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The relationship between ferritin levels and oxidative stress parameters in serum of β-thalassemia major patients

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1. Introduction

β-thalassemia is the most common genetic disorder worldwide. Typically, moderate to severe anemia, caused by hemolysis and ineffective erythropoiesis [1] characterizes β-thalassemia. More than %3 of world population carries thalassemia genes with highest incidence up to %40 in South East Asia [2]. β-thalassemia major (TM) is the most severe type of thalassemia and it is also the most common inherited blood disorder and a major public health problem in Cyprus [3]. Patients with TM are severely anemic and require life-long blood transfusions for survival. Repeated blood transfusions in patients with TM lead to iron accumulation in various organs [4,5] (see Figs. 1 and 2).

Ferritin is a cytoplasmic protein that stores iron as ferric and the liver is the major storage organ of iron. In various studies carried out with TM patients, iron overload is shown to first appear in liver when serum ferritin levels exceed 1000 μg/dL. In addition, iron deposition further occurs in other organs including heart, spleen and pancreas as the level of ferritin increases. Ferritin is the most convenient laboratory test available to estimate body iron stores [6]. The accumulation of toxic quantities of iron leads to the formation of reactive oxygen species (ROS), which induces oxidative stress [7].

Oxidative stress, defined as disruption of the equilibrium between pro-and anti-oxidant systems, is important in the pathology of many diseases [8,9]. When the oxidant-antioxidant balance is disturbed, macromolecules, namely lipids, proteins and nucleic acids of the cells are damaged [10,11]. Oxidative stress and possible consequential accelerated apoptosis may contribute to shortened erythrocyte life span [12]. Auto-oxidation of globin chains, iron overload and low levels of adult hemoglobin (HbA1) enhance the oxidative damage in TM patients [13]; [14]. Secondary iron overload is still a major concern in homozygous TM, even though iron chelation therapy determined considerable progress in the treatment of TM disease [15].

This study was planned for the first time to evaluate the relationship between oxidative stress parameters and ferritin levels in serum of transfusion dependent TM patients, which are under regular iron chelation therapy. For this reason, patients with TM were grouped according to serum ferritin levels and pro-oxidant and antioxidant parameters were determined.

2. Materials and methods

2.1. Study design

45 transfusion dependent TM patients followed up in Thalassemia Center of Dr. Burhan Nalbantoğlu State Hospital in an age range of 18–55 were investigated in this study. These patients received approximately 15 mL of packed red blood cells per kilogram body weight in order to maintain hemoglobin levels above 10 g per dL. Desferoxamine (DFO) was the basic chelation regimen for all TM patients. The Near East University Ethics Review Board approved the study protocol. The patients were divided into three groups according to their serum ferritin levels. Each patient group were consisted of 8 male and 7 female subjects and their ferritin levels were < 1000 ng/mL, 1000–3000 ng/mL and > 3000 ng/mL, respectively. Patients were regularly interviewed and examined by a staff of physicians twice a month. Serum ferritin levels together with the biochemical parameters were measured every three months. In addition, cardiac, endocrinologic, serologic and hepatologic evaluations screening were performed for TM patients once a year. Control group consisted of 8 male and 7 female healthy subjects (age 18–55) with normal ferritin levels who were not taking any medication. Patients and control subjects were not using any antioxidant agents.

2.2. Blood samples

Blood samples were obtained following an overnight fasting. The blood samples were collected just before the transfusion from the TM patients in dry tubes and after clotting, serum was separated by centrifugation at 1300g for 15 min and stored at −80 °C until tested.

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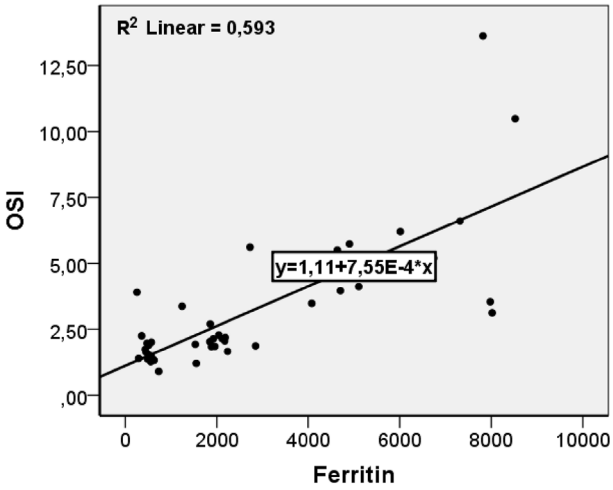

Fig. 1. Correlation between OSI and Ferritin in TM patients.

2.3. Laboratory assays

Serum ferritin levels were tested with an auto analyzer (Abbott immunoanalyzer USA). Complete blood count was done using Sysmex XP-300™ Automated Hematology Analyzer.

Total oxidant status (TOS) and total antioxidant status (TAS) were determined by using commercial kits obtained from Rel Assay Diagnostics (Gaziantep, Turkey). TOS and TAS values were expressed as μmol H_2O_2 Eq/L and μmol Trolox Eq/L, respectively. Oxidative stress index (OSI) values were calculated by using the formula (the ratio of TOS level to TAS level ×100).

