PLASMA IRON IS ASSOCIATED WITH LIPID PEROXIDATION IN WOMEN

Farshad Amirkhizi[(1)], Fereydoun Siassi[(2)], Sara Minaie[(3)], Mahmoud Djalali[(4)] Abbas Rahimi[(5)], Ahmad Reza Dorosty[(6)], Maryam Chamari[(7)]

Abstract

INTRODUCTION: It has been suggested that the risk of coronary heart disease increases with increase of body iron stores. Free iron catalyzes the generation of free radicals and free radicals promote the oxidation of lipids. The aim of this cross-sectional study was to examine the association of plasma iron and factors that could affect its levels (antioxidant enzymes), with the concentration of plasma malondialdehyde (MDA) as a marker of lipid peroxidation.

METHODS: In this study, 160 women aged 20-45 years were randomly selected. A medical history was obtained for each subject prior to enrolment. We assessed lipid peroxidation and the activity of antioxidant enzymes by measuring the concentration of plasma MDA and the activities of erythrocyte copper zinc superoxide dismutase (CuZn-SOD) and glutathione peroxidase (GPX).

RESULTS: Our results show that those in the highest tertile of plasma iron were at least twice as likely to have higher plasma MDA levels. Among the factors affecting plasma iron levels, we found that the upper tertile of erythrocyte CuZn-SOD was inversely associated with higher plasma iron. No associations were found between the highest TIBC and MDA levels. There was no significant association between GPX and plasma iron.

CONCLUSIONS: These findings support the concept that iron, as an important transition metal, might contribute to atherogenesis, along with the classic risk factors. A longitudinal study should confirm whether or not these MDA levels are connected to vascular disease and mortality.

Keywords: Plasma iron, Lipid peroxidation, Antioxidant enzymes, Women.

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Introduction

Iron is an essential metal in the human body. It is required for many metabolic functions, such as oxygen transport and enzymatic reactions. Most iron present in living organisms is tightly complexed with proteins, although some may be present as low-molecular-weight complexes in soluble pools.[1] The free iron is noxious to cells because it catalyzes the generation of hydroxyl radicals (•OH) from superoxide (O2•-) and hydrogen peroxide (H_2O_2) via the Fenton reaction.[2] The highly reactive hydroxyl radicals subsequently cause lipid peroxidation, DNA breaks and degradation of other macromolecules, leading to cell damage or death, which is implicated in the aging process and many human diseases.[3] The data also indicated that O2•- can release iron from ferritin, providing free iron to catalyze the peroxidation of cell membranes.[4] Therefore O2•- causes an increase in the internal pool of free iron that may increase oxidative stress which, in turn, increases the free iron concentration.

Even with all of the experimental evidence showing that ferrous iron (Fe^{2+}) promotes lipid peroxidation both in vivo and in vitro,[5,6] epidemiological literature on this association in humans is scarce and inconsistent.[7,8]

(1) Farshad Amirkhizi M.Sc. Nutritionist, Nutrition and Biochemistry Dept., School of Public Health, Tehran University of Medical Sciences (TUMS), Tehran, Iran.
(2) Fereydoun Siassi Ph.D. Associate professor, Nutrition and Biochemistry Dept., School of Public Health, TTUMS, Tehran, Iran.
(3) Sara Minaie MSc. Nutritionist, Nutrition and Biochemistry Dept., School of Public Health, TUMS, Tehran, Iran.
(4) Mahmoud Djalali Ph.D. Nutritionist, Nutrition and Biochemistry Dept., School of Public Health, TUMS, Tehran, Iran.
(5) Abbas Rahimi Ph.D. Associate Professor, Epidemiology and Biostatistics Dept., School of Public Health, TUMS, Tehran, Iran.
(6) Ahmad Reza Dorosty Ph.D. Assistant Professor, Nutrition and Biochemistry Dept., School of Public Health, TUMS, Tehran, Iran.
(7) Maryam Chamari M.Sc. Nutritionist, Nutrition and Biochemistry, Dept., School of Public Health, TUMS, Tehran, Iran.

