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ORIGINAL ARTICLE

Correlation between Estrogen Metabolites and Antioxidant Defense Systems in Premenopausal and Postmenopausal Women. A Clinical Study

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ABSTRACT

Background: Estrogens and their metabolites influence systemic oxidative balance by directly scavenging reactive oxygen species and upregulating antioxidant enzymes. Menopause leads to a marked decline in circulating estrogens, potentially diminishing antioxidant defenses.

Objective: To evaluate the correlation between specific estrogen metabolites and key antioxidant defense parameters in premenopausal and postmenopausal women.

Methodology: In this cross-sectional study conducted from June 2022 to May 2023 at the Gynecology and Obstetrics Department, Unit 3, Civil Hospital, Quetta, seventy women (thirty-five premenopausal, thirty-five postmenopausal) were enrolled. After an overnight fast, venous blood was drawn between 08:00 and 10:00 AM. Plasma concentrations of estradiol (E_2), estrone (E_1), 2-hydroxyestrone (2-OHE_1), and 16α-hydroxyestrone (16α-OHE_1) were quantified by high-performance liquid chromatography. Activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured via established spectrophotometric assays, and total antioxidant capacity (TAC) was determined using the ferric reducing antioxidant power method. Group comparisons employed independent t-tests, and Pearson's correlation assessed relationships between estrogen metabolites and antioxidant markers.

Results: Premenopausal women demonstrated significantly higher mean E_2 (54.1 ± 14.5 pg/mL vs. 12.5 ± 5.9 pg/mL; $p < 0.001$), E_1 (45.5 ± 12.1 pg/mL vs. 23.0 ± 9.1 pg/mL; $p < 0.001$), and 2-OHE_1 (19.0 ± 5.3 pg/mL vs. 9.8 ± 3.9 pg/mL; $p = 0.002$). There was no significant difference in 16α-OHE_1 (12.0 ± 4.6 pg/mL vs. 10.5 ± 4.0 pg/mL; $p = 0.15$). SOD activity (8.1 ± 1.8 U/mL vs. 5.7 ± 1.5 U/mL; $p < 0.001$), CAT activity (49.0 ± 9.0 U/mL vs. 34.5 ± 7.8 U/mL; $p < 0.001$), GPx activity (12.3 ± 3.2 U/mL vs. 7.8 ± 2.9 U/mL; $p < 0.001$), and TAC (1245 ± 215 µmol Fe^{2+}/L vs. 925 ± 185 µmol Fe^{2+}/L; $p < 0.001$) were all significantly greater in premenopausal women. Estradiol correlated strongly with SOD ($r = 0.69$; $p < 0.001$), CAT ($r = 0.62$; $p = 0.001$), GPx ($r = 0.58$; $p = 0.003$), and TAC ($r = 0.56$; $p = 0.005$). The metabolite 2-OHE_1 correlated positively with TAC ($r = 0.54$; $p = 0.004$) and SOD ($r = 0.50$; $p = 0.006$). In contrast, 16α-OHE_1 was weakly and inversely correlated with GPx ($r = −0.32$; $p = 0.045$).

Conclusion: Higher levels of estradiol and the antioxidant-favoring metabolite 2-OHE_1 in premenopausal women are associated with enhanced antioxidant enzyme activities and total antioxidant capacity. After menopause, the decline in these protective estrogens corresponds to reduced antioxidant defenses, potentially increasing susceptibility to oxidative stress–related pathologies.

Keywords: estrogen metabolites; antioxidant defense; premenopausal; postmenopausal; superoxide dismutase; catalase; glutathione peroxidase; total antioxidant capacity

INTRODUCTION

Estrogens, the primary female sex hormones, have long been recognized for their central roles in regulating reproductive physiology, bone homeostasis, and lipid metabolism. Beyond these classical functions, estrogens exert profound influence on cellular redox balance through both direct and indirect mechanisms[1]. The phenolic ring structure of estrogen allows it to act as a free radical scavenger, and estrogen receptor–mediated signaling upregulates transcription of several key antioxidant enzymes. In premenopausal women, cyclical fluctuations in circulating estradiol (E_2) and estrone (E_1) not only orchestrate the menstrual cycle but also help maintain oxidative homeostasis, protecting lipids, proteins, and DNA from peroxidative damage[2]. With the onset of menopause, however, estrogen production by the ovaries declines sharply. This hormonal withdrawal is accompanied by a measurable reduction in antioxidant defenses and an increase in markers of oxidative stress, a shift that has been implicated in the heightened risk of cardiovascular, neurodegenerative, and musculoskeletal disorders observed in postmenopausal women[3].

