

Effects of menopause on serum oxidant status and lipid profile in Mosul city

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ABSTRACT

Objectives: To study the effect of menopause associated with estrogen deficiency on lipid peroxidation products, such as malondialdehyde (MDA) with evaluation of some antioxidants like, glutathione (GSH) and its relation to lipoprotein levels in women living in Mosul City.

Design: Case-control study.

Setting: The study was carried out in Al-Salam Teaching Hospital in Mosul City, during the period from January ٢٠٠٨ to April ٢٠٠٨.

Patients and Methods: A total of ٢٧ women aged ٢٠–٤٥ years were reported to be premenopausal and ٤٢ women aged ٤٥–٦٠ years were recorded to be postmenopausal. Blood samples were collected for both groups. The assessments of serum malondialdehyde (MDA), glutathione (GSH), estrogen, arylesterase, calcium, total cholesterol, triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) were done.

Results: There were significant increase in MDA level in women after menopause in comparison with premenopausal age. On the other hand, GSH, estrogen, arylesterase, and calcium levels were significantly decreased. In respect to lipids, total cholesterol, TG, and LDL-c, were significantly increased in opposite to HDL-c, in which was decreased significantly in postmenopausal women in relation to premenopausal subjects.

Conclusion: The increase of MDA and the decrease of antioxidants concentrations like glutathione, estrogen and HDL-c in postmenopausal women could contribute to acceleration of the cellular oxidative damage.

Keywords: Menopause, oxidative stress, antioxidants, malondialdehyde.

الخلاصة

أهداف البحث: دراسة تأثير سن اليأس المصاحبة لانخفاض الاستروجين في نواتج البيروكسدة المتمثلة بالمالونديالدهيد مع قياس بعض مضادات الأكسدة كالكلوتاتايون وعلاقتها بمستويات البروتينات الدهنية في النساء في مدينة الموصل.

التصميم: دراسة عينية مقارنة.

مكان إجراء البحث والإطار الزمني: نفذت الدراسة في مستشفى السلام التعليمي بمدينة الموصل، خلال الفترة من كانون الثاني ٢٠٠٨ ولغاية نيسان ٢٠٠٨.

المرضى وطرق العمل: تضمنت هذه الدراسة ٢٧ امرأة قبل سن اليأس تراوحت أعمارهم من ٢٠-٢٥ سنة، و ٤٢ امرأة بعد سن اليأس تراوحت أعمارهم من ٤٥-٦٠ سنة. أخذت عينات الدم. تم في المجموعتين قياس كل من مستويات مصل دم المالونديالدهيد والكلوتاتايون وهرمون الأستروجين والأريل أستريز والكالسيوم وكمية الكوليستيرول الكلي والشحوم الثلاثية والبروتين الدهني واطى الكثافة والبروتين الدهني عالي الكثافة.

النتائج: أظهرت نتائج هذه الدراسة وجود زيادة معنوية في مستوى المالونديالدهيد في النساء بعد سن اليأس مقارنة مع تلك المجموعة قبل سن اليأس. من ناحية أخرى، فقد انخفضت معنويًا مستويات كل من الكلوتاتايون وهرمون الأستروجين والأريل أستريز والكالسيوم. وفيما يتعلق بالدهون فقد ازدادت معنويًا مستويات كل من الكوليستيرول الكلي والشحوم الثلاثية والبروتين الدهني واطى الكثافة وانخفض البروتين الدهني عالي الكثافة في النساء بعد سن اليأس قياسًا للأفراد قبل سن اليأس.

الاستنتاج: إن زيادة المالونديالدهيد وانخفاض مستويات مضادات الأكسدة كالكلوتاتايون والأستروجين والبروتين الدهني عالي الكثافة في النساء بعد سن اليأس يمكن أن يساهم في تعجيل التخريب الخلوي التأكسدي.