Corresponding author: Farshad Amirkhizi
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The same controversy exists on the role of iron stores in atherogenesis, and we have found an equal number of studies suggesting a detrimental[9,10] and a beneficial role for iron.[11,12]

The aim of this cross-sectional study was to examine the association of plasma iron with lipid peroxidation and erythrocyte cytoprotective enzymes activity in women. Therefore, lipid peroxidation and erythrocyte cytoprotection were assessed by measuring the concentrations of plasma malondialdehyde (MDA) and the activities of erythrocyte copper zinc-superoxide dismutase (CuZn-SOD) and glutathione peroxidase (GPX) in a selected group of menstruating women (20-45 years) in Kerman Province, Iran.

Materials and methods

In this study, 160 women aged 20-45 years (mean age: 31.5 years) were randomly selected. A medical history was obtained for each subject prior to enrolment. We excluded pregnant and lactating women and subjects with history of cancer, cardiovascular disease, diabetes, hypertension, renal or liver diseases, and those taking vitamin or mineral supplements.

Venous blood samples were drawn from subjects between 8:00 and 12:00 AM. Blood samples were centrifuged at 3000 rpm for 15 minutes. The buffy coat was removed and the remaining erythrocytes were drawn from the bottom, washed three times in cold saline solution (9.0 g/l NaCl) and hemolyzed by addition of an equal volume of ice-cold demineralized ultrapure water to yield a hemolysate. The subjects' plasma and hemolysates were stored at -70 ºC until analysis.

Plasma MDA concentrations were assayed by measurement of thiobarbituric acid reactive substances (TBARS) according to the Satoh method.[13] The pink chromogen produced by the reaction of thiobarbituric acid and MDA was measured at 530 nm. In order to express enzyme activity per gram hemoglobin (Hb), Hb concentration was measured in the hemolysates with a standard kit involving the cyanmethemoglobin method (Drabkin's method).

GPX (GPX, E.C.1.11.1.9) activity was measured according Paglia and Valentine method[14] and SOD (E.C.1.15.1.1) activity was assayed by RAN-SOD kit (cat. NO.SD 125).

Plasma iron was measured with a model 911 automatic analyzer (Hitachi Ltd, Mito, Japan) by using a colorimetric method without deproteinization, and plasma total iron binding capacity (TIBC) levels were measured by automatic analyzer (Kodak Ektachem 500) using the colorimetric method and standard kits.

The statistical analyses were performed with SPSS 12.5 for Windows. Goodness of fit to normal distribution was investigated by probit plots and the Kolmogrov test. When the distribution of variables was skewed, the natural logarithm of each value was used in the statistical test.

Study participants were classified into tertiles of plasma iron levels and plasma MDA levels. Comparisons of group means were made by a one-way analysis of variance (ANOVA). Differences were considered significant at $P<0.05$.

Results

Mean values of iron and TIBC levels according to tertiles of MDA are shown in Table 1. Subjects in the highest tertile of MDA presented the highest levels of plasma iron.

TABLE 1. Mean iron and TIBC plasma levels according to tertiles of MDA and odds ratio for the upper tertile of plasma iron or TIBC of being in the highest tertile of plasma MDA levels

	MDA <0.8 µmol/L (n=48)	MDA 0.8-2.5 µmol/L (n=57)	MDA > 2.5 µmol/L (n=55)	OR (95% CI)
MDA (µmol/L)	0.43±0.19	1.74±0.7	3.9±0.9‡	
Iron (µg/dL)	78.9±24.8	85.3±27.6	96.3±32.7†	2.5 (1.2-4.9)
TIBC (µg/dL)	251.1±41.8	249.2±38.2	260.4±40.6	1.4 (0.7-2.8)

Values are mean ± SD; OR: Odds ratio; CI: Confidence interval; †P<0.05; ‡P<0.001

TABLE 2. SOD and GPX in erythrocytes according to tertiles of plasma iron levels and odds ratio for the upper tertile of SOD or GPX of being in the highest tertile of plasma iron levels

	Plasma iron (µg/dL) <67	Plasma iron (µg/dL) ≥67<91	Plasma iron (µg/dL) ≥91	OR (95% CI)
Plasma iron (µg/dL)	56.1±9.8	79.4±5.7	114.2±15.2	
CuZn-SOD (U/gHb)	25.3±7.3	26.8±8.2	23.2±6.4†	0.46 (0.21-0.98)
GPX (U/gHb)	3.2±1.4	3.5±1.2	3.6±1.7	1.21 (0.74-2.51)

