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Oxidative stress and labile plasmatic iron in anemic patients following blood therapy

FernandesMS *et al*. Biochemical markers in politransfused subjects

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**Abstract**

**AIM:** Io determine the plasmatic iron content and evaluated the oxidative stress markers in subjects receiving blood therapy.

**METHODS:** 39 individuals with unspecified anemia receiving blood transfusion and 15 healthy subjects were included in the study. Anemic subjects were divided into 3 subgroups: (1) subgroup that received up to five blood transfusions (*n =* 14); (2) subgroup that received from five to ten transfusions (*n =* 11); and (3) subgroup that received more than ten transfusions (*n =* 14). Blood samples were collected by venous arm puncture and stored into tubes containing heparin. The plasma and cells were separated by centrifugation and were subsequently used for analyses. Statistical analysis was performed using Kruskal-Wallis analysis of variance followed by Dunn’s multiple comparison test when appropriated.

**RESULTS:** The eletrophoretic hemoglobin profile of the subjects included in this study indicates that neither of patients presented hemoglobinopathy. Labile plasmatic iron (LPI), ferritin, protein carbonyl, thiobarbithuric acid-reactive substances (TBARS) and dichlorofluorescindiacetate (DCFH-DA) oxidation were significantly higher (*P <* 0.05), whereas total -SH levels were significantly lower (*P <* 0.05) in anemic subjects compared to controls. Additionally, the activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were significantly lower (*P <* 0.05) in the transfused subjects. Antioxidant enzymes activity and total–SH levels were found to be positively and significantly (*P <* 0.05) correlated, being negatively correlated with the levels of protein carbonyl and TBARS. In contrast, protein carbonyl and TBARS were found to be positively and significantly (*P <* 0.05) correlated. Altogether these data confirms the involvement of the oxidative stress (OS) in patients following therapy with repeated blood transfusions.

**CONCLUSION:** Our data reveals that the changes in the OS markers are tightly correlated with LPI, ferritin and the number of transfusions.

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**Key words***:* Politransfused subjects; Antioxidant enzymes; Oxidative stress; Labile iron content

**Core tip:** Here, the readers will find important information regarding iron accumulation, and its correlation with the oxidative damage markers, in anemic subjects following blood therapy. This research, regarding iron accumulation and its associated toxicology, is remarkable because the mechanism(s) involved on its mode of action are not fully understood. Thus, our data are extremely important for all scientific community, especially from biomedical field, that search regarding the involvement of iron overload on the development of human diseases.

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**INTRODUCTION**

Iron is an essential element of cells and it participates in various cellular processes due to its ability to accept and donate electrons, interconverting between the Fe3+ and Fe2+ forms[1]. However, this redox property renders iron potentially toxic in biological systems, where the labile plasmatic iron (LPI) represents a component of non-transferrin-bound iron (NTBI), which is redox-active, chelatable and capable of permeating into organs inducing tissue iron overload[2]. Thus, the pathologically relevant fraction of NTBI, also referred to as LPI, makes it an accessible diagnostic marker of iron overload and cell toxicity[2]. Moreover LPI could participate in Fenton reaction, generating a large amount of reactive oxygen species (ROS)[3]. Thus, in order to prevent ROS over production, circulating and intracellular free iron are tightly regulated by binding to the transferrin, ferritin and other proteins[4,5]. However, in some situations the iron balance may be disturbed. Indeed, iron overload could occur under some conditions, such as in several chronic anemia, secondary to repeated blood transfusions, and following increased gastrointestinal absorption[6]. So, subjects undergoing repeated blood transfusions are believed to be at risk of toxicity associated with iron overload[7].

