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Stress Factors Increase Osteoporosis: A Comparative Assessment of Osteocalcin and Cortisol Levels in Menopausal Women

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ABSTRACT

Osteoporosis, a consequence of menopause in the biological cycle of women, emerges with the conclusion of reproductive capabilities. Hormonal changes during this phase contribute to the development of the disease. The study evaluated the relationship between stress, salivary cortisol levels, and osteocalcin, in postmenopausal women with osteoporosis.

The study involved a total of 60 postmenopausal volunteers diagnosed with osteoporosis. Stress levels were assessed using the NIH stress score system to evaluate cortisol levels. Saliva and blood samples were analyzed using the LC-MS/MS and ELISA methods. Statistical analyses, including the Wilcoxon Signed Rank test, paired samples t-tests, and correlation analyses, were conducted using IBM SPSS Statistics 21.0. A significance level of $p<0.05$ was considered.

In comparing stress scores between the first/second weeks, a statistically significant difference was observed ($z=4.795, p<0.001$), indicating a higher mean stress score in the second week. Cortisol levels showed a significant increase from the 1st week(27.58 ± 3.97) to the 2nd week (29.99 ± 2.44)($t=4.412, p<0.001$). Osteocalcin values exhibited a significant difference between the 1st week(21.04 ± 0.98) and the 2nd week (24.22 ± 1.44)($t=9.656$, $p<0.001$). Examining participant variations, the mean difference in stress scores was 7.73 ± 2.23, the mean difference in cortisol levels was 2.41 ± 2.99, and the mean difference in osteocalcin levels was 3.18 ± 1.81. A weak positive statistically significant relationship was found between stress score difference and cortisol difference ($r=0.363$, $p=0.049$). In contrast, an intermediate-level positive statistically significant relationship was observed between osteocalcin difference and cortisol difference ($r=0.586$, $p=0.001$). Findings demonstrate the intricate relationships between stress, cortisol levels, and osteocalcin.

Contrary to some existing findings, our study suggests that menopause, as a stress-inducing factor, leads to an increase in bone metabolism markers, including cortisol. Insights contribute to a more comprehensive understanding of the interplay between stress, hormonal changes, and osteoporosis in postmenopausal women.

Keywords: Menopause, Osteocalcin, Osteoporosis, Salivary Cortisol, Stress.

Introduction

Menopause, a physiological event, is inevitable for every woman during the latter stages of her life. Hormonal decline due to reproductive system deprivation plays a significant role in some metabolic functions. Menopause, the cessation of ovulation and the completion of the reproductive process, is associated with the loss of follicles in the female reproductive system (1–3). During this stage, there is a decrease in estradiol production, follicle-stimulating hormone (FSH) concentration, and the number of oocytes (4). One critical aspect of menopause is its multifactorial nature, leading to varied results. As body functions change, metabolic activities decline. One of the most severe consequences of menopause is osteoporosis, a condition many women confront. Decreased hormonal activity during this phase leads to bone loss, resulting in severe bone fractures. Estrogen's protective role against bone loss was first identified in the 1960s, and it was noted that this protection was replaced by bone loss after menopause, leading to osteoporosis (5–8). Even though the causes of osteoporosis are only partially understood, changes in hormonal levels associated with menopause have indicated that the activity of bone metabolism also varies. Osteocalcin (Ocn), a biomarker, that measures bone metabolism, can provide information about bone resorption (3, 7). This biomarker, frequently measured due to its calcium-binding properties of the carboxyl groups on it, inhibits the deposition of hydroxyapatite crystals.

Moreover, Ocn acts as a chemoattractive agent in the precursor synthesis for osteoclast orientation.

