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ASSESSMENT OF ANTIOXIDANT LEVELS AND ACUTE PHASE REACTANTS IN POSTMENOPAUSAL WOMEN

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ABSTRACT

Menopause induces hormonal changes, potentially leading to oxidative stress and inflammation, contributing to various health risks. This study aimed to investigate the relationship between oxidative stress, inflammation, and menopause-related physiological changes in women. A comparative cross-sectional study was conducted on 100 women aged ≥ 30 years, subdivided into postmenopausal (PMP), premenopausal (PRM), and reproductive women age (RWA). Serum levels of oxidative stress and antioxidant markers (SOD, MDA, GPx, CAT, H_2O_2) and acute phase reactants (CRP, SAA) were measured using standard spectrophotometric and immuno assay techniques. Statistical analysis was performed using SPSS 25.0. PMP women exhibited significantly higher levels of oxidative stress markers (MDA, H_2O_2) and acute phase reactants (CRP, SAA) compared to PRM and RWA ($p<0.05$). Conversely, enzymatic antioxidants (SOD, GPx, CAT) were significantly lower in PMP women ($p<0.05$). CRP showed superior diagnostic performance than SAA, with higher AUROC values (0.937 vs. 0.737). Menopause is associated with increased oxidative stress and inflammation, elevating the risk of cardiovascular diseases, osteoporosis and metabolic disorders. Early interventions targeting oxidative stress and inflammation are recommended to mitigate menopause-related health risks. Regular health screenings and lifestyle modifications emphasizing antioxidant-rich diets and physical activity are essential for women transitioning through menopause.

Keywords: menopause, inflammation, oxidative stress, acute phase reactants, antioxidants.

INTRODUCTION

Menopause represents a significant physiological transition in a woman's life, marking the cessation of menstrual cycles and the end of reproductive capacity. This natural process typically occurs around the age of 50 but can vary widely among individuals (Harlow *et al*., 2012; Voedisch *et al*., 2021). While menopause is a normal part of aging, it is accompanied by various hormonal, metabolic, and physiological changes that can impact women's health and well-being (Jeong & Park, 2022; Pandey *et al*., 2010). Despite its universal occurrence, menopause remains an understudied and often overlooked aspect of women's health research (Hooper *et al*., 2022).

The transition into menopause is characterized by hormonal fluctuations, particularly a decline in estrogen levels, which plays a crucial role in maintaining bone health, cardiovascular function, and metabolic homeostasis (Tuomisto *et al*., 2012; Vasikaran *et al*., 2023). This decline in estrogen is associated with an increased risk of various health conditions, including osteoporosis, cardiovascular disease, insulin resistance, and oxidative stress (Park & Lee, 2020). Additionally, menopause is often accompanied by symptoms such as hot flashes, mood changes, and vaginal dryness, which can significantly impact quality of life (Whiteley *et al*., 2013).

Despite the profound impact of menopause on women's health, there remains a gap in understanding the mechanisms underlying the increased risk of chronic diseases during this stage of life. Furthermore, limited research has focused

on elucidating the role of oxidative stress and inflammation in mediating the adverse health outcomes associated with menopause (Effendy & Shuid, 2014). Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, has been implicated in the pathogenesis of various age-related diseases, including cardiovascular disease and osteoporosis (Pizzino *et al*., 2017). However, the specific contributions of oxidative stress to the health outcomes of menopausal women are not fully understood.

This study aims to address these knowledge gaps by investigating the relationship between menopause, oxidative stress, inflammation, and their impact on women's health. The overarching goal is to enhance our understanding of the biological mechanisms underlying the increased risk of chronic diseases in menopausal women and identify potential targets for preventive and therapeutic interventions.