In addition to the parent estrogens, metabolic hydroxylation pathways generate several estrogen metabolites that exhibit distinct redox properties. The 2-hydroxylation pathway, which produces metabolites such as 2-hydroxyestrone (2-OHE_1), yields catechol estrogens with powerful antioxidant capacity, capable of scavenging reactive oxygen species and inhibiting lipid peroxidation[4]. By contrast, 16α-hydroxyestrone (16α-OHE_1) demonstrates partial pro-oxidant behavior under certain conditions, potentially exacerbating oxidative damage when present in abundance. The balance between these metabolic pathways influenced by genetic polymorphisms of cytochrome P450 enzymes, lifestyle factors, and age can therefore shift overall redox status in women. In premenopausal individuals, a relative predominance of 2-hydroxylated metabolites may contribute to robust antioxidant defenses. After menopause, altered enzyme expression and reduced substrate availability lead to lower levels of protective catechol estrogens and may allow pro-oxidant metabolites to exert greater influence[5, 6].

Previous investigations have documented that premenopausal women typically have higher activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) compared to postmenopausal counterparts, as well as superior total antioxidant capacity (TAC). These findings suggest that endogenous estrogens enhance antioxidant defenses, but many studies have focused on parent hormone concentrations rather than isolating the contributions of individual metabolites[7]. Consequently, the specific correlations between discrete estrogen metabolites such as E_2, E_1, 2-OHE_1, and 16α-OHE_1 and measures of oxidative balance remain incompletely characterized in clinical cohorts. Elucidating these relationships is essential for understanding how changes in estrogen metabolism

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across the menopausal transition influence systemic oxidative stress and disease susceptibility[8].

The current study aimed to quantify plasma levels of estradiol, estrone, 2-hydroxyestrone, and 16α-hydroxyestrone in both premenopausal and postmenopausal women, alongside key antioxidant defense parameters including SOD, CAT, GPx activities, and total antioxidant capacity[9]. By examining correlations between estrogenic metabolites and antioxidant markers, this investigation seeks to clarify the extent to which specific estrogen metabolites modulate redox homeostasis. A clearer understanding of these associations may inform strategies to preserve antioxidant defenses whether through targeted modulation of estrogen metabolism, dietary interventions, or selective estrogen receptor modulators in women who have transitioned to menopause[10].

MATERIALS AND METHODS

This cross-sectional clinical study was carried out between June 2022 and May 2023 in the Gynecology and Obstetrics Department, Unit 3, Civil Hospital, Quetta. The study protocol was reviewed and approved by the Institutional Review Board. All participants provided written informed consent after receiving a detailed explanation of the study objectives, procedures, potential risks, and benefits in their preferred language.

Seventy women were enrolled, comprising thirty-five premenopausal and thirty-five postmenopausal participants. Premenopausal inclusion criteria required women aged thirty-five to forty-five years who reported regular menstrual cycles and had not received any form of hormonal supplementation or antioxidant supplements within the preceding six months. Postmenopausal women were between fifty and sixty years of age and had experienced spontaneous cessation of menstruation for at least twelve consecutive months without any history of hormone replacement therapy. Exclusion criteria for both groups included current smoking, diagnosis of diabetes mellitus, cardiovascular disease, chronic kidney disease, autoimmune disorders, active malignancy, acute or chronic infections, renal or hepatic impairment as identified by routine laboratory screening and current use of lipid-lowering, antihypertensive, hypoglycemic, or other medications known to influence oxidative stress or estrogen metabolism.

Participants attended a single outpatient visit in the morning, between 08:00 and 10:00 AM, after an overnight fast of at least ten hours. Demographic and reproductive histories were recorded using a standardized questionnaire administered by trained research staff. Anthropometric measurements were performed according to World Health Organization guidelines: height was measured to the nearest 0.1 cm using a stadiometer, and weight was obtained to the nearest 0.1 kg on a calibrated digital scale. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Blood pressure measurements were taken in the seated position after five minutes of rest using an automated sphygmomanometer; two consecutive readings were obtained and averaged for both systolic and diastolic values.

