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Original Article

Status of trace elements and antioxidants in premenopausal and postmenopausal phase of life: a comparative study

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Abstract: The aim of the study was to determine the extent of free radical damage in the form of oxidative stress, the antioxidant status and correlate with trace element levels in postmenopausal females as compared to premenopausal females. Participants between the ages of 30-60 years were recruited for the study and status of antioxidant enzymes and trace metals level was determined. The serum Calcium (Ca) levels after menopause was higher than that of the premenopausal group (P<0.001). The changes in copper (Cu) and Zinc (Zn) between the groups were not significant (p>0.05). In postmenopausal women, antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GP_x), catalase (CAT) significantly decreased (P<0.001) in postmenopausal women showing oxidative stress in the cells. Concentrations of vitamin-C pointed out a significant decrease (P<0.05) in postmenopausal women when compared with premenopausal women. In conclusion.

Keywords: Trace elements, menopause, oxidative stress

Introduction

Menopause is a natural step in the process of aging [1]. Women face various physiological, psychological and sociological changes that impair quality of life during menopause [2]. The risk of nutritional disturbances, particularly trace elements and vitamin deficiencies is high during menopause. Several trace elements are essential in bone metabolism [3]. 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 [4]. It has been observed that there is increased production of free radicals after menopause which is due to sudden alterations in hormonal status [2, 5]. There is enhanced oxidative stress and decreased antioxidant defense in postmenopausal females as compared to premenopausal females which can play an important role in the pathogenesis of the various diseases related to menopause [6-9].

Free radicals are potentially harmful to almost all the biomolecules including lipids, carbohydrates and proteins [7, 8, 10-16]. The lipids of cell membranes are favorite 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 [17]. 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 (GP_x), catalase (CAT) or non-enzymatic which includes Vitamin C and Vitamin E [5, 17, 18]. The progressive loss of estrogen and its protective effects, combined with deficient endogenous antioxidant results in oxidative stress [19].

The decrease in sex steroid hormones during menopause in women causes a number of disturbances in the metabolism of different organs. Also, the risk of osteoporosis, cardiovascular disease, impairment of glucose meta-

Table 1. Subject characteristics of all participants

Parameters	Subject	
	Premenopausal (Control group)	Postmenopausal (Study group)
	N=50	N=50
Age (years)	38.5 ± 3.6	55.5 ± 7.3
Height (cm)	152.31 ± 10.12	155.12 ± 12.51
Weight (kg)	62.64 ± 9.39	63.46 ± 8.01
Systolic BP (mm Hg)	129 ± 6.38	132 ± 9.32*
Diastolic BP (mm Hg)	80 ± 6.5	84 ± 4.45*

*P<0.05 (significant).

Table 2. Status of antioxidant enzymes in pre- and postmenopausal women

Parameters	Subject	
	Premenopausal (Control group)	Postmenopausal (Study group)
	N=50	N=50
SOD (IU/mg prot)	11.12 ± 2.89	7.15 ± 2.31**
CAT (IU/mg prot)	7.31 ± 1.16	5.12 ± 1.13**
GP_x (nmol/mg prot)	12.15 ± 1.23	8.89 ± 1.81**
Vitamin C (mg/dl)	2.51 ± 0.32	1.21 ± 0.08*
Vitamin E (mg/dl)	2.11 ± 0.91	1. 99 ± 0.34

*P<0.05 (significant) and **P<0.001 (highly significant).

Table 3. Status of metals in pre- and postmenopausal women

Parameters	Subject	
	Premenopausal (Control group)	Postmenopausal (Study group)
	N=50	N=50
Zn (mg/dl)	1.31 ± 0. 21	1.38 ± 0.65
Cu (mg/dl)	0.31 ± 0.07	0.29 ± 0.031
Ca (mg/dl)	9.5 ± 2.12	13.32 ± 1.12**

**P<0.001 (highly significant).

bolism, and breast cancer are increased during this time [20].

Several trace elements, particularly Ca, Mg, Cu, Mn, and Zn are essential in bone metabolism [21]. Some trace minerals are cofactors of many enzymes. Selenium (Se) is a cofactor of glutathione peroxidase, one of the most important enzymes of the free radical defense enzyme. Zn and Cu molecules are integrated elements of superoxide dismutase (Cu/Zn SOD). Manganese superoxide-dismutase (Mn SOD) is a major enzyme responsible for detoxification of ROS in the mitochondria [22]. Reactive oxygen species (ROS) and lipid peroxide, which are produced by a free radical chain reaction, have been implicated in the pathogenesis of a variety of condition, including menopause [10, 23]. Estrogen deficiency in post menopausal women may associate with postprandial hyerlipidemia, and could limit peripheral glucose up take [24]. In this study, our aim was to find out if there is any relation between menopause, oxidative stress, and trace elements as enhanced oxidative stress may be a reason for increased tissue damage and other physiological symptoms that women face after menopause.

