

(13) ANTIOXIDANT STATUS IN PERI-MENOPAUSAL AND POST-MENOPAUSAL WOMEN

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ABSTRACT

BACKGROUND & OBJECTIVES: Menopause is a natural step in the process of ageing and free oxygen radicals have been proposed as important causative agents of ageing. The antioxidant system seems to be affected in post-menopausal women due to deficiency of estrogen, which is a powerful antioxidant. In view of this background, present study was carried out to find correlation between menopause and antioxidant status, if any.

METHODS: Status of antioxidants was determined by spectrophotometric estimation of Superoxide Dismutase (SOD), and Ascorbic Acid in blood/serum of 105 women. The subjects consisted of 35 women of three distinct groups, namely, reproductive age, peri-menopausal, and post-menopausal. Data obtained was analyzed by student's t- test, ANOVA, and Pearson's correlation coefficient (r). $P < 0.05$ was considered significant.

RESULTS: Peri- and post-menopausal women exhibited significantly low erythrocyte SOD and serum ascorbic acid, as compared to the women of reproductive age.

INTERPRETATION & CONCLUSION: Findings of this study corroborate the hypothesis that gradual loss of ovarian function is associated with a concomitant decrease in antioxidant status."

KEY WORDS: ANTIOXIDANTS, ASCORBIC ACID, MENOPAUSE, OXIDATIVE STRESS, SUPEROXIDE DISMUTASE

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INTRODUCTION

The aerobic life-style offers great advantages, but is fraught with danger¹ – as is evident by the fact that accumulation of oxygen-derived free radicals (oxidative stress) is one of the established and thoroughly studied mechanisms of cell injury. Free radicals are responsible for widespread and indiscriminate oxidation and peroxidation of lipids, denaturation of proteins, depolymerization of polysaccharides, breakage and modification of DNA or any other cell structure, causing cell death or organ damage². Antioxidants help to defend the body against free radical attack and human body has got well developed endogenous antioxidant defense system like cellular enzymes and vitamins. Vitamin C forms the first line of antioxidant defence in human plasma exposed to a variety of oxidant insults³. A group of antioxidants

present in RBC that prevent lipid peroxidation consists of SOD, GPX, catalase and reduced glutathione⁴.

Oxidative stress influences the entire reproductive lifespan of a woman and even thereafter i.e. menopause⁵. Menopause is a natural step in the process of ageing and free oxygen radicals have been proposed as important causative agents of ageing⁶. Estrogens have free radical scavenging structures⁷ and have been shown to have *in vitro* antioxidant effects on membrane phospholipid peroxidation⁸. The process of ageing is enhanced due to the damage caused by free radicals; hence menopausal women are proposed to develop oxidative stress because of estrogen deficiency and advancing age⁹.

In recent years, the medical problems and health care of women have received increasing attention; and several studies have highlighted the alterations in antioxidant status in postmenopausal women. Earlier studies had reported moderately decreased levels of ascorbic acid in postmenopausal women in comparison to healthy controls. However, there is fair measure of ambiguity as regards the effect of menopause on SOD levels. In view of this scientific background, present study was carried out to evaluate the antioxidant status both in peri-menopausal and post-menopausal women as compared to the women of reproductive age. Further, we had attempted to determine that whether the decline in antioxidant status in post-menopausal women is primarily due to the natural process of aging or due to gradual decrease in ovarian function.

MATERIALS & METHODS

This study was conducted in the Department of Physiology, S. M. S. Medical College & attached group of hospitals, Jaipur. The study was conducted on 105 women volunteers, selected from medical & paramedical staff, and healthy attendants of patients. The subjects were categorized into following groups:

Group I: Reproductive age group women (n = 35); normally menstruating women of age 21 – 40 years. This group was further subdivided on the basis of age of the subjects into two sub-groups. This sub-grouping was done to ascertain the effect of ageing alone on the antioxidant status as women of both these sub-groups were menstruating normally.

Sub-Group A: Normally menstruating women of 21 – 30 years, (n=17).

Sub-Group B: Normally menstruating women of 31 – 40 years, (n=18).