A total of ten milliliters of venous blood was drawn from the antecubital vein under aseptic conditions, using a 21-gauge needle. Two milliliters were collected into an EDTA-containing tube for complete blood count and basic biochemical screening to exclude subclinical anemia, leukocytosis, or hepatic and renal dysfunction. The remaining eight milliliters were drawn into lithium-heparinized tubes, immediately placed on ice, and transported to the central laboratory of Civil Hospital Quetta within fifteen minutes of collection. Upon arrival, samples were centrifuged at 3,000 × g for ten minutes at 4 °C. Plasma was aliquoted in 0.5 mL volumes into cryovials, labeled with anonymized alphanumeric codes, and stored at −80 °C until batch assays were performed; laboratory personnel remained blinded to participant group assignments throughout sample processing and analysis.

Quantification of plasma estrogen metabolites namely estradiol (E_2), estrone (E_1), 2-hydroxyestrone (2-OHE_1), and 16α-hydroxyestrone (16α-OHE_1) was performed using high-performance liquid chromatography (HPLC) with UV detection. Before analysis, each frozen plasma aliquot was thawed on ice and vortexed gently. A 500 µL aliquot of plasma was subjected to solid-phase extraction using preconditioned C18 cartridges (Sep-Pak, Waters Corporation). Cartridges were conditioned with 3 mL of methanol followed by 3 mL of water. Plasma was acidified to pH 2.0 by adding 50 µL of 1 M hydrochloric acid, then loaded onto the cartridge under controlled vacuum at a rate not exceeding 1 mL/min. After loading, cartridges were washed sequentially with 3 mL of water and 3 mL of 20 % methanol in water to remove hydrophilic impurities. Elution of estrogen metabolites was achieved with 3 mL of pure methanol; eluates were evaporated to dryness under a gentle stream of nitrogen at 37 °C. Dried residues were reconstituted in 200 µL of mobile phase (acetonitrile:water, 60:40 v/v, with 0.1 % formic acid) before injection. Chromatographic separation was accomplished on an HPLC system (Model 2695, Waters Corporation) fitted with a reverse-phase C18 column (XBridge, 250 × 4.6 mm, 5 µm particle size) maintained at 30 °C. Isocratic elution was performed at 1.0 mL/min using the above mobile phase. A 50 µL injection volume was applied for each sample. Detection wavelengths were set at 280 nm for E_2 and E_1, and 260 nm for 2-OHE_1 and 16α-OHE_1. Calibration curves were prepared using serial dilutions of certified reference standards (Sigma-Aldrich) ranging from 5 to 200 pg/mL. All standards and plasma samples underwent identical extraction and processing steps to account for matrix effects. The lower limit of detection for each analyte was 5 pg/mL, and limits of quantification were 15 pg/mL. Inter- and intra-assay coefficients of variation were determined by measuring quality-control samples at low, medium, and high concentrations, yielding values below 8 %.

Antioxidant enzyme activities and total antioxidant capacity were assessed in thawed plasma samples in duplicate, immediately after thawing and gentle mixing. Superoxide dismutase (SOD) activity was measured by the pyrogallol autoxidation method. In brief, 50 µL of plasma was mixed with 2.95 mL of Tris-HCl buffer (50 mM, pH 8.2) containing 0.1 mM EDTA. The reaction was initiated by adding 200 µL of freshly prepared 3 mM pyrogallol solution, and absorbance changes at 420 nm were recorded at 25 °C every 30 seconds over a 5-minute interval using a UV–visible spectrophotometer (Model UV-1800, Shimadzu). One unit of SOD activity was defined as the amount of enzyme required to inhibit the rate of pyrogallol autoxidation by 50 %, and results were expressed as units per milliliter of plasma (U/mL).

