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Lipid Peroxidation and Lipid Metabolism in Postmenopausal Women

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Abstract: The aims of this study were to determine the degree of lipid peroxidation in the sera of postmenopausal women and compare this with the lipid peroxidation of a premenopausal group, and to see if there was any correlation between lipid parameters and lipid peroxidation within the groups. We assayed the lipid profiles and malondialdehyde (MDA), a marker for lipid peroxidation, in a postmenopausal group (n=57, mean age=49.4±6.6), an age-matched male group (n=21, mean age=48.5±6.7) and a control group (n=31, mean age=27.7±5.2). MDA was measured as thiobarbituric acid reactive substances (TBARS) using a modified version of Yagi's method. MDA was significantly higher ($p<0.001$) in the postmenopausal group (2.61±0.69 nmol/ml) than the control group (1.66±0.79 nmol/ml) and the age-matched male group (1.35±0.4 nmol/ml). There was a significant correlation between age and lipid peroxides in the women (r=0.665) but not in the men. However, the correlation between lipid peroxides and total cholesterol, as well as triglycerides, HDL- and LDL-Cholesterol were not statistically significant within the two groups. This study suggests that aging and lack of oestrogen may be responsible for the increase in the MDA levels of postmenopausal women. Further studies should be carried out to explain the higher levels of MDA in postmenopausal women and assess the effectiveness of treatment with diet and hormone replacement therapy both with and without antioxidant supplementation.

Key Words: Malondialdehyde, postmenopausal women, lipid peroxidation.

Introduction

Coronary artery disease (CAD) is the leading cause of death in women as well as men. Risk factors such as hyperlipidemia, smoking, hypertension and diabetes account for less than 5% of the incidence of CAD. This suggests that there are a number of major CAD risk factors still to be identified (1). More postmenopausal women have high levels of plasma cholesterol than men of the same age (2). Hypercholesterolemia has potential importance as a major risk factor for CAD in postmenopausal women. In addition, the major component of the plasma total cholesterol that increases the risk of CAD is the cholesterol in LDL (3).

Several authors have suggested that the peroxidative modification of LDL is an important factor in the formation of atherosclerotic changes. This might help to explain the frequent occurrence of disease in normolipidemic people (4, 5, 6). These suggestions lead to the hypothesis that lipid peroxide may impair various functions of the endothelial cells and cause primary lesions in the vessel wall. Studies have demonstrated that lipid peroxidation is a complex process associated with a number of pathologic phenomena, such as increased membrane rigidity, atherosclerosis, carcinogenesis and myocardial infarction (7). Malondialdehyde (MDA) production is recognized a a marker for lipid peroxide and as an end-product of peroxidation (8).

The aim of this study was to determine the degree of lipid peroxidation in the sera of postmenopausal women and compare it with those of a premenopausal (control) group and a male group and see if any correlation existed between lipid parameters and lipid peroxidation within the groups.

Materials and Methods

Blood samples for the measurement of triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and blood MDA were taken from consenting women and men

	Premenopausal women (control) (n=31)	Postmenopausal women (n=57)	Age-matched male group (n=21)
Age	27.7±5.2	49.4±6.6	48.5±5.7
T.cholesterol (mg/dl)	178.3±41.6	220.4±42.8*	184.2±36.7
Triglyceride (mg/dl	117.8±31.2	121.6±28.7	140.2±31.8
HDL-cholesterol (mg/dl)	44.5±8.3	50.0±11.6	46.9±9.0
LDL-cholesterol (mg/dl)	82.0±28.5	145.2±35.0**	110.1±34.0
MDA (nmol/ml)	1.66±0.79	2.61±0.69***	1.35±0.4

Table 1. Significant differences between postmenopausal group and premenopausal and age-matched male groups are indicated by: *(p<0.001), **(p<0.001), ***(p<0.001)

Parameter	T.cholesterol < 200 mg/dl (n=17)	T.cholesterol > 200 mg/dl (n=40)
Total cholesterol (mg/dl)	171.5±27.0	240.9±26.9
MDA nmol/ml	2.64±0.71	2.59±0.70

Parameter	Triglyceride < 150 mg/dl (n=43)	Triglyceride > 150 mg/dl (n=43)
Triglyceride (mg/dl)	97.4±26.2	194.2±46.6
MDA nmol/ml	2.58±0.76	2.64±0.56

Parameter	HDL-chol. > 35 mg/dl (n=54)	HDL-chol. < 35 mg/dl (n=3)
HDL-cholesterol (mg/dl)	50.9±9.5	32.6±2.3
MDA nmol/ml	2.53±0.55	2.66±0.62

Parameter	LDL-chol. < 150 mg/dl (n=34)	LDL-chol. > 150 mg/dl (n=23)
LDL-cholesterol (mg/dl)	122.4±19.7	177.9±427.2
MDA nmol/ml	2.50±0.58	2.71±0.64

Table 2. No correlation between MDA and total cholesterol or triglyceride and HDL-cholesterol and LDL cholesterol values.

after fasting overnight. The following clinical points were recorded: age, history of diabetes mellitus, liver and renal disease, family history of ischaemic heart disease or peripheral arterial disease and medication. Those with histories of metabolic disorders, abnormal liver function and renal failure, essential hypertension or myocardial infarction were excluded from the control and male groups as well as from the postmenopausal group.