Menopause is the time in a woman's life when her period stops. It usually occurs naturally, most often after the age of ٤٠. Menopause happens because the woman's ovary stops producing the hormones estrogen and progesterone.^١

The relative estrogen deprivation in postmenopausal women is associated with physiological changes and increased risk of several diseases, including cardiovascular diseases.^٢ Estrogen has beneficial effects on the lipid profile, such as significant elevations in high density lipoprotein cholesterol and reductions in low density lipoprotein cholesterol reported.^٣ However, the available evidence suggests that in vivo physiological concentrations of estrogen may have a modest antioxidant activity.^٤

Oxidative stress has been implicated in the pathogenesis of ageing and menopause, and can arise through the increased production of lipid peroxides representing by malondialdehyde (MDA) level and/or a deficiency of antioxidant defense.^٥ On this bases, and because there are no previous reports concerning MDA levels in postmenopausal women related to premenopausal age; the study was planned to investigate the effects of the menopause on serum MDA concentration and antioxidants, including estrogen level and glutathione, in addition to lipid profile parameter in women living in Mosul City.

Subjects and methods

Experimental Design

A total of ٢٢ women aged ٢٠-٤٠ years were reported to be premenopausal and ٤٢ women aged ٤٠-٦٠ years were recorded to be postmenopausal from January ٢٠٠٨ to April ٢٠٠٨. Informations on menopausal status was based on self-report. Women who were premenopausal were asked whether their menstrual periods had stopped during the past year and, if yes, when their last period was and what the reason was (naturally, by surgery, or by radiation/medication). Generally, the onset of natural menopause was defined as the age at the last menstrual period prior to stopping menstruation for ١٢ months.^٦ Accordingly, women who had undergone a spontaneous menopause as demonstrated by amenorrhea volunteered to participate in this study as postmenopausal women.^٧

This study was conducted in Al-Salam Teaching Hospital, Mosul City, and approved by the local institutional review board, and all of the participants provided written informed consent. None of the patients had a history of

previous hormone replacement therapy, and smokers, diabetics, patients with chronic inflammatory conditions, hepatic or respiratory disease, and those on antioxidants, vitamin or other medication were excluded from the study. Each woman underwent a comprehensive history and physical examination including a standard electrocardiogram.

Blood sampling and analysis

All blood samples were collected after a ١٢-hour overnight fast. Venous blood samples from each woman obtained from the antecubital vein between ٨-١١ a.m. was drawn into plain test tubes. After centrifugation of the blood for ١٠ minutes at ٣٠٠٠ g, the serum obtained was stored in several aliquots at -٢٠ C^٨ until assay. The readings of the measured parameters were done at clinical biochemical laboratory in Mosul Technical Institute.

Oxidant-antioxidant serum capacity

To assess the association between lipid peroxidation and the menopause, the level of serum MDA was determined by a modified procedure using the thiobarbituric acid reaction substance (TBARS) methods.^٩ Serum glutathione (GSH), on the other hand, was determined by a modified procedure utilizing Ellman's reagent.^{١٠}

Serum estradiol

Estrogen hormone was analyzed by MINIVIDAS analyzer for the quantitative measurement of ١٧β-estradiol in serum, using the ELFA technique (Enzyme Linked Fluorescent Assay).^{١١}

Serum calcium

Serum total calcium was determined colorimetrically, without deproteinization, using a kit purchased from Syrbio (Cat No. ٢٤٠٣). The absorbance of standard and samples against blank were measured at ٦١٢ nm.^{١٢}

Measurement of arylesterase activity

Measurement of arylesterase activity in serum was performed using the colorimetric method at ٢٢٠ nm.^{١٣}

Serum lipid profile

Serum levels of total cholesterol,^{١٤} triglycerides (TG),^{١٥} and high-density lipoprotein cholesterol (HDL-c)^{١٦} were measured by enzymatic colorimetric methods with a spectrophotometer according to the manufacturer instructions. The concentration of low density lipoprotein cholesterol (LDL-c) was calculated according to the following formula:^{١٧}

$$C\ LDL = C\ serum - C\ HDL - TG/5$$

Where, C = concentration of cholesterol in LDL, HDL, in serum, expressed in mg/dl.

