection, who were diagnosed with parasitosis and whose infection treatment had not been completed yet, those who had a history of allergy or developed an allergic reaction a new to iron therapy, individuals who used any iron preparations or vitamins before, and/or at the time of diagnosis were excluded from the study.

Parenteral iron therapy was administered to patients with iron deficiency who could not receive oral iron therapy or during preparation for the operation. Candidates for parenteral iron therapy include socially incompatible, mentally retarded individuals, non-compliant children, patients with malabsorption, intolerance to oral iron therapy despite dosage adjustments, underlying chronic inflammatory bowel diseases, severe iron deficiency, chronic uncontrolled bleeding, acute diarrhea, and whose iron absorption is impaired because of an gastrointestinal disorder.[12-14]

The patients were divided into 4 subgroups: (First group: Oral treatment [p.o.] group with 20 patients); Second group: Intramuscular treatment [i.m.] group with 20 patients); Third group: Intravenous treatment [i.v.] group with 20 patients); Fourth group: Healthy control [h.c.] group 20 patients). Blood and urine samples were taken from all patients totally 4 times; just before the treatment, at the 24th hour of treatment, at the first week of treatment and at the third month of the treatment.

The study protocol has been approved (TF.1258) by the research institute's committee on human research. Written consent was obtained from the parents of the patients.

Procedures

Iron deficiency was considered in patients with low serum ferritin (<12 ng/ml) levels, and those with low hemoglobin (Hb) levels based on their age, and decreased serum iron content whose serum transferrin saturations and ferritin concentrations were $\leq 16\%$ and <12 ng/ml, respectively. Oral iron therapy was given as 2 to 4 divided daily doses of 4 to 6 mg/kg, ferrous sulfate (Ferro-Sanol® Adeka) on an empty stomach for 3 months.[9] As parenteral iron therapy i.v. ferrous iron sucrose (Venofer® ampoule, Abdi İbrahim), or i.m. ferrous hydroxide polymaltose (Ferrum Haussman® ampoule, Abdi İbrahim) was used. Parenteral (i.m., i.v.) doses of iron therapy were calculated with the following formula according to the Hb level and weight at the time of diagnosis.[14,15]

$$\text{Total iron dose (mg)} = (\text{targetHb - actual Hb}\,[\text{g}/\text{dL}]$$
$$\text{x80 mlxbody weight (kg)x0.034}$$

$$\text{Total dose of IV iron (mg)}$$
$$= \text{Total iron dose} + 20\%(\text{total iron dose})$$

Diphenhydramine and acetaminophen were given 30 minutes before i.v. iron treatment. According to the Hb level, 20 mg/ml iron in 75 to 100 ml 0.9% NaCl was given as an inttravenous infusion for longer than 2 hours. Infusion rate was adjusted slowly within the first 10 minutes.[10] Infusions were completed within an average of 5 days. On the other hand, i.m. iron treatment was administered as injections into alternate buttocks for an average of 4 days.

Blood and urine samples were obtained from all patients immediately before, and at 24 hours, 1 week, and 3 months of the treatment.[6,14,16,17] Blood samples drawn to determine complete blood counts, peripheral smear, reticulocyte counts, serum iron, iron binding capacity, and ferritin (F) levels were pipetted into EDTA containing tubes. The samples were properly stored at $-20°C$ until the time of analysis. All serum and urine samples were examined for 8-OHdG levels and compared with those of the control group.

Data analysis. The 8-OHdG levels in urine and blood samples were measured using an EIA kit (catalog no: 589320; Cayman Chemical Campany, Ann Arbor, USA), and all samples were diluted to 1:50 as stated in the manufacturer's catalog. The minimum detection limit of the kit was 33 pg/ml. The intra-assay CV values (coefficient of variation) were indicated as 4.7% and 11.6%, when the inter-assay CV values were 4.5%, and 10.7%, respectively. Values were given by converting pg/ml to ng/ml.[18,19]

Statistical analyses. Statistical analysis was evaluated using SPSS 16.0 program. Data were given as mean $\pm$ SD. One-way analysis of variance (ANOVA) and post-ANOVA tests, Tukey-B and Scheffe tests were used for comparison between groups. Values of $P < .05$ were considered statistically significant.

Scientific Research Project Unit of our university (University of Firat Faculty of Medicine) provided financial support (Approval: TF.1258) for our study. Written consent was obtained from the parents of the patients.