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Therefore, Ocn is recognized as a bone matrix protein that inhibits bone formation and resorption (7). The BGLAP gene, synthesized by osteoblasts, encodes Ocn, a protein of 49 amino acids. The bone matrix contains over 70% of Ocn, with the remainder circulating. The serum concentration of Ocn has been used as an osteogenesis biomarker, representing osteoblast activity. The World Health Organization (WHO) defines osteoporosis when the bone mineral density (BMD) measured by dual-energy X-ray absorptiometry (DEXA) scanning is less than -2.5 standard deviations from the mean value (T-score) for young adults of the same age and sex. In menopausal women who experience hormonal changes, there is a notable increase in metabolic activity due to stress. Given the significant impact of stress in today's society, a patient's comprehension of disease-triggering factors, coupled with alterations in their quality of life conditions, potentially hasten the progression of the disease (4, 9, 10). This study evaluated the impact of stress on osteoporosis. During the planning of the study, on the day of the osteoporosis diagnosis, patients were educated about the disease and asked to schedule a follow-up visit. The follow-up process and the period in between were examined. The increase in stress was monitored using cortisol and stress scores, investigating how these changes influenced the Ocn biomarker.

Materials and Methods

This study was approved by the Kafkas University Clinical Research Ethics Committee (Kars, Türkiye) (80576354-050-99/26). The 60 volunteers over 55 (57.4±2) diagnosed with osteoporosis during the menopausal period with a BMI of 23.1±1.3 were enrolled.

All patients' socioeconomic situations were evaluated. The values were compared to the control group on the first day and the first week. People with demographically low education and economic backgrounds were included in the study. All patients were non-smokers. Accordingly, stress levels were determined using the NIH stress score system to assess cortisol levels.

Table 1 outlines the specific criteria used to select participants for a study investigating the relationship between stress, cortisol levels, Ocn, and osteoporosis in postmenopausal women. The criteria aim to ensure the homogeneity of the study population and the reliability of the findings by minimizing confounding variables.

Sample Collection and Laboratory Analysis Methods: Saliva and blood samples were taken from the patients. These samples were analyzed within 14 days of collection. Saliva samples from 60 volunteers were obtained between 8 and 9 a.m. after brushing their teeth to remove food, blood, and smoke residue and to avoid contamination. During saliva collection, the sample stick was placed in the volunteer's mouth and chewed for approximately 60 seconds to obtain the saliva.

The swab containing the collected saliva was then deposited into a tube and centrifuged at 1000 x g for 2 minutes. Samples were collected in a Salivette® tube and stored at -80°C until use. Before analysis, samples were centrifuged (10 min, 2000 g) to remove particles.

The LC-MS/MS method used the XEVO TQD triple quadrupole mass spectrometer (Milford, Massachusetts, USA) and the dual pump Waters Acquity UPLC Class I system (Milford, Massachusetts, USA). Separation was performed on an Acquity UPLC C18 column (2.1 x 50 mm, 1.7 μm; Waters) at 30°C. The flow rate was 0.4 mL/min with an injection volume of 5μL. Mobile phases were 10 mM ammonium formate and 0.1% formic acid (A) in methanol and 10 mM ammonium formate and 0.1% formic acid (B) in water. Cortisol and IS were detected in the multiple reaction monitoring mode of tandem mass spectrometry: Cortisol, m/z 363.2>121 and IS, m/z 367.25>121.

Blood samples were taken intravenously at the same time that the saliva was collected. They were centrifuged at 4000 x rpm, and the serum samples were stored at -80 degrees until use. Serum samples were then analyzed with the Ocn Human ELISA Kit KAQ1381 (Thermo, USA). The relationship between the biomarkers and the stress score was statistically evaluated with paired t-tests and Pearson and Spearman correlation tests. These tests were performed using the differences between variables.

Statistical Analyses: The normal distribution suitability of continuous variables in the study was assessed graphically and through the Shapiro-Wilks test. It was determined that continuous variables adhered to a normal distribution except for the 1st Week Stress Score. Descriptive statistics were presented as Mean±SD (standard deviation) and Median (Minimum-Maximum) values.

