Oxidative stress in post menopausal women with cardiovascular risk factors

Subrat Kumar Tripathy[1,*], Viyatprajna Acharya[2], Sarthak Ranjan Nayak[3], Pramila Kumari Mishra[4]

[1]Assitant Professor, [2]Professor, [3]Assistant Professor, Department of Biochemistry, IMS & SUM Hospital, SOA University, Bhubaneswar.
[4]Professor, Department of Biochemistry, MKCG Medical College, Berhampur.

***Corresponding Author:**
E-mail: subrat49@rediffmail.com

Abstract

Background: Menopause is a normal occurrence in the life of every woman. As the life expectancy of woman all over the world now increases, a woman has to spend almost 1/3rd of her life in menopause years. Along with the increase in life expectancy the cardiovascular disease is increasing.

Objectives: To study the association between oxidative stress and various cardiovascular risk factors in post-menopausal women.

Methods: 50 postmenopausal women with cardiovascular risk factors like hyperglycemia, hypertension, high Body Mass Index and Hyperlipidaemia were selected as cases and compared with 50 age matched apparently healthy controls. Malon-di-aldehyde (MDA) was taken as the marker for oxidative stress and vitamin E & vitamin C were taken to assess antioxidant status. Student's t-test was applied to compare different parameters between the groups and Pearson's correlation was applied to see the correlation with different parameters in the case group.

Results: It was found that MDA values in postmenopausal women with cardiovascular risk factors were significantly higher as compared to the post menopausal women without cardiovascular risk factors ($p < 0.001$). There is significant decrease in vitamin E and C values in cases (p is < 0.001 and < 0.01 respectively). Comparing the various oxidative markers in cases and control it was found that MDA values have a significant negative correlation with vitamin E and C ($r= -0.4$ and $r= -0.7$ respectively). Whereas correlation between changes in Vitamin E and vitamin C was positive but not significant ($r= +0.2$, $p >0.05$).

Conclusion: Antioxidant medication and other measures can be adopted to reduce oxidative stress in post menopausal women with cardiovascular risk factors and reduce the morbidity and mortality associated with cardiovascular disease.

Key words: Menopause, Malon-di-aldehyde (MDA), Vitamin E, Vitamin C, Cardiovascular risk factors.

Access this article online	
Quick Response Code:	**Website:** www.innovativepublication.com
	DOI: 10.5958/2394-6377.2016.00018.6

Introduction

Menopause is a normal occurrence in the life of every woman. It is defined as permanent cessation of menses. By convention the diagnosis of menopause is not made until the individual had 12 months of amenorrhea.[1] The hormonal changes in a postmeno-pausal woman are rise in Follicular Stimulating Hormone (FSH) and Lutenizing Hormone (LH) level and a fall in estrogen level. The major consequences of menopause are related primarily to this estrogen deficiency. Principal health concerns of post menopausal woman include vasomotor symptoms, urogenital atrophy, osteoporosis, cardiovascular disease, cancer and other sexual problems. As the life expectancy of woman all over the world now increases, a woman have to spend almost one third of her life in menopause years. Along with the increase in life expectancy the cardiovascular disease is increasing and now it is one of the leading causes of death throughout both in developed and developing countries. Most cardiovascular diseases results due to atherosclerosis involving the major vessels. The risk factors are same for both men and women. However when controlling the risk factors, men have a high risk of developing coronary heart disease over 3.5 times than that of women.[2]

Cardiovascular diseases, especially atherosclerosis are the results of multiple metabolic changes that interact with each other like; adverse changes in circulating lipid and lipoprotein profile, oxidation of low density lipoprotein (LDL), endothelial injury and dysfunction etc. After menopause the risk of coronary heart diseases double for women as the atherogenic lipids by the age of 60 years reach greater than those of men. Prospective studies have documented a strong relation between total cholesterol and coronary heart disease in women. Adiposity of trunk or high body mass index (BMI) is also a risk factor for coronary heart disease in women and is associated with a relatively androgenic hormonal state, hypertension and disorders of lipid and carbohydrate metabolism. Central fat distribution in women is positively correlated with increase in total cholesterol, Triglyceride (TG), low density lipoprotein (LDL) and negatively with High Density Lipoprotein (HDL).[3]