Accordingly, it was shown that elevated tissue iron can overwhelm the protective mechanisms leading to an increase in iron complexes with small molecules, such as nucleotides and citrate, in the serum of these patients and also within cytoplasm and organelles[8,9]. Furthermore, repeated blood transfusions increase the levels of iron, being available to generate catalytically active complexes, and consequently free radicals and oxidative damage[9]. Thus, the labile iron promotes free radicals formation that culminates in the oxidation of biomolecules. Accordingly, iron overload in humans and in experimental animals seems to be associated with oxidative stress[10]. Indeed, it is known that an imbalance in the oxidant/antioxidant status of the cell is associated with oxidative stress, leading to important cellular macromolecules modifications resulting in cell damage[11]. Thus, oxidative stress is believed to be the phenomenal factors determining cell injury in patients with iron overload[12]. Hence, the end-result of the oxidation reactions is the formation of lipid peroxides and protein carbonyls, damaged deoxyribonucleic acid (DNA) bases, and mitochondrial dysfunction[13]. Additionally, individuals with an iron overload also demonstrate impaired antioxidant defenses[6]. Accordingly, the long-term consequences of chronic iron overload is an organ injury, which could contribute to the initiation and development of several metabolic disorders, such as endocrinopathies, diabetes mellitus, cirrhosis, hypogonadism and heart failure[14].

In general, oxidative damage of biomolecules can be counteracted by enzymatic as well as non-enzymatic defenses. Indeed, humans are provided with several biological mechanisms to defend against intracellular oxidative stress. One of the most important mechanisms involves the actions of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)[15]. In spite of well-developed antioxidant defense system, cells can be oxidatively damaged under some pathological conditions[11].

We hypothesize that oxidative stress can be correlated with LPI in anemic patients following therapy with repeated blood transfusions. Moreover, to the best of our knowledge, data about labile iron accumulation in anemic subjects receiving repeated blood transfusion and its correlation with the oxidative damage markers are scarce in the literature. Therefore, in order to verify this hypothesis, we evaluated the oxidative stress markers and the activity of the enzymatic antioxidant defenses in the blood of patients receiving repeated transfusions and in control subjects (not transfused). Additionally, we determined the LPI and ferritin levels in these subjects and correlated both parameters with other evaluated markers.

**MATERIAL AND METHODS**

***Chemicals***

1,1,3,3-tetramethoxypropane, 2-thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), 5,5- dithiobis (2-nitrobenzoic acid) (DTNB), trichloroacetic acid (TCA), 2’,7’-dichlorofluorescin diacetate (DCHF-DA), 2,4-Dinitrophenylhydrazine (DNPH), were purchased from Sigma, St. Louis, MI, USA. The kit for iron determination was obtained from Bio-Systems, kits for SOD and GPx from Randox® Laboratories, United Kingdom, and kit for protein determination from BioClin. All the other chemicals were commercial products of the highest purity grade available.

***Subjects***

This study was approved by the Ethics Committee in Research of Universidade Federal do Pampa (UNIPAMPA). Altogether 39 individuals with unspecified anemia receiving blood transfusion and 15 healthy subjects (blood donors) from the Banco de Sangue do Municipio de Uruguaiana were included in the study. Since most of our patients were male, the female patients were excluded from this study. Thus, both anemic and control healthy individuals were male. Anemic individuals, selected for the study, presented in their medical records according to the International Classification of Diseases (ICD): anemia unspecified - ICD 10: D64.9, and did not present other diagnosed disease, such as cancer, renal failure, hepatic disease, blood loss or others. Thus, anemic patients included in this study were individuals with unspecified anemia; that received the blood therapy during last year prior to collection (*i.e*., no more than 12 mo from the first transfusion until sample collection to analysis). Additionally, it is important to mention here that the sample collection was done before a new transfusion, namely clinical screening. The anemic subjects were divided into 3 subgroups: (1) subgroup that received less than five (< 5) blood transfusions (*n =* 14); (2) subgroup that received from five to ten blood transfusions (*n =* 11); and (3) subgroup that received more than ten blood transfusions (*n =* 14).

***Sample collection***

Blood from either controls or anemic subjects were collected by venous arm puncture and stored into tubes containing heparin. The plasma and cells were separated by centrifugation at 1500 rpm for 10 min and were subsequently used for biochemical analyses. All biochemical assays were done in duplicate or triplicate, depending on availability of samples.

***Analysis of hemoglobin***

The electrophoretic analysis of hemoglobin was performed using the Minicap (Sebia, Norcross, France) according to the manufacturer's instructions, running controls with each test as described previously. The Minicap system uses the principle of capillary electrophoresis in free solution. Charged molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific pH. Separation also occurs according to the electrolyte pH and electro-osmotic flow. Electropherograms were expressed with divided zones from Z1 to Z15 based on standardizing the location of HbA according to previous described[16].