Group II: Peri-menopausal women (n = 35); women of the age group 41 – 45 years. Both normally menstruating, and women with some sort of menstrual disorders e.g. irregular menses, menorrhagia, etc. were included in this group; but women experiencing amenorrhea were excluded. (Some authors denote this stage as peri-menopause while others refer it to as pre-menopause.)

Group III: Postmenopausal women (n = 35): women who had at least one year of amenorrhea, and were not receiving hormone replacement therapy; age: 46 – 50 years.

All subjects were screened for their general and medical history, especially menstrual and reproductive. The subjects suffering from hypertension, cardiovascular diseases, diabetes, venereal diseases and/or showing any pathology (including carcinoma) were excluded from the study. Women taking oral contraceptives, antioxidants or any other drug were also excluded from the study. Clinical examination was carried out with the aid and advice of a competent gynaecologist. Using aseptic techniques, 5 ml venous blood was drawn from the antecubital vein of the volunteers after informed consent. We evaluated the status of an enzymatic antioxidant – superoxide dismutase (SOD), and a non-enzymatic antioxidant – ascorbic acid (Vitamin C) in the blood/sera of the subjects to find out the correlation, if any, between menopause and level of antioxidants.

SUPEROXIDE DISMUTASE [SOD] ESTIMATION

Ransod kit, manufactured by Randox Lab. Ltd, was used for the estimation of SOD activity in the erythrocytes. 0.5 mL of whole blood was centrifuged for 10 minutes at 3000 rpm, and the plasma was aspirated off. Erythrocytes were washed four times with 3.0 mL of 0.9% NaCl solution and were centrifuged for 10 minutes at 3000 rpm after each wash. Lysate was prepared by suitable dilution with cold redistilled water and was further diluted with 0.01 mol/L phosphate buffer (pH 7.0). Thus prepared samples and standards were used for the estimation of SOD activity in U/mL by the following procedure. 0.05 mL of prepared standard/sample was added to 1.7 mL of mixed substrate and mixed well. Then, 0.25 mL of xanthine oxidase was added to the above mixture and mixed well. Absorbance was measured on a spectrophotometer at 505 nm at 37°C against air. Rate of reaction was determined by measuring initial absorbance (A_1) after 30 seconds and the final absorbance (A_2) after 3 minutes of measuring A_1 . As the reaction of Sample diluent (S1: 0.0 U/mL) was uninhibited, therefore its rate was deemed to be 100%. To calculate % inhibition of other standards/samples, their rates were converted into percentages of the sample diluent rate, and subtracted from 100%. A curve was plotted between percentage inhibition of each standard against Log_{10} (standard conc. in SOD U/mL). Percentage inhibition of sample was used to obtain Log_{10} (sample conc. in SOD U/mL) values from the standard curve and antilogarithms were taken of the values obtained by the curve to obtain sample conc. in SOD U/mL¹⁰.

ASCORBIC ACID ESTIMATION

Serum ascorbic acid was estimated by the method reported by Natelson S and Natelson EA, 1980¹¹. 0.2 ml of serum was added to 0.8 ml of 10% TCA and mixed well. Tubes were allowed to stand for 5 minutes and were centrifuged at 2000 rpm. 0.5 ml of supernatant was transferred to a small test tube and 0.2 ml of DNPH reagent was added. Tubes were stoppered and incubated at 37°C for 3 hours. Tubes were then chilled on ice-bath and 0.8 ml of cold 65% H₂SO₄ was added, and mixed well. Tubes were allowed to stand for 30 minutes at R.T. Blank comprised of 0.5% TCA, treated as for serum. Standard comprised of the 1.0 mg/dl ascorbic acid. Absorbance was read against the blank at 520 nm on a spectrophotometer.

STATISTICAL ANALYSIS

Data obtained for various parameters was subjected to statistical analysis. Arithmetic means and standard deviations were calculated to compute 't values' (student's t- test). ANOVA test (analysis of variance) was also employed and 'F values' (Fischer's test) were calculated. On the basis of t and F

values 'P' (probability) was determined to establish the significance of variance of individual parameters among the various groups studied. $P < 0.05$ was considered significant for all tests¹².