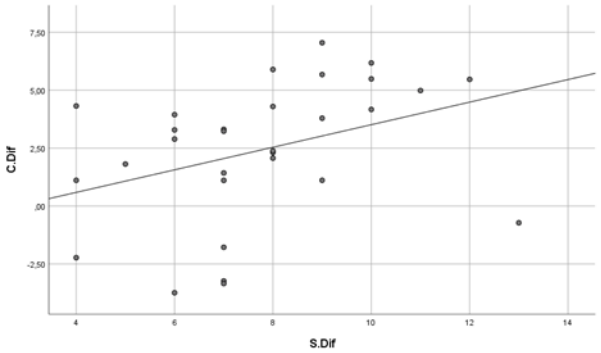

Fig. 1. Correlation Graph between Stress Score Difference (S.Dif) and Cortisol Difference (C. Dif.)

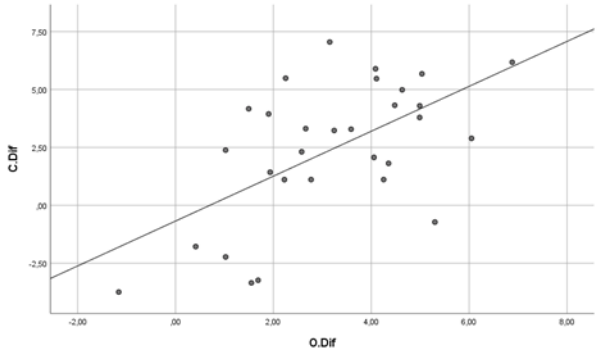

Fig. 2. Correlation Graph between Osteocalcin Difference (O. Dif.) and Cortisol Difference (C. Dif.)

For the comparison of 1st Week Stress Score and 2nd Week Stress Score values, the Wilcoxon Signed Rank test was employed. Paired Samples t-tests were used to compare 1st Week Stress Cortisol, 2nd Week Cortisol, 1st Week Stress Ocn, and 2nd Week Ocn values.

Pearson correlation coefficients were provided for the correlation analysis among the difference values of stress, cortisol, and Ocn.

IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) and MS-Excel 2007 programs were utilized for statistical analyses and calculations. A significance level of $p < 0.05$ was considered.

Results

Comparison of 1st Week and 2nd Week Stress Scores revealed a statistically significant difference ($z=4.795$, $p<0.001$). The mean score for stress in the second week was higher compared to the first week (Table 2).

The mean value of Cortisol in the first week was 27.58±3.97, and in the second week, it was 29.99±2.44. A statistically significant difference was found between 1st and 2nd-week Cortisol values ($t=4.412$, $p<0.001$) (Table 3).

The mean value of Ocn in the first week was 21.04±0.98; in the second week, it was 24.22±1.44. A statistically significant difference was found between 1st and 2nd-week Ocn values ($t=9.656$, $p<0.001$) (Table 4).

Examining the discrepancies between the first and second weeks for study participants, the mean difference in stress scores was 7.73±2.23, in cortisol levels, was 2.41±2.99, and in Ocn levels was 3.18±1.81 (Table 5).

A weak positive statistically significant relationship was found between Stress score difference and Cortisol difference ($r=0.363$, $p=0.049$). An intermediate-level positive statistically significant relationship was found between Ocn difference and Cortisol difference ($r=0.586$, $p=0.001$) (Table 6).

Evaluating the results of our study, Figure 1 illustrates the correlation between stress score difference and cortisol difference.

Taking into account the findings of our study, Figure 2 presents the correlation between Ocn difference and cortisol difference.

Discussion

Osteoporosis, a multifactorial disease, could cause stress for the patient, particularly in light of physiological conditions that mirror the manifestations of the disease. The common belief among the people is that with osteoporosis, their bones will soon be broken, which will cause their death. The selection of the control group should have been among the people with osteoporosis who have yet to be informed about their disease, which is completely unethical and impossible. Therefore, to reveal the course of stress on the disease, we aimed to evaluate the blood cortisol levels and Ocn values of the diagnosed patients in the progressing period. For this reason the most appropriate way to examine the stress Ocn levels was measured on the day of diagnosis and the following days. It is our theory that an increase in stress increases bone turnover metabolism. Therefore, we investigated the cortisol levels in saliva, which is more reliable for stress biomarkers, and Ocn levels in the blood. The study measured how hormonal processes are altered by the onset of anxiety. In other words, the effect of hormonal levels and stress