***Measurement of labile plasmatic iron***

The labile plasmatic iron (LPI) is used here according to the previously validated convention[17] and refers to iron non-heme bound, iron non-ferritin bound and iron non-transferrin-bound (NTBI), in other words, refers to the free iron. The LPI content was determined by its reactivity with ferrozine, in the presence of the denaturant sodium dodecyl sulfate and the reducing agents ascorbate and sodium metabisulphite, according to previously described[18,19].

***Ferritin***

Content ferritin was determined as described by Bernard and Lauwerys[20]. Serum ferritin causes agglutination of latex particles coated with anti-human ferritin. The latex particles-agglutination is proportional to the concentration of ferritin and can be measured by turbidimetry. The results were expressed as µg/L ferritin.

***Protein carbonyl determination***

Content protein carbonyl was determined as described by Levine *et al*[21]. The carbonyl protein presence is indicative of oxidation. Plasma samples were added to 0.2 mL of trichloroacetic acid (TCA), 10% and placed on ice for 5 minutes. After centrifugation (5 min), was added 1 mL of 2,4-dinitrophenylhydrazine (DNPH – 10 mmol/L) in 2 mol/L HCl to samples or 1 mL of 2 mol/L HCl in white tubes. Thereafter, tubes were incubated for 90 min at 37 ºC. Finally, proteins were dissolved in 6M guanidine and interference was removed after washing with ethanol-ethyl acetate 1:1 (v / v). The extent of the damage was estimated by reading absorbance at 370 nm. The results were expressed as nmol cabonyl/mg protein.

***Determination of TBARS levels***

Thiobarbituric acid reactive substances (TBARS) were determined in plasma by the method of Ohkawa *et al*[22]. In brief, samples were incubated at 100 °C for 60 min in acidic medium containing 0.45% sodium dodecyl sulfate and 0.6% thiobarbituric acid. After centrifugation, the reaction product was determined at 532 nm using 1,1,3,3-tetramethoxypropaneas standard and the results were expressed as nmol MDA/mg protein.

***Total thiol (Total –SH) determination***

Plasmatic total -SH were determined as described by Ellman[23]. The colorimetric assay was carried out in 1 mol/L phosphate buffer, pH 7.4. A standard curve using glutathione was constructed in order to calculate the total -SH content in samples. Total -SH content was expressed as nmol total –SH /mg protein.

***Determination of DCHF-DA oxidation***

The determination of intracellular oxidant production was based on 2’,7’-dichlorofluorescin diacetate (DCHF-DA) cleavage to 2’,7’-dichlorofluorescin (DCHF) that can be oxidized to the fluorescent compound 2’,7’-dichlorofluorescein by ROS according to previously described[24]. The plasma sample was diluted (1: 10) in Tris/HCl 10 mM buffer. Then, 50 µL of diluted plasma was incubated in 10 mmol/L Tris/HCl buffer and 10 mol/L DCHF-DA at 37 ° C for 30 min. The DCF fluorescence intensity emission was measured using a Perkin-Elmer spectrofluorometer at an excitation wavelength of 488 nm and an emission wavelength of 520 nm, 20 min after the addition of DCHF-DA to the medium. The results were expressed as arbitrary fluorescence unit (AFU).

***Catalase activity***

Catalase (CAT) activity was measured by the method previously described[25]. Packed erythrocytes were hemolyzed by adding 100 volumes of distilled water, then 20 μL of this hemolyzed sample was added to a cuvette and the reaction was started by the addition of 100 μL of freshly prepared 300 mmol/L H2O2 in phosphate buffer (50 mmol/L, pH 7.0) to give a final volume of 1 mL. The rate of H2O2 decomposition was measured by a spectrophotometer at 240 nm for a duration of 2 min. The CAT activity was expressed as UI/mg protein.

***Superoxide dismutase activity***

Superoxide dismutase (SOD) activity was measured in erythrocytes using the Kit RANSOD® (Randox Laboratories, United Kingdom). This method employs xanthine and xanthine oxidase to produce superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-feniltetrazol chloride (INT) to form compound formazan red. The superoxide dismutase activity was measured by the degree of inhibition of this reaction at 505 nm. The SOD activity was expressed as UI/mg protein.

***Glutathione peroxidase activity***

Glutathione peroxidase (GPx) activity was determined in erythrocytes using the Kit RANSEL® (Randox Laboratories, UK), according to the method previously described[26]. The GPx activity was expressed as UI/mg protein.