Catalase (CAT) activity was determined by monitoring the decomposition of hydrogen peroxide (H_2O_2) following the method described by Aebi. A 100 µL aliquot of plasma was mixed with 2.9 mL of phosphate buffer (50 mM, pH 7.0) pre-equilibrated at 25 °C. The reaction was initiated by adding 100 µL of freshly prepared 10 mM H_2O_2, and absorbance at 240 nm was recorded at 15-second intervals for 3 minutes. The molar extinction coefficient for H_2O_2 (43.6 M^{-1} cm^{-1}) was used to calculate the rate of decomposition, and one unit of catalase activity was defined as the amount of enzyme decomposing 1 µmol of H_2O_2 per minute under the assay conditions, expressed in U/mL of plasma.

Glutathione peroxidase (GPx) activity was measured using the coupled enzymatic assay described by Paglia and Valentine. Plasma (100 µL) was combined with 2.4 mL of phosphate buffer (100 mM, pH 7.4) containing 1 mM EDTA, 0.6 mM reduced glutathione (GSH), 0.2 mM NADPH, and 1 U/mL glutathione reductase. After a 5-minute equilibration at 25 °C, the reaction was initiated by adding 0.1 mL of 10 mM H_2O_2. The oxidation of NADPH was monitored at 340 nm over 3 minutes. One unit of GPx activity was defined as the amount of enzyme that oxidizes 1 µmol of NADPH per minute, and results were reported in U/mL.

Total antioxidant capacity (TAC) was assessed by the ferric reducing antioxidant power (FRAP) assay. Fresh FRAP reagent was prepared daily by mixing 300 mM acetate buffer (pH 3.6), 10

mM 2,4,6-tripyridyl-S-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1. A 300 µL aliquot of plasma was mixed with 2.7 mL of the FRAP reagent and incubated at 37 °C for exactly 10 minutes, after which absorbance at 593 nm was measured. A standard curve was constructed using ferrous sulfate solutions ranging from 100 to 1,000 µM. Results were expressed as µmol Fe^{2+} equivalents per liter of plasma. All reagents were freshly prepared, and intra-assay coefficients of variation were below 5 %.

Data were analyzed using SPSS for Windows, version 26.0 (IBM Corp., Armonk, NY). Continuous variables were first tested for normality using the Shapiro–Wilk test. Normally distributed variables are presented as mean ± standard deviation (SD), while non-normal variables are presented as median with interquartile range (IQR). Comparisons between premenopausal and postmenopausal groups for normally distributed variables were conducted using independent Student's t-tests; non-normal data were analyzed using the Mann–Whitney U test. Categorical variables were compared using the chi-square test.

Correlations between individual estrogen metabolite concentrations (E_2, E_1, 2-OHE_1, and 16α-OHE_1) and antioxidant defense markers (SOD, CAT, GPx, and TAC) were evaluated using Pearson's correlation coefficient for normally distributed variables. For variables violating normality assumptions, Spearman's rank correlation was employed. Correlation coefficients (r) are reported alongside two-tailed p-values, with $p < 0.05$ considered statistically significant. Prior to correlation analyses, scatterplots and standardized residuals were inspected to identify outliers; any data point with a residual exceeding ±3 SD was reviewed and excluded only if a clear procedural or measurement error was confirmed. Multicollinearity among predictors was assessed via variance inflation factors when constructing exploratory multiple linear regression models to adjust for potential confounders such as age, BMI, and blood pressure.

Quality control measures included double data entry, random verification of 10 % of records, and routine calibration of laboratory equipment. Laboratory personnel conducting assays remained blinded to participant group assignments to minimize measurement bias.

RESULTS

Demographic and Clinical Characteristics: Seventy women were included in the final analysis, with thirty-five premenopausal and thirty-five postmenopausal participants. As shown in Table 1, the mean age of the premenopausal group was 40.3 ± 3.2 years, whereas that of the postmenopausal group was 54.8 ± 2.9 years ($p < 0.001$). There was no significant difference in body mass index (BMI) between premenopausal (24.7 ± 2.8 kg/m²) and postmenopausal women (25.6 ± 3.1 kg/m²; $p = 0.28$). Both systolic and diastolic blood pressures were comparable between groups ($p > 0.05$), as were fasting blood glucose levels. These findings indicate that, aside from age, the two cohorts were well matched in terms of BMI, blood pressure, and glycemic status, minimizing potential confounding influences on antioxidant parameters.

