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ORIGINAL ARTICLE

Assessment of Psychological Status and Oxidative Stress in Postmenopausal Women: A Cross-Sectional Study

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Objectives: During menopause, women may experience some physical changes that can affect their psychological status. Oxidative stress also increases with menopause, as decreasing levels of estrogen reinforce the deterioration of antioxidant status. To assess total antioxidative capacity, malondialdehyde, superoxide dismutase, and glutathione peroxidase and the correlation between psychological status and oxidative stress in postmenopausal women.

Methods: This cross-sectional study was undertaken on 100 postmenopausal women (40–59 years). Blood malondialdehyde, superoxide dismutase, glutathione peroxidase, and total antioxidative capacity were analyzed, and psychological status was assessed using Rosenberg's self-esteem test, Zung's self-rating anxiety scale, and Zung's self-rating depression scale.

Results: Of the women, 38.0% had mild to moderate symptoms of anxiety (mean ± standard deviation, 50.15 ± 4.89), 21.0% showed depression, and 19.0% had low self-esteem. Twelve percent of the study population had mild to moderate symptoms of all three disorders. A statistically significant difference was found in the scores of anxiety and self-esteem ($P = 0.001$ and $P < 0.001$ in women with and without psychological disturbances, respectively). With regard to oxidative stress parameters, only superoxide dismutase levels showed a statistically significant difference ($P = 0.001$), with lower levels found in women with psychological disturbance.

Conclusions: Women with depression, anxiety, and low self-esteem are in an oxidative challenge, which might be associated with estrogen depletion. A lower superoxide dismutase level is associated with higher depression and anxiety scores in postmenopausal women.

Key Words: Antioxidant activity, Anxiety, Depression, Superoxide dismutase, Postmenopause

INTRODUCTION

Though a natural process during menopause some women experience physical changes that may impact upon their psychological and social status, affecting their quality of life. Women may not be able to deal with their psychological and social status because of these physiological changes [1,2]. Ovarian aging leads to unpredictable production of estrogen unless it reaches a permanent low level [3] which causes various vasomotor symptoms which interfere with sleep leading to anxiety and depression that are highly prevalent due to hormonal changes [4-6]. As reported by World Health Organization that by 2020 the second leading cause of diseases will be depression [7]. Women experience psychological distress through menopause but the reason for depression, anxiety and psychological instability cannot be attributed to menopause status alone.

Antioxidant status and lipid metabolism both deteriorated with decreased levels of estrogen which reinforces risk of oxidative stress related diseases in menopause [8]. The estrogens capacity to prevent scavenging of free radical, to neutralize effect of increased reactive oxygen species (ROS) and production of antioxidant

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molecules decrease in menopause. Brain cells along with many other tissues of the body are also affected by depleted levels of estrogen. Normal aerobic metabolism leads to production of biomolecules which with aging accumulated in cells resulting into malfunction of human cells and this is an unavoidable side effect as oxidative stress. Occurrence of oxidative stress is because of inability of antioxidant system to effectively cope with the production of ROS and free radicals. Under normal conditions, antioxidants clear ROS either by preventing the production of oxidizing agents or by decreasing the oxidizing effects of the oxidizing agents and also estrogen which is a sex hormone, have capability to scavenge free radical [9].

During postmenopausal period women suffer episodes of depression, anxiety and low self-esteem contributes to oxidative stress by blocking antioxidants [10] and these symptoms are considered as prooxidants.

However, the relationship between psychological status and oxidative stress which includes enzymes of oxidative stress and total antioxidative capacity (TAC) is little understood in postmenopausal women. Several studies support an association [11] but others do not [12], causing a controversy.

Therefore, aim of this study was to determine the relationship between psychological status and oxidative stress in postmenopausal women. The prime objectives of the study are to assess oxidative stress by measuring TAC, malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (Gpx) in postmenopausal women and correlation between psychological status and oxidative stress in postmenopausal women.

MATERIALS AND METHODS

Study design and subjects

The study was approved by N.K.P. Salve Institute of Medical Sciences Institutional Ethics Committee (approval No. IEC/NKPSIMS/1/2017), and informed consent was obtained from all individual participants included in the study.

