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Research Article

A Correlation Study between Lipid Peroxidation and Dyslipidemia in Postmenopausal Women

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Abstract: In spite of having lower incidence of coronary artery disease (CAD) in the reproductive age group, the incidence of CAD among postmenopausal females and age matched males are almost the same. This is due to alteration in the lipids and lipoproteins levels during postmenopausewhich contributes to development of CAD. Recent studies have shown that oxidative stress plays an important role in development of CAD. Increased production of reactive oxygen species (ROS) leads to increased lipid peroxidation resulting in dyslipidemia. Malondialdehyde (MDA), a marker of lipid peroxidation, is thought to play an important role in development of atherosclerosis. In this view, this case control study was undertaken to determine the MDA levels in the membrane of erythrocytes of postmenopausal women and to study the relationship between increased lipid peroxidation and degree of dyslipidemia. Fasting lipid profile, erythrocyte MDA levels were determined in 50 women in late reproductive phase and 50 postmenopausal females. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) 17.0 version (SPSS Inc., Chicago, IL, USA). This study showed that the MDA levels were significantly increased in postmenopausal women ($p < 0.01$). This increase showed a significant strong positive correlation with increasing TC/HDL ratio($r = 0.653$, $p < 0.05$). Therefore, increase oxidative stress during menopause may contributes to the development of cardiovascular complications. Therefore, measuring the MDA level during the postmenopausal phase would help to early diagnosis and prevention of life threatening complication.
Keywords: Postmenopause, Malondialdehyde, Dyslipidemia, Atherogenic index.

INTRODUCTION

Oxidative stress, defined as a measure of steady levels of reactive oxygen species (ROS) or oxygen radical in the biological system, is a resultant of overproduction of free radicals i.e. reactive oxygen species (ROS), which exceeds the body's antioxidant defense mechanisms. Normally, antioxidants neutralize ROS and thus help to prevent over exposure from oxidative stress [1]. Lipids are among the primary targets of oxidative stress [2]. Excessive ROS production results in lipid peroxidation of the cellular structures which is thought to play an important role in pathogenesis of many degenerative diseases, such as atherosclerosis, oxidative damage to DNA, aging, carcinogenesis, and atherosclerosis predisposing to development of Coronary Artery Disease (CAD) [3]. Incidence of CAD in women in reproductive age group is less when compared with men [4]. These women seem to tolerate classical cardiovascular (CVD) risk factors such as smoking, high levels of cholesterol, hypertension, and excessive weight better than men do [4, 5]. During menopause, the levels of lipids and their transporting lipoproteins are altered that results in an increase in the incidence of CAD [4]. Women undergoing surgical menopause or experiencing premature ovarian failure and not treated for estrogen deficiency have a two-fold risk of CAD than the women attaining menopause by around 50 years [6]. All this has been seen as an evidence that estrogens protect women from CAD. Moreover, estrogen is shown to have antioxidant property and hence deficiency of estrogen predisposes them to oxidative damage [7, 8]. The medical problems and health care of women have gained increasing attention in recent years and studies have reported the alterations in antioxidant status in postmenopausal women. In view of this, this study was carried out to determine the degree of lipid peroxidation in postmenopausal women and compare it with those in reproductive age group and see if any correlation existed between alteration in lipid parameters and lipid peroxidation within the groups.

Malondialdehyde (MDA), also referred to as TBARS (TBA Reacting Substances), is one of the lipid peroxidation products frequently used to determine the oxidant/antioxidant balance in individuals as they are stable and easily measurable [3].