TG = serum triglycerides concentration, expressed in mg/dl

Statistical analysis

Data are presented as mean ± standard deviation of the mean (SDM). Statistical analysis was performed by Independent-Samples t-test with SSPS version 10.0 software. A value of P<0.001 indicates statistical significance.

(P<0.001) in MDA concentration when compared with premenopausal women. On the other hand, women after menopause showed significant decrease (P<0.001) in glutathione content in comparison with those before menopause, as shown in Figure 1.

Estrogen and associated factors

The levels of measured parameters, estrogen, arylesterase, and calcium of both groups before and after menopause are depicted in table 1. Serum estrogen, arylesterase, and calcium levels of the women in the study group after menopause were significantly lower (P<0.001) than in the other before menopause.

Results

Oxidants-antioxidant capacity of serum

The effect of menopause on lipid peroxidation by-product, MDA is shown in Figure 1. Menopause produced significant increase

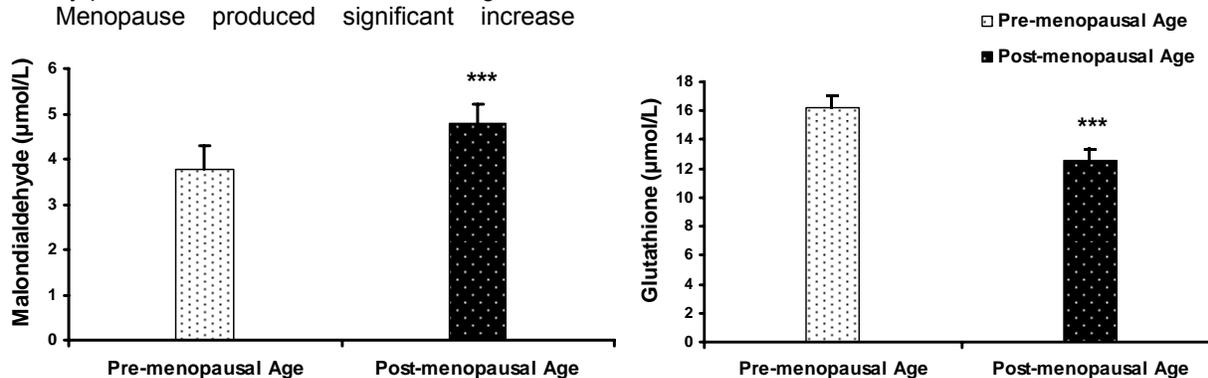


Figure 1. Effect of Menopause In Malondialdehyde (a) and Glutathione Level (b). Data are means ± SD from 27 women for pre-menopausal age, and 27 women for post-menopausal age. The stars on histograms indicate significant difference at p ≤ 0.001.

Table 1: Effects of Menopause In Estrogen Level And It's Related Factors.

Parameters Treatments	Estrogen	Erylesterase	Calcium
	(pg/ml)	(μ/ml)	(mmol/L)
Pre-Menopausal Age (n = 27)	21.6 ± 4.222	133.31 ± 20.261	2.31 ± 0.317
Post-Menopausal Age (n = 27)	*** 14.51 ± 1.191	*** 87.49 ± 7.421	*** 1.79 ± 0.228

- Values are expressed as means ± SD.
- *** Significantly different as compared with respective control at (p<0.001)

Table 2: Effects of Menopause In Lipid Profile

Parameters Treatments	Total Cholesterol	Triglyceride	Low-Density Lipoprotein Cholesterol	High-Density Lipoprotein Cholesterol
	(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)
Pre-Menopausal Age (n = 27)	4.07 ± 0.068	1.30 ± 0.187	3.39 ± 0.008	0.86 ± 0.008
Post-Menopausal Age (n = 22)	*** 6.36 ± 0.073	*** 2.78 ± 0.013	*** 0.26 ± 0.067	*** 0.73 ± 0.007

- Values are expressed as means ± SD.
- *** Significantly different as compared with respective control at (p<0.001)

Lipid profile

Serum lipids are reported in Table 2. In postmenopausal women, serum total cholesterol, TG, and LDL-c levels were significantly higher (p<0.001) than in premenopausal women. Conversely, HDL-c level was decreased significantly in postmenopausal women when compared with women before menopause.