Results

Demographic data of the cases and formation of 8-OHdG are shown in Table 1 and Figure 1. Hb (Table 2, Figure 2), serum iron (Table 3, Figure 3), ferritin (Table 4, Figure 4) serum (Table 5, Figure 5), and urine 8-OHdG (Table 6, Figure 5) levels are shown in indicated tables and figures. Serum (Table 7, Figure 5) and urine (Table 8, Figure 5) 8-OHdG levels in the groups with total iron deficiency anemia (oral, i.m., i.v. treatment
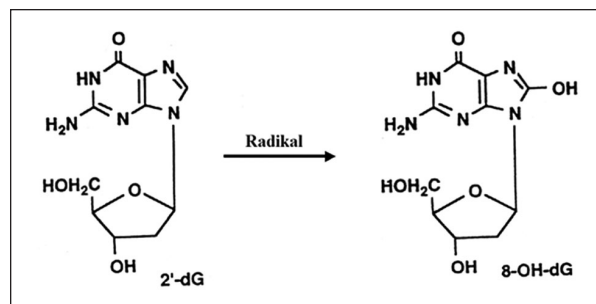
Figure 1. Formation of 8-Hidroxy-2'-deoxyguanosine.

groups) and the control groups are shown in indicated tables and figures.

The level of 8-OHdG in both serum and urine reached its highest value at the 24th hour of treatment (Tables 7 and 8). Serum 8-OHdG level was not different from healthy ones at diagnosis. In the i.v. treatment group, higher values were obtained from both treatment groups after the 24th hour. At third month, a lower than normal value was found in the p.o. treatment group (Table 5, Figure 5). In IDA, serum 8-OHdG level (Table 7) and urine 8-OHdG metabolic product; shows that oxidative stress is increased (Table 8).

Urine 8-OHdG level was higher at the 24th hour in the i.v. treatment group. But after that, urine 8-OHdG level was at the same in all treatment groups (Table 6, Figure 5). Oxidative system tries to turn the iron, which rises rapidly, into a more harmless state, especially in the 24th hour, in the i.v. treatment group. Then, oxidative metabolism is affected at the same level in all treatment groups.

Discussion

There is controversy about the susceptibility to evoked oxidative stress of cells in IDA. Therefore the potential oxidative damage generated by the participation of Fe in redox reactions might be due to improper Fe compartmentalization and trafficking during IDA rather than its total accumulation in the body.[2,3]

Nearly 100 oxidative DNA base damage have been identified. The most commonly encountered and well known mutagenicity is associated with 8-OHdG. The 8-OHdG is a mutagen formed in DNA by endogenous or exogenous ROS produced during normal oxidative metabolism. Guanine is the base that has the lowest ionization potential among DNA components and is most susceptible to oxidation.[5,10,11] Recently, an elevation of blood 8-OHdG was observed in neurodegenerative diseases.[20] The treatments for IDA we administer to children should not cause these serious risks.

Table 2. Hb Values Before, and At the End of 24 Hour, 1 Week, and 3 Months of Treatment.

Groups	Pretreatment (n=20) (g/dL, mean ± SD) (min-max)	24 hour (n=20) (g/dL, mean ± SD) (min-max)	1 week (n=20) (g/dL, mean ± SD) (min-max)	3 months (n=20) (g/dL, mean ± SD) (min-max)	*P
Group I (p.o.)	(1) 10.7 ± 0.90 (9.4-12)	(5) 11.5 ± 1.16 (9.5-14.5)	(8) 11.2 ± 1.10 (9-13)	(11) 12.5 ± 0.99 (11-14.9)	**1-11 P<.05** **5-11 P<.05** **8-11 P<.05**
Group II (i.m.)	(2) 10.6 ± 2.13 (5.5-12.5)	(6) 10.8 ± 2.19 (5.2-14.0)	(9) 11.6 ± 1.87 (8-14)	(12) 12.7 ± 0.97 (10.2-14.2)	**2-12 P<.05** **6-12 P<.05**
Group III (i.v.)	(3) 9.4 ± 2.2 (5.4-12.4)	(7) 9.9 ± 2.46 (4.3-14.4)	(10) 10.1 ± 1.70 (7-14)	(13) 12.7 ± 1.08 (11.0-14.9)	**3-13 P<.05** **7-13 P<.05** **10-13 P<.05**
Group IV (control)	(4) 13.1 ± 0.87 (11.7-15)				
**P	1-4 P<.05 2-4 P<.05 3-4 P<.05	4-7 P<.05 5-7 P<.05 4-5 P<.05 4-6 P<.05	4-8 P<.05 4-9 P<.05 4-10 P<.05 9-10 P<.05	11-4 NS 12-4 NS 13-4 NS	