MATERIALS AND METHODS

Study population

This case control study was carried out at Rohilkhand Medical College and Hospital, Bareilly on patients attending Gynaecology OPD for treatment of menopausal complains. The study was approved by the Institutional Ethics Committee and consent was obtained from the patients to participate. 100 patients between 35 to 55 years of age were included in the study. Based on the menstrual history and STRAW +10 criteria, they were divided into 2 groups: Group I (late reproductive phase group) with regular menstrual cycles and Group II (postmenopause group) with amenorrhoea over a year [9]. Each group had 50 participants. Patients with undiagnosed vaginal bleeding, cardiac disease, liver disorder, diabetes mellitus, breast tumour or other malignancies, history of stroke or transient ischemic attack, history of hormonal treatment or soya bean derived products in previous 12 months were excluded from the study.

Biochemical analysis

A venous blood sample was collected after a fasting period of 10 – 12 hours. Then the sample was centrifuged and serum was used for the analysis. Determination of the lipid profile was carried out by Erba 360 auto analyzer using enzymatic methods such as CHOD-PAP method for total cholesterol (TC), GPO-PAP method for triglycerides (TG) and precipitation method for High Density Lipoprotein – Cholesterol (HDLC-C) and Low Density Lipoprotein – Cholesterol (LDL-C).

Determination of Malondialdehyde

Erythrocyte MDA concentration was determined using the method described by Jain *et al*. [10].

Preparation of malondialdehyde standard

Standard Malondialdehyde was prepared from 1, 1, 3, 3- tetramethoxy propane or MDA bisacetal ($C_{11}H_24O_4$, 99%, Mol. Wt. 164.2, density 0.997). Fresh standards were prepared. 0.166 ml of MDA bisacetal was made up to 100 mL with 1% H_2SO_4. It was mixed and kept at room temperature (in dark) for 2 hours. This gave 10mM MDA solution, which was further diluted with 1% H_2SO_4 to get solutions with different MDA concentrations [32].

Procedure

Blood was collected in the EDTA (Ethylenediaminetetraacetic acid) tubes and was centrifuged at 2000g for 7 minutes. Plasma and the buffy coat were discarded. Then the cells were washed with cold 0.15M NaCl solution for two times after 1 - 10 dilutions. The volume of the packed cell was seen with a graduated centrifuge tube or an ordinary pipette with a wide tip. 0.2 ml of packed cells was suspended in 0.8 ml phosphate buffer saline (PBS) and 0.025 ml butylated hydroxy toluene (BHT). To this 0.5ml of 30% tricholroacetic acid (TCA) was added. Then the tubes were vortexed and were allowed to stand on ice for 2 hours. Then centrifugation was done at 2000 for 15 minutes. One ml of the supernatant was transferred into another tube and 0.075 ml of 0.1 M EDTA and freshly prepared 0.25ml of 1% thiobarbituric acid (TBA) were added. Contents were mixed and kept in a boiling water bath for 15 minutes. After that the tubes were cooled to room temperature. Absorbance was measured at 532 nm and 600nm using spectrophotometer. Absorbance at 600nm was subtracted from absorbance at 532 nm. TBARS value was calculated from the standard graph and expressed as nanomoles/gram of hemoglobin [32].

Statistical analysis

All values of analyzed parameters were expressed as mean ± S.D. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 17.0 version (SPSS Inc., Chicago, IL, USA). As the data showed normal distribution, independent t test was used to compare the mean values in the two groups. Pearson's correlation was applied to correlate between the parameters. A p-value of <0.05 was considered statistically significant.

RESULTS

The mean age of women in the late reproductive group was 36.49 ± 5.41 years and 46.91 ± 3.67 years in the postmenopausal group. In the postmenopausal women, the mean age of attaining menopause was 45.53 ± 3.13 years. Table 1 shows the lipid profile and MDA levels of the women in both the groups. The postmenopausal women had a statistically significant increased levels of TC ($p< 0.05$), LDL-C ($p< 0.01$) and significantly decreased levels on HDL-C ($p< 0.05$) when compared to women in late reproductive phase. The serum TG levels although increased in postmenopausal women was statistically not significant. TC/HDL cholesterol ratio was significantly higher in postmenopausal women in comparison to late reproductive age group women ($p< 0.01$). MDA levels were significantly elevated in postmenopausal women and correlated positively ($r = 0.653$; $p < 0.05$) increased TC/HDL-C ratio (Fig. 1).

