# ASSOCIATION BETWEEN BLOOD DONATION FREQUENCY, ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION

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**Abstract-** Iron is a pro-oxidant cofactor that may be linked to atherosclerosis progression. Free iron catalyzes the generation of free radicals and free radicals promote the oxidation of lipids. Reduction of body iron stores secondary to blood donation has been hypothesized to reduce lipid peroxidation. The aim of this study was to evaluate the association between blood donation and antioxidant enzymes and lipid peroxidation product malondialdehyde (MDA). We investigated hemoglobin (Hb), serum ferritin, MDA level, glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities in the whole blood of 150 male volunteer blood donors aged from 30 to 60 years attending Tehran Blood Transfusion Center. Subjects were divided into 5 groups according to the frequency of blood donation per year. With increasing the number of blood donation in a year, the body iron stores, GPX activity and serum MDA level were significantly reduced ($P < 0.05$) but SOD activity was significantly increased ($P < 0.05$). High-frequency blood donors had evidence of decreased body iron stores, decreased lipid peroxidation and enhanced activity of antioxidant enzymes when compared with low-frequency donors.



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**Key words:** Blood donation, superoxide dismutase, glutathione peroxidase, ferritin, malondialdehyde

## INTRODUCTION

Although iron deficiency is one of the most common nutritional problems existing today around the world, iron excess has recently gained attention owing to epidemiologic evidence suggesting its association with cardiovascular disease, cancer, and other diseases (1). The plausible explanation for this association is iron's prooxidant property in generating free radicals (2, 3). Iron is essential in the human diet and is needed for many important physiologic functions when bound to hemoglobin, myoglobin, cytochromes, several enzymes, and nonheme iron proteins (4). The bound iron is transported in the body by transferrin, while excess iron is stored as ferritin. When the ferritin is saturated, another storage iron protein called hemosiderin is formed (5). The formation of hemosiderin is eventually controlled. During the course of metabolism, a superoxide anion is produced (5). Normally the superoxide anion is converted by the ubiquitous enzyme superoxide

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dismutase (SOD) to produce $H_2O_2$ (6). The $H_2O_2$ is converted to innocuous compounds by the action of catalase (CAT) and peroxidase. But if free iron is available it reacts with $H_2O_2$ to produce hydroxyl radical which is an extremely reactive species and is known to have the ability to react with cellular constituents including amino acid residues and purine/pyrimidine bases in DNA; hydroxyl radicals are also able to attack cell membrane lipids causing lipid peroxidation, inactivation of enzymes and initiation of peroxidation (1, 7).

The availability of $Fe^{2+}$ is made possible by the reaction of superoxide radical entering the ferritin core through the hydrophobic channel causing reduction of $Fe^{3+}$ to $Fe^{2+}$ (8, 9). Malondialdehyde (MDA) is a metabolic product of peroxidative reactions (auto-oxidative) of lipids exposed to oxygen (7, 10). SOD, GPX (glutathione peroxidase), CAT, $O_2^{\circ -}$ and MDA play very important roles in the metabolism in human (7, 10). Both significantly decreased SOD, GPX and CAT, and markedly increased $O_2^{\circ -}$ and MDA can cause metabolic disorders and increase oxidative damage and oxidative stress in human body (1, 11, 12).

The present study aims to investigate whether blood donation might reduction of body iron stores, decrease oxidative stress and increase activity of antioxidative enzyme, and to explore its possible mechanism.

# MATERIALS AND METHODS

The reference population consisted of 150 male volunteer blood donors aged from 30 to 60 years attending Tehran Blood Transfusion Center. The review board and ethical committee of our institution approved the trial. We obtained informed consent from all participants.

The inclusion criteria included no history of minor surgery during the previous 72 hours, no major surgery within 6 months, no current infectious or noninfectious disease, notably cardiovascular disease, diabetes, polycythemia vera, no addiction to drugs and/or alcohol, no history of tattoo in the previous 6 months, no history of nutritional supplement intake for the previous 3 months, minimum weight of 50 Kg, systolic and diastolic blood pressure between 100-180 mmHg and 50-100 mmHg, respectively. Subjects were divided into 5 groups according to the frequency of blood donation per year; *i. e.* 0, 1, 2, 3 and 4 with 30, 27, 30, 30 and 30 persons in each group, respectively, which were matched for age, height, monthly income and weight. To collect data on general health, a questionnaire was completed for age, height, body mass index [BMI = weight (Kg)/height ($m^2$)], weight, number of blood donation per year, medications, medical history, duration from the last donation, literacy and occupation.