*P: Statistically significant difference according to days of treatment.
**P: Statistically significant difference between different treatment groups.
NS: Any statistically significant difference does not exist.
The bold text in Table 2 indicates $p<0.05$. It shows statistical significance.
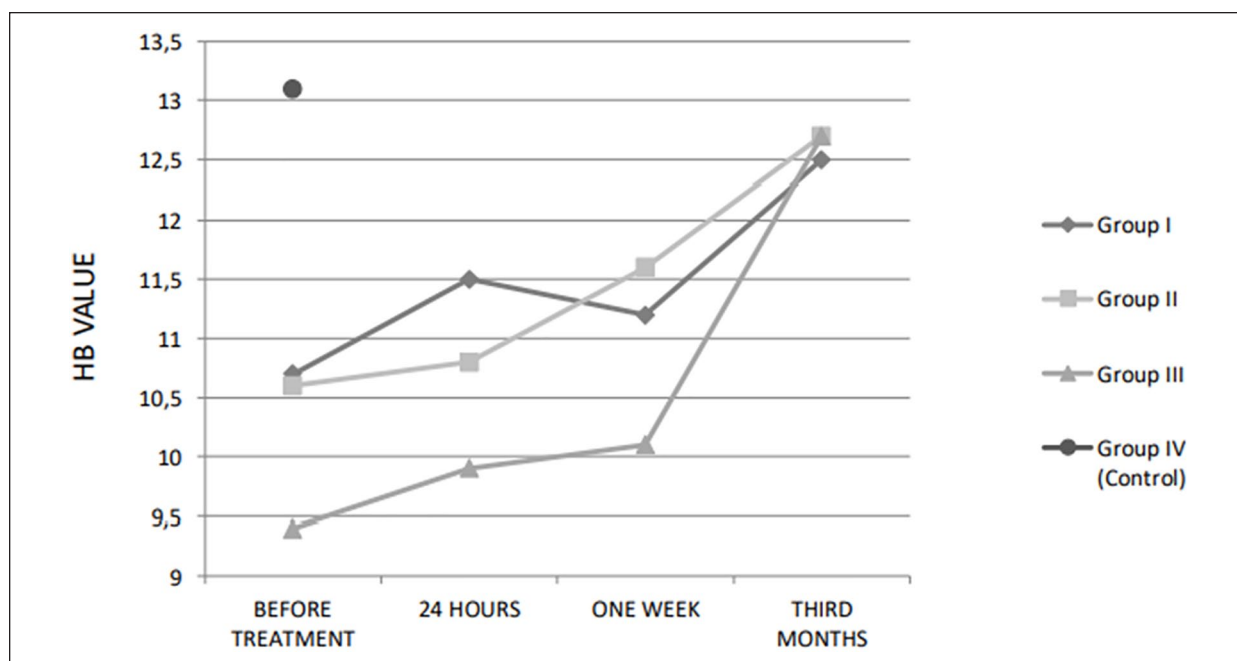
Figure 2. Hb values before, and at the end of 24 hour, 1 week, and 3 months of treatment.

Free radicals can contribute to the physiological reactions of the cell at low or moderate concentrations. Many reactions that result in free radical formation protect the cell against oxidative stress while maintaining the redox balance of the cells. These dual features are well defined. For example, while cancer cells function as secondary intracellular messengers that maintain oncogenic phenotypes, they can also exert an antitumor

Table 3. Serum iron Values Before, and At the End of 24 Hour, 1 Week, and 3 Months of Treatment.

Groups	Pretreatment (n = 20) (μg/dL mean ± SD) (min-max)	24 hour (n = 20) (μg/dL mean ± SD) (min-max)	1 week (n = 20) (μg/dL mean ± SD) (min-max)	3 months (n = 20) (μg/dL mean ± SD) (min-max)	*P
Group I (p.o.)	**(1)** 35.6 ± 19.6 (9.0-50)	**(5)** 112 ± 81,76 (12-182)	**(8)** 70.5 ± 53.65 (11-195)	**(11)** 68.8 ± 18.93 (25-125)	**1-5 P<.05** **5-8 P<.05** **5-11 P<0.05**
Group II (i.m.)	**(2)** 38.4 ± 23.53 (9.0-53.9)	**(6)** 98 ± 91.53 (19-377)	**(9)** 57.9 ± 22.12 (20-95)	**(12)** 59.3 ± 20.43 (28-124)	**2-6 P<.05**
Group III (i.v.)	**(3)** 25.3 ± 27.11 (10-58.8)	**(7)** 186 ± 137.3 (13-401)	**(10)** 79.8 ± 88.10 (11-420)	**(13)** 62.9 ± 14.43 (26-85)	**3-7 P<.05** **7-10 P<.05** **7-13 P<.05**
Group IV (control)	**(4)** 71 ± 26.32 (34-153)				
**P	**1-4 P<.05** **2-4 P<.05** **3-4 P<.05**	**4-7 P<.05** **6-7 P<.05**	8-4 NS 9-4 NS 10-4 NS	11-4 NS 12-4 NS 13-4 NS	