The cross sectional study was undertaken on 100 postmenopausal women in the age group ranging from 40 to 59 years old in the Department of Biochemistry at N.K.P. Salve Institute of Medical Sciences & Research Centre and Lata Mangeshkar Hospital after taking informed consent, from January 2018 to December 2019. Postmenopausal women having amenorrhea of at least 12 months and or serum estrogen levels below 25 pg/mL and FSH levels higher than 50 mU/mL were included in the study. Participants were selected using random sampling method. All women suffering from diabetes mellitus, endocrine disorders, kidney diseases, cardiac diseases, women on hormone therapy and women on antioxidant supplementation were excluded from the study. The research protocol was approved by ethics committee of the institution. To assess participants health status complete clinical history along with blood pressure measurements was taken and their complete blood count, blood glucose were estimated.

Measurement of oxidative stress

In ethylene diamine tetra acetic acid (EDTA) bottles and plain bulbs 5 mL sample of venous blood was collected and centrifugation of samples were done for 10 minutes. Randox laboratory method was used to measure MDA. The principle of method was based on that under acidic conditions condensation of lipid peroxide with 1-methyl-2-phenylindole (MPI) occur resulting in the formation of a red chromophore. In this method proteins are precipitated to remove water soluble MPI reactive substance to specifically obtain lipid peroxide in plasma. Lipid peroxide levels were expressed in the form of MDA. Standard used was of tetramethoxypropane, which is converted quantitatively to MDA. Reagents used in this methodology were MPI from National Chemicals (Vadodara, India) and methanol, acetonitrile, and hydrochloric acid were from Loba Chemie (Mumbai, India).

Spectrophotometeric kit method was used for the estimation erythrocytic GPx by using RANSEL kit of Randox Laboratories (County Antrim, UK). The mechanism of action being that glutathione oxidation catalysed by GPx. Immediate conversion of oxidized glutathione to reduced glutathione occurred with concomitant oxidation of NADPH to NADP in the presence of glutathione reductase and NADPH. In Systronic UV-Vis 117 Spectrophotometer at 340 nm reduced absorbance was measured.

By using RANSEL kit, by spectrophotometeric enzymatic kit method SOD was estimated from hemolysate. In this principle generation of superoxide radicals occurred after conversion of xanthine and xanthine oxidase which in turn react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. Systronic UV-Vis 117 Spectrophotometer was used to measure SOD activity at 505 nm by the degree of inhibition of this reaction.

TAC was measured by using QAYEE-BIO ELISA kit (Qayee Bio-Technology, Shanghai, China) which uses a double antibody sandwich enzyme linked immunosorbent one step process assay. In this procedure, test, standard and HRP-labeled TAC antibodies were added to enzyme wells which were pre-coated with TAC antibody. Microplate ELISA reader (450 nm) was used to take the absorbance.

Psychological status assessment

Rating of postmenopausal women were done using validated self-assessment questionnaires. Evaluation of self-esteem was done using Rosenberg's self-esteem test. The minimum score possible was 0 point and the maximum score was 40 point. Cut-off score below 15 points indicated low self-esteem [13]. To assess anxiety Zung's self-rating anxiety scale (SAS) was used. The SAS test is self-administered, with each response using a 4-point scale, from "little of the time" to "most of the time." There are 20 questions with 15 increasing anxiety level questions and 5 decreasing anxiety questions. There are two formats, self-evaluations and clinical evaluations. Cut-off score above 45 points indicated anxiety [14]. Depression was determined by Zung's self-rating depression scale (SDS). The SDS is a short self-administered survey to quantify the depressed status of a patient. There are 20 items on the scale that rate the four common characteristics of depression: the pervasive effect, the physiological equivalents, other disturbances, and psychomotor activities. There are ten positively worded and ten negatively worded questions. Each question is scored on a scale of 1–4 (a little of the time, some of the time, good part of the time, most of the time). The scores range from 20–80. SDS score above 45 indicted suffering from depression [15].