MATERIALS AND METHODS

Study Design and Participants

This comparative cross-sectional study enrolled a total of 100 women aged 30 years and above, randomly selected from the Owo metropolis in Nigeria. The participants were categorized into three groups: 60 postmenopausal (PMP) women aged between 50 and 65 years, 20 premenopausal (PRM) women aged between 40 and 49 years, and 20 women in the reproductive women age (RWA) group (30-40 years). Participants' medical history and personal data were collected through a comprehensive questionnaire after obtaining approval from the hospital's ethical committee. The study was conducted between January and June 2022.

Consent and Ethical Clearance

All participants provided written informed consent after receiving detailed information about the research protocols. Ethical clearance for the study was obtained from the Ethical Review Committee of the Federal Medical Center, Owo (Reference Number: FMC/OW/380/VOL.CL/184).

Sample Size Determination

The sample size was determined using Fischer's formula (Taherdoost, 2018) for cross-sectional studies. Based on a prevalence of menopause in Nigeria of 7% (Inyang-Etoh *et al*., 2018), a two-tailed significance level of 1.96, and a level of significance set at 5%, the minimum sample size required was calculated to be 100.

Inclusion and Exclusion Criteria

Inclusion criteria included PMP women aged 50-65 years with a minimum of one year of amenorrhea, PRM women aged 40-49 years, and women in the RWA group (30-40 years). Also, Women who provided written consent to participate in the study. Exclusion criteria comprised subjects with hypertension, cardiovascular diseases, diabetes, venereal diseases, those taking oral contraceptives or antioxidants, and pregnant women.

Sample Collection and Storage

Venous blood samples (5ml) were collected from each participant using standard procedures and dispensed into sterile plain bottles. The samples were centrifuged at 4000 rpm for 5 minutes to obtain serum, which was then stored at -20°C until analyzed.

Analytical Methods

Serum levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) were measured using spectrophotometric method as described by (Atere & Osadolor, 2017). The serum c-reactive protein (CRP), and serum amyloid A (SAA) were measured by ELISA kit from Melsin Medical Company, USA.

Statistical Analysis

The statistical analysis was performed the Statistical Package for the Social Sciences (SPSS) version 25.0 0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to compare groups, and correlations analysis was used to assess associations between variables. The area under the receiver operating characteristic curve (AUROC) of each marker (CRP and SAA) was compared using pair-wise comparison on a sensitivity plot. Data were presented as mean ± standard deviation, and significance was set at a 95% confidence interval with $p < 0.05$ considered statistically significant.

RESULTS

The mean age of PMP women was 56.23 ± 3.90 years, while PRM women had a mean age of 44.40 ± 2.19 years, and women in the RWA had a mean age of 33.90 ± 2.10 years. When comparing demographic data among the groups, significant differences were observed. PMP and PRM women had significantly higher mean systolic blood pressure (SBP), compared to women in the RWA ($p < 0.05$) (table 1). In the same way, significant differences were observed when acute phase reactants (CRP and SAA) were compared between the groups depicted in figures 1-2. The mean CRP and SAA levels of PMP and PRM women were significantly higher than those of women in the RWA ($p < 0.05$). Furthermore, it was observed that the levels of CRP and SAA were considerably elevated in the PMP group in comparison to the PRM group ($p < 0.05$).

Comparing oxidative stress and antioxidant indices among the groups revealed significant differences. PMP and PRM women exhibited significantly higher levels of MDA and H_2O_2 and lower levels of SOD, GPx, and CAT compared to women in the RWA ($p < 0.05$). Further analysis using post hoc tests confirmed these differences between PMP, PRM, and RWA groups (figure 3).

Correlation analysis showed significant negative correlations between acute phase reactants (CRP and SAA) and oxidative stress parameters (MDA and GPx) among PMP women ($p < 0.05$) (figure 4). However, there were no significant correlations among PRM women (table 2). The diagnostic performance of CRP and SAA was evaluated using receiver operating characteristic (ROC) curves, with CRP demonstrating a higher area under the curve (AUROC) compared to SAA, indicating its superior diagnostic utility ($p < 0.05$) (figure 5).

