



## Status of antioxidant enzymes and trace metals in postmenopausal women

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**OBJECTIVE(S)** : To know the status of antioxidant enzymes in postmenopausal women and to find their correlation with metals.

**METHOD(S)** : Plasma malondialdehyde (PMDA) which is a marker of lipid peroxidation, estradiol, status of antioxidant enzymes, trace metals and lipid profile level were estimated in the blood of postmenopausal women (n=50) and compared with those in age matched premenopausal women treated as control (n=50).

**RESULTS** : In post menopausal women, there was a significant decrease in estradiol, reduced glutathione, glutathione peroxidase, superoxide dismutase and high density lipoprotein cholesterol (HDL-C) and a significant increase in PMDA, catalase, zinc (Zn) total cholesterol (Tc), triglyceride (Tg), very low density lipoprotein (VLDL), and low density lipoprotein (LDL) when compared to these in the control group. The changes in copper (Cu) and iron (Fe) between the groups were nonsignificant. Superoxide dismutase showed an inverse correlation with Zn

**CONCLUSION(S)** : Menopause is associated with oxidative stress as indicated by increase in lipid peroxidation and lipid parameters except HDL. Antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPX) decrease while catalase (CAT) increases in postmenopausal women showing oxidative stress in the cells.

**Key words** : antioxidant enzymes, estradiol, plasma malondialdehyde

### Introduction

Menopause is associated with a wide variety of physical and psychological symptoms. It is a gradual three-stage process that concludes with the end of periods and reproductive life. Women experience menstrual bleeding during menopause and perimenopause. When a woman's menstruation has ceased spontaneously at least for a year it is postmenopause<sup>1</sup>. In post-menopause, ovaries stop making estrogen hormone. The antioxidant enzyme (AOE) system seems to be affected in this phase due to deficiency of estrogen, which has got antioxidant properties. The beneficial effects of estrogens might be attributable to their free radical scavenging structures<sup>2</sup>. Another benefit of estrogen is that they decrease low density lipoprotein (LDL) cholesterol and increase high density lipoprotein (HDL) cholesterol affecting lipid metabolism. Estrogen also plays a role in the increased production of neurotrophic growth factors, which modulate

neuronal growth survival and aging. Menopause is a natural step in the process of aging. Free oxygen radicals have been proposed as important causative agents of aging<sup>3</sup>. Aging increases because of free radical damage. Hence menopausal women develop oxidative stress (OS) because of estrogen deficiency and advancing age, accompanied with age related changes.

The human RBC has an effective mechanism to prevent and neutralize this OS induced damage. This is accomplished by a set of antioxidant enzymes as glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD). These enzymes are present as metallozymes. SOD is a metalloprotein present as Cu-Zn SOD in which Cu is the catalytic metal and Zn helps to maintain the enzyme structure. CAT is a hemeprotein, catalyzing the decomposition of H<sub>2</sub>O<sub>2</sub> to water and oxygen. GPX is a selenoenzyme, which catalyzes the degradation of H<sub>2</sub>O<sub>2</sub> and hydroperoxides at the expense of reduced glutathione (GSH)<sup>4</sup>.

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Our study in menopausal women was aimed to find out the status of AOE and lipid profile, and correlate levels of AOE with trace metals.

## Material and Methods

The study was carried out in 50 postmenopausal women and 50 premenopausal women of the age group of 40-55 years. All patients were taken from gynecological out patient department. Pre-menopausal women were treated as control group. Post-menopausal women had at least one year of amenorrhea. None had received estrogen therapy or any supportive treatment for menopausal symptoms for at least 6 months prior to the study. Written consent was taken from the women, and all ethical measures were followed prior to the study.

The blood samples were analyzed for plasma lipid peroxidation<sup>5</sup>, reduced glutathione<sup>6</sup> and antioxidant enzymes like glutathione peroxidase<sup>7</sup>, catalase<sup>8</sup> and superoxide dismutase<sup>9</sup>. Lipid profile was done by standard kit method

**Table 1. Subject characteristics**

Characteristics	Premenopausal (Control group) N=50	Postmenopausal (Study group) N=50
Age (years)	45.9 ± 2.8 <sup>a</sup>	47.7 ± 4.4 <sup>a</sup>
Systolic blood pressure (mm Hg)	127 ± 2 <sup>b</sup>	135 ± 4.1 <sup>b</sup>
Diastolic blood pressure (mm Hg)	79 ± 2 <sup>c</sup>	85 ± 2.1 <sup>c</sup>