Table 1: Baseline Demographic and Clinical Characteristics of Study Participants (n = 70)

Parameter	Premenopausal (n = 35)	Postmenopausal (n = 35)	p-value
Age (years), mean ± SD	40.3 ± 3.2	54.8 ± 2.9	< 0.001
Body Mass Index (kg/m²), mean ± SD	24.7 ± 2.8	25.6 ± 3.1	0.28
Systolic Blood Pressure (mmHg), mean ± SD	117.9 ± 9.3	120.8 ± 10.0	0.24
Diastolic Blood Pressure (mmHg), mean ± SD	76.5 ± 7.1	78.2 ± 7.6	0.32
Fasting Blood Glucose (mg/dL), mean ± SD	89.8 ± 8.2	91.2 ± 8.9	0.45
Duration Since Menopause (years), mean ± SD		5.4 ± 2.0	

Table 2: Plasma Estrogen Metabolite Concentrations (pg/mL)

Metabolite	Premenopausal (n = 35)	Postmenopausal (n = 35)	p-value
Estradiol (E_2), mean ± SD	54.1 ± 14.5	12.5 ± 5.9	< 0.001
Estrone (E_1), mean ± SD	45.5 ± 12.1	23.0 ± 9.1	< 0.001
2-Hydroxyestrone (2-OHE_1), mean ± SD	19.0 ± 5.3	9.8 ± 3.9	0.002
16α-Hydroxyestrone (16α-OHE_1), mean ± SD	12.0 ± 4.6	10.5 ± 4.0	0.15

Table 1 demonstrates that, aside from the expected age difference ($p < 0.001$), there were no statistically significant differences between premenopausal and postmenopausal women with respect to BMI ($p = 0.28$), systolic blood pressure ($p = 0.24$), diastolic blood pressure ($p = 0.32$), or fasting blood glucose ($p = 0.45$). By matching groups on these clinical parameters, potential confounding effects on the oxidative stress markers and estrogen metabolism were minimized.

Plasma Estrogen Metabolite Concentrations: Plasma concentrations of estradiol (E_2), estrone (E_1), 2-hydroxyestrone (2-OHE_1), and 16α-hydroxyestrone (16α-OHE_1) are presented in Table 2. Premenopausal women exhibited significantly higher mean E_2 levels (54.1 ± 14.5 pg/mL) compared to postmenopausal women (12.5 ± 5.9 pg/mL; $p < 0.001$). Likewise, mean E_1 was elevated in the premenopausal cohort (45.5 ± 12.1 pg/mL) relative to the postmenopausal cohort (23.0 ± 9.1 pg/mL; $p < 0.001$). The 2-hydroxylated metabolite 2-OHE_1 was also significantly higher in premenopausal participants (19.0 ± 5.3 pg/mL) than in postmenopausal participants (9.8 ± 3.9 pg/mL; $p = 0.002$). Conversely, 16α-OHE_1 concentrations did not differ significantly between groups (12.0 ± 4.6 pg/mL vs. 10.5 ± 4.0 pg/mL; $p = 0.15$). These results confirm a marked decline in parent estrogens and the antioxidant-favoring catechol estrogen 2-OHE_1 after menopause, while 16α-hydroxylation remains relatively unchanged.

Table 2 illustrates that premenopausal women had approximately fourfold higher estradiol levels and nearly twofold higher estrone levels compared to postmenopausal women (both $p < 0.001$). The 2-OHE_1 metabolite, which is known for its antioxidant properties, was also significantly elevated in premenopausal participants ($p = 0.002$). In contrast, 16α-OHE_1 levels did not show a statistically significant difference ($p = 0.15$), suggesting that the proportion of 16α-hydroxylation remains relatively stable across menopausal status despite lower overall estrogen production.

Antioxidant Defense Markers: Activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), along with total antioxidant capacity (TAC), are summarized in Table 3. Premenopausal women displayed significantly higher mean SOD activity (8.1 ± 1.8 U/mL) than postmenopausal women (5.7 ± 1.5 U/mL; $p < 0.001$). Catalase activity was also elevated in the premenopausal group (49.0 ± 9.0 U/mL) compared to the postmenopausal group (34.5 ± 7.8 U/mL; $p < 0.001$). Similarly, GPx activity in premenopausal women (12.3 ± 3.2 U/mL) was markedly greater than in postmenopausal women (7.8 ± 2.9 U/mL; $p < 0.001$). Total antioxidant capacity, measured as µmol Fe^{2+} equivalents/L, was significantly higher among premenopausal participants (1,245 ± 215 µmol/L) than postmenopausal participants (925 ± 185 µmol/L; $p < 0.001$). These data indicate that premenopausal women possess a substantially more robust antioxidant defense profile.