8-epi-prostaglandin F2 alpha (8- epi-PGF 2α), advanced oxidation protein products (AOPPs), 8-hydroxy-deoxyguanosine (8-OHdG), coenzyme Q10, α-tocopherol and extracellular superoxide dismutase (EC-SOD) levels were measured using ELISA kits (Yehua Biological Technology, Shanghai).

2.4. Statistical analysis

Data was analyzed with a commercially available statistics software package (IBM SPSS for Windows v. 21.0, Chicago, USA). Results were expressed as mean ± SD. Group's comparisons involved analysis of variance (One-way ANOVA) and Pearson correlation analysis was carried out.

3. Results

The patients were divided into three groups according to their serum ferritin levels as < 1000 ng/mL (G1), 1000–3000 ng/mL (G2) and > 3000 ng/mL (G3), respectively. Ferritin levels and hematological characteristics of these groups are shown in Table 1. According to this, erythrocyte counts, hemoglobin levels and hematocrit values together with mean corpuscular hemoglobin concentration decreased in all groups of TM patients. However, mean corpuscular volume did not change in TM groups as compared to controls.

TOS, OSI, 8-epiPGF 2α AOPPs, 8-OHdG levels together with EC-SOD levels increased in serum of TM groups as compared to the controls. However, TAS, α-tocopherol and coenzyme Q10 were found to decrease. These changes in oxidative stress parameters were parallel to the increase in ferritin levels in TM groups (Table 2). Indeed, serum ferritin levels were positively correlated with TOS, OSI, 8-epiPGF 2α AOPPs, 8-OHdG and EC-SOD levels in TM patients. However, there were negative correlations between the serum ferritin and TAS, α-tocopherol and coenzyme Q10 levels in TM patients(Table 3).

4. Discussion

Oxidative stress is defined as a dynamic imbalance between the amounts of ROS generated in the body and levels of anti-oxidants, which protect against their deleterious effects [16]. When this balance is disturbed, oxidative damage takes place in the macromolecules such as lipids, proteins, and nucleic acids [11,17]. In patients of TM, repeated transfusions result in excessive iron accumulation in the body, since they require frequent blood transfusions to combat the anemia. This iron overload triggers the extreme production of ROS leading oxidative stress. This stress may disturb the normal physiology of the cells and organs. For example, the rapid apoptosis and ineffective erythropoiesis may appear due to the oxidative damage to erythrocytes in TM patients. Therefore, antioxidant potential of these patients gains importance. In this study, we wanted to investigate the relationship between pro- and anti-oxidant parameters and ferritin levels, an indicator of iron accumulation, in transfusion dependent TM patients (see Table 4).

TOS, TAS and OSI are the mostly used parameters to evaluate the serum oxidative stress in clinical trials. Increased TOS [1] and decreased TAS [15] levels were reported in serum of patients with TM. In this study, serum TOS levels were detected to increase gradually in TM patients grouped according to ferritin levels. These elevated values of TOS may appear in response to oxidative stress, possibly induced by the multiple blood transfusions. Indeed, we detected a positive significant correlation between ferritin and TOS values of TM patients. Contrarily, in our study, TAS levels decreased gradually and negative correlation was found between TAS and ferritin levels. The reduced TAS of TM patients might be due to an increased utilization of antioxidants in order to counterbalance the effects of ROS, which may be involved in the pathological consequences of TM and contribute to the gradual development of organ damage [18].

Oxidative stress influences lipids, proteins and DNA. Therefore, oxidative changes in these molecules can be detected by various measurements. Isoprostanes (IsoP) are prostaglandin isomers produced in vivo from polyunsaturated fatty acids (mainly arachidonic acid) by a free radical catalyzed mechanism. 8-epi PGF 2α is nowadays in common use and researchers have proven that they are reliable oxidative stress markers [19]. Proteins are also susceptible to free radical damage and AOPPs are the products of protein damage induced by oxidative stress [20,21]. On the other hand, oxidized and damaged DNA in the form of 8-OHdG is used for the detection of DNA oxidative damage. In patients with TM, 8-epi PGF 2α [22], AOPPs [9,23] and 8-OHdG [24] levels were found elevated as compared to controls. In this study, these oxidation products also increased gradually in patients and showed positive correlation with ferritin levels.

On the other hand, to protect against the oxidative damages, protective antioxidant molecules exist in the organism. We measured the levels of some non-enzymatic and enzymatic antioxidants in TM patients with different ferritin levels. Some investigators have detected low coenzyme Q_{10} [25] and vitamin E [26] levels in TM patients. These results are in accordance with our findings. Additionally, negative correlation was found between ferritin and coenzymeQ_{10}/α-tocopherol levels in TM patients. Vitamin E is the most important fat-soluble antioxidant and considered as the primary step for antioxidant defense system [27]. In thalassemia, rapid consumption of vitamin E occurs while neutralizing oxidative damage in the pathological erythrocyte membranes and in other tissues. On the other hand, extracellular superoxide dismutase (SOD), are the first barriers to the change of the internal environment influenced by the increase of free radicals and abundant stress, creating super active oxygen. However, much of the data obtained from TM patients are conflicting [4,28]: [29,30]. In a research carried out in Jakarta, it has been shown that the antioxidant enzymes in TM patients are affected some factors such as the daily diet, the area and the iron chelation management [2]. This might be the cause for the conflicting results obtained in various studies. However, in