Original Article

Oxidative Stress and Antioxidant Status in Premenopausal and Postmenopausal Women: A Comparative Cross-sectional Study

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Abstract

Background: Menopause is a physiological transition associated with hormonal changes and age-related systemic alterations. Emerging evidence suggests that oxidative balance may be disrupted during this period, although the extent and pattern of oxidative stress (OS) in menopausal women remain incompletely defined. **Aim:** To compare OS markers and antioxidant parameters between premenopausal and postmenopausal women. **Methods:** This cross-sectional study included 100 women: 50 premenopausal (aged 18–45 years) and 50 postmenopausal (aged 45–65 years). Serum levels of arylesterase (ARE), paraoxonase (PON), thiols, total antioxidant status (TAS), and total oxidant status were measured using spectrophotometric methods. The OS index was calculated from total oxidant and antioxidant status. Statistical analysis was performed using SPSS version 25.0, with $P < 0.05$ considered statistically significant. **Results:** Total oxidant status and OS index were significantly higher in postmenopausal women compared with premenopausal women. In contrast, antioxidant parameters—including PON, ARE, TAS, and thiol levels—were significantly lower in the postmenopausal group ($P < 0.05$). **Conclusion:** Menopause is associated with increased OS and reduced antioxidant capacity. This altered redox balance may contribute to the heightened risk of age-related conditions observed in postmenopausal women and highlights the potential value of monitoring OS markers during the menopausal transition.

Keywords: *Antioxidant enzymes, biomarker, lipid peroxidation, menopause, oxidative stress*

Introduction

Menopause marks one of the many steps a woman goes through in aging. It marks the point of no return for a woman's ability to reproduce.[1-3] The menopause transition can cause several changes in a woman's physical, social, and psychological health. There are changes in mood, sleep, and cognition. There can be vasomotor changes that can lead to the development of chronic ailments, which in turn can lead to changes in functioning in day-to-day activities.[4]

Oxidative stress (OS) increases with age and has been reported to be related to estrogen levels in women. A decline in estrogen levels after menopause causes an increase in OS.[5] There are many studies conducted that show the deficiency of antioxidant age, which leads to an oxidative burden in the body.[6-10]

Hot flashes, night sweats, mood and sleep disorders, memory and concentration issues, depression and irritability, bone and joint problems, and muscle wasting are just a few symptoms that menopausal women may experience. Additionally, diseases such as sexual dysfunction, urinary incontinence, an increased risk of fractures, cardiovascular disease, reproductive cancers, and osteoporosis have been shown to be associated with menopause.[11-13]

Both paraoxonase (PON) and arylesterase (ARE) have similar functions and exhibit antioxidant

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properties.[14,15] Measuring individually the numerous oxidant and antioxidant molecules in the body is not practical. Therefore, it is more useful and significant to measure a sample's total oxidant status (TOS) and total antioxidant status (TAS).[16] Thiols exhibit antioxidant properties by reacting with free radicals. Erel and Neselioglu developed an innovative, automated method to facilitate the autonomous measurement of thiol groups.[17] Measuring OS levels in postmenopausal women are important for determining its contribution to menopause-related diseases such as cardiovascular disease, hypertension, vasomotor disorders, depression, and diabetes.[1]

Despite increasing evidence that OS is altered during the menopausal period, available studies have largely focused on isolated biomarkers or have yielded inconsistent findings regarding global oxidant–antioxidant balance. Moreover, data directly comparing comprehensive OS indices—including PON, ARE, thiols, TOS, and TAS—between premenopausal and postmenopausal women remain limited. Therefore, the present study was designed to address this gap by systematically comparing oxidant and antioxidant parameters in premenopausal and postmenopausal women. Additionally, the study aimed to evaluate the relationships among these biomarkers to provide a more integrated understanding of redox status during the menopausal transition.