Table 1: Comparison of mean demographic data (Age, BMI, SBP and DBP) in Postmenopausal, Premenopausal and Reproductive Women Age groups

	PMP (n=60)	PRM (n=20)	RWA (n=20)	P-Value
Age (Years)	56.23±3.90 [a,c]	44.40±2.19 [a,b]	33.90±2.10 [b,c]	0.000*
BMI (Kg/m^2)	30.98±5.52	29.17±6.45	28.19±6.72	0.151
SBP (mmHg)	137.53±15.23 [a]	132.65±13.64 [a]	123.15±7.87 [b,c]	0.000*
DBP (mmHg)	83.78±7.33 [a]	84.20±9.58	79.65±7.10 [b]	0.097

* significant at $p \leq 0.05$

a = significantly different from RWA, b = significantly different from postmenopausal group, c = significantly different from premenopausal group

Key: n=sample size, BMI= Body mass Index, SBP= Systolic blood pressure, DBP = Diastolic blood pressure

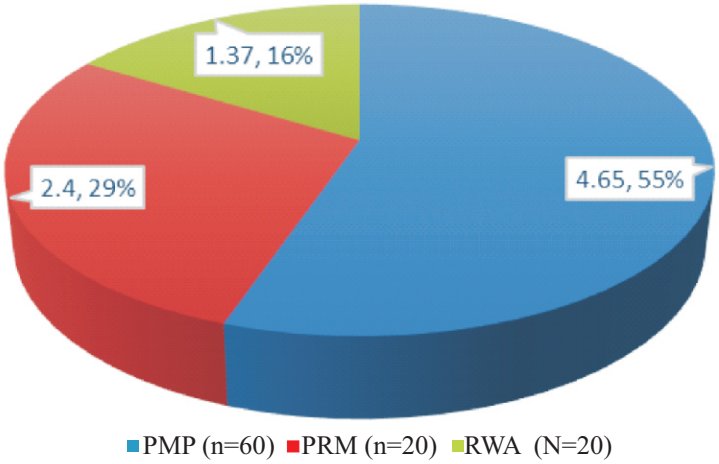


Figure 1: CRP (mg/dl) expression in Postmenopausal, Premenopausal and Reproductive Women Age groups