The subjects were grouped as follows:

- premenopause group (n=31, mean age=27.7±5.2)
- postmenopause group (n=57, mean age=49.4±6.6)
- age-matched male group (n=21, mean age=48.5±6.7)
- male group 1 (n=21, mean age=40.0±5.9)
- male group 2 (n=18, mean age=61.5±5.8)

Lipid Measurements

-Lipid peroxides

MDA concentrations were measured as TBARS (Thiobarbiuric Acid Reactive Substances) according to a modified version of Yagi's (9) and Satoh's (10) methods. In brief, 0.5 ml serum was mixed with 4 ml 1/12 N H_2SO_4

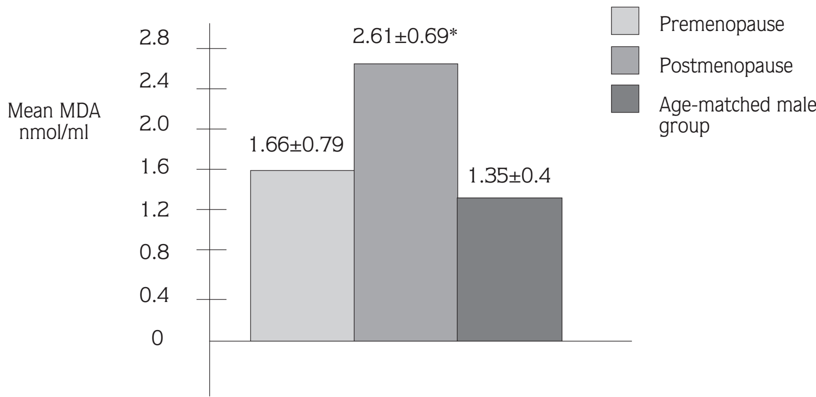

Figure 1. The mean values of MDA for premenopause, postmenopause and age-matched male groups *(P<0.001) Postmenopause group versus premenopause and age-matched male groups

mg/dl

	T.cholesterol	Triglyceride	HDL-cholesterol	LDL-cholesterol
Premenopause	178.3±41.6	117.8±31.2	44.5±8.3	82.0±28.5
Postmenopause	220.4±42.8	121.6±28.7	50.0±11.6	145.2±35.0
Age-matched male group	184.2±36.7	140.2±31.8	46.9±9.0	110.1±34.0

Figure 2. The mean values of t.cholesterol, triglyceride, HDL and LDL-cholesterol for premenopause, postmenopause and age-matched male groups.

in a centrifuge tube and shaken gently. After 0.5 ml 10% phosphotungstic acid was added to the tube and it was left to stand at room temparature for 5 minutes, the mixture was centrifuged at 3.000 rpm for 10 minutes. The supernatant was discarded and the sediment was mixed with 2.5 ml 1/12 N H_2SO_4. The centrifugation was repeated and the supernatant was discarded again. The sediment was resuspended in 2.5 ml 1/12 N H_2SO_4 and in fresh 3 ml 0.2% thiobarbituric acid reagent, mixed thoroughly and heated in a bath of boiling water for one hour. After cooling in cold water, the resulting chromogen was extracted with 3.0 ml n-butyl alcohol by vigorous shaking. The organic phase was separated by centrifugation at 3000 rpm for 10 minutes, and its absorbancy was recorded at a wavelength of 530 nm. The level of absorbancy was converted into nmol/ml MDA from a standard curve generated with 1,1,3,3-tetraethoxy-propane (SIGMA). The standard curve was linear up to 10 nmol/ml malondialdehyde. The intra-assay precision of this method was 3.1% (n=10) and the interassay precision was 6.4% in three differet assays.

Total cholesterol and triglycerides

These were determined using enzymatic methods, using bioMérieux kits in a Dacos analyzer.

HDL, LDL-cholesterol

HDL-cholesterol was measured with the colorimetric method using the Biocon test. LDL-cholesterol was calculated with the Friedwald formula.

Statistical Analysis

The means and standard deviations were calculated for each parameter. Student's unpaired t-test and the Mann Whitney-U test were used in the statistical analysis.

Results

The results are summarized in Tables 1 and 2 and Figures 1 and 2. The total cholesterol, LDL-cholesterol and MDA levels were significantly higher in the postmenopausal group than in the control and age-matched male groups (Table 1). There was no significant difference between the triglyceride and HDL-cholesterol levels of the three groups. MDA was positively correlated with total cholesterol (r=0.572) and LDL-cholesterol in the women (r=0.690). There was also a significant correlation between the age means of the women and the mean lipid peroxide values (r=0.665). However, this was not true for the men. No significant positive correlation was observed between the triglyceride and total cholesterol values in the postmenopausal group or the controls. The postmenopausal group had normal and pathological values in terms of lipid parameters, and no significant correlation was observed between MDA and total cholesterol, or triglycerides, and HDL-and LDL-cholesterol values (Table 2). Although there was a mean age difference of 21.5 years between the two male groups (40.5±5.9 vs 61.5±5.8), no statistically significant difference was found in the MDA levels of the two groups (1.32±0.3 vs 1.45±0.3 nmol/ml).

