



ORIGINAL RESEARCH PAPER

Medical Science

ATHEROGENIC INDICATOR AS A VALUABLE PREDICTOR OF CARDIOVASCULAR RISK AMONG POSTMENOPAUSAL FEMALE POPULACE IN NORTH KARNATAKA

KEY WORDS: Lipid profile, CVD, post menopausal, pre menopausal

S. Kulkarni

Senior Lecturer, Department of Biochemistry, USM-KLE IMP, Belgaum-590010, Karnataka, India.

Dr. P. Gupta*

Assistant Professor, Department of Biochemistry, L.N. Medical College. Sarvdharma complex, Kolar Road, Bhopal-462042, Madhya Pradesh, India *Corresponding Author

Avinash Math

Senior Lecturer, Department of Biochemistry, USM-KLE IMP, Belgaum-590010, Karnataka, India.

ABSTRACT

Impulsive termination of women's menstrual cycle for a year is a post menopause period¹. During the menopausal stage, ovaries discontinue the synthesis of estrogen hormone. Estrogen has dual effect after menopausal in women's life – primary on Lipid profile (body weight), changes in Lipid profile leads to various metabolic diseases like CVD, Hypertension etc and secondary on bone metabolism. Women undergo 2 different stages of bone loss – phase one, begins at menopause, is rapid, transient. Whereas 2 phase is slow, subsequent phase continues indefinitely. Both phases lead to bone loss². As we are aware that CVD is a major health burden worldwide. Therefore this study was conducted to assess the atherogenic markers as a valuable predictor of cardiovascular risk in pre and post menopausal women.

Methods: The pilot study was carried out jointly by Department of Biochemistry & Obstetrics and Gynecology and was approved by IEC of J. N. Medical College & Dr. P.K. Charitable Hospital, Belgaum. Total of 60 apparently healthy female subjects participated in this study, consisting of pre menopausal women as control (n=30) and post menopausal women as cases (n=30). Atherogenic Marker and Oxidative Stress marker were analysed and compared between both groups by standard biochemical methods.

Results: As compared to pre menopausal, post menopausal women showed increased levels of serum Total Cholesterol, Triglycerides (TAG), Malondialdehyde (MDA), and decrease level of High Density Lipoproteins-Cholesterol (HDL-C) and Vitamin C which were statistically significant. There was no difference observed of vitamin E levels in both the groups.

Conclusion: Increased levels of Total Cholesterol, LDL-C, TAG & MDA and decreased levels of HDL-C & Vitamin C represent post menopausal women are susceptible towards CVD.

INTRODUCTION:

Cardiovascular disease (CVD) is the leading cause of morbidity among males and females. But in females incidence of CVD is reduced when compared to age-matched males and present with CVD 10 years later than men (3). Protection against CVD in female is associated with sex hormone levels as the incidence and severity of CVD increases in women postmenopause. [4] Women's reproductive life has an array of 6 phases. It starts from premenarche stage, followed by the reproductive, premenopausal; the early and late menopausal transition stage; menopause and at last postmenopausal stage. Premenarche phase involves changes in the body to initiate the reproductive cycle. The reproductive cycle will start from the reproductive stage and continue till menopause. As female reaches to her menopausal fertility decline via a decrease in the number of ovarian follicles produced⁵.

These change in reproductive potential is the consequence of a decline in the production of hormones by the ovaries, which causes physical manifestations. This physical manifestation has a negative impact on quality of life of menopausal women⁶. Women suffer stress, anxiety and sometimes depression. As the stage advances from menopausal to postmenopausal and ovaries ceased estrogen hormone synthesis, women become prone to metabolic disorders like cardiovascular diseases. The prevalence of cardiovascular disease after menopause may be partly caused by altered serum lipid profile and increased oxidant stress that occur following the menopausal transition^{7,8 & 9}. Despite the extensive research on the effects of sex hormones on lipid and lipoprotein metabolism^{10,11} along with oxidative stress, it is still not clear whether changes in sex steroid concentrations are related to changes in lipid concentrations or it is independent of sex hormone, which is associated with menopausal status. Cardiovascular diseases(CVD) is a multifaceted disease. Low-density lipoprotein (LDL) cholesterol concentration consider as prime culprit of cardiovascular disease risk. Though, nowadays several lipoprotein ratios or "atherogenic indices" have been considered as a better predictive marker of CVD. These lipoprotein ratios can provide information on peril factors which are complicated to measure by regular analyses in addition to they may represent a better image of the metabolic and clinical interactions between lipid fractions¹².

Therefore, the aim of this study is to establish differences in lipoprotein ratios as cardiovascular risk factors in women who were in menopause and in women who underwent regular menstruation and to investigate the relationship between the menopausal status with plasma lipid concentrations and oxidative stress.