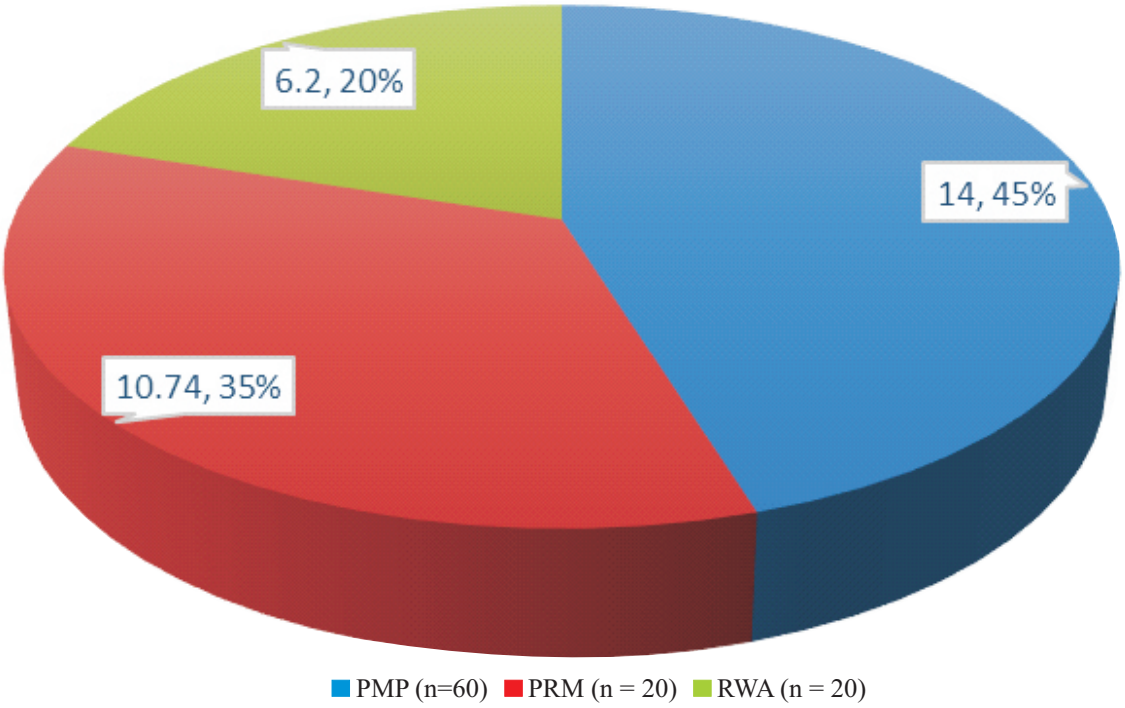


Figure 2: SAA (µg/mL) expression in Postmenopausal, Premenopausal and Reproductive Women Age groups

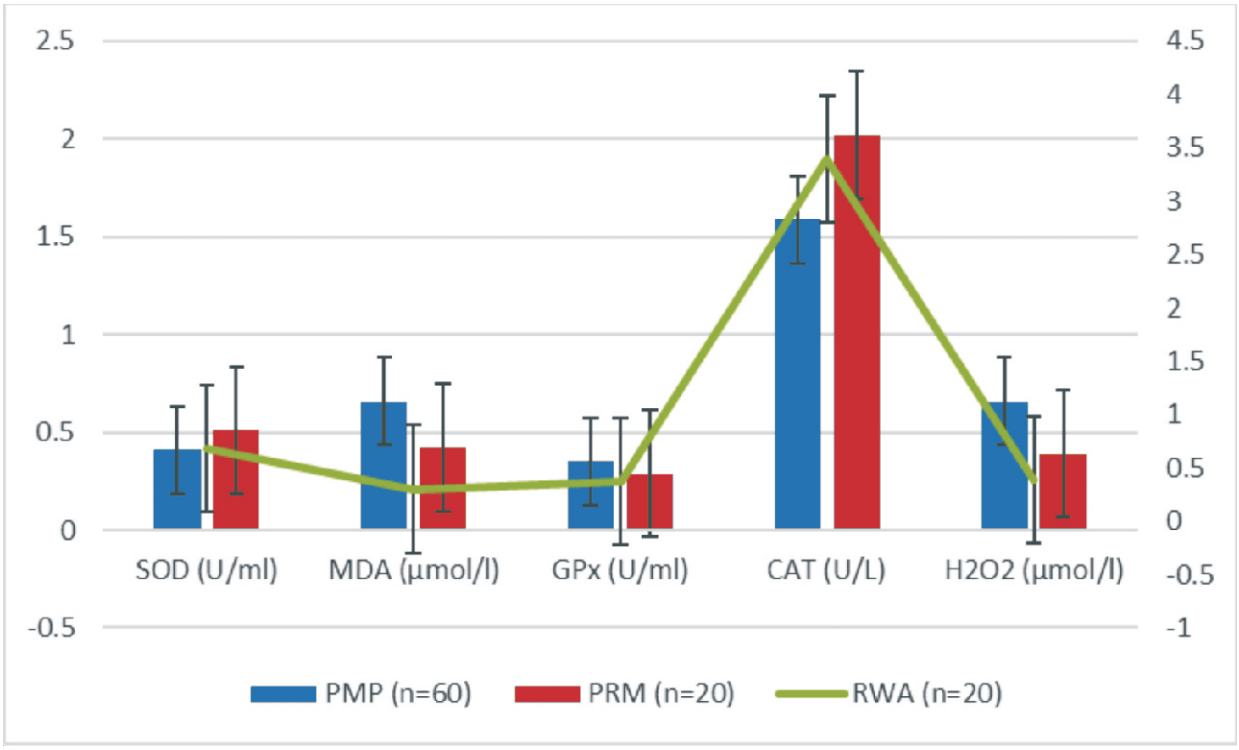


Figure 3: Comparison of oxidation stress and antioxidants indices (SOD, MDA, GPx, CAT and H_2O_2) in Postmenopausal, Premenopausal and Reproductive Women Age groups