*P: Statistically significant difference according to days of treatment.
**P: Statistically significant difference between different treatment groups.
NS: Any statistically significant difference does not exist.
The bold text in Table 3 indicates p<0.05. It shows statistical significance.
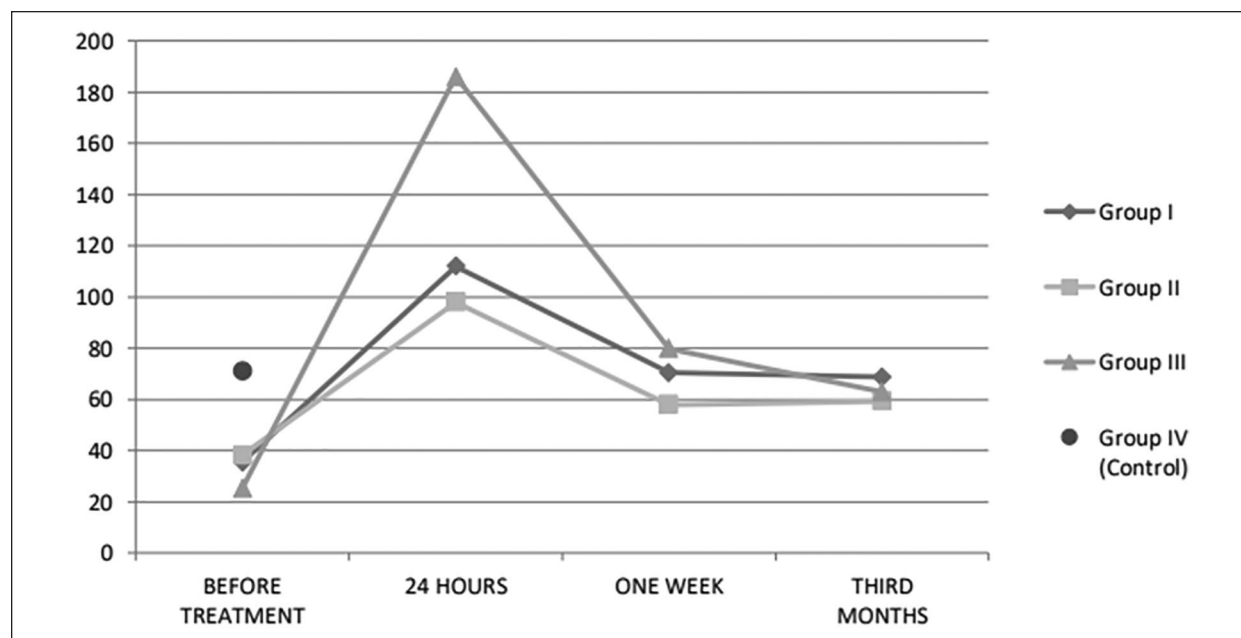
Figure 3. Serum iron values before, and at the end of 24 hour, 1 week, and 3 months of treatment.

effect by stimulating cellular apoptosis. Therefore, the increase in oxidant molecules which can rise up to a certain level, is again balanced by natural antioxidants that are always present at a certain level in the body.[21]

Iron unbound to protein increases the formation of free oxygen radicals. It is suggested that DNA in every

cell of the human body is exposed to oxidative damage 10^3 times a day. Thanks to the balance between DNA damage and repair, very low levels of damage are also detected in healthy individuals.[22]

The first lesion that occurs with oxidative damage in DNA is DNA strand break. It does not always show

**Table 4.** Serum Ferritin Values Before, and At the End of 24 Hour, 1 Week, and 3 Months of Treatment.

Groups	Pretreatment (n = 20) (ng/ml, mean ± SD) (min-max)	24 hour (n = 20) (ng/ml, mean ± SD) (min-max)	1 week (n = 20) (ng/ml, mean ± SD) (min-max)	2 months (n = 20) (ng/ml, mean ± SD) (min-max)	*P
Group I (p.o.)	**(1)** 8.7 ± 5.52 (0.5-18)	**(5)** 40.9 ± 25 (1.7-242)	**(8)** 35.8 ± 19.11 (11.3-236)	**(11)** 30.7 ± 13.77 (4.2-59)	**1-5 P < .05** **1-8 P < .05** **1-11 P < .05**
Group II (i.m.)	**(2)** 7.7 ± 5.92 (1-67)	**(6)** 65.6 ± 70 (2.5-290)	**(9)** 91.3 ± 74.21 (15.4-328)	**(12)** 28.1 ± 17.02 (10-120)	**2-6 P < .05** **2-9 P < .05** **9-12 P < .05**
Group III (i.v.)	**(3)** 7.6 ± 15.23 (0-85)	**(7)** 78.0 ± 68 (0-264)	**(10)** 140 ± 98.40 (14.3-409)	**(13)** 44.2 ± 44.01 (2.4-167)	**3-7 P < .05** **3-10 P < .05** **7-10 P < .05**
Group IV (control)	**(4)** 24.7 ± 16.8 (10-78)				**10-13 P < .05**
**P	**1-4 P < .05** **2-4 P < .05** **3-4 P < .05**	5-4 NS 6-4 NS 7-4 NS	**4-9 P < .05** **4-10 P < .05** **8-10 P < .05**	11-4 NS 12-4 NS 13-4 NS	

