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# OXIDATIVE STRESS & PARAOXONASE ACTIVITY IN POST MENOPAUSAL WOMEN

Biochemistry

**Mrinal Gupta** MD Demonstrator Department of Biochemistry, Dr. Baba Saheb Ambedkar Medical college & hospital, Rohini, Delhi -110085

**Priyanka Datta*** MD Demonstrator Department of Biochemistry, Nil Ratan Sircar Medical college & hospital, Kolkata, West Bengal – 700014 *Corresponding Author

**Pragna Rao** MD , PhD Dean & Professor of Biochemistry Dept. Of Biochemistry, Kasturba Medical College, Manipal, Manipal University, Karnataka, India

## ABSTRACT

**BACKGROUND**- Menopause can lead to alterations in women's well being, with changes in the oxidative status of postmenopausal women.
**OBJECTIVES**- To determine the extent of free radical damage in the form of oxidative stress (MDA), the antioxidant property of HDL (paraoxonase) in post menopausal women compared with healthy controls.
**MATERIALS & METHODS**- The study was conducted on 30 post menopausal women with no history of hypertension and diabetes and 30 healthy premenopausal controls. Mean age of post menopausal women was 41.1 ± 1.2 years whereas for pre menopausal women was 48.3 ± 1.5 years. Informed consent was taken. Serum MDA levels were estimated by Kei-Satoh's method, serum paraoxonase by spectrophotometric method using paraoxon as a substrate.
**RESULTS**- On evaluation there was increase in MDA levels which was statistically significant and there was decrease in serum paraoxonase in post menopausal women which was also statistically significant.
**CONCLUSIONS**- We hypothesize that increased susceptibility of post menopausal women to accelerated atherogenesis is due to increased oxidative stress & decreased antioxidant effect of HDL but further studies with larger sample size should be taken up to prove our hypothesis.

## KEYWORDS

Atherogenesis, diabetes, HDL, menopause, MDA, paraoxonase

## INTRODUCTION

Menopause is defined as having occurred when a woman has not had any menstrual cycles for a year and gradual decrease in ovarian production of estrogen and other hormones .[1]The time after menopause is called post menopause, a phase that lasts for the rest of a woman's life. Apart from changes associated with increasing age, women are exposed to changes such as vasomotor symptoms affect most women during the menopausal transition but their severity as well as duration varies widely between women. Hot flushes, though exact cause is not known, are reported in up to 85% of menopausal women. [2]Decrease in estrogen or menopause leads to resetting of the thermoregulatory system which can lead to hot flushes. Multiple studies confirm that about 27% to 60% of women report moderate to severe symptoms of vaginal dryness or dyspareunia in association with menopause.[3,4] Women report more trouble sleeping as they enter into the menopausal phase both by self-report as well as by actigraphy .[5,6] Dwindling estrogen levels during the menopause have been linked to endothelial dysfunction and larger vessel diameters, markers of early adverse vascular changes.[7-11]

Malondialdehyde (MDA) is a major metabolite of arachidonic acid (20:4) [fatty acid with 20 carbons and four double-bonds]. [12] It is a reliable marker of free radical mediated lipid peroxidation or the oxidative damage. *Lipid peroxidation* is a process in which reactive oxygen species (ROS) result in the oxidative deterioration of lipids. Free radical is a molecule that contains one or more unpaired electrons in its outer orbital and is capable of creating havoc in living systems. Oxidative damage is initiated when the double bonds in fatty acids of membrane lipids are attacked by oxygen derived free radicals, particularly by -OH extensive member leading to the formation of products like aldehydes like malondialdehyde, ketones or polymerisation products. MDA has been used for many years as a for lipid peroxidation because of its ability to react with thiobarbituric acid to form an intensely colored chromogen .[13]

Paraoxonase (PON) (EC number – 3.1.8.1) is a hydrolase enzyme belonging to the class of group A aryl dialkylphosphatases. As it is measured by using paraoxon as its substrate (O,O-diethyl, O-p-nitrophenyl phosphate), it has been widely accepted to refer to this enzyme family as "paraoxonase".[14] It is reported that the paraoxonase/arylesterase enzyme family is composed of three forms (PON1, PON2 and PON3) .[15]These are located next to each other on chromosome 7 in humans. Paraoxonase is known to hydrolyze oxidized phospholipids and protect lipoproteins (LDL and HDL) and membranes from oxidative modifications.[16]

The aim of the study was to find out if there is any relation between menopause and oxidative stress as well as antioxidant defenses by way of estimating paraoxonase activity in post menopausal women.

## MATERIALS & METHODS

Our study was conducted from March-November 2014.The study was conducted on 60 human subjects, out of which 30 were menopausal females with no history of hypertension and diabetes & 30 were pre menopausal healthy controls, coming for regular health check up. A narrow range of age group was taken to reduce the effect of aging as it is an independent factor which can increase oxidative stress. Mean age of post menopausal women was 41.1 ± 1.2 years whereas for pre menopausal women it was 48.3 ± 1.5 years. Study was conducted after taking informed consent from the patient and ethical committee approval of the institution.

### *Samples*

Under aseptic condition early morning fasting 4 ml blood sample was collected, in plain red capped vaccutainers. Collected blood was allowed to clot & centrifuged at 2000 rpm for 15 mins for separation of serum. All assays were performed on serum.