Discussion

The higher prevalence of diseases in postmenopausal women could be related to an increased oxidative stress, which is due to unbalanced pro-oxidant/antioxidant Equilibrium. As a consequence of oxidative stress, lipid peroxidation (a major indicator of oxidative stress) can occur. In this study, measured serum MDA as a marker of free radical-mediated lipid peroxidation. MDA was increased significantly after menopause compared to premenopausal levels. This is in line with results by Asada et al. which showed that serum lipid peroxide levels of

normal premenopausal women were lower than those of normal postmenopausal women, and that bilateral ovariectomy significantly increases serum lipid peroxide in premenopausal women.

On the other hand, a significant decrease in serum GSH concentration was noticed in women after menopause in comparison with premenopausal age. This indicates the presence of oxidative status, which may alter a variety of cell body functions. The alterations of serum GSH system components in women after menopause may be in part explained by the changes in the GSH-related enzyme levels. Lipid peroxides are quickly metabolized to a less toxic hydroxy derivative by glutathione peroxidase (GSH-PX), which uses glutathione as a proton donor to reduce hydrogen peroxide to water with the production of glutathione disulfide (GSSG). GSSG is recycled back to GSH by glutathione reductase using NADPH from the hexose monophosphate shunt. (Figure 2).

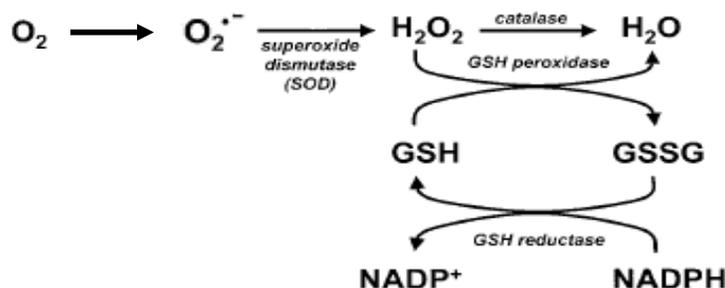


Figure 2: Pathways of reactive oxygen species (ROS) production and clearance. GSH, glutathione; GSSG, glutathione disulfide.

Estrogens, like GSH, have a substantial capacity to inhibit lipid peroxidation caused by free radicals.^{١١} Therefore, estrogen has been reported to have antioxidant capabilities,^١ and the circulating concentrations of estrogen decreased, as reported in the present study. The relative estrogen deprivation in postmenopausal women is associated with physiological changes and increased risk of several diseases, including cardiovascular disease,^١ which is presumably mediated through increased oxidative stress as assessed by the enhanced free radical superoxide production in the vessel wall.^{١٢}

The study of the present work was supported by Massafra et al.^{١٣} who suggested that a lack of ovarian estrogen production in women with amenorrhoea, was associated with reduced protection against oxidative stress.

One of the most important factor that make to facilitate the anti-oxidative action of estrogen and high density lipoprotein-cholesterol is arylesterase/Paraoxonase enzyme.^{١٤} To be able to function as potential antioxidants, estrogens first need to be converted to estrogen esters in a reaction catalyzed by plasma lecithin/cholesterol acyltransferase. After esterification, estrogens are able to incorporate in the lipoprotein particles. By this way, estrogen could provide antioxidant protection.^{١٤}

Human serum arylesterase is able to hydrolyze lipid hydroperoxides and organophosphates which include neurotoxins that are widely encountered in the diet and household,^{١٥} and to delay or inhibit the initiation of oxidation induced by metal ions on lipoproteins.^{١٦}

In the present study, serum arylesterase enzyme activity was decreased in association with a significant decrease of estrogen level after menopause. Our findings are in good agreement with a recently published study that showed, a more potent reduction of serum arylesterase enzyme activity after surgical menopause, whereas estrogen replacement therapy reverses these effects.^{١٧} Moreover, Sutherland et al.^{١٨} showed that arylesterase activity is abnormally low in postmenopausal women with type ٢ diabetes and increases during estrogen replacement therapy.