Data analysis

Data were expressed as the mean ± standard deviation of oxidative stress parameters and as percentage of psychological tests of postmenopausal women. Women were divided into two subgroups based on psychological disturbances. Group 1 was postmenopausal women without psychological disturbances (n = 52) and Group 2 was postmenopausal women with psychological disturbances (n = 48). Consideration of psychological disturbance was done if postmenopausal women had either anxiety, depression and low self-esteem or all three symptoms according to the respective cut-off values. Test for equality of variance was conducted using Student's *t* test with 95% confidence interval and results were compared using mean score and standard deviation of oxidative parameters ad psychological status of two groups. Pearson's correlation analysis were calculated to study the association between psychological tests score and oxidative stress using SOD levels as oxidative stress marker as SOD showed the significant difference among the oxidative stress parameters. Association between SOD as dependent variable and psychological status as independent variable was analyzed. The level of statistical significance was set at a two tailed *P* value of less than 0.05. Data was analyzed using statistical software Epi Info (ver. 7; Centers for Disease Control and Prevention [CDC], Atlanta, GA, USA).

RESULTS

The distribution of women in the category of mental health showed that 38.0% (n = 38) women had mild to moderate symptoms of anxiety with mean and standard deviation of 50.15 ± 4.89 while distribution for depression and low self-esteem was 21.0% (n = 21) and 19.0%

Table 1. Number, mean scores, and standard deviation in postmenopausal women (n = 100)

Parameter	Postmenopausal women without psychological disturbance (n = 52)	Postmenopausal women with psychological disturbance (n = 48)	*P* value
Zung's self-rating anxiety scale (anxiety > 45)	35.51 ± 4.16	46.25 ± 9.15	0.001*
Zung's self-rating depression scale (depression > 45)	36.09 ± 6.92	44.68 ± 9.86	0.013*
Rosenberg's self-esteem (low self-esteem < 15)	22.25 ± 1.90	18.60 ± 5.48	< 0.001*
MDA (nmol/L)	3.25 ± 0.75	2.79 ± 0.87	0.33
SOD (U/mL)	117.68 ± 94.76	95.19 ± 60.03	0.001*
GPx (U/L)	3,484.27 ± 724.35	3,594.58 ± 618.57	0.28
TAC (ng/mL)	208.05 ± 202.51	152.29 ± 155.07	0.67

MDA: malondialdehyde, SOD: superoxide dismutase, GPx: glutathione peroxidase, TAC: total antioxidative capacity.
**P* value < 0.05 is statistically significance.

(n = 19) mean and standard deviation of 53.57 ± 2.58 and 12.26 ± 1.44, respectively. Our data also revealed that 12.0% (n = 12) of the population study had mild to moderate symptoms of all three disorders. Oxidative stress parameters and psychological characterstics of the postmenopausal women included in the analysis are depicted in Table 1. Statistically significant difference was found in the score of anxiety and self-esteem with $P < 0.001$ in both the groups. Regarding oxidative stress parameters only SOD levels showed statistically significant difference ($P = 0.001$) with SOD levels less in women with psychological disturbance.

As shown in Table 2 with respect to oxidative stress parameters in women with anxiety and without anxiety no significant difference was observed in MDA and GPx levels. However, SOD and TAC showed slightly statistical significant difference ($P = 0.03$).

Table 3 indicating mean and standard deviation of oxidative parameters in patients with and without depression. No significant difference in the *P* valuewas observed except in the levels of SOD which showed highly statistically significant difference in the *P* values with decreased levels of SOD in women with depression.

Table 4 depicted mean and standard deviation of oxidative parameters in patients with and without low self-esteem. Only highly statistically significant difference in the *P* values was observed in the levels of SOD which showed decreased levels of SOD in women with low self-esteem.