Patients and Methods

Study population

The Ethics Committee of the Faculty of Medicine at Çanakkale Onsekiz Mart University granted ethical approval for our study on July 26, 2023 (Decision No. 2023/10-06). All procedures followed the ethical principles outlined in the Helsinki Declaration. Each participant signed a written informed consent form before joining the study. Our case-control study was conducted in the Department of Obstetrics and Gynecology at the Çanakkale Onsekiz Mart University Faculty of Medicine in Turkey from July 2023 to May 2025.

Sample size and power

We calculated the required sample size for this study using G*Power software (version 3.1.2). This analysis determined that we needed 100 participants.[11,12] This calculation was based on a confidence level of 95% ($\alpha = 0.05$) and an expected effect size of 0.75.

The premenopausal group included 50 women aged 18 to 45 with normal menstrual cycles. The postmenopausal group included 50 women aged 45 to 65 who had not had a period for at least 12 months.

Exclusion criteria

Women were excluded from participating in the study if they had a medical history of chronic conditions such as hypertension, diabetes mellitus, cardiovascular disease, kidney disease, a previous stroke, or any neurodegenerative disease. Additionally, women using oral contraceptives or hormone replacement therapy and/or women with irregular periods are also excluded.

Biochemical analyses

We collected peripheral venous blood samples from all participants to measure oxidative and antioxidant biomarkers, including thiol, PON, ARE, TAS, and TOS. Serum was extracted from the blood samples by centrifuging them for 20 minutes at 2,000 RPM. The collected serum samples were stored at −70°C prior to biochemical analysis.

Thiol measurement

We measured thiol levels using a commercial kit (Rel Assay Diagnostics, Turkey) and a new spectrophotometric method developed by Neselioglu and Erel. Sodium borohydride broke down the disulfide bonds in the samples into useful thiol groups. Residual borohydride was then neutralized with formaldehyde. Native (NT), total (TT), and disulfide (DS) thiol levels were measured using DTNB reaction chemistry.[18]

Paraoxonase and arylesterase activity

Standard commercial test kits (Rel Assay Diagnostics, Turkey) were used to determine the enzymatic activities of PON and ARE. The hydrolysis rate of paraoxon was measured spectrophotometrically at 412 nm to evaluate PON activity, and the results were expressed in units per liter of serum (U/L). Phenylacetate was used as a substrate to evaluate ARE activity, and enzymatic activity was estimated by measuring phenol production. Results were also provided in U/L.

Total antioxidant status and total oxidant status analysis

TAS was determined using a colorimetric technique based on its interaction with the ABTS radical cation. Results were expressed as millimoles of Trolox equivalents per liter (mmol Trolox equiv/L).[19] In parallel, TOS was quantified by evaluating the oxidation of a ferrous ion–o-dianisidine complex, which yields a colored ferric ion–xylenol orange complex. The resultant chromogenic reaction was spectrophotometrically evaluated, and absorbance readings were directly linked to the oxidant level. TOS levels were reported as micromoles of hydrogen peroxide equivalents per liter (μmol H_2O_2 equiv/L).[20]

Oxidative stress index

A dimensionless ratio computed from the TOS and TAS levels, the OSI is a measure of OS.

Statistical analysis

We performed statistical evaluations using SPSS software (IBM Corp. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA: IBM Corp). We presented quantitative data as mean values with corresponding standard deviations. We examined the normality of data distributions using the Kolmogorov–Smirnov test. For group comparisons of continuous variables, we applied nonparametric methods, including the Mann–Whitney *U*-test for two-group analyses and the Kruskal-Wallis test for comparisons involving more than two groups. We assessed categorical variables using the Chi-square test. When multiple comparisons were necessary, we used Duncan's *post hoc* test. We defined statistical significance as a *P* value below 0.05.