## Laboratory tests

Just before blood donation, 10 mL venous blood sample was taken and divided into EDTA and non EDTA tubes. In the EDTA tubes plasma was separated and erythrocytes were washed three times with 9 g/l NaCl solution and then lysed with cold deionized water. Cell membranes were removed by centrifugation at 4000 ×g for 5 min at 4° C and the supernates were used for determining antioxidant enzyme activity. The Hb concentration was also determined in the hemolysates, which were stored in -70° C until enzyme assays. The non EDTA samples were kept for a maximum of one hour at room temperature and were centrifuged at 2500 g at room temperature for 20 minutes. The serum was recovered and transferred to a fresh tube for determination of serum ferritin. Serum ferritin was determined using radioimmunoassay (RIA) (Immunotech, France) and Hb was determined using cyanmethemoglobin method.

## Enzymatic determination of SOD (EC 1. 15. 1. 1)

SOD activity was assayed by kit RANSOD (cat. No. SD 125). The role of SOD is to accelerate the dismutation of the toxic superoxide radical ($O_2^{\circ -}$), to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthinoxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (INT) to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the condition of the assay. Enzyme activity was expressed as SOD units/gHb.

Table 1. Selected characteristics of the subjects under study*

| Variable | Number of blood donation per year | | | | |
|---|---|---|---|---|---|
| | 0 time (n= 30) | 1 time (n= 27) | 2 times (n= 30) | 3 times (n= 30) | 4 times (n= 30) |
| Age (yr) | 42.10 ± 8.06 | 43.62 ± 7.98 | 42.00 ± 7.64 | 42.27 ± 10.52 | 39.93 ± 7.77 |
| Height (cm) | 1.72 ± 0.05 | 1.73 ± 0.05 | 1.74 ± 0.05 | 1.74 ± 0.05 | 1.73 ± 0.05 |
| Weight (kg) | 82.17 ± 10.39 | 82.89 ± 10.82 | 86.20 ± 13.67 | 86.10 ± 14.23 | 2.90 ± 13.05 |
| BMI | 27.41 ± 2.36 | 27.47 ± 2.05 | 28.28 ± 3.04 | 28.25 ± 3.25 | 27.42 ± 2.92 |

Abbreviation: BMI, body mass index.
*Data are given as mean ± SEM.

## Enzymatic determination of GPX (EC 1. 11. 1. 9)

GPX activity was assayed by kit Cayman (cat No. 703102). The Cayman Chemical Glutathione peroxidase Assay Kit measures GPX activity indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione (GSSG), produced upon reduction of hydroperoxide by GPX is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. Under condition in which the GPX activity is rate limiting, the rate of decrease in $A_{340}$ is directly proportional to the GPX activity in the sample. Enzyme activity was expressed as GPX units/gHb.

## Determination of MDA level

Lipid peroxidation was estimated by measurement of thiobarbituric acid reactive substances (TBARS) in plasma according to Satoh method (13). The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde was measured at 530 nm.

## DATA analyses

Results were expressed as mean ± standard error of mean (SEM). Data were subjected Kruskal-Wallis H test and one-way analysis of variance (ANOVA) and followed, if justified, by the resulting statistical probability (*i. e.* $P \leq 0.05$), by LSD test. Correlations were evaluated using Pearson's correlation coefficient. The predetermined upper limit of significance throughout this investigation was $P < 0.05$. All statistical analyses were done using Windows XP/SPSS 11. 5.

# RESULTS

There was no significant difference in weight, height, body mass index (BMI), and age between groups (Table 1). However, with increasing the number of blood donation in a year, the levels of Hb and serum ferritin of cases were significantly decreased ($P < 0.001$) (Table 2).

In subjects, serum ferritin showed significantly correlation with age ($r = 0.181$, $P < 0.05$). Frequency of blood donation per year was also inversely correlated with Hb ($r = -5.54$, $P < 0.001$) and serum ferritin ($r = -6.01$, $P < 0.001$). With increasing the number of blood donation in a year, GPX activity and serum MDA of subjects were significantly reduced but erythrocyte Cu-Zn SOD was significantly increased ($P < 0.05$) (Table 2). SOD showed significant correlation with BMI ($r = -0.179$, $P < 0.05$) and with number of blood donation in a year ($r = 2.145$, $P < 0.05$). Furthermore, frequency of blood donation per year was also inversely correlated with MDA ($r = -3.15$, $P < 0.001$) and GPX activity ($r = -2.73$, $P < 0.001$).