In the univariate analyses by calculating Pearson's correlation coefficient between SOD levels and anxiety ($P = 0.30$), between SOD levels and depression scores ($P = 0.99$) and for the self-esteem score and SOD levels the *P*

Table 2. Comparison of oxidative stress parameters between postmenopausal women with anxiety and without anxiety

Parameter	Postmenopausal women without anxiety (n = 62)	Postmenopausal women with anxiety (n = 38)	*P* value
MDA (nmol/L)	3.19 ± 0.78	2.76 ± 0.88	0.40
SOD (U/mL)	113.38 ± 88.98	96.28 ± 63.69	0.03*
GPx (U/L)	3,531.43 ± 703.28	3,546.67 ± 633.94	0.50
TAC (ng/mL)	211.18 ± 197.88	132.51 ± 143.78	0.03*

MDA: malondialdehyde, SOD: superoxide dismutase, GPx: glutathione peroxidase, TAC: total antioxidative capacity.
**P* value < 0.05 is statistically significance.

Table 3. Comparison of oxidative stress parameters between postmenopausal women with and without depression

Parameter	Postmenopausal women without depression (n = 79)	Postmenopausal women with depression (n = 21)	*P* value
MDA (nmol/L)	3.14 ± 0.87	2.63 ± 0.61	0.07
SOD (U/mL)	111.79 ± 87.93	88.40 ± 37.31	0.001*
GPx (U/L)	3,488.45 ± 702.55	3,720.67 ± 531.63	0.15
TAC (ng/mL)	192.29 ± 192.68	139.87 ± 133.78	0.06

MDA: malondialdehyde, SOD: superoxide dismutase, GPx: glutathione peroxidase, TAC: total antioxidative capacity.
**P* value < 0.05 is statistically significance.

Table 4. Comparison of oxidative stress parameters between postmenopausal women with and without low self-esteem

Parameter	Postmenopausal women without low self-esteem (n = 81)	Postmenopausal women with low self-esteem (n = 19)	*P* value
MDA (nmol/L)	3.15 ± 0.79	2.51 ± 0.87	0.55
SOD (U/mL)	115.11 ± 85.70	71.82 ± 36.21	0.001*
GPx (U/L)	3,518.54 ± 689.84	3,616.85 ± 615.87	0.60
TAC (ng/mL)	183.16 ± 189.97	173.29 ± 151.06	0.27

MDA: malondialdehyde, SOD: superoxide dismutase, GPx: glutathione peroxidase, TAC: total antioxidative capacity.
**P* value < 0.05 is statistically significance.

Table 5. Pearson's correlation coefficient between SOD levels and anxiety score, depression score, and self-esteem

	r	*P* value
SOD and anxiety score	–0.17	0.30
SOD and depression score	–0.001	0.99
SOD and self-esteem score	0.03	0.89

SOD: superoxide dismutase, r: correlation coefficient, *P*: probability value.

valueis 0.89 indicted that no statistically significant correlation was found between women with psychological disturbance and values of SOD levels (Table 5).

DISCUSSION

The results indicated that not all postmenopausal women experience high levels of anxiety, low self-esteem and depression. The present study investigated the association between SOD, TAC, and other oxidative parameters and mental disorders among the postmenopausal women. During menopause various psycho-neuro-endocrine relationships in social adaptation is identified as many of the hormonal alteration influence psychological status like changes in self-esteem and mood states [16]. In this study the proportion of women with anxiety score, depression score and low self-esteem levels were 38.0%, 21.0%, and 19.0% respectively and total 48.0% post-menopausal women reported psychological distress with either one of the symptoms or presented with all the symptoms of distress. Our results are consistent with research study by Freeman et al. [17] who observed that during menopausal transition there can be two and half times chances of developing depressive disorder without previous history of depression as compared with premenopausal women. The results of research by Jafari et al. [18] are similar to our study and they indicated that postmenopausal women experienced higher level of anxiety and depression scores and lower levels of mental health, quality of life and vitality and thus their results confirmed all the five hypothesis. As reported by Neugarten and Kraines [19] those women who have exhibited with signs of low self-esteem near menopause usually complaining of psychological disturbance. Before the beginning of menopause anxiety, depression and insomnia are most common psychological symptoms. Psychological symptoms such as depression can be indirectly caused by vasomotor symptoms with night sweats leading to chronic fatigue, sleep disturbances and hence responsible for depression.