A free radical is defined as a chemical species possessing an unpaired electron. All free radicals are extremely reactive and will seek out and take an electron in any way possible. In the process of acquiring an electron, the free radicals are capable of damaging most bio molecules including proteins, carbohydrates, lipid and nucleic acids. The free radical damage lipid peroxidation is a process generally occurring at low levels in all cells and tissues. It involves oxidative conversion of unsaturated fatty acids to primary products known as lipid hydroperoxides and a variety of secondary metabolites including malondialdehyde (MDA). The primary reaction site of lipid peroxidation involves membrane associated polyunsaturated fatty acids (PUFA) and cholesterol, which alter cell membrane fluidity and permeability of cell membrane and may eventually induce widespread membrane damage.[4] Extensive tissue lipid peroxidation is often shown by evidence of increase blood peroxide level in both free and LDL bound form.

Materials and Methods

The study was conducted on the out patients of the Department of Medicine and Department of Obstetrics and Gynecology of the hospital. Out of various cardiovascular risk factors four risk factors are chosen for the study. They were Fasting Blood sugar(FBS) ≥ 100 mg/dl, Systolic Blood pressure (BP) ≥ 140 and diastolic BP ≥ 90 mm of Hg, BMI ≥ 25kg/m^2 , total cholesterol ≥ 200 mg/dl, TG ≥ 150mg/dl and HDL ≤ 40mg/dl.

50 postmenopausal women having any of the above risk factor were selected for the study from the outpatient department of medicine and gynecology. Another 50 post menopausal women were selected from the staff of the college and some from the nearby localities without any known cardiovascular risk factors. All the 100 women selected for the study were in a narrow age range of 45 to 54 years to reduce the influence of age on the study. Any woman having one or more of the above selected risk factors were taken as a case. Women with any other risk factors like family history of cardiovascular diseases (CVD), previous CVD were excluded from the study. Again women having history of smoking, alcohol intake, major illness in recent pass, major surgery in recent past and taking antioxidant medication were excluded from the study. All study subjects were informed about the study and their consent was taken before the general and biochemical examination. The above study was cleared from the ethical committee of the institution.

All subjects of the study were undergone through general examination and 10 ml of blood was taken from each individual through antecubital venipuncture. Serum was separated after 30 min standing and then through centrifuge. For blood glucose estimation 2 ml of above blood was transfer to another vial containing sodium fluoride. Care was taken to do all biochemical test on the same day of collection and if not the blood sample was stored in deep refrigerator with -80 ^{0}C. FBS, Serum total cholesterol, TG, HDL was measured by COBAS INTEGRA 400 AUTOANALYZER by enzymatic colorimeter methods.[5] Blood pressure was measured by standard sphygmomanometer with left arm supine position 3 time half hour interval and mean BP was calculated. BMI was calculated by dividing the body weight in kilogram by the square of height in meter. Serum MDA was measured by *KEI Satoh et al* method. MDA when react with barbituric acid in acidic medium forms trimethine coloured substance. This has a maximum absorbance at 532 nm, whose reading was taken by a spectrophotometer.[6]

Vitamin C was measured by converting it first into dehydro-ascorbic acid and then was coupled with 2,4-dinitrophenyl hydrazine in presence of thiourea as mild reducing agent to produce 2,4- dinitrophenyl hydrazone which is later converted to a red-coloured compound by sulphuric acid and measured colorimetrically.[7]For measurement of vitamin E it was first extracted into xylene and then converting it to a red coloured compound with α,α-dipyridil. A correction for the carotene is made after adding ferric chloride and reading was taken at 520 nm in a colorimeter.[8]

Results were analysed by Microsoft excel and SPSS 15.0.