Values are mean ± SD; OR: Odds ratio; CI: Confidence interval; †P<0.05

Those in the highest tertile of MDA also had the highest mean values of TIBC but differences were not statistically significant. The odds ratio of being in the highest tertile of plasma MDA for those in the upper tertile of plasma iron and TIBC are also presented in Table 1 and the results showed that subjects with highest levels of plasma iron were at least twice as likely to have higher plasma MDA levels (OR=2.5). No associations were found between the highest TIBC and MDA levels. Mean values of the antioxidant enzymes CuZn-SOD and GPX in erythrocytes according to the tertiles of plasma iron and the odds of the highest tertile of CuZn-SOD or GPX for being in the highest tertile of plasma iron levels are presented in Table 2. The upper tertile of CuZn-SOD was inversely associated with higher plasma iron levels. There was no significant association between GPX and plasma iron.

Discussion

In this cross-sectional study, plasma iron levels were positively associated with lipid peroxidation. Few studies have examined the association of plasma iron levels and plasma MDA in human populations. Results from a study in patients with acute myocardial infarction showed an association of higher iron status and increased lipid peroxidation.[15] In another study, the results suggested that iron and copper status may be associated with lipid peroxidation in subjects without metal overload.[16]

These results support other experimental studies (in vivo and in vitro) which report that iron may accumulate along the negatively charged lipid bilayer and subsequently cause membrane damage,[17] or those which describe dietary iron as the main predictor of increases in liver thiobarbituric acid reactive substance (TBARS),[18] and a substantial hepatic lipid peroxidation after iron overload,[19] but they are not in concordance with other studies.[11-20] However, a great part of the discrepancy may result from the variability in the methods used, the population analyzed, study outcomes or types of epidemiological design.

After we confirmed a positive association between plasma iron levels and plasma MDA, we attempted to identify factors associated with plasma iron levels. Lasheras et al. observed that the variability of plasma MDA was mostly determined by levels of the free radical scavenging enzyme CuZn-SOD in erythrocytes,[21] which regulates the concentration of the superoxide anion by converting it to hydrogen peroxide. Recent results have led to the proposal that superoxide plays an additional role in oxidative stress, that of increasing the level of intracellular 'free' iron ions,[22,23] as well as acting as a reducing agent in the Fenton reaction. Therefore, we speculate an association between the activity of erythrocyte CuZn-SOD and plasma iron levels. We observed that subjects in the highest tertile of plasma iron levels had the lowest level of CuZn-SOD. This observation was in line with our first hypothesis: the less CuZn-SOD, the more O2•-, which elevates iron levels. To our knowledge, this is the first study to determine whether CuZn-SOD predicts changes in plasma iron and TIBC levels in an apparently healthy women, and therefore we could not compare our data with other epidemiological studies in women. Nevertheless, we did not observe changes of plasma iron with the GPX activity. Our results were in agreement, however, with those of Armutcu, who found a negative correlation between serum iron levels and CuZn-SOD activity in iron miners.[24] Over 90% of serum iron-binding capacity is accounted for by the iron transport protein transferrin. Apart from its function as an iron-transporting protein, transferrin has long been known to be an antioxidant and its antioxidant property is believed to be related to its capacity to bind iron.[25] Transferrin decreases the generation of free oxygen radicals stimulated by iron, by inhibiting free iron transport. Cooper and Liao found no relation between TIBC and the incidence of coronary heart disease; on the other hand, iron was inversely associated with MI in women and iron and transferrin saturation was inversely associated with coronary heart disease in both sexes.[26] In our study, we also found no relation between the plasma TIBC and MDA levels.

These findings support the concept that iron, as an important transition metal, might contribute to atherogenesis, along with the classic risk factors. The results are also in agreement with the concept that iron overload would elevate the risk of coronary artery disease by promoting the lipid peroxidation. A longitudinal study should confirm whether these MDA levels are connected to vascular disease and mortality.

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