RESULTS

Mean values of the parameters studied are presented in table: 1, and the significance of difference computed by student's t test, between the various groups of the subjects is depicted in table: 2. Superoxide dismutase levels were found to be low in both peri-menopausal women and post-menopausal women in comparison to women of reproductive age. The decrease was, however, more marked in post-menopausal women. Further, difference between SOD levels of women of reproductive age and post-menopausal women was statistically significant while that between women of reproductive age women and pre-menopausal women was not significant. Similarly, peri-menopausal and post-menopausal women differed insignificantly in this regard.

TABLE 1: SOD and Ascorbic Acid Values (Mean \pm S.D.) of various groups of the subjects

| Parameters | Groups | | |
|-----------------------|------------------------|-----------------------|-----------------------|
| | Reproductive Age Women | Peri-Menopausal Women | Post-Menopausal Women |
| SOD (U/mL) | 179.56 \pm 82.69 | 155.84 \pm 57.48 | 139.15 \pm 54.31 |
| Ascorbic Acid (mg/dL) | 1.01 \pm 0.24 | 0.90 \pm 0.19 | 0.79 \pm 0.22 |

TABLE 2: SOD and Ascorbic Acid Values Compared across the three groups of subjects studied

| Parameters | Groups Compared | | |
|-----------------------|------------------------|------------------------|-------------------------|
| | Rep. Age v/s Peri-Meno | Rep. Age v/s Post-Meno | Peri-Meno v/s Post-Meno |
| SOD (U/mL) | > 0.05 | < 0.05* | > 0.05 |
| Ascorbic Acid (mg/dL) | < 0.05* | < 0.001* | > 0.05 |

* Significant

Serum ascorbic acid levels were also significantly decreased in peri-menopausal and post-menopausal women in comparison to those observed in women of reproductive age. Post-menopausal women exhibited lowest ascorbic acid levels. Non-significant variation was observed in pre-menopausal and post-menopausal women.

Analysis of variance (ANOVA) showed that - within group variations, of parameters studied, were significantly less in comparison to the inter-group differences, as depicted in table no.: 3. These findings reaffirm the significance of variance deduced by student's t test and indicate that the

subjects of the three groups possess some sort of intra-group uniformity in regard to the antioxidant levels, apart from the similarities arising out of menstrual behaviour. Moreover, a positive and significant correlation was observed between the two antioxidants studied, as illustrated by figure: 1.

Table 3: Analysis of Variance (ANOVA) in the parameter s studied

| Parameter | F Value | P Value |
|---------------|---------|---------|
| SOD | 5.56 | < 0.05* |
| Ascorbic Acid | 5.86 | < 0.05* |

* Significant

Fig. 1: Correlation between SOD and Ascorbic Acid Levels of all the subjects

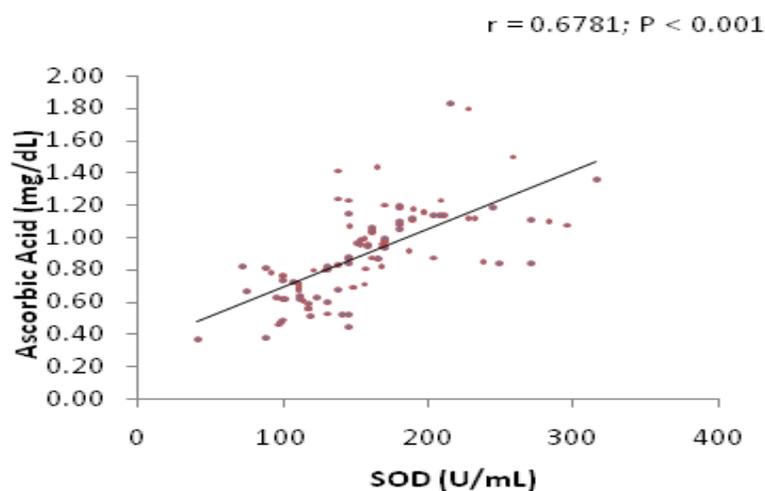


Table 4 depicts the mean values of SOD and Ascorbic acid in the two sub-groups of women of reproductive age. Although there was a difference of almost 10 years in the mean age of the subjects of the two sub-groups, yet no significant difference was observed in either SOD or Ascorbic acid levels of the two sub-groups.

