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ORIGINAL ARTICLE

Role Of Free Radicals In Menopausal Distress

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ABSTRACT

Aim: The aim of the study was to determine the extent of free radical damage in the form of oxidative stress and the antioxidant status in postmenopausal females as compared to premenopausal females.

Methods: 100 female subjects (age group 40-50 years) were studied of which 50 were premenopausal and 50 postmenopausal. Oxidative stress was assessed by estimating malondialdehyde (MDA), a lipid peroxidation product in the form of thiobarbituric acid reactive substances. Antioxidants in the form of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), vitamin C and E were measured in both the groups.

Results: On comparative evaluation, SOD, GPx and vitamin C levels were significantly decreased ($p < 0.001$) whereas MDA and CAT levels were significantly increased ($p < 0.001$) in postmenopausal females as compared to premenopausal females. However there was no significant difference in levels of vitamin E in both the groups ($p > 0.05$).

Conclusion: Postmenopausal females are exposed to greater risk of oxidative stress as compared to premenopausal females and many of the health problems of menopause may be related to increased oxidative damage.

Key Words: Menopause, Free radicals, Lipid peroxidation, Antioxidants.

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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]. During menopause women face various physiological, psychological and sociological alterations that impair quality of life or may be life threatening. The adverse effects of menopause are attributed to decrease in estrogen level which leads to alterations in lipid profile, body mass index, insulin levels and also to increased risk of hypertension, cardiovascular diseases, osteoporosis, diabetes mellitus, cancer and other degenerative changes in postmenopausal females. It has been observed that there is increased production of free radicals after menopause which is due to sudden alterations in hormonal status [2]. These free radicals lead to enhanced oxidative stress. Free radicals are potentially harmful to almost all the biomolecules including lipids, carbohydrates and proteins [3]. 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 [4]. There are certain naturally occurring antioxidants in our body which neutralize the effects of these free radicals thereby protecting the body against their deleterious effects. These antioxidants can be enzymatic e.g. superoxide dismutase (SOD), Glutathione peroxidase (GPx), catalase (CAT) or non-enzymatic which includes Vitamin C and Vitamin E [5].

In this study an effort was done to find out if there is any 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. To assess the level of oxidative stress we measured malondialdehyde, the end product of lipid peroxidation reaction that is produced as a result of damage to cell membrane lipids by free radicals. To determine the extent of protection against these oxidants, we estimated the levels of antioxidants (SOD, GPx, CAT,

Vitamin C and E) in the erythrocytes and plasma of pre- and post-menopausal females.

Materials and Methods

To conduct this study, 100 female subjects were selected in the age group of 40-50 years out of which 50 were premenopausal (with no history of menstrual irregularities) and 50 were postmenopausal (having menopause for more than one year). 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. The study was conducted in the department of Physiology, Adesh Institute of Medical Sciences and Research, Bathinda with approval of ethical committee. All the selected subjects were healthy and were not suffering from any disease like hypertension, diabetes mellitus, rheumatoid arthritis, malignancy, collagen disorders, or any other disease that could affect the oxidant status of the body. Also none of the subjects was on any medication or taking vitamins or mineral supplements. A detailed history of all the subjects included in the study was taken and their general physical and systemic examination was done. Informed consent was taken from all the subjects.

For assessment of various parameters, 10 ml of blood was collected under aseptic conditions from the antecubital vein of the subjects in heparinized vials. The Erythrocytes were washed with cold isotonic saline and used for estimation of SOD, GPx and CAT and the plasma was used for estimation of estradiol, MDA, vitamin E and vitamin C. Malondialdehyde (MDA) was used as an indicator of lipid peroxidation and was estimated in terms of thiobarbituric acid reactive species (TBARS) by the method of Satoh [6]. SOD activity was assayed based on the method of Marklund and Marklund [7]. The activity of Glutathione Peroxidase was measured as described by Paglia and Valentine (8). CAT was measured colorimetrically by the method of Sinha (9). Concentration of vitamin C was estimated in plasma according to the method of Natelson [10] and concentration of vitamin E was estimated by method of Baker and Frank [11]. Serum estradiol level was estimated by using ELISA kits (Diametra, Italy) according to manufacturer protocol.

Statistical analysis was carried out by Student's paired't'-test. The data were expressed as Mean ± SD and the p value < 0.05 was taken as significant.