*P: Statistically significant difference according to days of treatment.
**P: Statistically significant difference between different treatment groups.
NS: Any statistically significant difference does not exist.
The bold text in Table 4 indicates $p < 0.05$. It shows statistical significance.
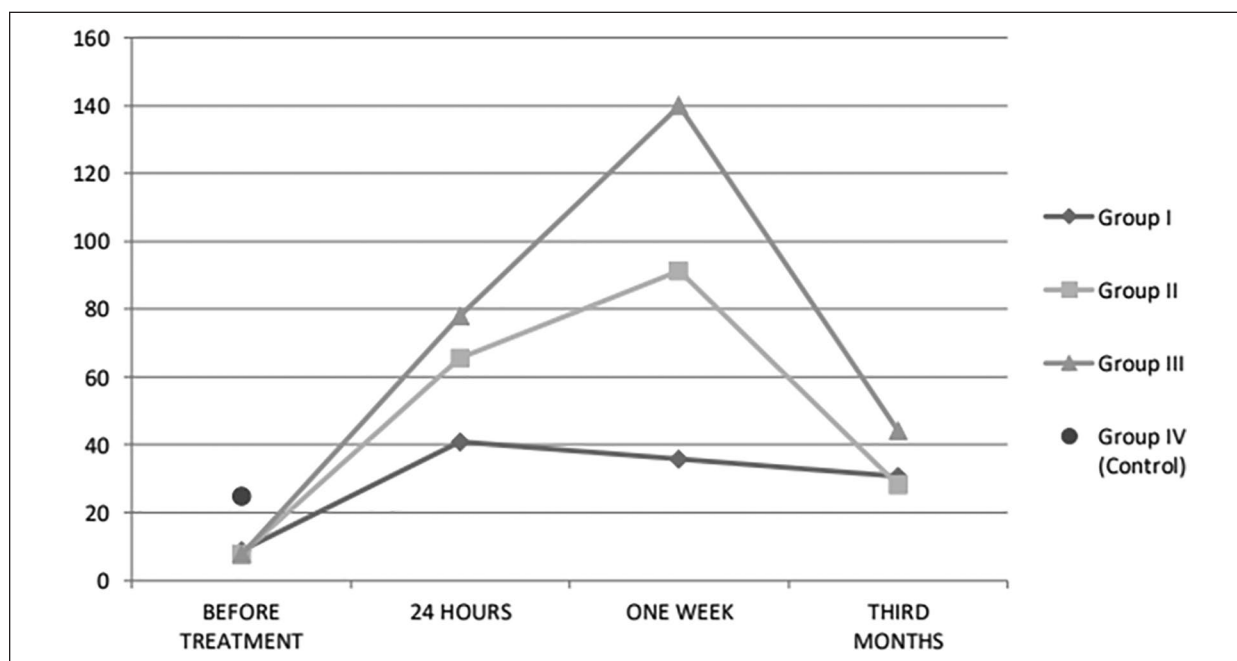
Figure 4. Serum ferritin values at the end of 24 hour, 1 week, and 3 months of treatment.

oxidative DNA damage. It has been shown that oxidative DNA base damage aggravates with increasing Fe and Cu ion concentrations and the use of copper and/or iron chelators in cells exposed to H_2O_2 prevents oxidative damage in DNA.[23] Improvement at 24 hours of oral iron therapy indicates that the physiological doses of iron prevent oxidative damage of IDA (Table 5, Figure 5). Nutrition is associated with oxidative metabolism, which besides production of energy is involved in a number of vital functions of the host. Fermented goat milk consumption

Table 5. Serum 8-OHdG Values Levels Before, and **At the End of 24 Hour**, 1 Week, and 3 Months of Treatment.