Results

The demographic and biochemical features of 100 participants—50 postmenopausal women and 50 healthy premenopausal women with normal menstrual cycles—are displayed in Table 1. The premenopausal group's average age was 34.6 ± 1.3 years, whereas the postmenopausal group's average age was 53.9 ± 1.2 years (P = 0.47). The mean body mass index (BMI) was 23.0 ± 4.4 for the postmenopausal group. Age, BMI, weight, triglycerides, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, glucose, insulin, uric acid, vitamin D, and vitamin B12 did not significantly differ across the groups ($P > 0.05$) [Table 1]. OS measurements for both groups are presented in Table 2. Antioxidant indicators, such as PON, TAS, natural thiols, and total thiols, were considerably lower ($P < 0.001$) in the postmenopausal group than in the premenopausal group. However, TOS and OSI values were significantly higher ($P < 0.001$). ARE and disulfide levels did not differ significantly between the two groups ($P > 0.05$).

Table 1: Demographic and clinical details of menopausal patients and healthy controls

	Post-menopause (*n*=50)	Pre-menopause (*n*=50)	*P*
Age (mean years±SD)	53.94±1.25	34.6±1.32	0.47
Weight (kg)	60.76±1.87	65.22±1.97	0.10
BMI (kg/m^2)	23.04±4.41	24.50±4.50	0.10
Triglyceride (mg/dl)	105.96±4.79	119.74±4.65	0.30
Total Cholesterol (mg/dl)	179.41±4.25	182.16±3.47	0.72
HDL-C (mg/dl)	53.58±1.33	55.66±1.87	0.36
LDL-C (mg/dl)	100.56±5.90	100.39±4.30	0.98
Glucose (mg/dl)	93.62±1.93	91.22±1.60	0.34
Insulin (lU/mL)	25.00±1.30	24.16±1.22	0.84
Uric acid	4.31±0.30	4.36±0.22	0.77
Vitamin D	21.41±1.01	21.49±1.34	0.96
Vitamin B12	404.42±2.51	406.62±2.49	0.95

Data are presented as mean and standard deviation. BMI=Body mass index, HDL-C=High density lipoprotein cholesterol, LDL-C=Low density lipoprotein cholesterol, SD=Standard deviation

Table 2: Analysis of oxidative stress parameters in menopausal patients and control groups

	Mean±SD		*P*
	Post-menopause	Pre-menopause	
PON (U/L)	172.04±16.31	243.04±19.41	<0.001
ARE (kU/L)	75.45±6.11	84.32±6.01	0.30
TAS (mmol Trolox Equivalent/L)	1.55±0.02	1.68±0.01	<0.001
TOS (µmol H_2O_2 Equiv/L)	97.04±4.77	71.16±6.14	<0.001
OSI	6.40±0.33	4.34±0.37	<0.001
Native thiol, µmol/L	102.01±6.82	157.49±6.90	<0.001
Total thiol, µmol/L	48.36±2.83	83.50±5.81	<0.001
Disulfide, µmol/L	30.01±2.71	37.33±2.91	0.07

PON=Paraoxonase, ARE=Arylesterase, TAS=Total antioxidant status, TOS=Total oxidant status, OSI=Oxidative stress index, SD=Standard deviation

Discussion

In addition to the hormonal changes that come with age, menopause includes physical, psychological, and cultural changes. It is a significant health issue for middle-aged women around the world and affects every woman. It is estimated that approximately 900,000 postmenopausal women will live in developing countries by 2030.[21] Therefore, women's health during menopause is very important for public health. Research published in the literature indicates that numerous diseases, such as diabetes, high blood pressure, osteoporosis, and cardiovascular disorders, are associated with OS experienced by menopausal women with estrogen deficiency.[22-24]

When endogenous antioxidant defense systems are unable to counteract reactive oxygen species (ROS) and free radicals generated during regular metabolic activities, OS results. An imbalance favoring oxidant production leads to excessive ROS accumulation, which disrupts physiological functions and metabolic homeostasis, particularly during aging. Biomarkers commonly used to evaluate OS include PON, ARE, TAS, TOS, and thiol groups.[1,25]

In our study, postmenopausal women had considerably lower levels of OS indicators (PON, TAS, natural thiol, and total thiol) than premenopausal women. However, TOS and OSI values were found to be significantly higher in postmenopausal women compared to premenopausal women.