***Protein determination***

The protein content is determined by the biuret method, through the Total Protein Kit Bioclin®, using bovine serum albumin as standard. The copper ions in an alkaline medium (biuret reagent) react with peptide, producing a purple color, whose intensity is proportional to the concentration of proteins in the samples being measured in a spectrophotometer at 545 nm.

***Statistical analysis***

All results are reported as median ± range and presented as box-plot graphics for the different groups of patients. Hemoglobin, LPI and Ferritin are presented as mean ± SD (Table I). Considering that variables do not present a normal distribution, as determined by Shapiro-Wilk test, statistical analysis was performed using Kruskal-Wallis analysis of variance followed by Dunn’s multiple comparison test when appropriated. *P <* 0.05 was considered significant. Spearman correlation between variables was also carried out.

**RESULTS**

Some characteristics of the subjects included in this study are presented in Table 1. The eletrophoretic hemoglobin profile of the subjects included in this study indicates that no individual presented hemoglobinopathy (100% had normal hemoglobin profile; data not shown). As expected, the LPI content and ferritin content were statistically (*P <* 0.05) higher in the transfused subjects when compared to control group (Table 1). Nonetheless, only subjects receiving > 5 transfusions presented LPI levels statistically significant from controls and only subjects receiving > 10 transfusions presented ferritin levels statistically significant from controls. Additionally, we found a statistically significant correlation between the number of transfusions, the LPI content and the ferritin content (Table 2).

The oxidative stress analyzed markers such as TBARS (Figure 1A), protein carbonyl (Figure 1B) and DCFH-DA oxidation (a marker ROS; Figure 1C) were significantly (*P <* 0.05) higher compared to the control group. In addition, total -SH levels (Figure 1D) were significantly (*P <* 0.05) lower in transfused subjects compared to controls. However, statistical analysis revealed that only subjects receiving > 10 transfusions presented statistically significant decrease in SH groups when compared to controls, while other evaluated markers were found to be altered in all groups of transfused subjects, independent from the number of transfusions (Figure 1).

Moreover, the activity of all the antioxidant enzymes reported here (CAT, SOD and GPx) were significantly (*P <* 0.05) lower in the transfused subjects than in controls (Figure 2). However, CAT and GPx activity did not differ from controls in the group that received < 5 transfusions (Figure 2A and C, respectively), whereas SOD activity was statistically different from controls only in the group that received > 10 transfusions (Figure 2B). Additionally, we also found statistically significant negative correlations between the number of transfusions and the activity of the antioxidant enzymes CAT, SOD, and GPx (Table 2).

Analyzing the groups of anemic and control subjects we found statistically significant correlations between the evaluated oxidative stress markers and the LPI content, except to DCDH-DA oxidation (Table 2). LPI was found negatively correlated to antioxidant enzymes activity and with total –SH, while it was positively correlated to the protein carbonyl and TBARS levels. Likewise, ferritin content was found to be negatively correlated to antioxidant enzymes activity and with total –SH and positively correlated to the protein carbonyl and TBARS levels. Other correlations are also presented in Table II. Indeed, it was found that antioxidant enzymes activity and total–SH levels were positively correlated, being negatively correlated with the levels of protein carbonyl and TBARS. In contrast, protein carbonyl and TBARS were found to be positively correlated (Table 2).

**DISCUSSION**

Our data are in accordance to a previous study showing that the increase in LPI content could lead to an increase in ROS generation, and consequently an increase in oxidative damage[12]. Additionally, based on data concerning hemoglobin profile (data not shown), we discarded hemoglobin disorders in these individuals. These data are extremely important to avoid misinterpretations, once it was previously shown that any imbalance between α and β chains of hemoglobin (α or β- thalassemia, respectively) plays a crucial role in oxidative stress[27]. Besides, we found literature data linking the observed levels of the various biomarkers, evaluated here, to health outcomes, such as in renal failure[28] and in breast cancer patients[29], just to name a few.

