



Long-term effect of intravenous iron carboxymaltose treatment on oxidative stress in women with iron deficiency anemia

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Abstract

This study aims to clarify the effects of intravenous iron supplementation on biomarkers for oxidative stress in women with iron deficiency anemia. This is a cross-sectional review of 40 healthy women and 40 women who underwent intravenous iron treatment due to anemia. Biochemical markers for oxidative stress were determined for both healthy controls and anemic patients. These markers were also evaluated at hour 1 and day 30 of intravenous iron treatment. The patients with anemia had significantly higher catalase activity and total oxidant status (TOS) but significantly lower nitrate and total anti-oxidant status (TAS) than the healthy controls ($p=0.0245$, $p<0.0001$, $p=0.0437$ and $p<0.0001$ respectively). At hour 1 of intravenous iron treatment, nitrate, nitrite, nitric oxide, total thiol and TAS values were significantly lower and TOS values were significantly higher than those before the administration of treatment ($p=0.0322$, $p=0.0003$, $p=0.0005$, $p<0.0001$, $p<0.0001$ and $p=0.004$). At day 30 of intravenous iron treatment, catalase activity, nitrate, total thiol and TOS values were significantly lower than those before the administration of treatment ($p=0.0332$, $p=0.0015$, $p=0.0391$ and $p<0.0001$ respectively) and at hour 1 of treatment ($p=0.0498$, $p<0.0001$, $p=0.0004$ and $p<0.0001$ respectively). At day 30 of intravenous iron treatment, nitric oxide and TAS values were significantly higher than those before the administration of treatment ($p=0.0480$ and $p=0.001$ respectively) and at hour 1 of treatment ($p<0.0001$ for both). Intravenous iron replacement prompts oxidative stress at hour 1 of infusion in adults with anemia but this increase resolves partially in the following 30 days.

Keywords: Anemia, iron, oxidative stress

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Introduction

The World Health Organization defines anemia as blood hemoglobin values of less than 12g/dl in women and 13g/dl in men. Generally, complete blood count, peripheral smear, reticulocyte count and serum iron indices are included in the evaluation of the cause of anemia. The lower blood hemoglobin, iron (<70.1 µg/l), ferritin (<30ng/l), transferrin saturation (<15%), and iron-binding capacity (>13.1 µmol/l) are the basic findings for the diagnosis of iron deficiency anemia. [1–3]. Iron deficiency and related anemia are commonly encountered in patients with chronic renal disease, chronic heart failure, inflammatory bowel diseases, malignancies and abnormal uterine bleeding [1,4]. Iron deficiency anemia can also affect patients who have undergone childbirth or surgery [4,5]. Intravenous iron treatment has been developed to correct the iron deficiency and related anemia [6]. The rationale behind this treatment is that the reticuloendothelial system (RES) immediately causes absorption of iron and it would allow quick provide to the bone marrow [7]. Commercially available intravenous iron products are essentially low molecular weight iron dextran/sucrose or isomaltoside; sodium ferric gluconate or carboxymaltose formulations [8]. These drugs using for replacement therapy are colloidal solutions that contained iron-oxyhydroxide nanoparticles with a carbohydrate ligand [7,8]. Iron-carbohydrate complexes with lower stability are largely dissolved in plasma and thus, iron molecules can leak into plasma before their uptake by the RES [9,10]. It has been hypothesized that this leak of “labile” iron can directly bind to plasma proteins so that oxidative stress is induced. Animal studies have shown that non-specific uptake of circulating iron by different organs might lead to inflammation and even tissue injury [11–14].

This study aims to clarify the short term and long term effects of intravenous iron replacement on biomarkers for oxidative stress in women with iron deficiency anemia.

Materials and Methods

The present study was approved by the Ethical Committee of Mugla Sitki Kocman University Medical School Hospital where it was undertaken between January 2020 and March 2020. This study was conducted in accordance with the guidelines of Helsinki Declaration so that all participants were informed about the study design and their written consents were obtained.