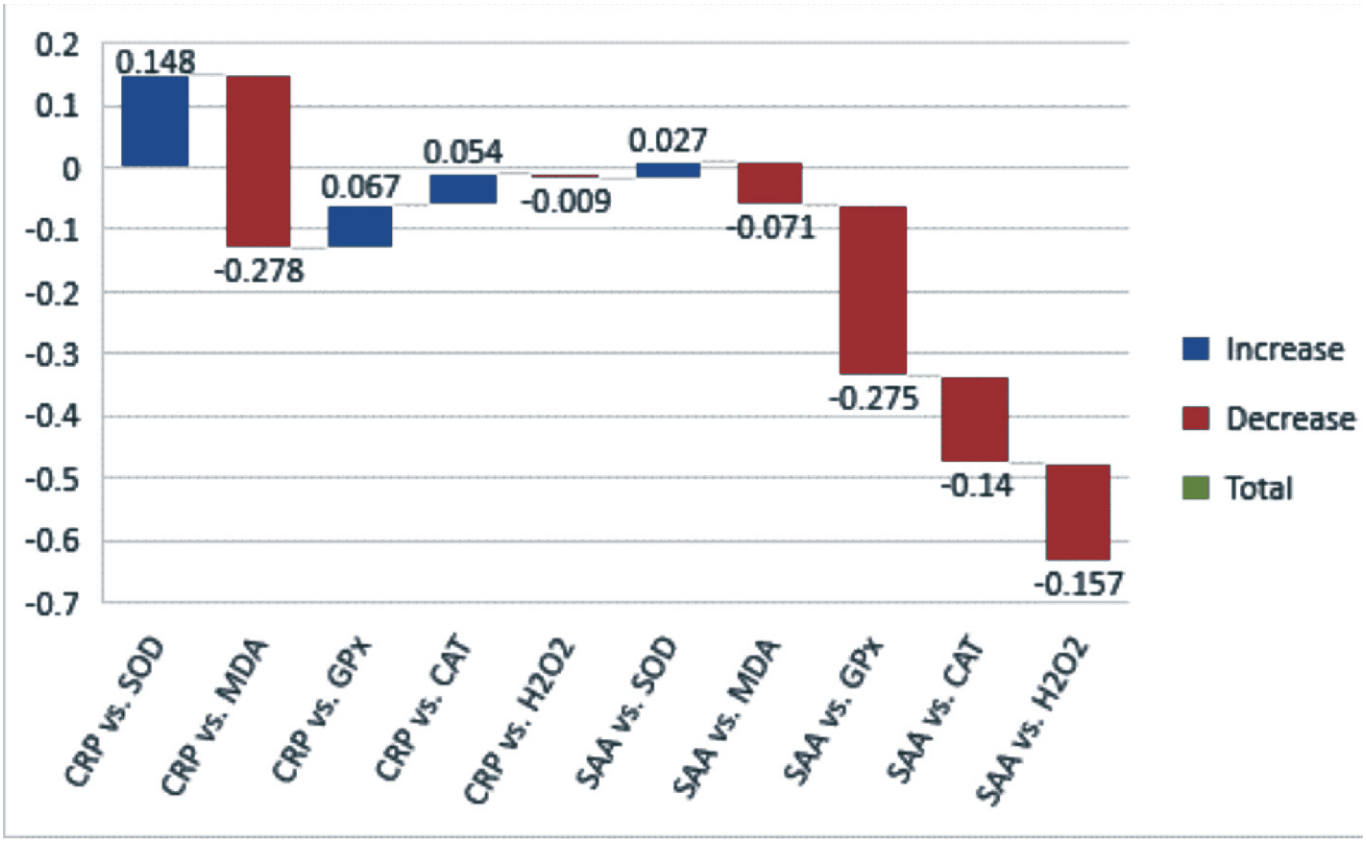