Results

Estimation of oxidative stress in post menopausal women it was found that the mean and standard deviation (SD) of MDA values of all control is 1.58±0.3 µmol/L where as the mean and SD of MDA values of the women with cardiovascular risk factor were 2.99±0.68 µmol/L. Now applying t-test to both cases and controls it was found that there is increase in oxidative stress in cases and the increase was significant (t- 8.55, $p < 0.001$). Now evaluating the mean and SD of vitamin E in control was 25.3±2.67 and cases was 20.54±1.64 and applying t-test to this change it was found that there is mean decrease in vitamin E level in cases and the decrease was significant t- 10.77, ($p< 0.001$).Similarly the vitamin C the mean and SD of control was 30.01±3.8 and for control was 26.71±3.02. Again there is decrease in vitamin C level in cases and the decrease was significant (t- 6.13, $p < 0.01$) **[Table-1].**

Comparing the various oxidative markers in control and cases it was found that MDA and vitamin E are negatively correlated (correlation coefficient r was -0.4 and the correlation was significant (t- 2.48, $p < 0.05$) Correlation between change in MDA and vitamin C was also negative($r = -0.7$) and significant (t- 4.9, $p < 0.01$. Whereas correlation between changes in Vitamin E and vitamin C was positive ($r= +0.2$) but was not significant (t- 1.4, $p >0.05$) **[Table-2].**

Table 1: Comparison of the levels of MDA, Vitamin E and C between cases and controls

Parameters	Mean±SD of Controls	Mean±SD of Cases	t	p
MDA (µmol/L)	1.58±0.3	2.99±0.68	8.55	< 0.001
Vit-E (µmol/L)	25.3±2.67	20.54±1.64	10.77	< 0.001
Vit-C (µmol/L)	30.01±3.8	26.71±3.02	6.13	< 0.01

Table 2: Correlation between MDA, Vitamin E and C levels in cases

Parameters	r	p
MDA & E	-0.4	< 0.01
MDA & C	-0.7	< 0.001
E & C	+0.2	> 0.05

Discussion

Present study was done to evaluate serum MDA, vitamin E and C for the detection of any alteration of oxidative stress in postmenopausal women with cardiovascular risk factors. This may explain role of oxidative stress in the development of cardiovascular disease in postmenopausal women and also highlight cardiovascular risk factors as predictor of the disease.

All the women selected for the study were in a narrow age range to minimize the effect of age on oxidative stress though cases had a slight higher mean age than controls and the difference in age was significant ($p < 0.05$) in our study. According to a study in Chinese women increasing age in women was associated with increased body mass index, waist-to-hip ratio, systolic and diastolic BP, fasting plasma total cholesterol, TG, LDL, apolipoprotein B and FBS. There was a progressive increase with age in the prevalence of glucose intolerance, hypertension, dyslipidaemia and obesity.[9] These findings suggest that age had an important and independent effect on cardiovascular risk in Chinese women and that, as in Caucasians, the onset of menopause might further increase this risk. These finding suggest that age has an important and independent effect on cardiovascular risk in women.

In our study FBS was taken as a risk factor for CVD and some of the cases have FBS as the sole risk factor or present in combination with other risk factors where as all controls have normal range of FBS. Hyperglycemia, which occurs during both type of diabetes, causes oxidative stress. Free fatty acids, which may be elevated during inadequate glycemic control, may also be contributory.[10] In vivo studies have revealed that oxidative stress caused by hyperglycemia occurs before the complications of diabetes become clinically evident. Wolff and Dean suggested that non enzymatic protein glycation, a mechanism proposed early on to account for glucose cytotoxicity was dependent on Reactive Oxygen Species (ROS) like superoxide and hydroxyl radical formation through transition metal-catalyzed glucose autoxidation.[11] Research in numerous laboratories has indicated that hyperglycemia activates several major, well-characterized biochemical pathways that play a significant role in the etiology of diabetic complications. These pathways include advanced glycation end products (AGEs) and receptors for AGE, protein kinase C and the polyol pathway.[12] Increasing evidence exists, suggesting an important role for oxidative stress in the pathogenesis and progression of hypertension in women via a decrease of Nitric oxide (NO) production after menopause.[13] The vasculature is a rich source of ROS, which under pathological conditions, plays an important role in vascular damage. There is growing evidence that increased oxidative stress and associated oxidative damage are mediators of vascular injury in cardiovascular pathologies, including hypertension and atherosclerosis. Increased production of superoxide anion and hydrogen peroxide has been demonstrated in experimental and human hypertension. This development has evoked considerable interest because of the possibilities that therapies targeted against reactive oxygen intermediates by decreasing generation of ROS and/or by increasing availability of antioxidants, may be useful in minimizing vascular injury and hypertensive end organ damage.