Discussion

Atherosclerosis is a multifactorial disease which is caused by both genetic and environmental factors. Hyperlipidemia, particularly hypercholesterolemia, is generally accepted as a risk factor for CAD. However, some authors suggest that not only increased concentrations of total or LDL-cholesterol, but also increased concentrations of lipid peroxides in the blood may be risk factors for atherosclerosis (5).

Incerased lipid peroxides may inhibit the formation of prostacyclin in the endothelia, which in turn may lead to an increase in platelet aggregation. They also reduce the activity of antithrombin II, and thus contribute to the acceleration of blood clotting processes. Lipid peroxides may cause oxidative modifications of lipoproteins, particularly LDL. Oxidized LDLs are effective ligands for macrophage scavenger receptors. Through these receptors, the macrophage can be overloaded with cholesterol to form foam cells. Oxidized LDLs are chemotactic for smooth muscle cells, drawing them into the intima. They also inhibit the outward migration of macrophage foam cells from subendothelial space, accounting for the accumulation of those cells within growing altheromas (1, 12).

Lipid peroxides are derived from the oxidation of polyunsaturated fatty acids and their esters and are capable of further lipid peroxide production by a free-radical chain reaction. MDA, which is one of the products of lipid peroxidation, has been the most extensively studied marker. Of the human plasma lipoproteins, low density lipoproteins are the most susceptible to peroxidation (11, 12), and this process results in many alterations in their composition and biological properties. The effects of lipid peroxides; endothelial cell damage, uncontrolled lipid uptake by macrophages, reduced endothelial prostacyclin synthesis and associated thrombogenicity, are strongly implicated in the pathogenesis of atherosclerosis (13).

In this study we measured MDA as TBARS

(Thiobarbituric Acid Reactive Substances). This method has been criticized for its lack of specificity, but it is one of the easiest and most frequently used methods. Moreover, there is a good correlation between the results obtained using Yagi's method and those achieved through HPLC (High Performance Liquid Chromatography) (14, 15).

There is a tendency for postmenopausal women to have hypercholesterolemia, which is a risk factor for CAD. It has been reported that hypercholesterolemia increases the level of oxygen-free radicals (OFRs), which would cause endothelial damage and establish a basis for the development and maintenance of atherosclerosis. The aim of this study was to determine the lipid peroxides, and thus shed light on the causes of atherosclerosis so that postmenopausal women liable to develop atherosclerotic disease can be monitored and strateiges for prevention.

The results of the study show that sera lipid peroxide (MDA), total cholesterol and LDL-cholesterol concentrations were significantly higher in the postmenopausal women, than in the premenopausal group ($p<0.001$). This could be due to a decrease in the activity of the scavenger system and a reduction in the number or activity of LDL receptors with age (23, 24). The MDA levels had a positive correlation ($r=0.665$) with age in the women when the means of the MDA and the ages of the two groups of women were considered. However, there was no correlation between MDA levels and age in males. Increased lipid peroxide levels in sera or plasma with aging have been reported by some authers (5, 10). Some other authors have reported that there is no correlation between MDA and age (4, 17). This supports the findings for men in this study. In addition, the MDA levels in the age-matched male group were significantly lower than in the postmenopausal group. These results support the claim that risk factors for CAD in women and the elevation of MDA in the postmenopausal group could be related to oestrogen deprivation in menopause rather than aging within the age range of this study group (25, 26). The results of a new study which will deal with women of the same age in both groups (premenopausal and postmenopausal) may explain whether aging or the menopause itself is responsible for increased lipid peroxidation in the postmenopausal period.

A significant correlation was found between MDA levels and total cholesterol, LDL-cholesterol and age. The mean age values of the control group and postmenopausal group were significantly different. To determine whether MDA had any correlation with t. cholesterol and LDL-cholesterol within the postmenopausal group, the group was divided into two subgroups according to normal and pathologic t.cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol values. However, no statistically significant correlations were found between lipid peroxide and t.cholesterol, or triglycerides and HDL-and LDL-cholesterol, although the results of some studies suggest that hypercholesterolemia increases the level of oxygen-free radicals (OFR). This discrepancy can be attributed to the LDL-cholesterol and total cholesterol values, which remained in the normal range in these study groups.

Some epidemiologic studies suggest that hormone replacement therapy (HRT) can be used in postmenopausal women with high LDL-cholesterol (2, 25). By lowering LDL-cholesterol and perhaps also lipid peroxides, the use of oestrogen can reduce the risk of CAD. Oestrogen therapy enhances receptor expression and thus reduces the need for drug therapy (19). However, patients with very high LDL-cholesterol levels should also be treated with drugs.

The results of this study suggest that aging and lack of oestrogen may be responsible for the increase in MDA levels. Further studies should be carried out to explain the increased lipid peroxidation in postmenopausal women and assess the effectiveness of treatment with diet and hormone replacement therapy, both with and without antioxidant supplementation.

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