Table 4: SOD and Ascorbic Acid Values in two sub-groups of Reproductive Age Women

| Parameters | Reproductive Age Women Sub Groups | | |
|-----------------------|-----------------------------------|-------------------------|---------|
| | A | B | P Value |
| | 21 – 30 years (n=17) | 31 – 40 years (n=18) | |
| SOD (U/mL) | 184.30 ± 76.79 | 175.09 ± 83.42 | > 0.05 |
| Ascorbic Acid (mg/dL) | 0.99 ± 0.18 | 1.02 ± 0.27 | > 0.05 |

DISCUSSION

Various studies conducted by different authors, exhibit a fair measure of ambiguity with regard to the effect of menopause on SOD levels. Shrivastava V *et al* reported that post-menopausal women had significantly lower concentrations of SOD in comparison to pre-menopausal women⁹. Krstevska M *et al* observed that SOD levels were decreased in peri-menopausal and post-menopausal women as compared with normally menstruating women; however, the variations were statistically non-significant¹³. Bednarek-Tupikowska G *et al* stated that SOD activity did not differ between pre-menopausal and post-menopausal women¹⁴. However, Gurdol F *et al* found increased SOD activity in menopausal women, but only at an older age¹⁵.

Relatively few studies have been conducted on the association of menopause and serum ascorbic acid concentration; however, studies reviewed for this study indicate that post-menopausal women exhibit lower serum ascorbic acid levels. Oner P *et al* found mean leukocyte and plasma ascorbate values in postmenopausal women significantly less but within acceptable ranges as compared to normally menstruating women¹⁶. According to these observations¹⁶, plasma and leukocyte ascorbic acid concentrations decreased after the cessation of ovarian hormone production. Vural P *et al* also reported a significant decrease in plasma ascorbic acid in postmenopausal women, in comparison to healthy control women¹⁷.

Mean ages of women of reproductive age, pre-menopausal women and post-menopausal women were 30.74 ± 5.72 years, 43.06 ± 1.49 years and 47.91 ± 1.40 years respectively. As subjects of the three groups were of different age groups, it is qualified to doubt that the differences observed among these groups may be due to differences in the age of the subjects because increased oxidative stress is an attribute of ageing. Since ovarian dysfunction and menopause are also implications of ageing, so to evaluate general increase in oxidative stress due to ageing - women of reproductive age were divided into two sub-groups i.e. sub-group A including women of 21–30 years while sub-group B comprising women of 31–40 years. The mean age of sub-group A was 25.76 ± 3.01 years, while that of sub-group B was 35.44 ± 2.94 years. However, no significant variation was observed between the two sub-groups of reproductive age women, in any of the analytes (Table: 4); in spite of difference of ten years in the mean age of subjects of the two sub-groups. Therefore, the decreased levels of antioxidants in post menopausal women as compared to the peri-menopausal women cannot be

attributed to ageing alone; the difference in mean age of the subjects of the two groups being less than five years.

Women with proper ovarian function (reproductive age) exhibited highest values of SOD and ascorbic acid, intermediate values of the two antioxidants studied were represented by women with mild ovarian dysfunction (peri-menopausal) while the lowest levels of SOD and ascorbic acid were observed in women suffering from ovarian dysfunction (post-menopausal).

This order indicates that ovarian dysfunction may be responsible for the decreased antioxidant status evident in peri-menopausal women and, more remarkably, in post-menopausal women.

CONCLUSION

Findings of this study corroborate the hypothesis that gradual loss of ovarian function is associated with a concomitant rise in oxidative stress as exhibited both by decreased levels of antioxidants in peri-, and post-menopausal women.

We suggest further studies on this issue which may involve larger sample size, additional parameters, and may also look into the nutritional aspects especially in reference to non-enzymatic anti-oxidants, so that the intricate relationship between menopause and oxidative stress is understood more clearly and such knowledge may contribute in attenuation of distress caused by menopause to half of the world's population.

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