Endothelial dysfunction is reported in obese women with high BMI; though the exact mechanism is not known yet. It was also found that obesity is associated with glucose intolerance, hypertension and hypertriglyceridaemia. All are important cardiovascular risk factors. Studies by Francesco Perticone et al. shows that there is increased oxidative stress in obese persons and vitamin C has beneficial role in obese to decrease its complications.[14]

Alteration of plasma lipids and lipoprotein level are common risk factors for CHD and increase cardiovascular mortality. Decrease HDL cholesterol

appears to be the major lipid risk factor in women, with increased HDL being negatively associated with subsequent coronary disease. The incidence of hyperlipidaemia increases after menopause. In fact more post menopausal women have high LDL cholesterol, than man of same age. TG also appears to be an independent risk factor in women. An extensive review of various studies shows that postmenopausal women tend to have more atherogenic lipoprotein profile showing higher level of total cholesterol, TG, LDL and VLDL.[15] Study by Rui-Li Yang et al has postulate that, the activities of superoxide dismutase and glutathione peroxidase decreased in higher lipid group compared with lower lipid group, and were even lower in hyperlipidemic subjects. There was a positive correlation between MDA and Atherogenic Index and appropriate support for enhancing antioxidant supply in higher lipid subjects may help prevent the course of the disease.[16] Hyperlipidaemia is associated with oxidized LDL and ultimate increase in lipid peroxidation products. Unsaturated fatty acids are known to have a crucial role in the pathogenesis of atherosclerosis. Lipid and lipoprotein metabolism is markedly altered in postmenopausal women. All these explain the significant increase in MDA values in postmenopausal women with cardiovascular risk factors in comparison with controls in our study. So increase in oxidative stress is both the effect of cardiovascular risk and the cause of development of further CVD.

Vitamin E is a fat-soluble antioxidant that stops the production of ROS formed when fat undergoes oxidation. Scientists are investigating whether, by limiting free-radical production and possibly through other mechanisms, vitamin E might help prevent or delay the chronic diseases associated with free radicals. Study by Nagyova A et al. shows decrease in cardiovascular disease risk in post menopausal women taking long term vitamin E supplementation.[17] In study by Kharb S et al. low vitamin E levels and raised MDA levels were observed in postmenopausal women as compared to pre-menopausal women suggesting that antioxidant vitamin may be important in preventing cardiovascular disease in these women.[18] According to the study by Perin Vural et al**.** the menopause is associated with an increase in oxidative stress and a decrease of some antioxidants, such as ascorbic acid, α-tocopherol, total thiols and erythrocyte GSH.[19] Decrease in serum vitamin E in postmenopausal women and in cardiovascular disease is also observed in some other studies.[20, 21] Our study too observe a significant fall in vitamin E in postmenopausal women with cardiovascular risk factors as vitamin E is a major antioxidant to fight the increase oxidative stress.

As a water-soluble antioxidant, vitamin C is in a unique position to "scavenge" aqueous peroxyl radicals before these destructive substances have a chance to damage the lipids. It works along with vitamin E and the enzyme glutathione peroxidase to stop free radical chain reactions. Vitamin C supports the cardiovascular system by facilitating fat metabolism and protecting tissues from free radical damage, and it assists the nervous system by converting certain amino acids into neurotransmitters. As an antioxidant and as a co-factor for collagen synthesis, vitamin C may play a number of roles in maintaining cardiovascular fitness. The fatty plaques that form in blood vessels, called atherosclerosis, are a major contributor to heart diseases. Vitamin C may prevent this plaque formation by inhibiting the oxidative modification of LDLs, according to a study conducted at the University of Texas Southwestern Medical Center. Beyond that, vitamin C may play a mitigating role in another aspect of atherosclerosis - the buildup and adhesion of platelets on vessel walls. The decrease in both vitamin E and C is mostly due to the fact that they are used up to decrease the increased oxidative stress.

All of these finding point out in one direction that with the increase in age and menopause the prevalence of cardiovascular risk factors increase in woman. Association of cardiovascular risk factors increases the oxidative stress in postmenopausal women working along with increase age and low estrogen level. With increase in oxidative stress there is simultaneous decrease in antioxidant status to counter act the oxidative damage.