Table 3: Antioxidant Defense Markers

Parameter	Premenopausal (n = 35)	Postmenopausal (n = 35)	p-value
SOD Activity (U/mL), mean ± SD	8.1 ± 1.8	5.7 ± 1.5	< 0.001
Catalase Activity (U/mL), mean ± SD	49.0 ± 9.0	34.5 ± 7.8	< 0.001
GPx Activity (U/mL), mean ± SD	12.3 ± 3.2	7.8 ± 2.9	< 0.001
TAC (μmol Fe^{2+} equivalents/L), mean ± SD	1245 ± 215	925 ± 185	< 0.001

Table 4: Correlation Coefficients between Estrogen Metabolites and Antioxidant Defense Markers (n = 70)

Metabolite	SOD Activity	Catalase Activity	GPx Activity	TAC
Estradiol (E_2)	0.69 (< 0.001)	0.62 (0.001)	0.58 (0.003)	0.56 (0.005)
Estrone (E_1)	0.47 (0.007)	0.43 (0.009)	0.41 (0.012)	0.39 (0.018)
2-OHE_1	0.50 (0.006)	0.44 (0.008)	0.36 (0.025)	0.54 (0.004)
16α-OHE_1	−0.20 (0.11)	−0.23 (0.08)	−0.32 (0.045)	−0.28 (0.06)

Values in parentheses represent p-values.

According to Table 3, premenopausal participants exhibited a 42 % higher SOD activity, a 42 % higher catalase activity, and a 58 % higher GPx activity relative to postmenopausal participants (all $p < 0.001$). The nearly 35 % greater TAC in the premenopausal group underscores the superior overall antioxidant capacity when estrogen levels are intact. These findings suggest that estradiol and related metabolites play a pivotal role in upregulating enzymatic defense mechanisms against reactive oxygen species.

Correlation between Estrogen Metabolites and Antioxidant Parameters: Pearson's correlation coefficients evaluating relationships between estrogen metabolites and antioxidant markers are displayed in Table 4. Estradiol (E_2) demonstrated a strong positive correlation with SOD activity ($r = 0.69$, $p < 0.001$), catalase activity ($r = 0.62$, $p = 0.001$), GPx activity ($r = 0.58$, $p = 0.003$), and TAC ($r = 0.56$, $p = 0.005$). Estrone (E_1) also correlated positively with catalase ($r = 0.43$, $p = 0.009$), GPx ($r = 0.41$, $p = 0.012$), and TAC ($r = 0.39$, $p = 0.018$). The 2-hydroxylated metabolite 2-OHE_1 showed significant positive correlations with TAC ($r = 0.54$, $p = 0.004$) and SOD ($r = 0.50$, $p = 0.006$), as well as moderate correlations with catalase ($r = 0.44$, $p = 0.008$) and GPx ($r = 0.36$, $p = 0.025$). In contrast, 16α-OHE_1 exhibited a weak inverse correlation with GPx activity ($r = −0.32$, $p = 0.045$) and non-significant trends toward inverse relationships with SOD ($r = −0.20$, $p = 0.11$), catalase ($r = −0.23$, $p = 0.08$), and TAC ($r = −0.28$, $p = 0.06$).

As depicted in Table 4, estradiol (E_2) exhibited the strongest positive correlations across all antioxidant parameters (all $p \leq 0.005$). This indicates that higher circulating E_2 levels are closely associated with enhanced enzymatic defense and overall antioxidant capacity. Similarly, 2-OHE_1 showed a particularly strong positive correlation with TAC ($r = 0.54$, $p = 0.004$), highlighting the importance of this catechol estrogen in bolstering total antioxidant status. Estrone (E_1) also demonstrated moderate positive correlations with catalase, GPx, and TAC (all $p < 0.02$), suggesting that even lower-affinity estrogens contribute meaningfully to antioxidant defenses. In contrast, 16α-OHE_1's weak but statistically significant inverse correlation with GPx ($r = −0.32$, $p = 0.045$) suggests a potential pro-oxidant influence of this metabolite when present at higher concentrations. The non-significant inverse trends of 16α-OHE_1 with SOD, catalase, and TAC ($p > 0.05$) further imply that elevated 16α-hydroxylation may tilt the redox balance toward oxidative stress, although larger sample sizes would be needed to confirm these associations conclusively.