MATERIALS AND METHODS

Sixty female subjects participated in this study, among them 30 pre menopausal women as control and 30 post menopausal women as cases have been compared. Participants that have crossed menopausal state are included as post menopausal subjects. All 60 subjects selected for this study were healthy and not suffering from any of the diseases like diabetes mellitus, renal disorder, cardiovascular disease, tuberculosis, anemia and hypertension. Informed consent had been collected from all the participants. For assessment of all parameters included in this study, a 10ml blood was collected in aseptic conditions from the antecubital vein of the subject in heparinized vials. 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 after fasting overnight. The present study was carried out jointly by Department of Biochemistry and Obstetrics and Gynaecology. This study was approved by ethical committee of J. N. Medical College and Dr. Prabhakar Kore's Charitable Hospital, Nehru Nagar, Belgaum. The subjects were grouped as follows: Premenopausal women (n= 30, mean age 30±5) and Post-menopausal women (n=30, mean age 49.4±6.6). Assessment of lipid profile such as Serum Total Cholesterol by Carr Dreker method¹³, Serum Triglyceride (TAG) by ERBA kit method in semi auto analyser¹⁴, Serum High Density Lipoprotein (HDL) by Lopez- Virella ML et al method¹⁵, Serum Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) by Friedwald's Formula¹⁶, Oxidative stress marker –MDA by Thio barbiturate method¹⁷, Estimation of plasma Vitamin C and Vitamin E by reagent method^{18,19}.

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:

As compared to Pre menopausal, Post menopausal women had increased levels of Total Cholesterol , Triglycerides, Low Density Lipoproteins-Cholesterol (LDL-C) , Very Low Density Lipoproteins-Cholesterol (VLDL-C), Malondialdehyde (MDA) and decrease level of High Density Lipoproteins(HDL-C), antioxidants Vitamin C which were statistically significant. There was no difference observed of vitamin E levels in both the groups. The levels of Total Cholesterol , Triglycerides, Low Density Lipoproteins-Cholesterol (LDL-C) ,Very Low Density Lipoproteins-Cholesterol (VLDL-C), High Density Lipoproteins –Cholesterol (HDL-C) in pre and post menopausal women are presented in Table No. 1 and levels of oxidative stress namely Malondialdehyde (MDA) and antioxidants (Vitamin C and Vitamin E) are presented in Table No. 2.

In the present study, post menopausal women had Total Cholesterol levels between 122 to 249 mg/dl whereas premenopausal women had a range of Total Cholesterol between 104 to 193 mg/dl. Levels of Triglyceride, VLDL-C and LDL-C in post menopausal women were between 54 to 207, 11 to 201 and 58 to 180 respectively. The reference value of Total Cholesterol in adults is 150-200 mg/dl and for Triglyceride normal levels range are from 40 to 145 mg/dl. The reference value for LDL-C is 130 to 160 mg/dl.

Levels of oxidative stress markers namely Malondialdehyde, Vitamin C and Vitamin E between in post menopausal women were between 7.2 to 16 mg/dl, 0.15 to 0.52 mg/dl and 0.66 to 1.4 mg/dl respectively.

DISCUSSION:

Women experience a lot of transformation in her life. When women reach to their early 50s, Menopause usually occurs resulting from the loss of ovarian and follicular activity causing permanent cessation of menstruation²⁰. The trouncing of ovarian and follicular activity and altered hormonal status shows a harmful effect in women's life. These disturbances not only enhance mental and physical stress but also make them susceptible to CVD and other metabolic diseases. Experience, sign and symptoms of menopause are different in each and every woman. These all changes are associated with decreased secretion of reproductive hormone namely estrogen. Estrogen is known to have cardio protective mechanisms because of its antioxidant properties²¹. After menopause, that is post menopausal phase, the deficiency of estrogen hormone predisposes women to increased free radical damage and atherosclerosis. In menopausal women, vasomotor disturbances are involved with oxidative stress²². Oxidative stress is defined as the imbalance between free radical damage (e.g. the oxidation of lipids) and antioxidant protection²³.

In postmenopausal and oophorectomized women, a decrease in the plasma concentration of estrogen leads to enhanced activity of lipoprotein lipase, which may increase the plasma LDL concentrations²⁴. Additionally, Arca et al. have suggested that impairment of the LDL receptor resulted from hypercholesterolemia was observed especially in post-menopausal women²⁵. These imbalances in post menopausal female become susceptible to CHD. As risk of CHD is difficult to quantify by routine investigation of lipid profile. Henceforth the ratio of lipid fractions has been used to predict the metabolic and clinical interaction between lipid fractions. The total cholesterol: HDL cholesterol ratio also known as the atherogenic or Castelli index and LDL-C : HDL-C are the two important lipoprotein ratio as an indicator of vascular risk which predictive value is greater than the isolated parameters.