Study Design

This is a cross-sectional analysis of 40 patients diagnosed with iron deficiency anemia and 40 healthy controls. All of the patients with iron deficiency anemia and healthy controls were adult females. The women with iron deficiency anemia underwent intravenous iron treatment (ferric carboxymaltose, 500 mg/10 ml) as soon as the diagnosis of anemia was made. Complete blood count, biochemical analyses and biochemical evaluation for oxidative stress were simultaneously performed for the patients with iron deficiency anemia and healthy controls. The biochemical evaluation for oxidative stress markers were reproduced at hour 1 and day 30 of intravenous iron treatment.

The patients with acute and chronic systemic diseases, the patients who have been already under treatment for iron deficiency anemia, the patients who were diagnosed with infections in the preceding four weeks, the patients who were using any drugs (including vitamin and mineral supplements), the patients with a habit of smoking and/or alcohol consumption, the patients with any dietary restriction, pregnant and breastfeeding women were excluded.

Laboratory Studies

Venous blood samples were retrieved in early morning after 8 hours of fasting. In order to avoid further oxidation, they were immediately centrifuged at 3600 g for 10 minutes after retrieval and then stored at -80 C for biochemical measurements. Complete blood count was made by means of an XN-1000 Sysmex hematology auto-analyzer and biochemical determinations were made by using Beckman Coulter Olympus AU 2700 system.

Plasma catalase activity was colorimetrically determined using an assay kit containing a stopping solution based on Jeong method [15]. The measurement of serum ferroxidase activity was based on the oxidation of o-dianizidine. Since serum ferroxidase activity was highest at pH: 5.1, serum was mixed with acetate buffer at this pH and then added to the dianizidine. As O-dianizide is oxidized by ferroxidase, alterations in color occur and the enzyme activity was measured kinetically at 460 nm [16].

Plasma nitrite/nitrate levels were measured by a modification of the procedure described by Braman and Hendrix [17] using the purge system of a Sievers Instruments Model 270B Nitric Oxide Analyzer (NOA 228; Sievers Instruments Inc., Boulder, CO, USA). Plasma

samples were diluted and deproteinized using chilled 100% ethanol (plasma/ethanol = 1:2 vol/vol), and a saturated solution of VC13 in 1 M HCl was prepared and filtered prior to use.

Total thiol level was measured by using an automatic measurement method defined by Erel and Neselioglu (Roche Hitachi Cobas c501 automatic analyzer, Roche Diagnostics, USA). In this method, dynamic disulfide bonds (-S-S-) are reduced to thiol groups (-SH) by NaBH₄ and then Elman's reagent was used to determine the amount of total thiol [18].

Total Antioxidant Status (TAC) [19] and Total Oxidant Status (TOS) [20]. Assay Kit (Rel Assay Diagnostics, TR) were used for measurements. Measurements were made by the manual method. The TAS results were offered as mmol Trolox Eq/L, however, TOS results were presented $\mu\text{mol H}_2\text{O}_2$ Eq/L for serum.

Statistical Analysis

Collected data were analyzed by Statistical Package for Social Sciences version 19.0 (SPSS Inc., SPSS IBM, Armonk, NY, USA). Continuous variables were expressed as mean \pm standard deviation whereas categorical variables were denoted as numbers of percentages. Shapiro Wilk test was used to analyze the distribution of data. Student t-test and Mann Whitney U test were used for the comparisons. Two-tailed p values less than 0.05 were accepted to be statistically significant.

Results

Table 1 compares the clinical and biochemical characteristics of the controls and anemic patients. The women with iron deficiency anemia had significantly lower hemoglobin, lower ferritin, higher platelet count, lower mean corpuscular volume, lower iron, higher erythrocyte sedimentation rate and higher C-reactive protein than the healthy controls ($p < 0.05$ for each).

Figure 1 shows that the patients with anemia had significantly higher plasma catalase activity than the healthy controls ($p = 0.0245$). Plasma catalase activity at day 30 of intravenous iron treatment was significantly lower than the plasma catalase activity before the administration of treatment ($p = 0.0332$) and at hour 1 of treatment ($p = 0.0498$). When compared to the hour 1 of intravenous iron treatment, serum ferroxidase activity was significantly lowered at day 30 of the treatment ($p = 0.0049$) (Figure 2).