Taking into account our results and those previously found, it is plausible to assume that, under blood transfusion therapy the excess of labile iron (catalytically active iron) must generate free radicals (ROS) via Fenton chemistry, resulting in oxidative damage to biomolecules *in vivo*[30]*.* Our assumption is further supported by previous report showing that iron-catalyzed ROS generation leads to an increase in the genomic instability in hematopoietic progenitor cells[31]. Moreover, in animal models it was shown that iron overload causes liver damage *via* both oxidative and nitrosative mechanisms[32]. Indeed, we assume that under repeated blood transfusions, the iron content increase to values that overwhelm the protective mechanisms, leading to an increase in the amount of iron available to form complexes with small molecules, the “catalytically active iron complexes”. Thus, we assume that ROS generated are responsible for the oxidation of DCFH-DA found in the transfused subjects, which is supported by previous report showing that overload with iron (ferric nitrilotriacetate) leads to an increase in oxidation of DCFH-DA in culture rat hepatocytes[33].

However, we found some changes in the oxidative stress parameters even in the absence of significant iron accumulation, suggesting that alterations in oxidative stress markers could precede iron accumulation in patients following blood therapy. Accordingly, it seems logical that the differences in other parameters, in the hemoglobin and ferritin levels for example, could potentially contribute to the different oxidative state among patients. So, it is hard to affirm that iron alone is the main responsible factor for these differences. Anyway, this point is extremely relevant and deserves further attention in future investigations.

Likewise, we found the levels of TBARS significantly increased in subjects receiving blood transfusions, which was positively correlated to LPI content, ferritin content and the number of transfusions. These findings are in accordance to previous reports showing that the levels of lipid peroxidation products were increased in beta-thalassaemic patients receiving blood transfusions[19] and in subjects with hepatic iron overload[6]. Moreover, we found a significantly increase in the protein carbonyl in the subjects receiving repeated blood transfusions, which was found to be significantly correlated with LPI content. Additionally, our data are in accordance to a previous paper showing a significant increase in the protein carbonyl content associated to iron overload[33].

We also found a significant reduction in total SH in the subjects receiving repeated blood transfusion, which are in accordance to previous report showing a decrease in thiol content in the liver of rats treated with iron[34]. Albeit not completely understood, we believed that thiols are oxidized (consumed/used) in these subjects due to oxidative stress status following iron overload. However, other possibility is that the iron could reacts non enzymatically with thiols in plasma to generate ROS, which directly lead to reduction of antioxidant capacity in plasma and the increased susceptibility of blood components to oxidation[35]. Thus, this thiol-dependent free radical generation by iron overload might be a potential contributing factor for the changes in the oxidative markers reported here. Our assumptions are supported by other previous study showing that oxygen radicals can be produced by iron-catalyzed auto-oxidation of cysteine or glutathione (GSH)[36]. So, the generated ROS (either by Fenton chemistry as well as *via* iron-catalyzed autooxidation of thiols) are the putative responsible for the oxidation of other biomolecules reported here, such as lipids and proteins.

Additionally, we found a severe decrease in the antioxidant enzymes activity in the subjects with iron overload, which are in accordance with previous reports[30,33]. In fact, we clearly show a statistically significant negative correlation among enzymes activity and the LPI, TBARS and protein carbonyl. In line with this, we suppose that the decrease in the antioxidant enzymatic activity further contributes to the oxidative stress condition. Indeed, according to Chakbraborty and Bhattacharyya the decrease in the antioxidant enzymes strongly contributes to the increase in the markers of oxidative stress (TBARS, protein carbonyl and ROS)[19]. Although our data do not support this supposition, we hypothesize that a decrease in the antioxidant enzymes reported here could, at least in part, be due to a decrease in their expression. Indeed, it was previously shown that both CAT and GPx were down regulated under oxidative stress conditions in human cells[37]. However, the detail mechanisms of regulation in the expression of antioxidant enzymes under iron overload remains to be better explored.

Considering the exposed, our data confirms the involvement of OS in patients following therapy with repeated blood transfusions. Additionally we found that the changes in the OS markers seem to be tightly correlated with iron content, ferritin and the number of transfusions. Thus, iron chelators that efficiently decrease the levels of labile iron are putative candidate to counteract the iron-induced ROS generation[38]. However, more studies are necessary to better understand the mechanism(s) associated to iron-induced oxidative changes, tentatively to minimize the side effects associated to blood transfusion therapy and uncertainly to improve some clinical benefits. Accordingly, antioxidant supplementation seems to be not so safe and may cause unfavorable effects to different patients, and therefore need to be better discussed since it is a controversial topic[39].