Table 1. Inclusion and Exclusion Criteria for the Study on Cortisol Levels, Osteocalcin, and Osteoporosis in Postmenopausal Women

Criteria Type	Criteria Detail
Inclusion Criteria	Postmenopausal women diagnosed with osteoporosis, as confirmed by bone mineral density (BMD) measured by dual-energy X-ray absorptiometry (DEXA) scanning, with a T-score less than -2.5
	Ages 55 years and above
	Non-smokers
	Willingness to participate and provide informed consent
	Ability to provide saliva and blood samples for cortisol and osteocalcin measurement.
	No new medications affecting cortisol/osteocalcin for 3 months prior
Exclusion Criteria	Pre-menopausal or perimenopausal women
	Women without a diagnosis of osteoporosis
	Women with a history of hormone replacement therapy or other medications known to affect bone density or cortisol levels within the last 6 months.
	Individuals with psychiatric conditions such as major depression, given its potential impact on cortisol levels and bone density.
	Patients with any metabolic bone diseases other than osteoporosis, such as hyperparathyroidism or Paget's disease.
	Women with severe systemic diseases that might affect bone metabolism, such as chronic renal failure or malabsorption syndromes.
	Current smokers
	Individuals with known endocrine disorders (e.g., Addison's disease, Cushing's syndrome) that could significantly influence cortisol levels.
	Participants currently undergoing treatment with glucocorticoids or other medications known to affect bone metabolism or cortisol levels, as these can significantly alter the study's outcome measures.
	Osteoporosis-specific treatments within the past 6 months
	Use of corticosteroid medications (including oral, inhaled, topical, or injectable forms) within the past 3 months, due to their potential impact on cortisol levels and bone metabolism.
	Use of bisphosphonates, selective estrogen receptor modulators (SERMs), or other osteoporosis-specific treatments within the past 6 months, as these could affect osteocalcin levels and bone turnover markers.
	Use of anticonvulsants, barbiturates, or other medications known to induce cytochrome P450 enzymes, potentially altering cortisol metabolism
	Use of psychiatric medications such as antidepressants, especially SSRIs, which can influence cortisol levels through stress pathway modulation.
	Subjects with recent fractures (within the past 6 months) since this could affect osteocalcin levels and the interpretation of bone turnover rates

Table 2. Comparison of 1st Week Stress Score and 2nd Week Stress Score Values

1st Week Stress Score	2nd Week Stress Score	Test Statistic*	
Mean±SD Median (Min-Max)	Mean±SD Median (Min-Max)	Z	p
12.90±1.09 13.0 (11-16)	20.63±1.69 21.0 (18-24)	z=4.795	<0.001

Table 3. Comparison of 1st Week Cortisol and 2nd Week Cortisol Values

1st Week Cortisol	2nd Week Cortisol	Test Statistic*	
Mean±SD	Mean±SD	t	p
27.58±3.97	29.99±2.44	t=4.412	<0.001

*t: Paired Samples T-test statistic

Table 4. Comparison of 1st Week Osteocalcin and 2nd Week Osteocalcin Values

1st Week Osteocalcin	2nd Week Osteocalcin	Test Statistic*	
Mean±SD	Mean±SD	t	p
21.04±0.98	24.22±1.44	t=9.656	<0.001

*t: Paired Samples T-test statistic

on the disease was investigated when the person was informed about the disease.

The study assessed how hormonal changes due to anxiety and stress impact osteoporosis. Specifically, the effect of hormonal levels and stress on the disease was investigated when the individual was made aware of their condition. The data were compared to a control group on the first day and during the first week. Participants were primarily from low educational and economic backgrounds. All patients were non-smokers. Stress levels were determined using the NIH stress score system to validate cortisol levels.

In the study by Akkus et al., a significant correlation was discovered between the duration of menopause and low bone density (11). The presence and duration of menopause are associated with osteoporosis.