Figure 3 demonstrates that the patients with anemia had significantly lower concentrations of plasma nitrate than the healthy controls ($p = 0.0437$). Plasma

nitrate concentrations at hour 1 of intravenous iron treatment were significantly lower than those before the administration of iron treatment ($p = 0.0322$). Plasma nitrate concentrations at day 30 of intravenous iron treatment were significantly higher than those before the administration of iron treatment ($p = 0.0015$) and at hour 1 of treatment ($p < 0.0001$).

Table 1. Clinical characteristics of the controls and anemic patients

	Control (n=40)	Anemia (n=40)
Age (years)	28.6 \pm 1.1	36.4 \pm 1.3
Hemoglobin (g/dL)	13.5 \pm 0.2	11.2 \pm 0.3*
Leukocyte count (x1000/ μL)	7.3 \pm 0.3	6.3 \pm 0.3
Platelet count (x1000/ μL)	248.3 \pm 7.8	309.9 \pm 11.7*
Mean corpuscular volume (fl)	86.8 \pm 4.6	74.7 \pm 3.9*
Glucose (mg/dL)	91.1 \pm 1.2	90.2 \pm 1.4
Urea (mg/dL)	21.6 \pm 0.8	21.8 \pm 1.3
Creatinine(mg/dL)	0.60 \pm 0.01	0.58 \pm 0.02
Albumin (g/dL)	4.6 \pm 0.10	4.57 \pm 0.04
Alanine transaminase (U/L)	15.3 \pm 1.4	14.2 \pm 1.4
Aspartate transaminase (U/L)	15.4 \pm 0.6	15.9 \pm 0.8
Lactate dehydrogenase (U/L)	168.6 \pm 3.6	163.7 \pm 5.7
Iron ($\mu\text{g/dL}$)	85.3 \pm 5.6	61.4 \pm 5.6*
Total iron binding capacity ($\mu\text{g/dL}$)	342.3 \pm 6.5	355.3 \pm 11.7
Transferrin saturation (%)	25.5 \pm 1.7	19.3 \pm 2.1
Ferritin (ng/ml)	33.9 \pm 3.1	7.43 \pm 0.8*
Vitamin B12 (pg/ml)	346.7 \pm 22.8	345.12 \pm 22.6
Erythrocyte sedimentation rate (mm)	8.6 \pm 0.9	13.6 \pm 1.6*
C-reactive protein (mg/L)	2.0 \pm 0.4	2.81 \pm 0.7*

* $p < 0.05$ was accepted to be statistically significant.