According to Ansar *et al*. and Rasheed *et al*., the postmenopausal group had considerably greater serum levels of malondialdehyde (MDA) than the premenopausal group.[6,26] These studies reported that the

decrease in estrogen levels after menopause is associated with an increase in MDA, a lipid peroxidation product, and OS.

Indirect indicators of OS during menopause include elevated oxidant levels and decreased antioxidant levels.[1,4] Our study's measurements of OS indicators (PON, ARE, TAS, TOS, and thiol groups) showed that menopausal women had higher levels of OS and lower antioxidant capacity. Therefore, rather than showing a clear causal relationship, changes in OS and antioxidant status seen in postmenopausal women may be linked to illnesses including depression, osteoporosis, diabetes, hypertension, and cardiovascular disease.

The current study assessed oxidant-antioxidant status by analyzing serum PON, ARE, TAS, TOS, and thiol groups. Further studies are needed to support our results, either positively or negatively, by increasing the sample size and including other markers of OS.

Limitations of the study

Due to the cross-sectional design, small sample size, and lack of demographic and sociodemographic information, a cause-and-effect relationship could not be established. Thus, more studies with larger sample sizes should be carried out to confirm our findings.

Conclusion

Postmenopausal women have impaired antioxidant defense systems and higher levels of oxidants in their bodies. This imbalance may make postmenopausal women more susceptible to disorders such as metabolic syndrome, osteoporosis, and cardiovascular disease.

Measuring OS markers in postmenopausal women may help diagnose high-risk women early and quickly execute an intervention strategy. Further studies involving larger populations are needed to assess the efficacy of hormone-based treatments or antioxidants aimed at reducing oxidative damage and influencing healthy aging during menopause.

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Conflicts of interest

There are no conflicts of interest.

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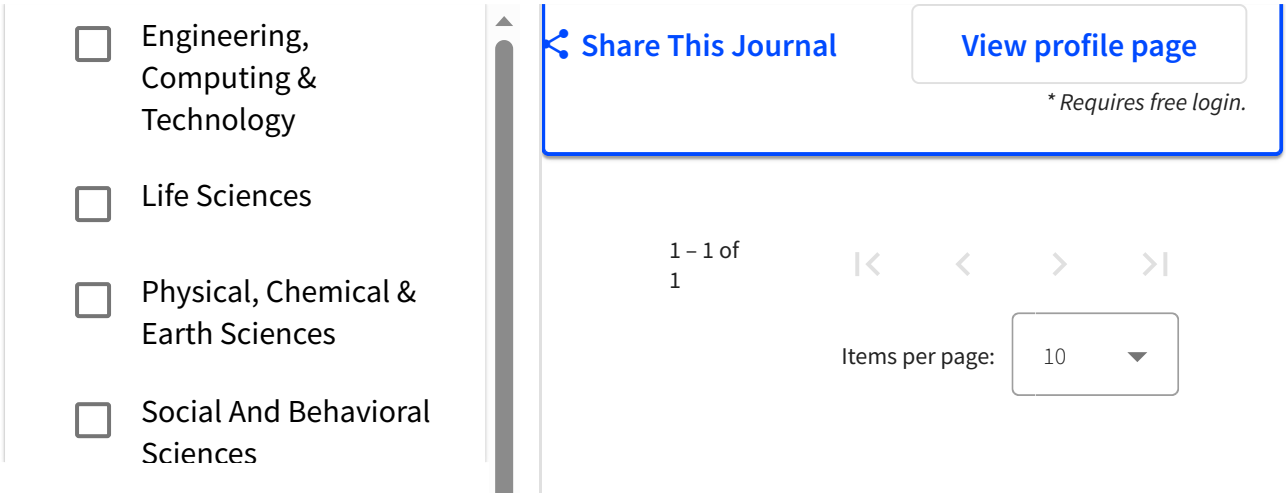
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