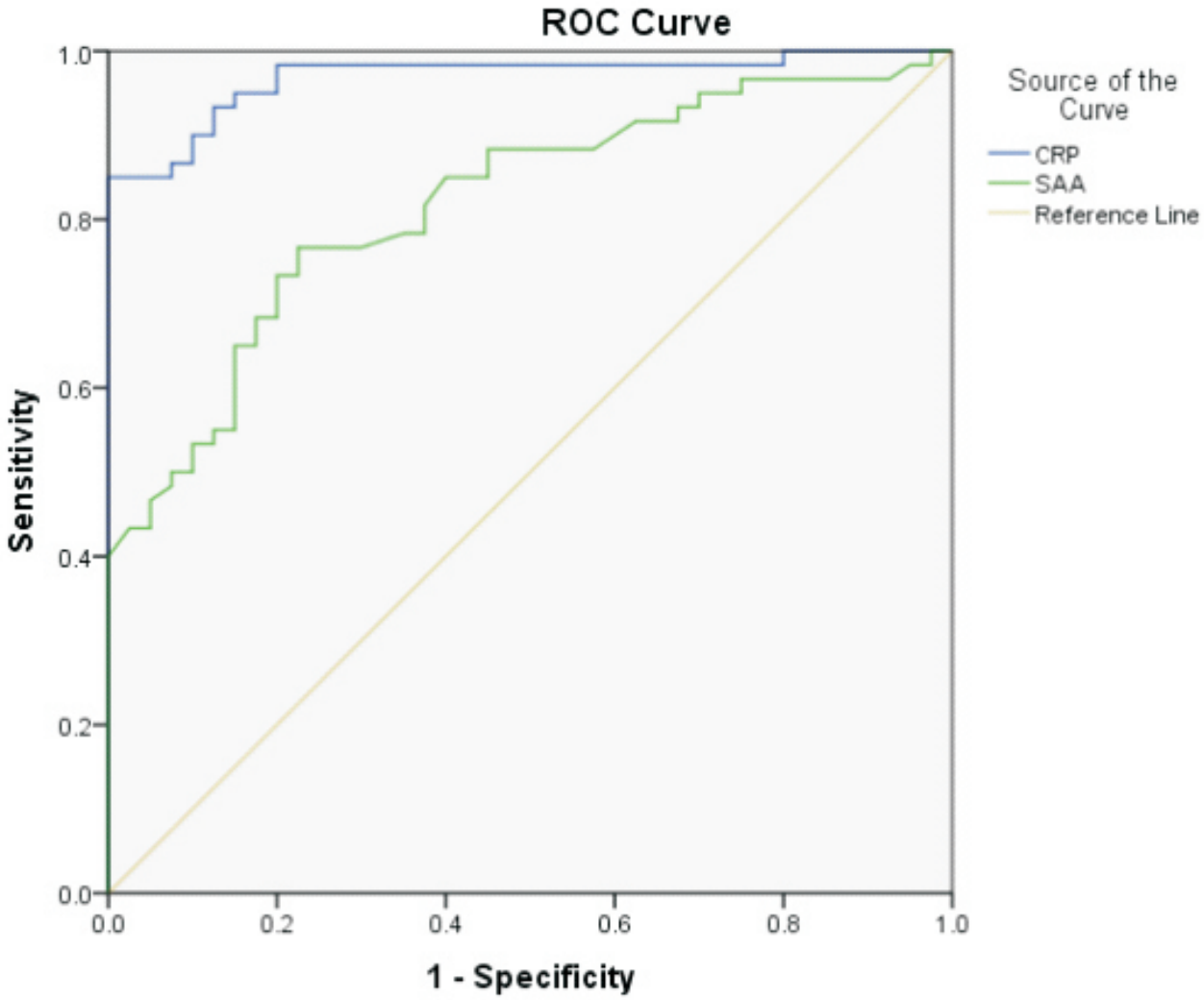