In summary, the results demonstrate (1) a clear decline in parent estrogens and antioxidant-favoring metabolites particularly E_2, E_1, and 2-OHE_1 after menopause (Table 2); (2) significantly reduced enzymatic antioxidant activities and total antioxidant capacity in postmenopausal women (Table 3); and (3) strong positive correlations between beneficial estrogen metabolites (E_2, E_1, 2-OHE_1) and antioxidant parameters, with a potential inverse effect of 16α-OHE_1 on GPx activity (Table 4). Together, these findings indicate that the menopause-associated decline in estrogen and its protective metabolites contributes substantially to diminished antioxidant defenses.

DISCUSSION

The present study investigated the relationship between estrogen metabolites and antioxidant defense systems in premenopausal and postmenopausal women, revealing several key findings. First, as shown in Table 2, estradiol (E_2), estrone (E_1), and 2-hydroxyestrone (2-OHE_1) concentrations were significantly higher in premenopausal participants. These differences reflect the expected decline in ovarian estrogen production after menopause. Notably, 2-OHE_1, a catechol estrogen recognized for potent radical-scavenging activity, was nearly twice as high in the premenopausal group[11]. In contrast, 16α-hydroxyestrone (16α-OHE_1) levels did not differ significantly between groups. This stability in 16α-OHE_1 suggests that, despite overall estrogen withdrawal, the relative fraction of 16α-hydroxylation remains unchanged, potentially favoring a shift toward pro-oxidant metabolite predominance after menopause[12].

Second, enzymatic antioxidant activities including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were markedly higher in premenopausal women (Table 3). Similarly, total antioxidant capacity (TAC) was approximately 35 % greater in the premenopausal group[13]. These findings align with previous reports demonstrating that endogenous estrogens upregulate antioxidant enzymes and bolster overall redox homeostasis. The phenolic structure of estradiol allows direct neutralization of reactive oxygen species (ROS), while estrogen receptor–mediated signaling increases transcription of genes encoding SOD, CAT, and GPx. Consequently, premenopausal women maintain superior enzymatic defenses compared to their postmenopausal counterparts, who experience diminished estrogenic stimuli[14].

Third, correlation analyses (Table 4) revealed strong positive associations between beneficial estrogen metabolites and antioxidant parameters. Estradiol exhibited the highest correlation coefficients with SOD ($r = 0.69$), catalase ($r = 0.62$), GPx ($r = 0.58$), and TAC ($r = 0.56$), indicating that fluctuations in E_2 concentrations closely parallel changes in enzymatic and total antioxidant defenses[15]. Estrone also correlated positively, albeit to a slightly lesser degree, with catalase ($r = 0.43$), GPx ($r = 0.41$), and TAC ($r = 0.39$). Importantly, 2-OHE_1 demonstrated a robust positive correlation with TAC ($r = 0.54$) and moderate correlations with SOD ($r = 0.50$), catalase ($r = 0.44$), and GPx ($r = 0.36$). These relationships highlight the antioxidant efficacy of catechol estrogens. In contrast, 16α-OHE_1 was weakly and inversely correlated with GPx activity ($r = −0.32$, $p = 0.045$) and showed non-significant inverse trends with other antioxidant markers. Such inverse associations imply a subtle pro-oxidant role for 16α-OHE_1 that may become more pronounced under conditions of metabolic stress or in older age, contributing to cumulative oxidative damage[16].

Taken together, these results suggest that the menopause-associated decline in estradiol and 2-OHE_1 is accompanied by a

proportional reduction in antioxidant defenses, predisposing postmenopausal women to increased oxidative stress and its related pathologies[17]. Indeed, epidemiological studies report a steep rise in cardiovascular events, osteoporosis, and neurodegenerative diseases following the menopausal transition, coinciding with diminished endogenous estrogen levels. The mechanistic insights from this study affirm that both parent estrogens and specific hydroxylated metabolites are central to maintaining redox homeostasis, and their depletion after menopause explains, in part, the vulnerability to oxidative stress–mediated tissue injury[18].

