Iron Body Status, Ceruloplasmin Levels and Oxidative Stress in Pre- and Postmenopausal Women at Baghdad City

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ABSTRACT

Background and Objectives: Estimation of different iron body status markers, ceruloplasmin and oxidative stress in pre- and postmenopausal women at Baghdad city.
Methods: This study was performed at the Chemistry and Biochemistry Department, College of medicine, Al-Nahrain University, Baghdad, Iraq from January 2009 to October 2009. The study consisted of 42 healthy women categorized into two groups premenopausal women (n= 22) aged 29.71±8.92 years and postmenopausal women (n=20) aged 53.33±4.61 years. Iron, total iron binding capacity (TIBC) were measured colorimetrically, ferritin levels were measured by enzyme linked immunosorbent assay (ELISA), ceruloplasmin level was estimated by radial immunodiffusion technique whereas malondialdehyde was quantified colorimetrically.
Results: Non-significant increase (P>0.05) in iron, ferritin levels, transferrin saturation percents in sera of postmenopausal women and non- significant increase (P>0.05) in total iron binding capacity (TIBC), transferrin, iron stores in sera of premenopausal women. Malondialdehyde increased non- significantly (P=0.44) in premenopausal women whereas ceruloplasmin increased significantly (P=0.000) in postmenopausal women group. On the other hand ferritin was correlated positively and significantly with body mass index (BMI) (r= 0.696; P < 0.05) and with ceruloplasmin levels (r=0.725; P<0.05) in pre- and postmenopausal women.
Conclusions: Hormonal changes which usually associated with menopause may be the cause of increased oxidative stress and consequent rise in ceruloplasmin levels as an antioxidant response
Key words: Iron, ferritin, transferrin receptor, malondialdehyde.

INTRODUCTION:

Ovarian failure occurs naturally between 45 and 55 years of age in American women [1]. When the depletion of oocytes and follicles occurs at the expected time, it is termed menopause [1]. Menopause is a natural inevitable event that results in elevation of FSH and LH levels with low levels of estrogen [1]. It is the permanent cessation of menstruation resulting from the loss of ovarian follicular function [2,3]. The major underlying pathophysiology of menopause is that loss of ovarian follicles. Ovarian primordial follicle number decrease steadily with increasing age up to about the age of 38, but their number then declines much during the last decade of reproductive life [2]. At the time of menopause itself, few if any follicles can be found in the ovaries. The ovary of the postmenopausal woman is reduced in size, weighs less than 2.5 g, and is wrinkled in appearance [2]. Iron is important in the human body because of its occurrence in many hemoproteins such as hemoglobin, myoglobin, and the cytochromes [4]. In the blood, iron is transported mainly bound to transferrin, each molecule of which binds two Fe^{+2} ions. Transferrin is normally about one-third saturated with iron. In tissues, iron is bound in ferritin and haemosiderin. Free iron is very toxic and protein binding allows iron to transported and stored in a non