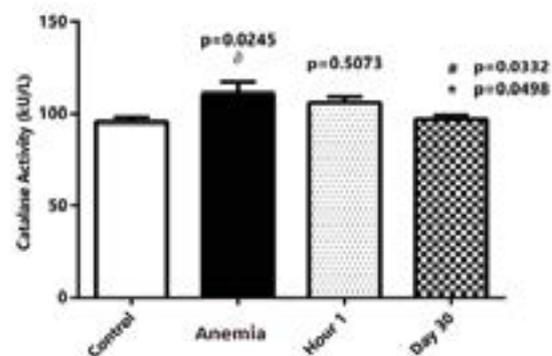


Figure 1. Catalase activity of the participants

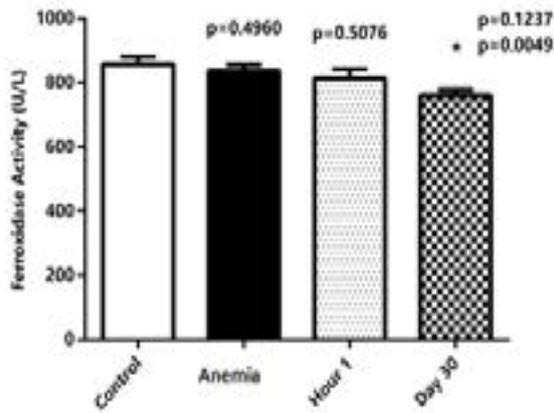


Figure 2. Ferroxidase activity of the participants

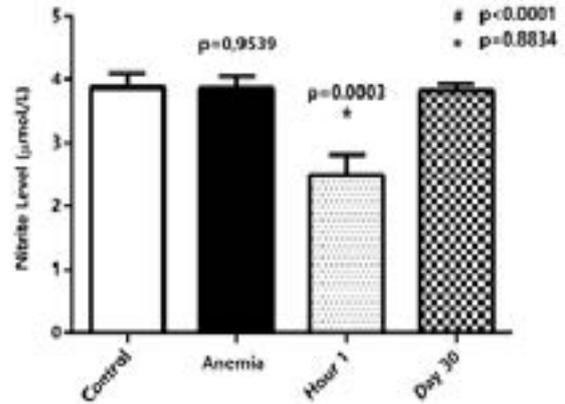


Figure 4. Nitrite levels of the participants

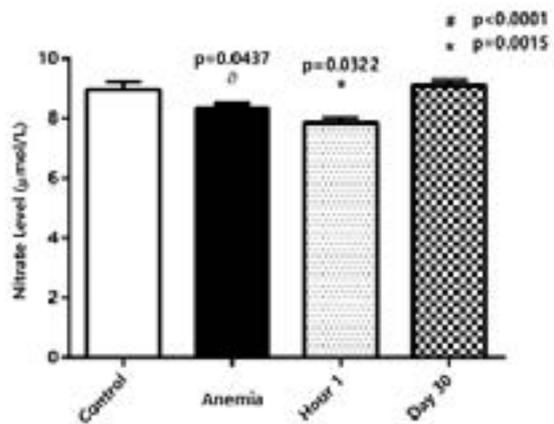


Figure 3. Nitrate levels of the participants

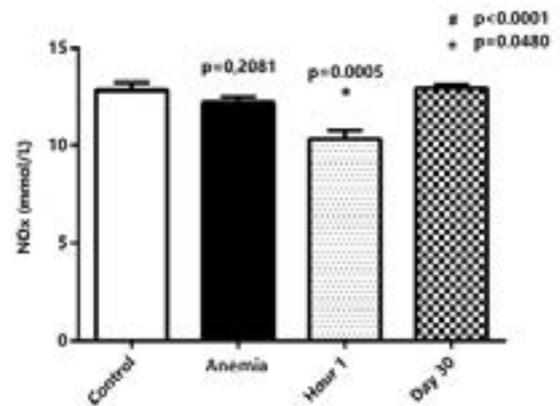


Figure 3. Nitrate levels of the participants

Figure 4 displays that plasma nitrite concentrations at hour 1 of intravenous iron treatment were significantly lower than those before the administration of iron treatment ($p=0.0003$). Plasma nitrite concentrations at day 30 of intravenous iron treatment were significantly higher than those at hour 1 of treatment ($p=0.0001$).

Plasma nitric oxide levels at hour 1 of intravenous iron treatment were significantly lower than those before the administration of treatment ($p=0.0005$). Plasma nitric oxide levels at day 30 of intravenous iron treatment were significantly higher than those before the administration of treatment ($p=0.0480$) and at hour 1 of treatment ($p<0.0001$) (Figure 5).

Serum total thiol levels at hour 1 of intravenous iron treatment were significantly lower than those before the administration of treatment ($p<0.0001$). Serum total thiol levels at day 30 of intravenous iron treatment were significantly lower than those before the administration of treatment ($p=0.0391$) and significantly higher than those at hour 1 of treatment ($p=0.0004$) (Figure 6).