<sup>a</sup> P<0.05 (significant)      <sup>b</sup> and <sup>c</sup> P<0.001 (highly significant)

**Table 2. Status of antioxidant enzymes, estradiol and metals in pre- and post-menopausal women**

	GSH U/dL	GPX U/dL Hb	CAT U/g protein/mL	SOD U/mg protein/mL	PMDA nmol/mL	Estradiol pg/mL	Fe mg/dL	Cu mg/dL	Zn mg/dL
Premenopausal (N=50)	30.48 ± 2.85 <sup>a</sup>	8.90 ± 0.49 <sup>b</sup>	6.04 ± 0.22 <sup>c</sup>	13.0 ± 0.14 <sup>d</sup>	3.0 ± 0.19 <sup>e</sup>	145.93 ± 16.82 <sup>f</sup>	38.0 ± 3.08 <sup>h</sup>	0.30 ± 0.04 <sup>i</sup>	1.10 ± 0.44 <sup>g</sup>
Postmenopausal (N=50)	14.26 ± 3.11 <sup>a</sup>	3.59 ± 1.37 <sup>b</sup>	7.51 ± 0.74 <sup>c</sup>	6.98 ± 0.78 <sup>d</sup>	5.97 ± 0.64 <sup>e</sup>	16.6 ± 20.0 <sup>f</sup>	38.67 ± 4.41 <sup>h</sup>	0.31 ± 0.73 <sup>k</sup>	1.46 ± 0.57 <sup>g</sup>

<sup>abdef</sup> P<0.001 Highly significant      <sup>g</sup> P<0.01 Significant      <sup>h</sup> Nonsignificant

**Table 3. Status of lipid profile in pre - and post menopausal women**

Type of Subject	Total cholesterol mmol/L	Triglyceride mmol/L	HDL-c mmol/L	LDL-c mmol/L	VLDL-c mmol/L
Premenopausal (N=50)	4.54±0.24	1.32±0.20	1.20±0.09	2.74±0.24	0.59±0.22
Postmenopausal (N=50)	5.20±0.57	2.06±0.38	1.07±0.13	3.16±0.54	0.93±1.73

All differences are highly significant (P<0.001)

(Span / Diaagnostic Ltd.), and estrogen was estimated by Omega Kit method. Metal analysis (copper, iron and zinc) was done by atomic absorption spectrophotometer [(AAS)-Model Analyst 100 Perkin Elmer USA]. For statistical analysis, post-menopausal women were compared to pre-menopausal women treated as control. Statistical analysis was done by using exact software package.

## Results

The levels of reduced glutathione, antioxidant enzymes as GPX, CAT, SOD, PMDA, estradiol; metals as Cu, Fe, Zn, and lipid profile in postmenopausal women were compared with those in premenopausal women treated as control.

Table 1 shows age and systolic and diastolic blood pressure of pre-and postmenopausal women.

Table 2 shows a significant decrease in GSH, GPX, SOD, estradiol (P<0.001) and a significant increase in CAT, PMDA (P<0.001) and Zn (P<0.01) in postmenopausal women as compared to those in premenopausal women. The changes in Cu and Fe were nonsignificant.

Table 3 shows a significant increase in lipid parameters like total cholesterol (TC), Tg, VLDL-c and LDL-c (P<0.001) in postmenopausal women when compared to those in the control group while HDL-c showed a significant decrease (P<0.001) in the same.

**Table 4. Correlation of antioxidant enzymes with trace metals in post - menopausal women**

	Fe	Cu	Zn
GPX	0.0629	-0.1962	-0.0556
CAT	0.1652	-0.0210	-0.0131
SOD	0.0271	-0.3212 <sup>a</sup>	-0.2845 <sup>a</sup>

Values expressed as correlation coefficient (r)

<sup>a</sup> P<0.05. Differences in other values are nonsignificant

Table 4 shows correlation values. An inverse correlation of SOD with Cu and Zn (P<0.05) is present. The correlation of other AOE's with metals was non-significant.

## Discussion

Menopausal phase in a woman's life is an important physiological phenomenon, which is associated with cessation, of menstrual cycle due to loss of ovarian function.