In conclusion, our data confirm the involvement of OS and its correlation with LPI and ferritin in unspecified anemic patients following therapy with repeated blood transfusions. However, we found some alterations on OS markers even in the absence of significant iron accumulation, which encourages us to further explore the changes in the OS parameters in subjects receiving blood therapy that occur previously to iron overload.

**COMMENTS**

***Background***

Iron is an essential element that participates in several metabolic activities of cells; however, it excess seems to be a major cause of oxidative stress (OS) in subjects undergoing blood transfusion therapy. Despite this, the relationship between plasmatic iron content, OS markers and the activity of the antioxidant enzymes, in anemic subjects receiving repeated blood transfusions, remains to be better characterized.

***Research frontiers***

Blood therapy has been used in the medical practice to treat, among others, anemic patients. However, the increase in the iron level in patients following blood therapy must be considered. Thus, the purpose of this research was to better understand the changes associated with OS markers in patients under blood therapy, tentatively, to prevent iron-supported oxidative damage in anemic subjects.

***Innovations and breakthroughs***

Previous data, concerning blood therapy, have shown an association among these practice and iron overload (and as a consequence association with oxidative changes) in different tissues. However, efficient therapies to prevent the side effects associated to repeated blood transfusions are not known. For that, elucidative studies regarding the plasmatic oxidative changes associated to iron overload are extremely necessary. Here, we found that plasmatic labile iron, protein carbonyl, TBARS and DCFH-DA oxidation were significantly higher, whereas Total -SH levels were significantly lower in anemic subjects compared to controls. Additionally, the activity of CAT, SOD and GPx were significantly lower in the transfused subjects. Moreover we found statistically significant correlations between the number of transfusions, plasmatic iron content, the OS markers and the activity of the antioxidant enzymes.

***Applications***

The study results suggest that is reasonable to suggest that antioxidants could be associated with blood therapy. Additionally, iron chelators that efficiently decrease the levels of labile iron are putative candidate to counteract the iron-induced ROS generation. However, more studies are necessary to better understand the mechanism(s) associated to iron-induced oxidative changes, tentatively to minimize the side effects associated to blood transfusion therapy and uncertainly to improve some clinical benefits.

***Peer review***

This is a study that contains important information regarding iron accumulation in anemic subjects receiving repeated blood transfusion and its correlation with the plasmatic oxidative damage markers in these subjects.

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**Figure 1**

B

****

Protein Carbonyl

TBARS

A

**nmol carbonyl/ mg protein**

a, e

10

300

**nmol MDA/ mg protein**

a

a

8

a

6

200

a

a

4

100

2

< 5 transfusions

Control

> 10 transfusions

5- 10 transfusions

< 5 transfusions

Control

0

0

> 10 transfusions

5- 10 transfusions

D

C

Total -SH

DCFH-DA Oxidation

**nmol Total -SH/ mg protein**

2,0

40

1,5

a

30

a, e, c

1,0

a

a

**AFU**

20

0,5

10

< 5 transfusions

Control

Control

0

0

> 10 transfusions

5- 10 transfusions

> 10 transfusions

5- 10 transfusions

< 5 transfusions

**Figure 1 The oxidative stress analyzed markers.** A: Protein carbonyl; B: Thiobarbithuric acid-reactive substances (TBARS); C: Dichlorofluorescindiacetate (DCFH-DA) oxidation; D: Total SH levels in different groups (controls (*n =* 15); up to five transfusions (*n =* 14); 5-10 transfusions (*n =* 11); over 10 transfusions (*n =* 14). a*P <* 0.05 *vs* control group; e*P <* 0.05 *vs* group up to five transfusion; c*P <* 0.05 *vs* group 5-10 transfusion by Kruskal-Wallis followed by Dunn’s multiple range test.