Figure 4: Correlation of acute phase reactant indices (CRP and SAA) with Oxidative and antioxidant indices (SOD, MDA, GPx, CAT and H_2O_2) in Postmenopausal Subjects

Table 2: Correlation of mean acute phase reactant indices (CRP and SAA) with Oxidative and antioxidant indices (SOD, MDA, GPx, CAT and H_2O_2) in Premenopausal Subjects

	CRP		SAA	
	r	p	r	p
SOD (U/ml)	-0.062	0.796	0.056	0.813
MDA (µmol/l)	0.040	0.867	0.053	0.825
GPx (U/ml)	-0.041	0.865	0.100	0.675
CAT (U/L)	-0.041	0.865	0.100	0.675
H2O2(µmol/l)	0.116	0.627	0.048	0.840



Diagonal segments are produced by ties.

Figure 5: The ROC Curve of blood levels of CRP and SAA as diagnostic tool in Postmenopausal subjects

DISCUSSION

Menopause marks a significant physiological transition in a woman's life, characterized by hormonal fluctuations that impact various metabolic processes. Our study corroborates existing literature indicating that menopause is associated with increased oxidative stress and altered inflammatory status (Badr Roomi *et al*., 2021; Talaulikar, 2022; Ugurlu *et al*., 2022). The decline in estrogen levels during menopause plays a pivotal role in the disruption of bone metabolism, leading to conditions such as osteoporosis (Adewole *et al*., 2021; Talaulikar, 2022). Furthermore, estrogen deficiency contributes to oxidative stress, as estrogen possesses antioxidant properties that mitigate ROS production (Pusparini *et al*., 2015). Consequently, the imbalance between oxidants and antioxidants observed in menopausal women predisposes them to various pathologies, including cardiovascular diseases, vasomotor disturbances, and metabolic disorders (Ansar *et al*., 2015; Zovari *et al*., 2020).

Our findings underscore the dysregulation of oxidative stress and antioxidant defense mechanisms in menopausal women. We observed elevated levels of MDA and H_2O_2, indicative of increased lipid peroxidation and ROS production, coupled with reduced activity of SOD, GPx, and CAT enzymes. This dysregulation suggests a diminished capacity to neutralize ROS, resulting in oxidative damage to cellular components (Atere *et al*., 2021; Bourgonje *et al*., 2020). These alterations in oxidative stress parameters highlight the need for targeted interventions to mitigate the adverse

health outcomes associated with menopause.

Additionally, our study revealed heightened inflammatory status in menopausal women, as evidenced by elevated levels of acute-phase reactants, including CRP and SAA. Inflammation is implicated in the pathogenesis of various diseases, particularly cardiovascular diseases, which exhibit a strong association with menopause (Pierce *et al*., 2009; Shahid *et al*., 2022). The correlation between CRP and SAA levels with oxidative stress parameters further highlights the interplay between inflammation and oxidative stress in menopausal physiology (Shahid *et al*., 2022). These findings emphasize the importance of addressing both oxidative stress and inflammation in managing the health of menopausal women. CRP also exhibited a higher AUROC than SAA, making it a more effective diagnostic tool. According to (Shahid *et al*., 2022), in univariate analysis, CRP outperforms SAA as the strongest risk factor of cardiovascular disease in postmenopausal women.

CONCLUSION

In conclusion, our study elucidates the multifaceted impact of menopause on oxidative stress, inflammatory status, and associated health outcomes. Strategies aimed at attenuating oxidative stress and inflammation, such as antioxidant supplementation and lifestyle modifications, may offer therapeutic benefits to mitigate the risk of chronic diseases in menopausal women. Furthermore, our findings underscore the importance of early intervention strategies targeting menopausal transitions to promote women's health across the lifespan.

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Conflict of interest: The authors declare that there are no conflicts of interest

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