Materials and methods

The study was carried out in 50 post-menopausal women (50-60 years) and 50 pre-menopausal women (30-40 years). 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.

The blood samples were analyzed for antioxidant enzymes like glutathione peroxidase, catalase and superoxide dismutase [25-27]. Metal analysis (copper, calcium and zinc) was done by atomic absorption spectrophotometer [(AAS)-Model Analyst 100 Perkin Elmer USA]. For statistical analysis, post-menopausal women were compared to premenopausal women treated as control.

Statistical analysis was done by using SPSS software. Results were expressed as the mean ± standard error of the mean (SEM). Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the Statistical Package for the Social Sciences (SPSS version 17.0). All *P* values are two-tailed and P<0.05 was considered significant for all statistical analysis in this study.

Results

The levels of antioxidant enzymes as GP_x, CAT, SOD; metals as Zn, Cu, Ca, in postmenopausal women were compared with those in premenopausal women treated as control.

Table 4. Correlation of antioxidant enzymes with trace metals in postmenopausal women

	Ca	Zn	Cu
GP_X	0.1238	-0.1834	-0.1387
CAT	0.1547	-0.0321	-0.0451
SOD	0.1176	-0.2176*	-0.3546*

Values expressed as correlation coefficient (r please clarify how it was calculated?) *P<0.05. Differences in other values are non significant.

Table 1 shows age, systolic and diastolic blood pressure of premenopausal women and postmenopausal women.

Table 2 shows a significant decrease in GP_X, SOD, and catalase (P<0.001) and also in vitamin C (P<0.05) levels in postmenopausal women as compared to premenopausal women. Changes in vitamin-E level were not significant.

Table 3 shows the levels of metals as Zn, Cu and Ca in postmenopausal women were compared with those in premenopausal women treated as control. The changes in Cu and Zn were non significant. However, there was a significant increase in the serum Ca levels (P<0.001).

Table 4 shows correlation of antioxidant enzymes with trace metals in postmenopausal women. An inverse correlation of antioxidants (AOEs) with Zn and Cu is present. An inverse correlation of SOD with Cu and Zn (P<0.05) is significant. The correlation of other AOEs with metals is 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 presence of oxidative stress can negatively impact a women's health in long term. 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 [4, 16, 28]. Free oxygen radicals have been proposed as important causative agents of aging. Aging increases because of free radical damage.

In postmenopause, ovaries stop making estrogen hormone and circulating concentrations of estrogen decrease. The antioxidant enzyme (AOE) system can also be altered due to deficiency of estrogen, which has got antioxidant properties. The human RBC has an effective mechanism to prevent and neutralize the oxidative stress induced damage. There are certain naturally occurring antioxidants in our body which neutralize the effects of these free radicals and protect the body against their deleterious effects.

The present study pointed out significant decrease (P<0.001) antioxidant enzyme (AOE) activities in postmenopausal women. The pathophysiology of menopause is attributed to decrease of estrogen. Antioxidants like SOD, glutathione peroxidase act to prevent lipid peroxidation. The antioxidants enzyme SOD, is the most important enzyme present virtually in all aerobic organism, catalyzes the dismutation of super oxide (O_2-) into oxygen and hydrogen peroxide (H_2O_2). GP_X is a selenoenzyme, which catalyzes the degradation of H_2O_2 and hydroperoxides at the expense of reduced glutathione (GSH). The catalase, the other antioxidant enzyme, catalyzes conversion of H_2O_2 into H_2O and O_2 [17]. This study, showed a significant decrease in the level of vitamin-C associated with non-significant decrease in the level of vitamin-E in postmenopausal women when compared with premenopasual women.

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. 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 [29]. This may result in decreased levels of vitamin C while maintaining the normal activity of vitamin E.

The present study showed that trace elements (Zn, Cu) status in postmenopausal women is not significantly different from that of premenopausal women. However, results of serum copper (Cu) in postmenopausal women pointed out decrease when compared with premenopausal women. These results were agree with previous studies which are pointed out a decrease of Cu concentration with advanced age [30]. Also, previous studies suggest that administration of estrogen replacement therapy is connected with increase in serum copper concentration

[31]. Also, menopause causes increased bone resorption, resulting in the mobilization of bone Zn along with an increased urinary Zn excretion with normal serum Zn levels [32]. In this study, significant increase in the serum Ca levels after menopause was observed, which may be explained by estrogen deficiency, which induces synthesis of cytokines by theosteoblasts, monocytes and the T-cells leading to modification of the reabsorption, excretion and the resorption of Ca, thus leading to increased circulating levels of Ca.

This study shows that there are changes in the serum biochemical profiles in postmenopausal women. It is evident from this study that there is enhanced oxidative stress and decreased antioxidant defense in postmenopausal females as compared to premenopausal females which can play an important role in the pathogenesis of the various diseases related to menopause. 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. Therefore, antioxidants, trace elements and vitamins can be given as supplements in postmenopausal women along with or as a substitute to hormone replacement therapy.

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Disclosure of conflict of interest

None.

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