Groups	Pretreatment (n = 20) (ng/ml, mean ± SD) (min-max)	24. hour (n = 20) (ng/ml, mean ± SD) (min-max)	1.week (n = 20) (ng/ml, mean ± SD) (min-max)	3. months (n = 20) (ng/ml, mean ± SD) (min-max)	*P
Group I (p.o)	**(1)** 10.8 ± 6.12 (4.0-29)	**(5)** 10.8 ± 8.08 (2.9-37.9)	**(8)** 5.5 ± 1.36 (3-8.2)	**(11)** 5.6 ± 2.34 (3.1-12)	**1-8 $P < .05$** **1-11 $P < .05$** **5-8 $P < .05$** **5-11 $P < 0.05$**
Group II (i.m.)	**(2)** 12.0 ± 7.01 (3.6-29)	**(6)** 7.2 ± 2.43 (3.9-12.9)	**(9)** 5.66 ± 1.26 (4.1-8.6)	**(12)** 8.3 ± 3.22 (5.5-16)	**2-6 $P < .05$** **2-9 $P < .05$** **2-12 $P < .05$**
Group III (i.v.)	**(3)** 12.7 ± 10.23 (4.0-41)	**(7)** 18.3 ± 10.50 (4.6-38.0)	**(10)** 12.7 ± 9.30 (3.1-36)	**(13)** 10.6 ± 4.70 (4.8-20)	**7-13 $P < .05$** **3-13 $P < .05$** **10-13 $P < .05$**
Group IV (control)	**(4)** 8.8 ± 4.5 (5.3-13.05)				**3-7 $P < .05$**
**P	NS	**5-7 P < .05** **6-7 P < .05** **4-7 P < .05**	**9-10 P < .05** **4-10 P = .05** **8-10 P < .05**	4-11 $P < .05$ 11-13 $P < .05$	

*P: Statistically significant difference according to days of treatment.
**P: Statistically significant difference between different treatment groups.
NS: Any statistically significant difference does not exist.
The bold text in Table 5 indicates $p < 0.05$. It shows statistical significance.
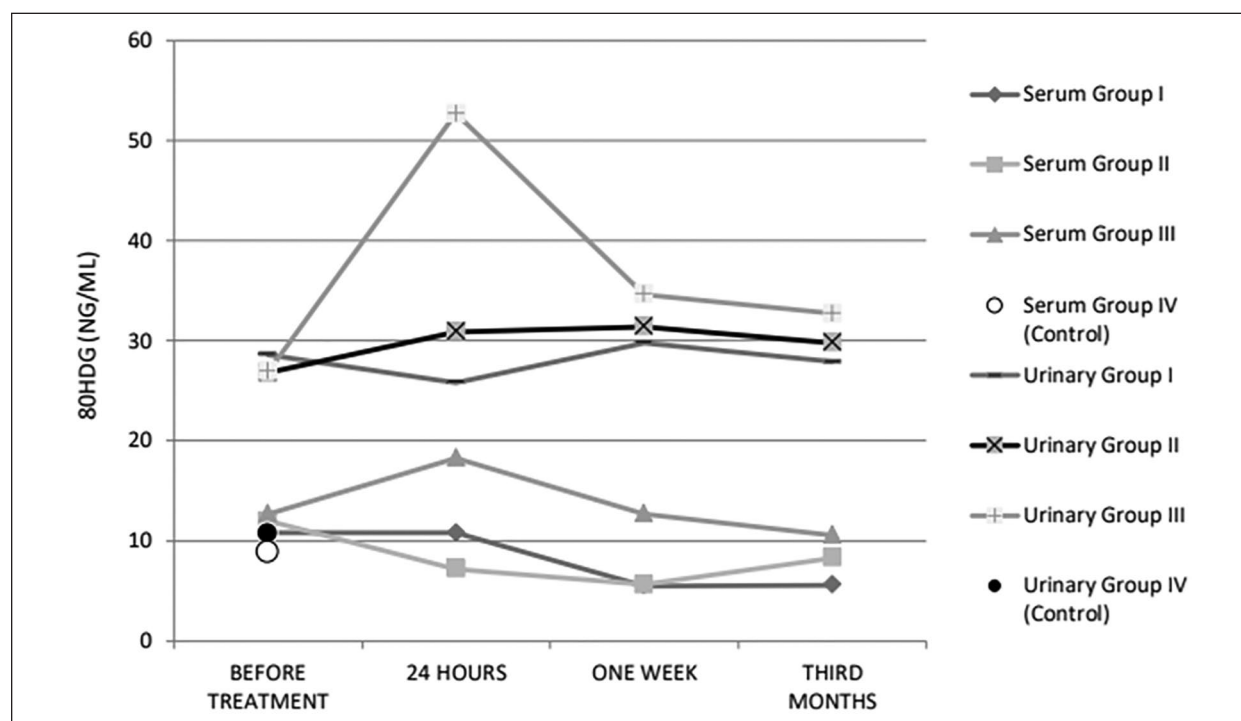
Figure 5. Serum, and urine 8-OHdG values before, and at the end of 24 hour, 1 week, and 3 months of treatment.

induces a protective increase in total antioxidant status together with lower oxidative damage biomarkers, revealing that the milk protects main cell bioconstituents from evoked oxidative damage during anemia recovery.[2]

The 8-OHdG is formed as a result of attacks of hydroxyl radical at the eighth carbon atom of the guanine.[5,10,11] Although the cause of increased urinary 8-OHdG levels in cancer patients is still unclear, this

Table 6. Urinary 8-OHdG Values Before, and At the End of 24 Hour, 1 Week, and 3 Months of Treatment.

