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"Study of antioxidant and lipid peroxidation status among postmenopausal women"

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Abstract

Present study was conducted in Department of Biochemistry Dr. SCGMC Nanded over the period of 18 months. Well informed written consent was taken from every study subject and it was ethically cleared from the institutional ethical committee. Study subjects includes 50 healthy premenopausal and 50 healthy postmenopausal women. We have studied the antioxidant status my measuring the values of antioxidant enzymes as glutathione peroxidase (GPx) and superoxide dismutase (SOD). Lipid peroxidation status was studied by measuring the malondialdehyde (MDA) level. One-way ANOVA was used for statistical analysis. We observed that the mean glutathione peroxidase (GPx) and superoxide dismutase (SOD) levels were decreasing from premenopausal to early postmenopausal and late postmenopausal women and mean MDA levels were increasing from premenopausal to early postmenopausal to late postmenopausal. The p value was<0.0001 and was statistically significant. At the end we conclude that in postmenopausal women the antioxidant defense decreases and oxidative stress increases as compared to premenopausal women and these parameters are directly proportional to menopausal duration.

Keywords: Antioxidant, Lipid peroxidation, Antioxidant enzyme (AOE) system, Glutathione peroxidase (GPx), Superoxide dismutas (SOD), Atherosclerosis, Malondialdehyde (MDA)

Introduction:

Menopause marks the time in a woman's life when her menstruation stops and she is no longer fertile due to depletion of ovarian follicles and gradual decrease in ovarian production of estrogen and other hormones[1]. In postmenopausal women, 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[2]. Menopause is a natural step in the process of ageing. Free oxygen radicals have been proposed as important causative agents of ageing[3]. The human RBC has an effective mechanism to prevent and neutralize this oxidative stress induced damage. This is accomplished by a set of antioxidant enzymes as glutathione peroxidase (GPx) and superoxide dismutase (SOD). These enzyes 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. Glutathione peroxidase is a selenoenzyme, which catalyzes the degradation of H_2O_2 and hydroperoxides at the expense of reduced glutathione (GSH) [4]. The lipids of cell membranes are favourite targets of the free radicals which get oxidized leading to lipid peroxidation. The lipid peroxidation is specifically dangerous for the cell as it propagates as a self-perpetuating chain reaction[5]. Peroxidative modification of LDL-C is an important factor in the formation of atherosclerotic changes. This might help to explain the frequent occurrence of coronary artery disease in normolipidemic people [6, 7, 8]. Lipid peroxidation is a complex process associated with a number of pathologic phenomena, such as increased membrane rigidity,

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atherosclerosis, carcinogenesis and myocardial infarction[9]. The malondialdehyde (MDA) production is recognized a marker for lipid peroxide and as an end-product of lipid peroxidation[10].

Present study was undertaken to find out the relation between menopause and oxidative stress, as enhanced oxidative stress may be a reason for increased tissue damage and other physiological symptoms that women face after menopause and also to find out the antioxidant status of these women.

Aims and objectives:

To determine the extent of free radical damage(in the form of lipid peroxidation product malondialdehyde) and antioxidant status(in the form of superoxide dismutase and glutathione peroxidase) in postmenopausal women and to compare it with premenopausal women.

Material and methods:

This study was conducted in Department of Biochemistry Dr. SCGMC Nanded over the period of 18 months. Each subject gave an informed written consent and was approved by an institutional ethical committee. In the study total 100 female subjects were included of which 50 were healthy premenopausal women who served as control and 50 were healthy postmenopausal women who served as cases. Postmenopausal women were divided in to two groups as early postmenopausal with menopause for less than 5 years and late postmenopausal with menopause for more than 5 years.

Inclusion criterion for cases

Healthy postmenopausal women with history of natural menopause (i.e. cessation of menstrual period for at least 1 year) without any major illness, DM, Hypertension, cardiovascular diseases etc.

Inclusion criterion for controls

Healthy premenopausal women without any menstrual irregularities & without any major illness, DM, Hypertension, cardiovascular diseases etc.

Parameters

In the present study following biochemical parameters were studied

Sr. no	Parameter	Method
1.	Glutathione peroxidase	UV method based on Paglia and Valentine
2.	Superoxide dismutase	Marklund S. ,Markuland G. , 1974, Modified by Nandi et al., 1998
3.	Malondialdehyde	Kei Satoh

SOD and MDA were analyzed on spectrophotometer while GPx was analyzed on Accustar semiautoanalyzer.