Previous studies had been mentioned that an increase in total cholesterol concentration and mainly LDL cholesterol could be considered as an atherogenic lipid marker, whereas decreased HDL cholesterol levels are associated with several risk factors, containing the components of the metabolic syndrome and probably involves independent risk. According to the previous studies these lipoprotein ratios that is Total cholesterol/ HDL-cholesterol and LDL-cholesterol/ HDL- cholesterol have the predictive capacity. Increased concentration of HDL – cholesterol is closely associated with plaque regression whereas the reduced

concentration of LDL- cholesterol may decrease the process of atherogenesis. Both these indices have important predictive value for a cardiovascular risk for a wide range of cholesterol concentration. The intention to rely on these lipoprotein ratios, especially total/HDL cholesterol, because they are grounded on two values directly measured in a clinical laboratory and it also specifies the association or proportion between the atherogenic and antiatherogenic lipid fraction, have strong predictive power for cardiovascular disease and cardiovascular risk reduction after intervention superior to that of classic lipid parameters.

In the present study we have found increased levels of LDL, Total Cholesterol and decreased levels of HDL cholesterol and there ratio that is LDL cholesterol: HDL cholesterol and Total cholesterol: HDL cholesterol are also increased in post menopausal female compared to the control group which indicates that the post menopausal female group have high chances to develop cardiovascular disease compared to pre menopausal female (control) group. This problem will enhance multiple times in presence of oxidative stress²⁶. Decreased antioxidant levels in oxidative stress enhance the action of free radicals. These free radicals cause oxidative destruction of polyunsaturated fatty acids (PUFAs) leads to Lipid peroxidation. The oxidizing free radicals attack polyunsaturated fatty acids (PUFAs) located in cell membranes²⁷. The self-perpetuating chain reaction of lipid peroxidation marks the cytotoxic and mutagenic products such as lipid peroxides and aldehydes like MDA²⁸. A study by S.S. Signorelli et al observed the increased level of Malonaldehyde in post-menopausal women compared with premenopausal women²⁹. In our study also we observed the higher levels of MDA in post-menopausal than in premenopausal women. Estrogen being reproductive hormone it also has antioxidant properties and its deficiency after menopause prejudices the body to increased free radical damage²¹. Therefore in the state of post-menopausal women, there are increased levels of free radicals or oxidants that outnumbered the antioxidants and leads to a high degree of oxidative stress.

The vitamin C (ascorbic acid) and E (-tocopherol) have a higher antioxidant capacity, which scavenges free radicals and neutralizes oxidative stress³⁰. In the present study, we observed a significant decrease in the levels of plasma vitamin C in postmenopausal females than in premenopausal females. There was no difference observed of vitamin E levels in both the groups.

Vitamin C plays a counter role for increased oxidative stress and to inhibit membrane lipid peroxidation. As a result, there is a decreased level of vitamin C with its increased consumption.

Vitamin E has antioxidant properties which neutralize reactive oxygen species and reduces oxidative DNA damage and genetic mutations³⁰. But the main function of vitamin C is to recycle and restore the antioxidant properties of vitamin E radical which results in decreased levels of vitamin C while maintaining the normal activity of vitamin E³¹.

CONCLUSION:

Compared to pre menopausal, in post menopausal women, increased level of Total Cholesterol(TC) , Triglyceride(TAG), Low Density Lipoprotein-Cholesterol (LDL-C), Very Low Density Lipoprotein-Cholesterol (VLDL-C), and decreased level of High Density Lipoprotein- Cholesterol (HDL-C), shows post menopausal women are susceptible towards Cardiovascular disease (CVD) whereas increased levels Malondialdehyde (MDA) and decreased level of Vitamin C, represent that post menopausal females have high oxidative stress, known key factor for various lifestyle disease and metabolic disorders.

RESULTS:

Table 1 :Atherogenic parameters in pre and post menopausal females.

Atherogenic Markers	Premenopausal women(control) (n=30)	Postmenopausal women (n=30)	T value	P value
Age	30±5	49.4±6.6		

TC (mg/dl)	156.56±16.4	196.3±31.9	5.9157	0.0001
TAG (mg/dl)	130.58±37.6	162.10±33.3	3.4373	0.0011
HDL-C(mg/dl)	42.62±8.22	35.96±6.79	3.4214	0.0011
LDL-C(mg/dl)	89.43±24.3	117.8±30.2	4.0007	0.0002
VLDL(mg/dl)	27.25±7.6	32.69±7.12	2.8611	0.0059
TG:HDL	3.21± 1.41	4.25±1.94	2.51	0.01
LDL:HDL	2.17± 0.83	3.403 ± 1.2	5.39	<0.001
TC:HDL	3.80 ± 0.96	5.71 ± 1.87	5.65	<0.001

In Table :1 -Lipid profile parameters, the total cholesterol, trigly ceride, LDL and VLDL values were significantly higher in Postmenopausal women when compared with control subjects of premenopausal group.

Table 2: lipid peroxidation and antioxidants

Markers	Premenopausal women(control) (n=30)	Postmenopausal women(n=30)	T value	P value
MDA	5.20±1.24	12.63±2.4	17.99	<0.001
Vit.C	0.879±0.13	0.322±0.10	17.26	<0.001
Vit. E	0.9±0.10	0.92±0.14	0.59	0.56

In Table 2: A lipid peroxidation and antioxidants level, the MDA level is significantly increased and vitamin C is decreased in Postmenopausal women when compared with control subjects of premenopausal group.

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