Groups	Pretreatment (n = 20) (ng/ml, mean ± SD) (min-max)	24 hour (n = 20) (ng/ml, mean ± SD) (min-max)	1 week (n = 20) (ng/ml, mean ± SD) (min-max)	3 months (n = 20) (ng/ml, mean ± SD) (min-max)	*P
Group I (p.o.)	(1) 28.6 ± 6.60 (18.3-35.7)	(5) 25.8 ± 29.8 (1.0-129.5)	(8) 29.8 ± 2.55 (22-35)	(11) 27.9 ± 4.96 (21.2-34.6)	NS
Group II (i.m.)	(2) 26.8 ± 8.11 (13.2-36.7)	(6) 30.9 ± 24.7 (0.52-89.4)	(9) 31.4 ± 2.23 (24-35)	(12) 29.8 ± 3.58 (20.5-34.0)	NS
Group III (i.v.)	(3) 26.9 ± 11.90 (8.5-54.6)	(7) 52.7 ± 44.1 (4.0-134)	(10) 34.6 ± 12.20 (18-63)	(13) 32.7 ± 5.92 (20.9-49)	**3-7 $P < .05$** **13-7 $P < .05$**
Group IV (control)	(4) 10.7 ± 8.1 (4.8-14.80)				
**P	**4-1 $P < .05$** **4-2 $P < .05$** **4-3 $P < .05$**	**4-7 $P < .05$** **5-7 $P < .05$**	**4-8 $P < .05$** **4-9 $P < .05$** **4-10 $P < .05$**	**4-11 $P < .05$** **11-13 $P < .05$** **4-12 $P < .05$** **4-13 $P < .05$**	

*P: Statistically significant difference according to days of treatment.
**P: Statistically significant difference between different treatment groups.
NS: Any statistically significant difference does not exist.
The bold text in Table 6 indicates $p < 0.05$. It shows statistical significance.

Table 7. Serum 8-OHdG Levels of the Patient and Control Groups.

	Pretreatment (ng/ml, mean ± SD) (min-max)	24 hour (ng/ml, mean ± SD) (min-max)	1 week (ng/ml, mean ± SD) (min-max)	3 months (ng/ml, mean ± SD) (min-max)	**p
Total IDA (Groups I, II, III) n = 60	(1) 11.8 ± 9.6 (3.65-11.40)	12.2 ± 9.0 (2.95-38.0)	8.0 ± 6.3 (3-36)	8.24 ± 4.04 (3.10-20.0)	$p > .05$
Control group (Group IV) n = 20	(2) 8.8 ± 4.5 (5.35-13.05)				$p > .05$
*p	**1-2 $P < .05$**				

*P: 1-2 $P < .05$.
**P: Statistically significant difference between different treatment groups.

marker may be a good prognostic indicator in cancer patients.[13]

In our study, in cases with IDA; serum (11.8 and 8.8 ng/ml) and urinary (27.4 and 10.7 ng/ml) levels of 8-OHdG which is the indicator of oxidant damage increased relative to the control group (Tables 7 and 8). At the first week and third month of oral treatment, a decrease in blood and urine levels of 8-OHdG was observed compared to pretreatment levels (Tables 5 and 6, Figure 5). This is an indication that IDA itself causes oxidative stress which improves with oral treatment (Table 8). In a study performed to determine oxidative DNA damage in children with IDA, after 12 weeks of oral Fe therapy, DNA strand breaks increased after treatment compared to baseline. There are some important limitations to this study. We only assessed 8-OHdG levels in serum but not in tissue such as leukocyte.[4]

Total antioxidant capacity (TAOC) was found to be decreased with iron therapy. In terms of creating oxidative stress; oral, i.m., and finally i.v. iron treatments have been recommended.[7] The 8-OHdG values in our study were fully compatible with these data. Detection of lower serum 8-OHdG levels even shortly after the oral

Table 8. Urinary 8-OHdG Levels of the Patient and Control Groups.