Table 1: Age, lipid profile and MDA levels of women in late reproductive age group and postmenopausal women

	Late reproductive group	Postmenopause group
Age (in years)	36.49 ± 5.41	46.91 ± 3.67
Total Cholesterol (mg/dL)	164.82 ± 28.40	192.93 ± 32.97*
Triglyceride (mg/dL)	116.22 ± 63.11	139.28 ± 51.08
HDL-C (mg/dL)	49.94 ± 11.12	41.40 ± 9.95*
LDL-C (mg/dL)	91.636 ± 27.05	123.67 ± 32.02**
TC/HDL-C ratio	3.39 ± 0.69	4.94 ± 1.45*
MDA (nmol/gm of Hb)	3.5212 ± 1.17	5.99 ± 2.27**

** p< 0.01 * p < 0.05

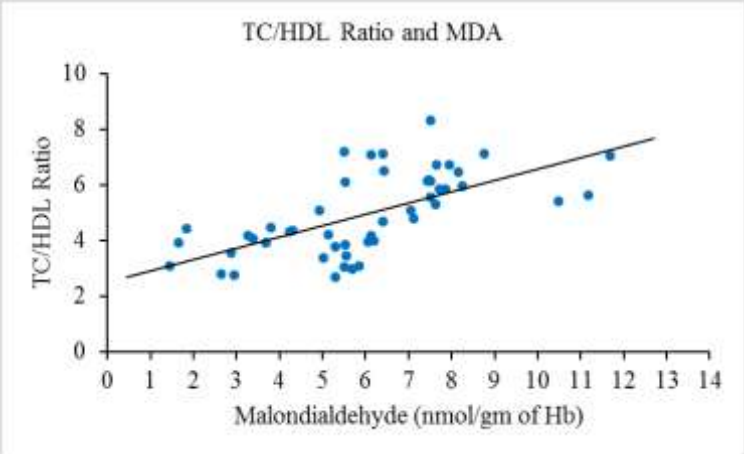

Fig. 1: Correlation between TC/HDL ratio and MDA levels in postmenopausal women (r = 0.653, p < 0.05)

DISCUSSION

In present era with increased life expectancy, women are likely to face long periods of menopause. The Indian menopause society in their third consensus meeting established that Indian women attain menopause much earlier than their counterparts in the western countries [11]. Additionally, there is an increased incidence and prevalence of premature menopause and increasing burden of surgical menopause resulting in a woman today spending almost a third of her life in menopause and that too with distressing clinical problems [3, 12-16]. It has been reported that postmenopausal women are four to eight times more likely to die of CAD than of any other disease [17]. It is therefore critical to understand the influence of menopause on lipid profile and oxidative stress in order to enhance our ability to identify targets for effective preventive measures. Lipids and lipoproteins are the established markers of vascular risks. National Cholesterol Education Programme (NCEP) has set the desirable levels for fasting lipid profile i.e.; total cholesterol below 200 mg/dL, serum triglyceride levels below 150 mg/dL, serum HDL cholesterol levels more than 60 mg/dL and LDL cholesterol level less than 100 mg/dL. The total cholesterol/high-density lipoprotein (TC/HDL) ratio is also used as an useful indicator of vascular risk. TC/HDL-C ratio greater than 4.5 indicates average cardiovascular risks in females [18].

Stage of Reproductive Aging Workshop (STRAW) has divided the reproductive age of an adult female into three phases i.e.; reproductive, the menopausal transition, and postmenopause. These three phases included a total of seven stages centred on the final menstrual period (FMP). The reproductive phase was divided into early, peak, and late. The menopausal transition phase consisted of early phase and late phase. Similarly postmenopausal phase consisted of early and late [9].