The deficiency of estrogen in postmenopausal women develops oxidative stress, due to release of free radical or reactive oxygen species (ROS) and becomes the cause of various pathologies like development of hypertension (Table 1). Estrogen is a powerful antioxidant, which prevents lipid peroxidation and change in lipid profile, as observed in premenopausal group (Table 3). In postmenopausal women aging has been associated with increased concentration of Tc, Tg, VLDL-c, and LDL-c and decreased concentration of HDL-c, all of which contributed to a more atherogenic lipid profile (Table 3). This finding is consistent with the earlier finding of Abbey et al<sup>10</sup>. The cardio protective effect of estrogen has been long related to its beneficial effect on cholesterol metabolism and deposition, contributing to inhibition of atherosclerotic plaque formation in arterial walls.

Estrogen lowers LDL-c by upregulating LDL receptors in the liver and enhancing LDL catabolism<sup>11</sup>. Estrogen use is associated with elevations in HDL by up to 25% and HDL seems to be the best predictor of coronary heart disease risk in women<sup>12</sup>.

The erythrocytes are an early model for studies of oxidative stress. They are a target for oxidative reaction because of their relatively high oxygen tension and presence of hemoglobin and a plasma membrane rich in polyunsaturated fatty acids (PUFA). A group of antioxidants present in RBC which prevents lipid peroxidation consists of SOD, GPX, catalase and reduced glutathione. In postmenopausal women in our study the levels of reduced glutathione, GPX, and SOD are found decreased along with increased catalase activity (Table 2). SOD is irreversibly inactivated by its product hydrogen peroxide, because exposure of intact erythrocytes

to H<sub>2</sub>O<sub>2</sub> resulted in inactivation of endogenous SOD activity in the concentration dependent manner, GSH and GPX are consumed in reducing excessive H<sub>2</sub>O<sub>2</sub> and hydro-peroxides originating from PUFA. Catalase activity is enhanced in the red cells, taking care of the disposal of H<sub>2</sub>O<sub>2</sub> in the cells particularly when GPX is significantly reduced<sup>13</sup>. Since all enzymes are metalloprotein, the level of metals in blood could be correlated with the activity of enzymes. Supplementation in diet can fulfill any deficiency of metal. The present study shows inverse correlation between SOD and ZN (Table 4). Zincemia observed in our study may be due to osteoporosis associated with post menopause<sup>14</sup>. No significant change is noted in the level of Cu and Fe in this stage.

It is therefore concluded that oxidative stress in postmenopausal of women causes potential oxidative injury in the cell, which causes pathology in this stage of life.

## References

- Porter M, Penney GC, Russell D et al. A population based survey of women's experience of the menopause. *Br J Obstet Gynecol* 1996; 103: 1025-8.
- Ruiz-Larrea MB, Martin C, Martinez R et al. Antioxidant activities of estrogens against aqueous and lipophilic radicals; differences between phenol and Catechol estrogens. *Chem Phys Lipids* 2000; 105: 179-88.
- Inal ME, Kanbak G, Sunal E. Antioxidant enzyme activities and malondialdehyde levels related to aging *Clin Chim Acta* 2001; 305: 75-80.
- Gutteridge JMC. Free radicals in disease processes: a compilation of cause and consequence. *Free Radic Res Common* 1993; 19: 141-58.
- Jean CD, Maryse T, Marie JF. Plasma malondialdehyde levels during myocardial infarction. *Clin Chim Acta* 1983; 129: 319-22.
- Beutler E, Duron O, Kelly BM. Improved method for determination of blood glutathione. *J Lab Clin Med* 1963; 61: 882-8.
- Hafeman DG, Sunde RA, Hoekstra WG. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *J Nature* 1971; 104: 580-7.
- Sinha KA. Colorimetric assay of Catalase. *Analytical Biochem* 1972; 47: 389-94.
- Mishra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase. *J Biol Chem.* 1972; 247: 3170-5.
- Abbey M, Owen A, Suzakawa M et al. Effects of menopause and hormone replacement therapy on plasma lipids, lipoproteins and LDL-receptor activity. *Maturitas* 1999; 33: 259-69.
- Walsh BW, Schiff I, Rosner B et al. Effects of postmenopausal estrogen replacement therapy on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med* 1991; 325: 1196-204.
- Bass KM, Newschaffer CJ, Klag et al. Plasma lipoprotein levels as predictors of cardiovascular death in women. *Arch Intern Med* 1993; 153: 2209-16.
- Kumar PA, Rajagopal G. Lipid peroxidation in erythrocytes of patients with type 2 diabetes mellitus. *Ind J Clinical Biochem* 2003; 18: 71-4.
- Contreras F, Simonovis N, Fouillious C et al. Zincuria and Zincemia in postmenopausal osteoporosis. *Science Direct-International congress series* 2002; 1237: 219-29.