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may require assessment when iron deficiency or overload is suspected or when the distribution or metabolism of iron is thought to be abnormal[5]. Plasma iron–binding capacity can be measured as a functional measurement of transferrin concentration, or transferrin can be measured by immunoassay and the iron –binding capacity calculated from it [5]. Knowing the plasma iron concentration, the transferrin saturation can then be calculated it is normally about 33% [5]. Measurement of plasma ferritin concentration is superior to plasma iron and iron –binding capacity for the assessment of body iron stores. In healthy individuals, plasma ferritin concentrations are usually within the range 20-300 µg/L [5]. The only known cause of a low concentration is a decrease in body iron stores. Concentrations less than 20 µg/L indicate depletion, and less than 12 µg/L suggest a complete absence of stored iron [5]. However, ferritin is an acute phase protein and patients with iron deficiency may have plasma ferritin concentrations within the reference range (e.g., up to 50-60 µg/L) when they are acutely ill [5]. Storage of iron occurs in most cells, but especially those of the liver, spleen and bone marrow. In these cells, the storage protein, apoferritin, forms a complex with iron (Fe^{+3}) known as ferritin. Normally, little ferritin is present in the blood. This amount increases, however, as iron stores increases. Therefore, the amount of ferritin in the blood is the sensitive indicator of the amount of iron in the body's stores. Iron can be drawn from ferritin stores, transported in the blood as transferrin, and taken up via receptor – mediated endocytosis by cells that require iron (e.g., by reticulocytes that are synthesizing hemoglobin) [6]. The transferrin receptor is a homo dimer with a molecular weight of 200 kDa. It spans the membrane and binds two molecules of transferrin. Genes responsible for transferrin and transferrin- receptor are on the chromosome 3 in humans. Transferrin receptor is present in almost all atoms of iron per minute [7]. Ceruloplasmin is a copper containing alpha-2 glycoprotein enzyme with a molecular weight of 160 kDa. It is synthesized in liver and contains six to eight copper atoms per molecule. Ceruloplasmin is also called ferroxidase, an enzyme which helps in the incorporation of iron into transferrin. Ceruloplasmin also has an important antioxidant action in plasma [8]. Also it is an acute- phase reactant. It is frequently elevated in inflammation, severe infection, and tissue damage and may be increased with some cancers [9,10]. Normal blood level of Ceruloplasmin is 25-50 mg/dL. It is estimated either by its oxidative property on phenylene diamine or by radial immunodiffusion [8]. This level is reduced in Wilson's disease, malnutrition, nephrosis and cirrhosis [8]. Chemical compounds and reactions capable of generating potential toxic oxygen species can be referred to as pro- oxidants. On the other hand, compounds and reactions disposing of these species, scavenging them, suppressing their formation, or opposing their actions are antioxidants and include compounds such as glutathione, ascorbic acid, Ceruloplasmin, and vitamin E [11]. In a normal cell, there is an appropriate pro-oxidant: oxidant balance. However, this balance can be shifted toward the pro-oxidants when production of oxygen species is increased greatly (e.g., following ingestion of certain chemicals or drugs) or when levels of antioxidants are diminished (e.g., by inactivation of enzymes involved in disposal of oxygen species and by conditions that cause low levels of the antioxidants. This state is called "oxidative stress" and can result in serious cell damage if the stress is massive or prolonged [11]. Iron is linked to the production of reactive oxygen species by two important reactions [11].:

1- Fenton reaction:

$$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + OH^{\cdot} + OH^{-}$$

2- Iron – catalyzed Haber – Weiss reaction:

$$O_2^{\cdot -} + H_2O_2 + Fe^{+2} \longrightarrow O_2 + OH^{\cdot} + OH^{-}$$

Oxidative stress is generally recognized as

degenerative diseases including atherosclerosis, hypertension, Alzheimer's disease, Parkinson's disease and cancer [12]. The aim of this study was to explore the association between iron status, Ceruloplasmin and oxidative stress in pre- and postmenopausal women in Baghdad city.

SUBJECTS AND METHODS:

Settings: This study was conducted at the department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad- Iraq between January 2009 to October 2009. The study design was approved by the Research Ethics Committee of Al-Nahrain Medical College.

Subjects: The study was performed on 22 premenopausal women with age (29.71 ±8.92) years and 20 postmenopausal women of (53.33±4.61) years old. Height and weight were recorded, and body mass index (BMI, $Kg/m^{2)}$ was calculated. None of women had cardiac, renal disease, diabetes or any endocrine disorder. No woman was taking any vitamin or food supplements, nor was on a weight reducing diet at the time of study.

Sampling: Non- fasting venous blood samples were drawn between 8:00 and 12:00 am. Blood samples were collected into plane Plastic tubes and centrifuged at 3000 rpm for 15 min at 4˚c and serum was separated for the assay laboratory parameters. Subject's serum was stored in - 70˚C until analysis.

Methods: The following tests were done: serum iron, serum ferritin, serum TIBC, serum transferrin, serum transferrin saturation, iron body stores, Ceruloplasmin and malondialdehyde (MDA). Serum iron was measured colorimetrically by releasing Fe^{+3} ions from binding proteins by acidification, reducing to Fe^{+2} by ascorbate and complexed with the color reagent (Ferrozine) [13]. Total iron binding capacity (TIBC) is determined by adding sufficient Fe^{+3} to saturate the binding sites on transferrin, with the excess iron removed by addition of $MgCO_3$ to precipitate any precipitated Fe^{+3}, the supernatant solution containing the soluble iron bound to proteins is analyzed for total iron content [13]. Serum iron and TIBC were measured using a commercial kit (Biomaghreb). The transferrin saturation percent was calculated by dividing serum iron over total iron binding capacity. Ferritin was measured by a solid phase enzyme linked immunosorbent assay (ELISA) method [13, 14]. Serum ferritin was measured using Biocheck commercial kit. Ceruloplasmin levels in this study were measured by a radial immunodiffusion method [10, 14]. Ceruloplasmin levels were measured using a BINDARID commercial kit (UK). Iron stores were estimated by the method of Cook et al.[15]. For women with serum ferritin concentrations above 12 µg/L, the method reduces to the following equation:

Iron stores (mg) = 400 x (ln SF – ln 12)

Where 400 is the proportionality constant, ln is the natural logarithm and SF is the serum ferritin in µg/L. all of the participants in this study had serum ferritin concentrations above 12 µg/L [16]. Lipid peroxidation was evaluated by the thiobarbituric acid reactive substances (TBARS) method. This method evaluated malondialdehyde (MDA) reactive products, the last product of lipid breakdown caused by oxidative stress. The optical density was determined at 532 nm [17].

Statistical analysis: The data of this study were analyzed by computer software program statistical package for social sciences (SPSS for windows, version 17, Chicago). Data were expressed as mean ± SD. Independent student's t test was used for data analysis. Correlations were assessed using Pearson correlation coefficient. Statistical tests were considered to be significant at the $P \leq 0.05$ level.

RESULTS:

Table (1) shows BMI and waist circumference (WC) in pre- and postmenopausal women. BMI values were

women than in premenopausal women group, whereas WC did not differ significantly (p=0.055) between the studied groups. From (Table 2), serum iron and serum ferritin were increased non-significantly (p=0.29, p=0.09 respectively) in premenopausal women, serum TIBC and transferrin also increased non-significantly (p=0.55, p=0.32 respectively) in premenopausal women while serum transferrin saturation percents was increased non-significantly (p=0.34) in (Table 3) illustrates the levels of ceruloplasmin and malondialdehyde in both studied groups. Malondialdehyde levels were increased non-significantly in premenopausal women (p= 0.44) while ceruloplasmin increased in a high significance level (p= 0.000) in postmenopausal women.Ferritin is correlated positively and significantly with BMI (r=0.696 ; P< 0.05) and with ceruloplasmin levels (r=0.725; P< 0.05) in pre and postmenopausal women as shown

Table 1: Study participants characteristics.

	Premenopausal women (n=22)	Postmenopausal women (n=20)	P-value
Age (years)	8.92±29.71	4.61±53.33	0.000
Body mass index (BMI) (Kg/m^2)	2.36±21.61	0.42±24.50	0.006
Waist circumference (WC)	5.78±70.22	11.32±86.75	0.055

Table 2: Serum iron, ferritin, TIBC, transferrin, transferrin saturation percents and iron body stores in studied groups.

	Premenopausal women (n=22)	Postmenopausal women (n=20)	P-value
Serum iron (µmole/L)	17.26 ±6.14	21.65 ± 6.30	0.29
Serum ferritin (ng/mL)	16.63 ±3.16	26.70 ±8.63	0.09
Serum TIBC (µmole/L)	56.6 ±8.92	52.06 ±12.91	0.55
Serum transferrin (g/L)	21.98 ±3.17	19.04 ±4.70	0.32
Serum transferrin saturation (%)	32.37±15.35	45.86±23.12	0.34
Iron body stores (mg)	153.47 ±82.38	301.76±144.55	0.13

Table 3: Ceruloplasmin and Malondialdehyde levels in pre- and postmenopausal women groups

	Premenopausal women (n=22)	Postmenopausal women (n=20)	P-value
Ceruloplasmin (mg/L)	**352.14 ± 51.03**	**530.0 ±38.96**	**0.00**
Malondialdehyde (µmole/L)	1.12 ± 0.48	0.86 ± 0.51	0.44

Statistically significant findings are highlighted in bold.

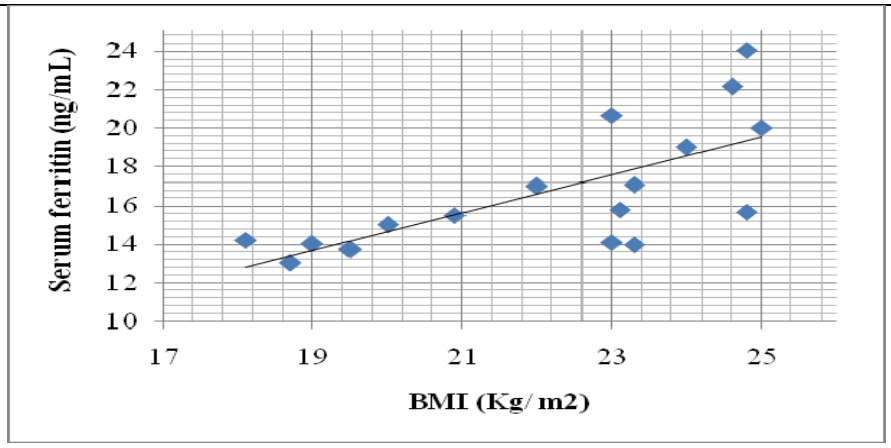

Figure 1: Scatter plot of serum ferritin levels versus BMI in pre- and postmenopausal women (r=0.696; P< 0.05).