Blood sample for estimating glutathione peroxidase was collected in separate heparin containing vial and in plain bulb for rest parameters. 5ml of venous blood was drawn under aseptic

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precaution using sterile disposable syringe and serum was separated by centrifugation and was used for analysis.

Observations:

TABLE NO. 1
Distribution of antioxidants among study subjects

Parameter		**Pre-M**	**Early-PM (**<5 year)	**Late-PM (**>5 year**)**
GPx (Unit/gm of Hb)	**Mean ± 2SD**	56.34 ± 8.88	53.77 ± 2.68	50.07 ± 1.9
	Range	47.46 – 65.22	51.09 – 56.45	48.17 – 51.97
SOD (Unit/ml)	**Mean ± 2SD**	4.25 ± 0.46	3.55 ± 0.32	2.62 ± 0.36
	Range	3.79 – 4.71	3.23 – 3.87	2.26 – 2.98

(One-way ANOVA, p value<0.0001, significant)

In premenopausal group the range of glutathione peroxidase was 47.46–65.22 Unit/gm of Hb and the range of superoxide dismutase was 3.79–4.71 Unit/ml. In early postmenopausal group the range of glutathione peroxidase was 51.09–56.45 Unit/gm of Hb and the range of superoxide dismutase was 3.23–3.87 Unit/ml. In late postmenopausal group the range of glutathione peroxidase was 48.17–51.97 Unit/gm of Hb and the range of superoxide dismutase was 2.26–2.98 Unit/ml.

The levels of glutathione peroxidase and superoxide dismutase were significantly decreased in both classes of postmenopausal women in comparison with premenopausal women with p value <0.0001

TABLE NO. 2
Distribution of lipid peroxidation product among Study subjects

Parameter		**Pre-M**	**Early-PM (**<5 year)	**Late-PM (**>5 year**)**
MDA (nmol/ml)	**Mean ± 2SD**	1.67 ± 0.42	2.35 ± 0.22	2.94 ± 0.64
	Range	1.25 – 2.09	2.13 – 2.57	2.3 – 3.58

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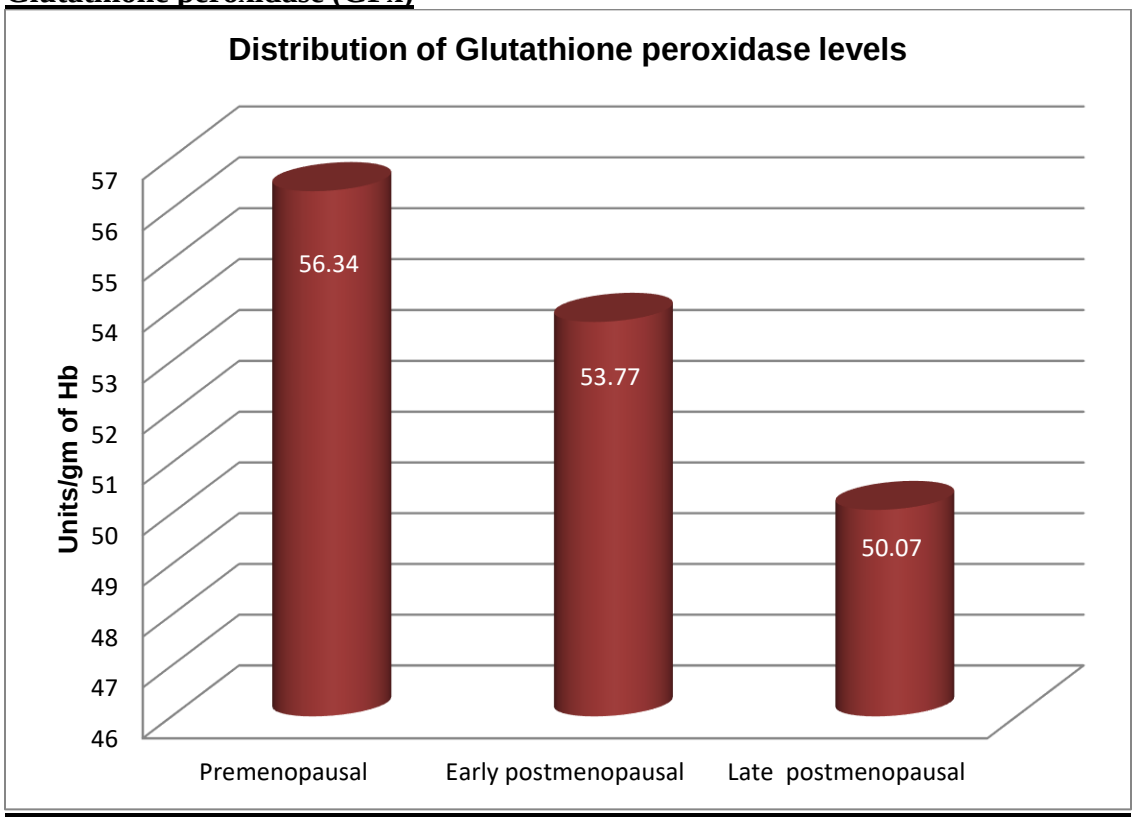
(One-way ANOVA, p value<0.0001, significant)