During postmenopausal period, oxidative stress is increased [20], as estrogen exhibit antioxidant property through its action either by acting on antioxidant enzyme activity or via intracellular signaling cascades and also by up-regulation of the gene expression of antioxidant enzymes [21]; therefore, due to altered estrogen postmenopausal women have diminished antioxidant capacity that leads to higher lipid per oxidation. Moreover factors like depression and anxiety act as pro-oxidant factors, and contribute to increased oxidative stress [22,23]. Hence, the objectives of this study was to assess the correlation between the oxidative stress with psychological status in the postmenopausal period. By using various tests we assessed psychological behaviour and tried to evaluate the effect of increased intensity of postmenopausal discomfort with higher oxidative stress levels. While analysing our results we noticed that women with depression or anxiety and low self-esteem that is those who were psychologically disturbed maybe susceptible to oxidative stress. Our results showed a statistically significant $P < 0.001$ between the SAS score for anxiety, SDS score for depression and Rosenberg's self-esteem score in postmenopausal women with and without psychological disturbances. Amongst the oxidative stress parameters there was statistically significant decreased SOD values in postmenopausal women with anxiety, depression and low self-esteem with $P < 0.001$. But in the current study we obtained no Pearson's correlation between anxiety, depression and low self-esteem and in postmenopausal women. No association was detected between TAC and tests scores. Supporting our study Stedile et al. [11] reported no association between TAC and depression and anxiety. However, Bouayed et al. [12] pointed out a link between oxidative stress and high-anxiety-related behaviour.

Depletion of antioxidants is an indirect marker of oxidative stress during menopause. SOD is the principal antioxidant enzyme. It scavenges superoxide anions by catalyzing the conversion of highly reactive superoxide radicals to less toxic hydrogen peroxide and decreases cell damage [24]. The decrease in the level of SOD in postmenopausal females may be due to its increased consumption to counteract the increased oxidative stress. Hence, increased oxidative stress and decreased antioxidant levels after menopause play an important role in the development of many disorders such as depression, anxiety. Thus our findings suggest that women with depression, anxiety and low self-esteem

are in oxidative challenge which may be associated to estrogen depletion and is considered to be a main antioxidant mechanism at both cerebral and systemic level. Our study depicted that lower SOD is associated with higher depression and higher anxiety scores in postmenopausal women and decreased levels of SOD can be related to initial phases of menopause. Thus long term follow studies should be needed to evaluate oxidative stress.

Therefore, those postmenopausal women who may be susceptible to develop oxidative stress should avail the benefits of a diet which is rich in antioxidants so that their psychological disturbances can be improved and effects of oxidative damage can be reduced which are produced because of free radicals. A cross sectional study on postmenopausal Chinese women featured that intake of whole plant food was negatively associated with depression score and intake of processed food was positively associated with stress and depression and negatively associated with scores of self-esteem [25]. Also study by Abshirini et al. [26] suggested that dietary TAC was inversely related with the depression, anxiety scores and lower MDA levels in postmenopausal women. Postmenopausal women should be screened for oxidative stress and women with psychological disturbance can be supplemented with hormonal therapy with estradiol or any other beneficial treatment. This will help them in improving their self-esteem and can prevent oxidative stress. As evidenced by Yazici et al. [27], there were improvement in the symptoms of anxiety and depression in menopausal women after introduction of hormone replacement therapy with 17beta-estradiol [27] and Sánchez Rodríguez et al. [28] observed that improvement in quality of life and reduction in the lipoperoxides levels occurred in postmenopausal women who were receiving estrogen therapy.

Hence, this work showed that increased SOD levels are associated with higher depression and anxiety in post-menopausal women and females who are prone to exaggerated oxidative stress should take the diet rich in antioxidants and can be supplemented with hormone therapy if needed to improved their psychological disturbances and to reduce oxidative stress which would be helpful to maintain healthy mental status.

Limitations of the study: As it is a cross-sectional study and sample size may not be representative, hence prospective studies are needed with representative samples size to confirm our outcome.

Also cause–effect association cannot be concluded from our study due to cross-sectional design of study. Thus more studies with larger sample size should be carried out for the confirmation of our findings.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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