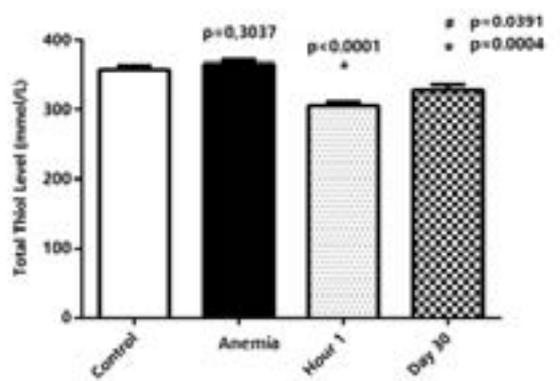


Figure 6. Total thiol levels of the participants

Figure 7 points out that the patients with anemia had significantly lower TAS than the healthy controls ($p<0.0001$). The TAS values at hour 1 of intravenous iron treatment were significantly lower than those before the administration of treatment ($p<0.0001$). The TAS values at day 30 of intravenous iron treatment

were significantly higher than those before the administration of treatment ($p=0.001$) and at hour 1 of treatment ($p<0.0001$).

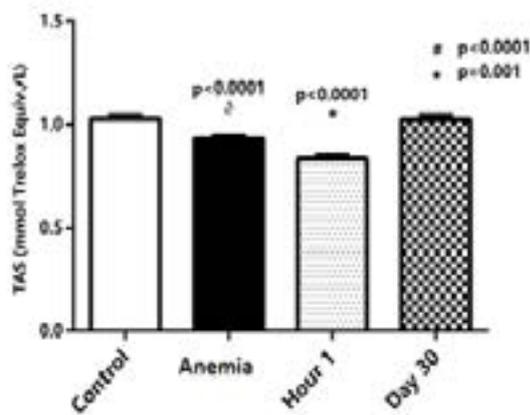


Figure 7. Total anti-oxidant status of the participants

Figure 8 indicates that the patients with anemia had significantly higher TOS than the healthy controls ($p<0.0001$). The TOS values at hour 1 of intravenous iron treatment were significantly higher than those before the administration of treatment ($p=0.004$). The TOS values at day 30 of intravenous iron treatment were significantly lower than those before the administration of treatment and at hour 1 of treatment ($p<0.0001$ for both).

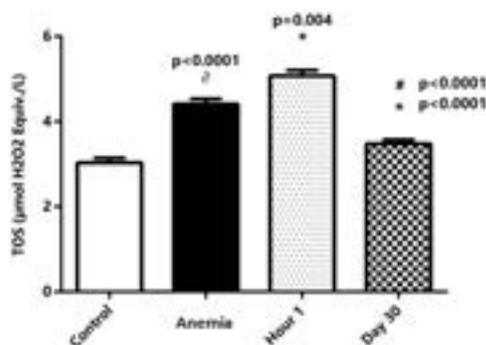


Figure 8. Total oxidant status of the participants

Discussion

Oxidative status refers to the equilibrium between oxidants and anti-oxidants in normally functioning cells [21]. If a pathological condition occurs, the equilibrium shifts towards the oxidants and oxidative stress emerges [22]. Oxidative stress causes oxidation in normally

functioning cells which eventually results in cell death and tissue injury [21,22].

Being the major pro-oxidants, reactive oxygen species are produced by mitochondria during energy generation [23]. The main anti-oxidant mechanisms consist of water or lipid-soluble molecules that counteract and eliminate reactive oxygen species [24]. Thiols are the principal anti-oxidant molecules of which reduced thiol glutathione is the most significant. Moreover, dynamic thiol/disulfide homeostasis has been addressed as an indicator for oxidative stress [25]. Catalase is an enzyme which neutralizes hydrogen peroxide and fatty acid radicals. Similarly, ferroxidase activity of ceruloplasmin is an anti-oxidant mechanism that hinders the formation of free radicals from iron by accelerating the oxidation of Fe²⁺ to Fe³⁺ [24].

Bioactive nitric oxide originating from the nitrate-nitrite-nitric oxide pathway might reduce the mitochondrial synthesis of reactive oxygen species so that cytotoxicity and apoptosis could be prevented [26]. Such a mechanism of cytoprotection related with the enhancement of nitrate-nitrite-nitric oxide pathway has been specified in ischemia-reperfusion injury [27]. Nitrite and nitrate are endogenously synthesized by quick oxidation of nitric oxide which is derived by nitric oxide synthase [24,26]. Plasma levels of nitrate or the sum of plasma nitrate and nitrite concentrations reflect the activity of nitric oxide synthase [26,27].