Results

[Table/Fig 1] shows the mean ± SD of the various parameters studied in premenopausal and postmenopausal women. The postmenopausal females were found to have significantly lower levels of estradiol as compared to premenopausal females. There was a statistically significant increase in MDA and CAT levels with decrease in levels of SOD, GPx and Vitamin C in postmenopausal women as compared to premenopausal women while there was no significant variation in Vitamin E levels in both the groups.

(Table/Fig 1) Estradiol, MDA, SOD, GPx, CAT, Vitamin-C and Vitamin-E levels in Study Groups

Parameters	(Pre-menopausal females) Mean ± SD	(Post-menopausal females) Mean ± SD
Estradiol (pg/ml)	217.75 ± 89.43	18.56 ± 9.91**
MDA (nmol/ml)	1.69 ± 0.57	4.68 ± 0.44**
SOD (U/g Hb)	5.68 ± 0.42	3.05 ± 0.26**
GPx (U/g Hb)	43.51 ± 2.66	18.11 ± 1.98**
CAT (KU/g Hb)	17.58 ±3.15	30.40 ± 2.79*
Vitamin-C (mg/dl)	1.73 ± 0.32	0.66 ± 0.16**
Vitamin-E (mg/dl)	1.23 ± 0.29	1.21 ± 0.23***

*** p<0.01, ** p<0.001, *** p >0.05 when compared with Pre-menopausal females**

Discussion

Menopause is characterized by a number of untoward effects like hot flushes, palpitations, angina pectoris, psychogenic alterations, senile vaginitis, osteoporosis, degenerative arthropathy, hirsutism, etc. [12]. The pathophysiology of menopause is attributed to decreased levels of estrogen. Estrogen is known to have antioxidant properties and its deficiency after menopause predisposes the body to increased free radical damage (13). Estrogen acts as an antioxidant by preventing oxidation of LDL lipoproteins, terminating the lipid peroxidation chain reaction and may also stimulate the action of other antioxidant enzymes [14].

In this study we observed that the levels of MDA were significantly increased in postmenopausal females as compared to premenopausal females. Similar results of increased lipid peroxidation have been observed in ovariectomized rats used as an oxidative stress model [15]. Lipid peroxidation occurs as a result of oxidative destruction of polyunsaturated fatty acids (PUFAs) located in cell membranes that are readily attacked by oxidizing radicals [4]. The lipid peroxidation proceeds as self-perpetuating chain reaction to form lipid peroxides and aldehydes like MDA which are cytotoxic and mutagenic [16]. Moreover, lipid peroxidation has been implicated in a wide range of tissue injuries and diseases e.g. atherosclerosis and coronary artery disease.

Antioxidants like SOD, GPx and Catalase are present in erythrocytes which prevent lipid peroxidation. The present study shows that levels of SOD and GPx were significantly decreased and that of CAT was increased in postmenopausal females. Similar reports of decreased antioxidant enzyme activities have been reported in postmenopausal females by other authors

[17]. The antioxidant enzyme superoxide dismutase (SOD), the most important enzyme present virtually in all aerobic organisms, catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Glutathione peroxidase is a selenoenzyme which catalyzes the reduction of hydroperoxides at the expense of reduced glutathione. Catalase is a primary antioxidant defense component that works to catalyze the decomposition of hydrogen peroxide to water, sharing this function with GPx [5]. SOD and GPx concentrations are reduced as they continuously work to neutralize the superoxides, hydrogen peroxide and hydroperoxides produced in increased amount after menopause. Levels of CAT were observed to be increased which may be explained by the fact that the activity of catalase increases when the level of GPx decreases because they both work together to perform similar task of neutralizing hydrogen peroxide [18].

We observed a significant decrease in the levels of plasma vitamin C in postmenopausal females as compared to premenopausal females while there was no difference in vitamin E levels in both the groups. The decrease in the levels of vitamin C in postmenopausal females might be due to its increased consumption to counteract the increased oxidative stress and to inhibit membrane lipid peroxidation. Because of antioxidant properties, vitamin E neutralizes reactive oxygen species and reduces oxidative DNA damage and genetic mutations [19]. Vitamin C can restore the antioxidant properties of oxidized vitamin E, suggesting that a main function of vitamin C is to recycle the vitamin E radical [20]. This may result in decreased levels of vitamin C while maintaining the normal activity of vitamin E.

Conclusion

It is evident from this study that there is enhanced oxidative stress and decreased antioxidant defence in postmenopausal females as compared to premenopausal females which can play an important role in the pathogenesis of the various diseases related to menopause. Therefore antioxidants in the form of micronutrients and vitamins can be given as supplements in postmenopausal women along with or as a substitute to hormone replacement therapy which itself is associated with serious side effects.

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