As age could be a confounding factor which alters the lipid profile and lipid peroxidation, we included women of higher age before the onset of menopausal features and postmenopause women who recently attained menopause. The mean age of attaining menopause in our study was 45.53 ± 3.13years, which is lower than the mean age at menopause in Caucasians, but it was within the normal range for the Indian population [11]. Many studies have been done to evaluate the changes in lipids and their transporting vehicle, the lipoproteins, in postmenopausal women. Most of the studies have shown that menopause in associated with higher levels of total cholesterol, serum triglyceride and LDL-C [20-22]. However data on HDL-C in postmenopausal women have been inconsistent as some studies have shown the levels to be unaffected [23, 24] whereas some have shown the levels to be reduced [25, 26]. Similar observation was seen in our study. We observed an increased levels of total cholesterol, triglyceride and LDL-C along with

reduced levels of HDL-C in postmenopausal women as compared to late reproductive age women (Table 1). Although the changes in total cholesterol ($p<0.05$), LDL-C ($P < 0.01$) and HDL-C ($p< 0.05$) were significant, the increase in triglyceride was not significant. Atherogenic index or the Total/HDL cholesterol ratio was calculated to assess the cardiovascular risk in women in both the groups. Higher Total/HDL cholesterol indicates higher cardiovascular risk [18]. In our study the women in the post menopause group had the statistically significant increased TC/HDL ratio ($p < 0.05$). Similar observation was made in other studies [26, 27]. Higher total cholesterol, triglyceride, LDL-C andatherogenic index may be attributed to the hormonal changes and failure of the follicular development due to reduced levels of estradiols in the postmenopausal phase [25]. These alteration in the lipid profile helps to explain the higher incidence of cardiovascular diseases in postmenopausal women in comparison the premenopausal women.

Oxidative stress occurs due to imbalance between the free radical and the defensive antioxidant system. Free radicals or the reactive oxygen species (ROS) when exceeds, results in damage of several biomolecules such as DNA, protein and lipids [19]. In our study we have used erythrocytes as a model to study oxidative stress. It is a target for oxidative reaction because of high oxygen tension and the presence of hemoglobin and plasma membrane rich in polyunsaturated fatty acids (PUFA) [3]. Of the lipoproteins, low density lipoproteins are the most susceptible to peroxidation resulting in 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 thepathogenesis of atherosclerosis [28]. Victorino *et al*. in their study concluded that postmenopausal women have decreased level of lipid peroxidation characterized by lower levels of MDA in comparison to premenopausal women [29]. However in our study we observed an increase in MDA levels in postmenopausal women which was statistically significant ($p< 0.01$). This was in accordance with various other studies [30, 31]. Increased lipid peroxidation in postmenopausal women is probably due to marked reduction in estrogen levels. At a higher concentrations, as seen in women in the reproductive age group, estrogen inhibits hydroxylation of DNA bases particularly guanine. However, at low concentrations, estrogen has a pro-oxidant action which contributes to increased concentration of inflammatory cytokines and products of lipid peroxidation [30]. As seen in Fig. 1, we also observed that the degree of lipid peroxidation and increase in TC/HDL cholesterol ratio showed a strong positive correlation ($r = 0.653$, $p < 0.05$) indicating that reduced levels of estrogen in the postmenopausal phase contributes to increased lipid peroxidation which in turn predisposes to cardiovascular risk.

CONCLUSION

Menopause is a significant stage marking the end of a woman's reproductive life. Although this process is physiological, it is accompanied by reduction in the levels of estrogen, which is known to have an antioxidant property. The deficiency of estrogen along with an increasing age results in an imbalance between the ROS and antioxidants causing an oxidative stress. This leads to the development of a variety of symptoms and pathologies that characterize menopause. Specifically, oxidative stress has been linked to an altered lipid profile and increased risk of cardiovascular disease. The results of our study suggest that aging along with deficiency of estrogen may be responsible for the increase in MDA levels and dyslipidemia. Further large population based prospective 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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