In addition to antioxidant action of estrogen, both estrogen and calcium have been shown to be beneficial in preventing osteoporosis in postmenopausal women. Therefore, women over the age of ٧٠ yr are at greatest risk for osteoporotic fractures.^{١٩}

Estrogen replacement therapy (ERT), on the other hand, has been associated with slower bone loss, and reduced fracture incidence in postmenopausal women.^{٢٠} Calcium supplementation has also been shown to reduce bone loss in postmenopausal women.^{٢١} Furthermore, Prestwood et al.^{٢٢} demonstrated that treatment with a combination of these two agents may be more effective in preserving bone mass than treatment with either estrogen or calcium alone.

To further explore the role of estrogen on serum calcium level, Nordin et al.^{٢٣} suggested that estrogens promote tubular reabsorption of filtered calcium in postmenopausal women particularly in those with osteoporosis. Therefore, menopause has been shown to be accompanied by an increase in calciuria.^{٢٤} In addition, estrogen has been shown to stimulate the active form of vitamin D $1,25(OH)_2D_3$, which in turn increases calcium intestinal absorption.^{٢٥} Moreover, calcium modulates estrogen metabolism, and increasing calcium intakes are associated with increasing concentrations of estrogen metabolites.^{٢٥}

There have been few reports on a relationship between calcium deficiency and antioxidant enzymes. A reduction in calcium level in the present study after menopause, appears to be associated with a decrease in antioxidants such as glutathione. Unlike to the present study, Itoh et al.^{٢٦} demonstrated that dietary calcium restriction increased the activities of certain antioxidant enzymes like superoxide dismutase (SOD), and GSH-PX in rat diaphragm but not of catalase. This suggests that dietary calcium restriction imposes oxidative stress in rat diaphragm.

The last part in this study concerned with lipid profile. In postmenopausal women, significantly increase in serum total cholesterol, TG, and LDL-c levels were observed in the present study. Conversely, HDL-c level was decreased significantly in postmenopausal subjects when compared with women before menopause.

Initial studies were done to determine the significance of the association of estrogens with lipoproteins on their antioxidant property. Cardiovascular disease is the leading cause of death in postmenopausal women over the age of ٥٠.^{٢٧} On the other hand, estrogen administration^{٢٨} caused a reduction in cardiovascular disease by ٥٠%. At least part of the cardiovascular benefits of estrogens are mediated through changes in lipoproteins,^{٢٩} particularly by increases in high density lipoproteins (HDL). However, ٧٥% of the beneficial effect of postmenopausal estrogen

therapy is not due to improvements in circulating lipid levels but may involve antioxidant protection.^{٤٧}

Recent studies have shown that estrogens inhibit oxidation of LDL-c in vitro and protect against DNA oxidative damage induced by hydrogen peroxide and arachidonic acid.^{٤٨} This can be achieved by inhibiting the generation of superoxide radicals and at higher concentration can scavenge hydroxyl radicals.^{٤٩}

In contrast to LDL-c, Abplanalp et al.^{٤٧} provide evidence that HDL could facilitate the antioxidant effect of estradiol-١٧β(E٢) through initial association, esterification and eventual transfer of E٢ esters to LDL. Furthermore, several lines of evidence suggest that the antioxidant effect of HDL is at least partially related to paraoxonase (PON), an enzyme associated with HDL surface (HDL-PON).^{٤٨}

In conclusion, oxidative stress and oxidation of lipoproteins and membranes have been implicated in the development of many diseases and ageing. On the basis of the present study, it might be hypothesized that the increase of MDA and the decrease of antioxidants activity like GSH, estrogen and HDL in postmenopausal women could contribute to acceleration of the cellular oxidative damage.

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