The range of malondialdehyde was 1.25–2.09 nmol/ml, 2.13–2.57nmol/ml, 2.3–3.58 nmol/ml in premenopausal, early postmenopausal and late postmenopausal groups respectively and the increase in the malondialdehyde level was statistically significant in early and late postmenopausal group as compared with premenopausal group with p value <0.0001.

Discussion:

This study had been done to determine the extent of free radical damage and antioxidant status in postmenopausal women in comparison with premenopausal women. We evaluated the free radical damage by estimation of MDA (malondialdehyde) which is lipid peroxidation product and the antioxidant status was evaluated by estimation of GPx (glutathione peroxidase) and SOD (superoxide dismutase). We had also seen the effect of duration of menopause on these parameters.

Glutathione peroxidase (GPx)



Graph No. 1: Distribution of glutathione peroxidase levels among study subjects

In premenopausal group the mean glutathione peroxidase level was 56.34±8.88 Unit/gm of Hb. In early postmenopausal group the mean glutathione peroxidase level was 53.77±2.68 Unit/gm of Hb.In late postmenopausal group the mean glutathione peroxidase level was 50.07±1.9 Unit/gm of Hb.

Thus the mean glutathione peroxidase levels were found to be decreasing from premenopausal to early postmenopausal to late postmenopausal. The p value was<0.0001 and was statistically significant.

Superoxide dismutase (SOD)