### *Assessments*

Malondialdehyde (MDA) was estimated by Kei-Satoh's method as described by Kei Satoh (1978) in his research study, thiobarbituric acid reactive substances (TBARS).[17]PON was estimated spectrophotometrically by spectrophotometric method as described in the research study by Schiavon R (1996).[18] Briefly, the assay mixture consisted of 500 µl of 2.2 mmol/l paraoxon substrate in 0.1 mol/l tris-HCl buffer, pH 8.0, containing 2 mmol/l $CaCl_2$ and 50 µl of fresh serum specimen. The absorbance was monitored at 405 nm, at 25 °C. The PON activity was expressed in nmols/ml/min.

## STATISTICALANALYSIS

Numerical values were reported in terms of mean and standard deviation. Statistical analysis of results was done by using student t test. P value <0.05 was considered statistically significant. Statistical analysis was done using the Statistical Package for Social Sciences (SPSS-17.0).

## RESULTS

**Table 1**: MDA & Paraoxonase activity in cases & controls As seen from table 1**,** there was increase in MDA levels in post menopausal women & the increase was significant statistically. Paraoxonase levels were significantly decreased in postmenopausal women compared to the pre menopausal women.

## DISCUSSION

Menopause can lead to alterations in women´s health, with excessive ROS formation and changes in the oxidative status of postmenopausal women. From table 1, we can see increased MDA in postmenopausal women. ROS (reactive oxygen species), formed during menopause, may react with a variety of molecules like lipids and macromolecules of connective tissue interfering with cell function. Malondialdehyde is produced during the attack of free radicals on membrane lipoproteins and polyunsaturated fatty acids.[19] It causes oxidative modification of LDL; Oxidized LDL is cytotoxic and could produce or promote endothelial dysfunction and the evolution of fatty streak that may contribute to the progression or initiation of atherosclerosis.[20] During oxidation initially minimally modified LDL is formed in the sub endothelial space. It can induce leukocyte-endothelial cell adhesion and promote secretion of monocyte chemo tactic protein-1 (MCP 1) and macrophage colony stimulating factor by the endothelium. This produces monocyte binding and migration into sub endothelial space, where macrophage colony stimulating factor promotes differentiation of monocytes into macrophages. Oxidized LDL can also promote atherogenesis by stimulating the expression of several other genes in the arterial wall such as interleukin 1 (IL 1)further leading to atherosclerosis.[21]

As seen from table 1, there was decrease in paraoxonase activity in post menopausal women compared to controls. Various studies have shown decreased HDL in postmenopausal women who can lead to decreased paraoxonase activity hence decreased anti oxidant protection.[22,23] Furthermore, there are studies related to HDL-independent antioxidant properties of PON1 and its effects on levels of hydroperoxides and platelet activating factor, which may also affect oxidative stress in tissues.[24-28] It has been suggested that subjects with low PON activity have a greater risk of developing diseases in which oxidative damage and lipid peroxidation are involved like atherosclerosis, compared with subjects with high PON activity.[29] PON which is co-present with HDL in the plasma prevents the oxidation of plasma lipoproteins. By re-metabolizing the broken down lipids, it prevents accumulation of lipid peroxides in HDL and LDL. Thus, HDL indirectly protects LDL from oxidation Some studies emphasize the homocysteine thiolactonase activity of the enzyme & it hydrolyses homocysteine thiolactone into homocysteine.[30] Homocysteine levels are directly proportional to homocysteine-thiolactone which is a toxic product.As homocysteine thiolactone is unstable compound and can bind to proteins at lysine residues; this N homocysteinylation of proteins alters protein's structure and increases its susceptibility to proteolysis. The decrease in paraoxonase activity initiates a positive feedback response, since reduced paraoxonase activity will cause further accumulation of homocysteine thiolactone and may augment protein homocysteinylation.[31] Furthermore, it has been demonstrated that the interaction between homocysteine thiolactone and low density lipoproteins (LDL) induces the formation of homocystamide-LDL adducts (Hcy-LDL). Structural and functional alterations of homocysteine thiolactone and low density lipoproteins have been described and it has been demonstrated that homocysteinylation could increase atherogenicity of LDL. [32] PON 2 which is not bound to HDL also has antioxidant characteristics and inhibits LDL oxidation. Besides, it inhibits the monocyte chemotaxis activated by oxidized LDL.[33] Based on the substrate spectrum elucidated by many lines of research, the PONs are now proposed to be lactonase enzymes. Oxidized metabolites of polyunsaturated fatty acids (PUFAs) could be physiological substrates of PONs, because the structure of many of these molecules is similar to that of lactones.[34]

As seen from the table, MDA levels are negatively correlated with paraoxonase activity in post menopausal women compared to controls. Various studies have shown decreased HDL levels due to falling estrogen levels in menopause which can lead to decreased paraoxonase activity which can further lead to less antioxidant protection leading to increased MDA levels. Hence, post-menopausal women with decreased anti oxidant defenses by way of decreased paraoxonase activity could be more prone to oxidative stress both of these factors can add up to make them more susceptible for atherosclerosis.

## CONCLUSION

We speculate that the enhanced LDL oxidation associated with associated with increased MDA formation & HDL dependent as well as independent dysfunction of paraoxonase can put post menopausal women at higher risk of developing atherosclerosis or CHD.Further studies with large sample size & patient samples from different strata should be taken up to substantiate these findings.

**Table 1: MDA & Paraoxonase activity in cases & controls**

| | Cases (Postmenopausal) | Controls (Premenopausal) |
|---|---|---|
| **n** | 30 | 30 |
| **Mean age *(Years)* (mean ± SD)** | 50 ± 2 | 41 ± 1.5 |
| **MDA (Mean ± SD) *(µmol/l)*** | 5.0 ± 0.3* | 1.7 ± 0.1 |
| **Paraoxonase activity (Mean ± SD) *(nmols/ml/min)*** | 2.9 ± 0.4 | 7.2 ± 0.4* |

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