It has been well established that hypoxia induces the production of reactive oxygen species. Similar to chronic obstructive pulmonary disease, iron deficiency anemia leads to tissue hypoxia which triggers the production of reactive oxygen species [28]. The deficiency of iron also interrupts the maintenance of sufficient ATP stores during oxidative phosphorylation in the mitochondria [29]. In order to adapt to hypoxia, transcriptional activator hypoxia-inducible factor-1 (HIF-1) is synthesized in patients with chronic anemia. This factor decreases mitochondrial mass and decelerates mitochondrial metabolism, reducing the production of reactive oxygen species [30].

The activity of the anti-oxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase is significantly lowered in adults with iron deficiency anemia than the healthy controls [31,32]. Additionally, iron deficiency anemia causes the upregulation of nitric oxide synthase and elevation of nitric oxide in otherwise healthy adults [32].

Complying with literature, the patients with anemia were found to have significantly higher TOS and

lower TAS than the healthy controls in this study. On the contrary, the patients with anemia had significantly higher plasma catalase activity and lower plasma nitrate level. This contradictory finding might be due to the relatively small cohort size, differences in measurement techniques and variations in the duration of iron deficiency anemia.

In vitro studies have demonstrated that parenteral iron therapy has a potential of releasing labile and redox-active iron and precipitate the formation of oxidants [33,34]. However, several animal studies have reported that intravenous iron treatment causes oxidative stress at varying severity [11–14]. Most of these animal studies have been performed with the iron doses that are higher than those used in clinical practice. It might be argued that these significantly higher doses are required to compensate for the shorter half-life of iron complexes in rodents [11,12].

A number of clinical studies have been conducted to evaluate how intravenous injection of various iron preparations would affect the biomarkers for oxidative stress [8–10]. Although these studies tend to report about the augmentative effects of intravenous iron complexes on oxidative stress, their findings should be interpreted carefully. The first reason is that hemodialysis patients make up the cohorts in the majority of the studies focusing on oxidative stress related with parenteral iron therapy. Since hemodialysis alone is a well-known underlying etiology for inflammation, oxidative stress and endothelial dysfunction, data about the oxidative stress markers in hemodialysis patients might have been biased. The second reason is the heterogeneity of the intravenously administered iron preparations. The majority of related studies have been performed with iron sucrose whereas only a few studies have been held with ferric gluconate, low molecular weight iron dextran, ferric carboxymaltose and iron sucrose similar complexes. The third reason is the heterogeneity in the selection of biomarkers for the assessment of oxidative stress. The fourth reason is the distinct distribution of various iron preparations in different tissues which end up with varying degrees of oxidative stress. For instance, iron sucrose similar complexes are concerned with significantly higher levels of oxidative stress in heart, liver and kidneys than iron sucrose.

As for the present study, the levels of TAS, nitrate, nitrite, nitric oxide, and total thiol were significantly reduced however; TOS levels were elevated in patients with anemia at hour 1 of intravenous iron treatment. At day 30 day of parenteral iron therapy, catalase ac-

tivity, nitrate, total thiol and TOS values were significantly lowered and nitric oxide and TAS values were significantly elevated when compared to hour 1 and time of diagnosis.

Conclusion

These findings suggest that parenteral iron therapy prompts oxidative stress in adults with iron deficiency anemia. Oxidative stress increases markedly in hour 1 of intravenous iron infusion but this increase appears as a transient alteration which undergoes a partial recovery spontaneously in the following 30 days. Therefore, it would be prudent to assume that the elevation in oxidative stress might be a temporary change in otherwise healthy patients receiving intravenous iron treatment due to anemia. The power of the present study is limited by relatively small cohort size, inability to study all biomarkers for the evaluation of oxidative stress and lack of long term data. Further research is warranted to clarify the effects of intravenous iron administration on biomarkers for oxidative stress in anemic adults.

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The author has no conflicts of interest to declare.

Conflict of interest

The author declared that this study has received no financial support.

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