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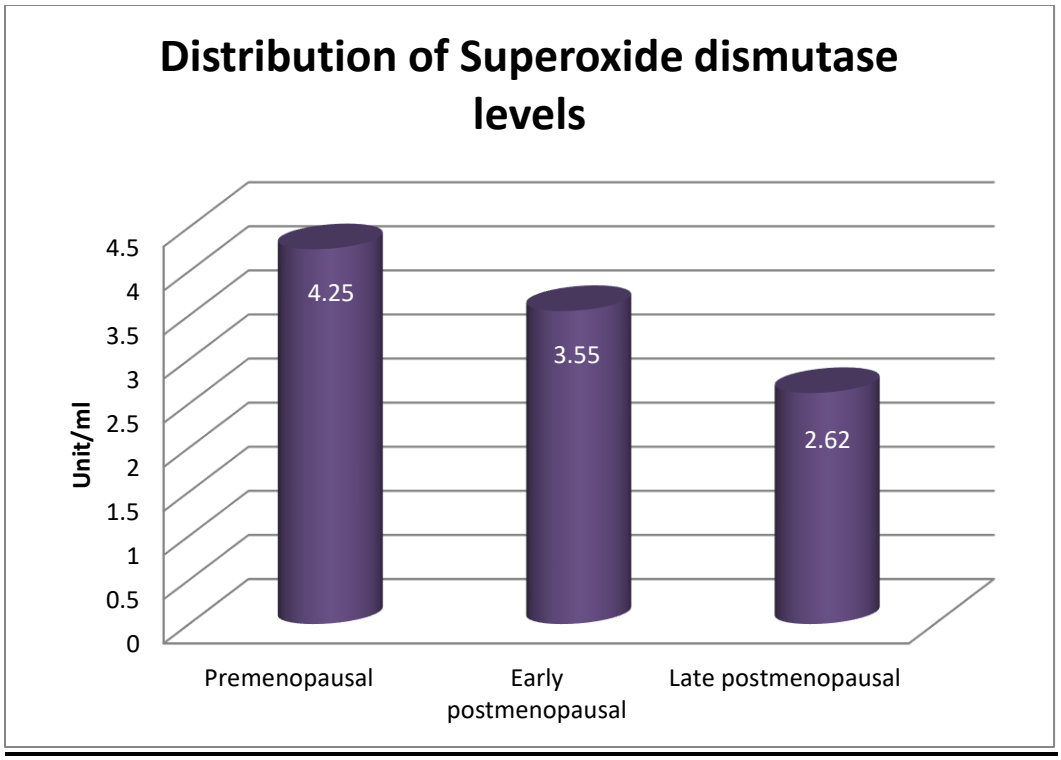
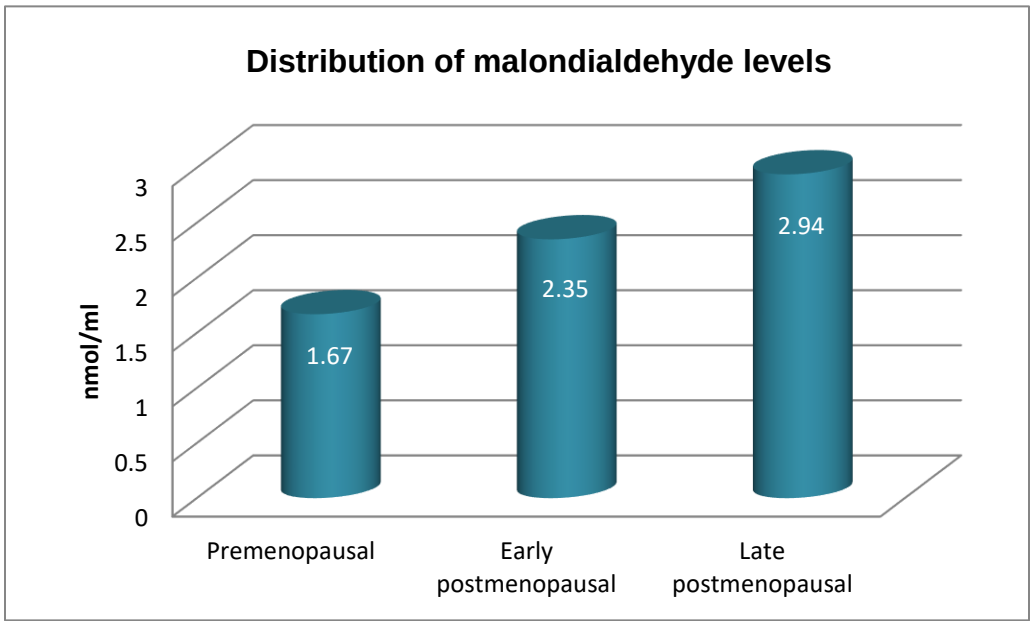


Graph No. 2: Distribution of superoxide dismutase levels among study subjects

In premenopausal group the mean superoxide dismutase level was 4.25±0.46 Unit/ml. In early postmenopausal group the mean superoxide dismutase level was 3.55±0.32 Unit/ml and in the late postmenopausal group the mean superoxide dismutase level was 2.62±0.72 Unit/ml. The mean superoxide dismutase levels were found to be decreasing from premenopausal to early postmenopausal and late postmenopausal. The p value was<0.0001 and was statistically significant.

Malondialdehyde (MDA)



Graph No. 3: Distribution of malondialdehyde levels among study subjects

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Mean malondialdehyde level was 1.67±0.42 nmol/ml in premenopausal group. In early postmenopausal group the mean malondialdehyde level was 2.35±0.22 nmol/ml. In late postmenopausal group the mean malondialdehyde level was 2.94±0.64 nmol/ml. Thus mean MDA levels were found to be increasing from premenopausal to early postmenopausal to late postmenopausal. The p value was<0.0001 and was statistically significant

Conclusion:

This study suggests that in postmenopausal women the antioxidant defense decreases and oxidative stress increases as compared to premenopausal women and these parameters are directly proportional to menopausal duration.

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