	Pretreatment (ng/ml, mean ± SD) (min-max)	24 hour (ng/ml, mean ± SD) (min-max)	1 week (ng/ml, mean ± SD) (min-max)	3 months (ng/ml, mean ± SD) (min-max)	**p
Total IDA (Groups I, II, III) n = 60	**(1)** 27.4 ± 8.1 (4.8-33.5)	36.5 ± 35.3 (0.5-134.9)	31.97 ± 75 (18-63)	30.1 ± 5.2 (20.55-49.10)	p > .05
Control group (Group IV) n = 20	**(2)** 10.7 ± 8.1 (4.8-14.80)				p > .05

*P 1-2 **P** < .05.
***p**: Statistically significant difference between different treatment groups

and i.m. treatment indicates that DNA damage was rapidly repaired. With i.v. treatment, more oxidative stress is induced relative to IDA (Tables 5 and 6, Figure 5, Tables 7 and 8). At the 24th hour in the i.v. treatment group, the levels of 8-OHdG, which is the indicator of oxidative damage in serum and urine were found to be significantly higher compared to other treatment groups ($P < .05$) (Tables 5 and 6, Figure 5). This finding supports the relationship between increased serum iron level and oxidative stress. Oxidative stress, appears to be elevated between 24 hours and 1 week of especially i.m. and i.v. treatment. This increase occurs at the same time with the increase in serum iron (Table 3, Figure 3) and ferritin (Table 4, Figure 4). Although ferritin levels at the 24th hour of the i.v. treatment were higher than the control group, any statistically significant difference was not found. Serum iron level appears to be more effective in determining the oxidative effect of treatment. Intravenous treatment most rapidly raised the serum iron level. Serum iron shows oxidant damage earlier than increased ferritin levels. In this regard, giving lower doses at less frequent intervals in parenteral treatment will reduce oxidant stress.

Increases in oxidative stress were not seen in hemodialysis (HD) patients receiving erythropoietin and treated with iron sucrose delivered via rapid injection or slow infusion every 2 weeks.[24] In rats with chronic renal failure, oxidative stress increased, and antioxidant enzymes decreased 13 weeks after a single dose (0.5 g/kg, i.v.) iron dextran injection.[17] Intravenous administration of high doses of iron preparations has been shown to lead to an increase in biologically active iron levels, development of oxidative stress and and then increase in lipid peroxidation.[25] Repeated i.v. iron administration for HD patients was associated with signs of increased oxidative DNA injury, as reflected by increased serum levels of 8-OHdG. As these changes were accompanied by increased serum ferritin levels, excess body iron stores might play an important role in oxidative

stress.[26,27] Dose-dependent increases in 8-OHdG levels were observed before, and 1, 2, and 4 hours after iron sucrose treatment given to hemodialysis patients at i.v. doses of 200, and 500 mg.[28] In our study, higher 8-OHdG levels were found at 24th hour of i.v. iron sucrose treatment ($P < .05$). Serum 8-OHdG levels in the third months of the i.v. treatment group weren't higher than the control group (Table 5, Figure 5). However, urine 8-OHdG levels increased in all 3 treatment groups was, even in the third month of treatment ($P < .05$) (Table 6). High 8-OHdG values in urine during all treatments were interpreted as an attempt to recover oxidant damage. Urine 8-OHdG level may be more significant in determining oxidant damage.

Oxidative stress and DNA damage in the lymphocytes of IDA patients were found to be increased. It is thought that increased oxidative stress and DNA damage may play a role in the pathogenesis of IDA.[29] In our study, higher pretreatment serum and urine 8-OHdG levels in our IDA groups ($P < .05$) compared to the control group support this point of view (Tables 7 and 8). There are also publications reporting that there is no increase in lipid peroxidation in IDA.[30] This finding showed us that IDA itself causes oxidative stress (Tables 5 and 6).

The half-life of 8-OHdG in the mouse liver was determined to be 11 minutes. The half-life of 8-OHdG, which was examined using gas chromatography-mass spectroscopy, was determined as 55.2 minutes in human lymphocytes.[17]

There is a linear correlation between oxygen consumption of tissues and baseline 8-OHdG levels. The 8-OHdG level is higher in kidneys than in other organs. It has been indicated that serum 8-OHdG levels will be underestimated, because they vary dependent on the severity of damage to DNA.[2] In our study, serum 8-OHdG levels were found to be lower than its simultaneously measured urinary levels. In our study, urine 8-OHdG levels were 3 times higher than its serum levels (Tables 5 and 6, Figure 5).

As a result, IDA causes oxidative stress. Therefore, it should be treated. IDA itself and its treatment affect the 8-OHdG level. Oral therapy is the most convenient, easily applied, and effective treatment with mild and few side effects. Apart from the shortness of the treatment period, parenteral treatment is not superior over oral therapy. In terms of development of oxidative stress, p.o. route should be priorly preferred in children, followed by i.m., and i.v. routes of administration. Especially i.v. iron application should only be applied if compelling indications exist. In parenteral treatment, it will be appropriate to give lower doses at less frequent intervals.

Author Contributions

All authors contributed to the study design and revised the manuscript which was drafted by Dr. Akarsu and Dr. Esen Ağar. All authors approved the final version submitted for publication.

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