Comparison with earlier literature underscores these conclusions. For example, in vitro experiments have shown that estradiol upregulates SOD and catalase expression in endothelial cells, reducing lipid peroxidation. Similarly, human studies have documented higher SOD, CAT, and GPx activities in premenopausal versus postmenopausal women, corroborating our findings[19]. The novel aspect of the current work is its parallel assessment of individual estrogen metabolites, establishing that 2-OHE_1 correlates most strongly with total antioxidant capacity, whereas 16α-OHE_1 may exert the opposite effect. Prior investigations of estrogen metabolism in postmenopausal women have primarily focused on parent hormones or broad measures of oxidative stress; by contrast, our study elucidates the nuanced contributions of distinct hydroxylation pathways[20].

Several limitations warrant consideration. The cross-sectional design limits causal inferences; longitudinal studies following women through the menopausal transition are needed to confirm temporal changes in estrogen metabolites and antioxidant defenses[21]. The sample size, though sufficient to detect moderate correlations, may not have been large enough to fully explore the subtleties of 16α-OHE_1's pro-oxidant tendencies or to examine interindividual variation in cytochrome P450 enzyme activity. Additionally, dietary intake of antioxidants and variations in lifestyle factors such as physical activity, smoking history, or psychosocial stress were not rigorously controlled, which could confound antioxidant measurements. Although smokers and women with known chronic diseases were excluded, subclinical nutritional differences may still have influenced TAC. Future research should incorporate detailed dietary logs or controlled feeding protocols to isolate the effects of estrogen metabolism on oxidative balance[22].

Furthermore, our study measured antioxidant parameters in plasma, providing a systemic snapshot of redox status. Tissue-specific assessments such as evaluating antioxidant enzyme expression in vascular endothelium, bone, or neural tissue could offer more precise insights into organ-level oxidative dynamics[23]. Genetic polymorphisms in estrogen-metabolizing enzymes (e.g., CYP1A1, CYP1B1, COMT) influence the ratio of 2- to 16α-hydroxylation and may therefore modulate individual susceptibility to oxidative stress. Incorporating genotyping into future studies could identify women at particular risk for redox imbalance and related diseases[24].

Clinically, these findings suggest that therapeutic strategies aimed at preserving or mimicking estrogen-mediated antioxidant effects may benefit postmenopausal women. Hormone replacement therapy (HRT) has been shown to partially restore antioxidant enzyme activities; however, concerns over adverse cardiovascular and oncologic outcomes necessitate caution. Selective estrogen receptor modulators (SERMs) or phytoestrogens that preferentially promote 2-hydroxylation pathways could offer antioxidant benefits with fewer risks[25]. Dietary interventions rich in phytoestrogenic compounds such as soy isoflavones have been associated with increased 2-OHE_1 production and improved redox markers. Antioxidant supplementation (e.g., vitamin E, vitamin C) may also help counteract estrogen deficiency–related oxidative stress, though clinical trials yield mixed results. Ultimately, individualized approaches that consider each woman's metabolic and genetic profile may optimize redox balance and mitigate menopause-associated morbidity[26].

CONCLUSION

In conclusion, the current study demonstrates that premenopausal women maintain higher concentrations of estradiol, estrone, and 2-hydroxyestrone, which correlate strongly with enhanced activities of SOD, catalase, GPx, and greater total antioxidant capacity. Conversely, postmenopausal women exhibit significantly reduced estrogen metabolites and antioxidant markers, underscoring a shift toward oxidative stress. The inverse correlation between 16α-hydroxyestrone and GPx suggests a subtle pro-oxidant influence of this metabolite after menopause. These findings highlight the pivotal role of estrogen metabolism in regulating systemic redox homeostasis and suggest that targeted modulation of estrogenic pathways whether through selective metabolism favoring 2-hydroxylation, phytoestrogenic diets, or tailored pharmacologic agents may restore antioxidant defenses and reduce oxidative stress–related disease risk in postmenopausal populations.

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