**Figure 2**

B

A

SOD

CAT

****

> 10 transfusions

5- 10 transfusions

< 5 transfusions

Control

Control

< 5 transfusions

5- 10 transfusions

> 10 transfusions

Control

< 5 transfusions

5- 10 transfusions

> 10 transfusions

**UI/ mg protein**

**UI/ mg protein**

**UI/ mg protein**

2000

1500

1000

500

0

0.20

0.15

0.10

0.05

0

2.5

2.0

1.5

1.0

a, e

a

a, e, c

a, e, c

a, e

GPx

C

**Figure 2 The antioxidant enzymes** **analyzed**.A: Catalase activity in different groups; B: Superoxide dismutase activity in different groups; C: Glutathione peroxidase activity in different groups (controls; group *<* 5 transfusion; group 5-10 transfusion and group > 10 transfusion). a*P <* 0.05 *vs* control group; e*P <* 0.05 *vs* group up to five transfusion; c*P <* 0.05 *vs* group 5-10 transfusion by Kruskal-Wallis followed by Dunn’s multiple range test.

**Table 1** **Clinical characteristics, biochemical and hematological parameters of the participants of the study**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Group control** | **Group < 5** **Transfusion** | **Group 5-10** **Transfusion** | **Group > 10** **Transfusion** |
| **Mean of age**1, yr | 40.1 (20-50) | 62.8 (24-92) | 64.8 (49-84) | 57.5 (24-74) |
| **Subjects** | 15 | 14 | 11 | 14 |
| **Mean of number Transfusion**1 | 0 (0) | 3.20 (2-4) | 7.17 (5-9) | 18.78 (14-26) |
| **Hemoglobin (g/dL)** 2 | 13.8 ± 0.5 | 7.5 ± 2.1 | 6.75 ± 0.5 | 4.9 ± 0.9 |
| **Labile iron content**2 | 108.9 ± 13.8 | 149.2 ± 45.1 | 216.2 ± 68.3a | 366.9 ± 68.5a |
| **Ferritin**2 | 219.6 ± 18.2 | 190.3 ± 11.7 | 221.2 ± 16.1 | 277.5 ± 27.5a |

1Results are expressed as mean (min - max). 2Results are expressed as mean ± SD. a *P <* 0.05 *vs* control by Dunn’s multiple range test.

**Table 2 Spearman correlations between biochemical and oxidative markers in subjects politransfused**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 　 | **LPI** | **Ferritin** | **GPx** | **SOD** | **CAT** | **TBARS** | **DCFH-DA oxidation** | **Carbonil** | **Total SH** |
|
| **Nº of transfusions** | 0.85691 | 0.79911 | -0.87961 | -0.71031 | -0.81431 | 0.51141 | 0.0111 | 0.57931 | -.55551 | 　 |
| *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | NS | *P <* 0.001 | *P <* 0.001 |
| **Total SH** | -0.41511 | -0.33541 | 0.48301 | 0.48491 | 0.54011 | -0.27901 | 0.0106 | -0.1164 |  |  |
| *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | NS | (NS) | - |
| **CARBONIL** | 0.55831 | 0.51221 | -0.56131 | -0.37131 | -0.48621 | 0.52081 | -0.06271 |  |  |  |
| *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | NS | - | - |
| **DCFH-DA oxidation** | -0.1291 | -0.0049 | 0.037 | -0.0446 | -0.0401 | -0.2293 |  |  |  |  |
| NS | NS | NS | NS | NS | *P <* 0.05 | - | - | - |
| **TBARS** | 0.49841 | 0.41441 | -0.46381 | -0.31131 | -0.3457 |  |  |  |  |  |
| *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | - | - | - | - |
| **CAT** | -0.72661 | -0.59441 | 0.89451 | 0.72511 |  |  |  |  |  |  |
| *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | - | - | - | - | - |
| **SOD** | -0.60851 | -0.57441 | 0.74431 |  |  |  |  |  |  |  |
| *P <* 0.001 | *P <* 0.001 | *P <* 0.001 | - | - | - | - | - | - |
| **GPx** | -0.79731 |  -0.71441 |  |  |  |  |  |  |  |  |
| *P <* 0.001 | *P <* 0.001 | - | - | - | - | - | - | - |
| **Ferritin** | 0.91121 | **-** | **-** | **-** | **-** | **-** | **-** | **-** | - |  |
| *P <* 0.001 |

TBARS: Thiobarbithuric acid-reactive substances; SH: Protein thiol groups; LPI: Labile plasmatic iron; Carbonil: Protein carbonil; ROS: Reactive oxygen species; CAT: Catalase; SOD: Superoxide dismutase; GPx: Glutatione peroxidase; NS: Not significant; 1Significant correlation.