Conclusion

These cardiovascular disease risk factors should be considered as high priority health problems in rural and low socioeconomic areas of developing communities like India. The study was to assess the cardiovascular risk factors and its effect on the low socio economic group of Indian population belonging to village and small town area. The study shows significant increase in oxidative stress and decrease in antioxidant status in the presence of cardiovascular risk factors. So it is highly recommended that intervention to modify the cardiovascular risk factors should be included in routine primary health care programs. Also anti oxidant medication may be beneficial to these women.

Conflict of Interest: None
Source of Support: Nil

References

1. Kumar P, Malhotra N, Menopause, Jeffcoates principle of Gynaecology, 7th ed. (2008) p 862-883.
2. Speroff L, Fritz MA, Menopause and perimenopausal transition, 7th ed. P 621-688.
3. Haarbo J, Hassager C, Riis BJ, Christiansen C, relation of body fat distribution to serum lipid and lipoprotein in elderly women, Atherosclerosis 80:57, 1989.
4. Kagan VE. Lipid peroxidation in biomembranes. Boca Raton,Florida: CRC Press, 1988: 13-14.
5. Method Manual, COBAS INTEGRA 400/700/800, Ed V4, 2008-02.
6. KEL Satoh: Clinical Chima Act. (1978): 90. P. 37-43.

7. Roe: Plasma Vitamin C by 2-4 dinitrophenyl hydrazone method, Harold Varley; Practical Clinical Biochemistry.(1961): 4th ed. P. 635.
8. Baker & Frank: Varleys Practical Clinical Biochemistry (1968); Ed. 6, p. 902.
9. The effect of age on cardiovascular risk factors in Chinese women*International Journal of Cardiology*, Volume 61, Issue 3, Pages 221-227**9.**
10. George L. King and Mary R. Loeken, J of Histochemistry and Cell Biology, V 122, Number 4/Oct, 2004, p 333-338.
11. Wolff SP, Dean RT: Glucose autoxidation and protein modification: the potential role of 'auto-oxidative glycosylation' in diabetes. *Biochem J* 245 :243 -250,1987
12. Stevens MJ, Obrosova I, Feldman EL, Greene DA: The sorbitol-osmotic and sorbitol-redox hypothesis. In *Diabetes Mellitus: A Fundamental and Clinical Text.* LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippincott Williams & Wilkins,2000 , p.972 -983
13. *Pialoux, Vincent; Brown, Allison D.; Leigh, Richard; Friedenreich, Christine M.; Poulin, Marc J.* Effect of Cardiorespiratory Fitness on Vascular Regulation and Oxidative Stress in Postmenopausal Women**.** Hypertension. 54(5):1014-1020, November 2009.
14. Francesco Perticone, Roberto Ceravolo, Mafalda Candigliota,Giorgio Ventura, Saverio Iacopino,Flora Sinopoli and Pier L. Mattioli, J of American Diabetic Association, V 50, p159-165.
15. Matthews KA, Meilahn E, Kuller LH et al. Menopause and risk factor for coronary heart disease, New Eng. J. Med, 1989, 321: 641-646.
16. Rui-Li Yang, Yong-Hui Shi, Gang Hao, Wu Li, and Guo-Wei Le, J Clin Biochem Nutr. 2008 November; 43(3): 154–158.
17. Nagyova A.; Mongiellova V.; Krivosikova Z.; Blazicek P.; Spustova V.; Gajdos M.; Dzurik R. Physiological research J, 2002, vol. 51, nº5, pp. 457-464
18. Kharb S, Singh V, Ghalaut PS, Singh GP; Biomedicine. 1999;19(2):113-5
19. Perin Vural, Cemil Akgül and Mukaddes Canbaz**,** Ann Clin Biochem 2005;42:220-223
20. M. Torun, N. Avci S. Yardim Journal of Clinical Pharmacy and Therapeutics, Volume 20 Issue 6, Pages 335 - 340
21. FJ Kok, AM de Bruijn, R Vermeeren, A Hofman, A van Laar, M de Bruin, RJ Hermus and HA Valkenburg, American Journal of Clinical Nutrition, Vol 45, 462-468.