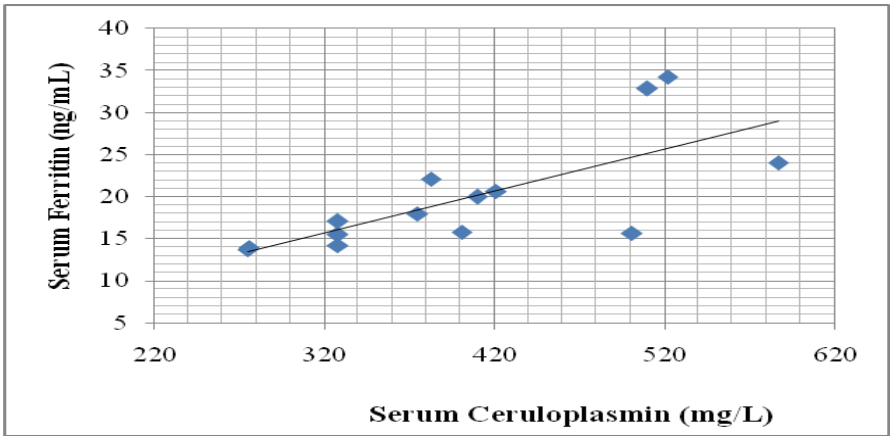

Figure 2: Scatter plot of serum ferritin levels versus ceruloplasmin in pre- and postmenopausal women (r=0.725; P< 0.05).

DISCUSSION:

Increasing susceptibility to oxidative stress is believed to be important in the development of aging and the pathogenesis of age- related diseases [18]. Iron is essential for life, but is also a highly reactive pro- oxidant that may be an important generator of oxidative stress [18]. It is generally considered that women, especially before menopause, have lower levels of iron stores than age- matched men [18]. Studies have shown that age is accompanied by increasing levels of serum ferritin [16]. This observation was in accordance with the results of this study. women may be attributed to that ferritin is an acute- phase reactant that increases in response to inflammation [19], and the age-associated increases in iron measured by serum ferritin may be confounded by the increased presence of chronic disease in the elderly [16,18]. Another explanation for high ferritin levels in postmenopausal women may be attributed to the "setpoint" hypothesis for iron stores. The setpoint theory states that healthy elderly individuals have different steady state levels of iron stores that are probably under genetic control. At steady state levels of iron stores, iron absorption is thought to be limited to amounts required

to replace basal losses. The age at which woman achieves her theoretical setpoint of iron stores may occur after her menopause. So postmenopausal women have no enough elapsed time to reach steady state levels of iron stores and therefore iron stores significantly increases in women with advancing in age [16,20]. The marginally significant relationship between iron stores or ferritin and body mass index is interesting and may be explained by that ferritin like C-reactive protein, is an inflammatory marker. Thus the positive association between BMI and plasma ferritin could be at least partly due to inflammation, which is linked to insulin resistance [21]. In this study the high significant increase (P=0.000) in serum ceruloplasmin levels of postmenopausal women was in different with findings reported previously by Johnson [22] who found that women during their menstrual years have higher ceruloplasmin levels mainly due to high estrogen levels which induce ceruloplasmin synthesis by the hepatic parenchymal cells [22]. This elevation of ceruloplasmin in postmenopausal women may be caused by the fact that ceruloplasmin like C- reactive protein (CRP) was acute phase protein which was elevated due to chronic inflammation associated with developing in age. Also there is an inverse association between ceruloplasmin and oxidative stress which was approved by this study this partly because that under physiologic conditions, ceruloplasmin is important in the control of membrane lipid oxidation probably by direct oxidation of cations, thus preventing their catalysis of lipid peroxidation and also because that cupric ions are reported to inhibit the production of singlet oxygen; this is of particular significance because of the latter's ability to cross the cell membrane and its high reactivity towards various biomolecules [22,23].

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