**Table 2.** Distribution of hematological parameters in subjects based on number of blood donation per year*

| Variable | Number of blood donation per year | | | | |
|---|---|---|---|---|---|
| | 0 time (n= 30) | 1 time (n= 27) | 2 times (n= 30) | 3 times (n= 30) | 4 times (n= 30) |
| HB (g/dl) | 15.65 ± 1.12 | 15.37 ± 0.94 | 15. 06 ± 1.43 | 14.40 ± 1.65 | 13.82 ± 1.75 |
| Ferritin (ng/dl) | 118.89 ± 70.84 | 87.70 ± 89.2 | 53. 5 ± 31.2 | 45.9 ± 29.7 | 39.10 ± 25.9 |
| MDA (nmol/ml) | 1.67 ± 0.63 | 1.48 ± 0.65 | 1.40 ± 0.64 | 1.29 ± 0.53 | 1.24 ± 0.36 |
| SOD (U/gHb) | 759 ± 72 | 761 ± 77 | 767 ± 113 | 772 ± 103 | 841 ± 171 |
| GPX (U/gHb) | 32.9 ± 16.7 | 28.9 ± 30.2 | 25.2 ± 18.5 | 19.03 ± 17.8 | 24.2 ± 15.2 |

Abbreviations: HB, hemoglobin; MDA, malondialdehyde; SOD, superoxide dismutase; GPX, glutathione peroxidase.
*Data are given as mean ± SD.

# DISCUSSION

The current findings demonstrate evidence of greater reduction of body iron stores, increase activity of SOD, decreased oxidative stress, and decrease lipid peroxidation in high frequency blood donors when compared with low frequency blood donors. Sullivan first proposed in 1981 that chronic iron depletion from menstrual bleeding rather than gonadal hormone milieu may account for the reduced risk of coronary heart disease in premenopausal women (14). The therapeutic corollary to Sullivan's initial hypothesis is that serial blood donation to mimic the severe reduction in iron stores found in menstruating females will reduce coronary heart disease risk. Blood donation is known to reduce total body iron stores as measured by serum ferritin levels. In healthy men, a single 500 mL whole blood donation results in a substantial loss of heme iron (≈200 to 250 mg) and 1 whole blood donation per year decreases serum ferritin levels by 44% (15, 16). We found an association between a decrease in iron status indices and frequency of blood donation. The markers of iron status, especially serum ferritin concentrations, were all significantly lower in high frequency blood donors and this finding is accord with previous reports (17).

Lipid peroxidation is a free radical-generating process which occurs on every membranous structure of the cell. Free radicals are known to be involved in human pathologies including atherosclerosis (18), cancer (19) and hypertension (20). Our finding of decreased MDA in high frequency blood donors is consistent with the hypothesis that reduction in iron stores is associated with decrease oxidative stress. Few studies have examined the association of plasma iron levels and plasma MDA in human population. Results from a study in patients with coronary artery disease, showed an association of higher serum ferritin with increase lipid peroxidation (21, 22). In another study, results suggested that iron and copper status may be associated with lipid peroxidation in subjects without metal overload (23). These results support other experimental studies reporting that iron may collect along the negatively charged lipid bilayer and promote lipid peroxidation and subsequently membrane damage (24).

Our results also showed a positive association between number of blood donation per year and activity of Cu/Zn SOD. Low frequency blood donors had higher ferritin contents than high frequency blood donors. Excessive free iron and free radicals, as strong oxidants, might interact directly with DNA in human, therefore damaging DNA, inhibiting and/or depressing DNA replication, and might destroy the active groups in molecular structures of vitamin C, vitamin E, β-carotene, SOD, CAT, GPX and others, thereby inactivating and deactivating them (7, 8, 9, 10, 25). Second mechanism by which blood donation can increased SOD activity is by decrease iron stores, because iron decrease Cu/Zn SOD activity, probably due to a negative effect on copper status (26). In this study, we found a negative association between BMI and Cu/Zn SOD activity. There are at least two ways by which obesity can decrease SOD activity. Obesity increases the mechanical and metabolic loads on the myocardium, thus increasing myocardial oxygen consumption. A negative consequence of the elevated myocardial oxygen consumption is the production of reactive oxygen species such as superoxide, hydroxyl radical and hydrogen peroxides from the increase mitochondrial respiration (27). The second mechanism by which obesity can independently cause decreased Cu/Zn SOD activity is by progressive and cumulative cell injury resulting from pressure from the large body mass cell injury cause the release of cytokines especially tumor necrosis factor alpha, which generates reactive oxygen species from the tissues (28) which destroy the active groups in molecular structures of antioxidant enzymes and decrease SOD activity.

Decrease erythrocyte GPX activity in high frequency blood donors was observed in this study. Reduction of erythrocyte GPX activity in high frequency blood donors may result in decrease iron stores because GPX is dependent on iron for its synthesis or the function of the enzyme is dependent on an iron-containing protein serving as an electron carrier (29).

To our knowledge, this is the first study to determine whether blood donation changes antioxidant enzymes and lipid peroxidation in blood donors, and therefore we could not compare our data with other epidemiological studies in human populations.

In conclusion, high frequency blood donation was associated with evidence of reduction of body iron stores, reduced oxidative stress and enhanced activity of antioxidant enzymes in voluntary blood donors. Additional clinical studies are required to evaluate the effect of blood donation on antioxidant enzyme activity and lipid peroxidation in blood donors.

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## Conflict of interests

The authors declare that they have no competing interests.

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