Ocn, a marker of bone turnover by mature osteoblasts, was negatively correlated with serum cortisone. However, unlike the S. Lee et al. (12) study, Cooper et al. (13) found Ocn levels were higher in people aged 61-73 years with high cortisol levels. Additionally, a 9.4 nmol/L increase in cortisone (1SD) was associated with a 6.7% reduction in Ocn levels (13).

In their study, Govender et al. (14) found that premenopausal patients diagnosed with major depression exhibited lower BMD, higher bone resorption, and elevated cortisol levels compared to patients without a depression diagnosis. The median 24-hour cortisol level in the psychiatric sample (8.334 ng/ml) was higher than that in the control group (6.45 ng/ml). This finding presents a different perspective on the relationship between stress factors and osteoporosis, emphasizing the role of depression as an additional stressor. In their study, Yazici et al. (15) suggested that major depressive disorders are associated with lower BMD without changes in cortisol levels. Osteoporosis may be related to changes in growth hormone, subclinical hypoestrogenism, subclinical thyroid abnormalities, vitamin D receptor genotype, inflammatory mediators, restricted physical activity, and nutritional deficiency. Coelho et al. (16) reported that osteoporosis was associated with the severity of depressive symptoms using the Beck Depression Inventory.

Contrary to this data, Yazici et al. (17) in their study showed that mild and moderate major depression did not cause osteoporosis, and they did not detect a difference between patient and control groups in terms of BMD, plasma cortisol levels, Ocn, and C-telopeptide levels. Yazici et al. (17) emphasized in their study that

Table 5. Stress, Osteocalcin, and Cortisol Differences and Descriptives

	Mean±SD	Median (Min-Max)
Stress Difference	7.73±2.23	7.50 (4—13)
Osteocalcin Difference (ng/mL)	3.18±1.81	3.19 (-1.16—6.88)
Cortisol Difference (nM)	2.41±2.99	3.06 (-3.74—7.05)

Table 6. Correlations of Differences in Cortisol, Osteocalcin, and Stress Levels. Stress Difference Osteocalcin Difference Cortisol Difference

		Stress Difference	Osteocalcin Difference	Cortisol Difference
Stress Difference	r	-	0.346	0.363
	p		0.061	0.049
Osteocalcin Difference	r	0.346	-	0.586
	p	0.061		0.001
Cortisol Difference	r	0.363	0.586	-
	p	0.049	0.001	

r: Pearson Correlation Coefficient

Cortisol is an important parameter in the development of osteoporosis and stated that they did not find hypercortisolism in their patients. This factor might explain the lack of significant differences in BMD between patients with major depression and control subjects in this study. Hypercortisolism might be associated with severe depression, and osteoporosis might be seen in those patients. Therefore, stress factors should be considered as an important effect on osteoporosis.

As a result, it has been observed that stress scoring is not a fair comparison for determining the degree of disease over Ocn, considering the person's socioeconomic level and lifestyle. In their study, Gur et al. (18) examined the relationship between education level and osteoporosis in postmenopausal women. They found decreased BMD with decreasing education levels and suggested a protective effect of increasing education levels on bone mineral density. While the mechanism here is not fully understood, it draws attention to the disadvantage of low education levels in coping with stress compared with our study's results.

However, although the stress score is not correlated, there is a clear correlation between salivary cortisol levels and Ocn, which are used as markers of unlikeability and, unlike other studies, show that the disease is affected by stress. This theory has been confirmed in various studies. In the study of Cortisol and Ocn, which was conducted to support our study and seems to be related to different hormonal diseases (12, 13), we have correlated menopause and stress, which significantly impact the disease and should be considered.

Stress is a factor that affects our lives and can also change the levels of metabolic diseases. Considering that a multifactorial disease such as osteoporosis may also be affected by stress, we have investigated the effect of stress on bone metabolism. As a result, in statistical evaluation, it is reported that the amount of Ocn, a bone resorption marker, increases with the increase in cortisol levels, that is, the stress level, and therefore, bone dissolution occurs. After this study, our main goal is to investigate the circadian rhythm and deoxypyridinoline, pyridoxine, and cortisol levels in detail.

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