The Effect of Oxidative Stress and Antioxidant Capacity on Postmenopausal Bone Mineral Densitometry

Postmenopozal Kemik Mineral Yoğunluğu Üzerine Oksidatif Stres ve Antioksidan Kapasitenin Etkisi

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ABSTRACT

Objectives: There are many mechanisms associated with the onset and development of osteoporosis.The purpose of our study is to investigate the relationship between the fasting blood sugar, lipid profile, bone mineral density (BMD) values, and total antioxidant status (TAS), total oxidant status (TOS) levels, and paraoxonase 1 (PON1), arylesterase (ARE) enzyme activities in postmenopausal women.

Materials and Methods: Our study included 95 patients diagnosed as being postmenopausal. The patients were divided into three study groups, which were the control (T-score>-1; n=33 with normal BMD, osteopenia (-2.5<T-score≤-1; n=31), and osteoporosis (T-score≤-2.5; n=31) groups. The sociodemographic characteristics and the clinical and laboratory findings of the groups were evaluated. Serum fasting blood sugar, cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL) levels were calculated with an autoanalyzer. Serum TAS and TOS levels, PON1 and ARE enzyme activities were calculated with calorimetric tests.

Results: It was determined that the duration of menopause, age, and PON1 enzyme activities were different between the groups (p<0.05), whereas other parameters were not different among groups (p>0.05). Accordingly; the comparison of the control group with the osteopenia and osteoporosis groups revealed that serum PON1 enzyme activity was significantly increased in the control group (p<0.05).

Conclusion: The results of the study suggest that increased age, duration of menopause, and oxidative stress, as well as decreased PON1 enzyme (an antioxidant enzyme) activity, lead to increased osteoporosis, independently of the lipid profile and serum fasting blood sugar.

Keywords: Postmenopausal osteoporosis, total antioxidant status, total oxidant status, paraoxonase 1, arylesterase

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ÖZET

Giriş: Osteoporozun başlangıcı ve gelişimi ile ilişkili birçok mekanizma bulunmaktadır. Çalışmamızın amacı, postmenopozal kadınlarda açlık kan şekeri, lipid profili, kemik mineral yoğunluğu değerleri ile total antioksidan kapasite (TAS), total oksidan kapasite (TOS) düzeyleri, paraoksonaz 1 (PON1) ve arilesteraz (ARE) aktiviteleri arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntemler: Çalışmamıza postmenopozal dönemdeki 95 hasta dahil edildi. Hastalar kontrol (T-skor>-1; normal kemik mineral yoğunluğu (KMY) ile n=33), osteopeni (-2.5<T-skor≤-1; n=31) ve osteoporoz ((T-score≤-2.5; n=31)) olmak üzere üç çalışma grubuna ayrıldı. Grupların sosyodemografik özellikleri ile klinik ve laboratuvar bulguları değerlendirildi. Serum açlık kan şekeri, kolesterol, trigliserit, yüksek yoğunluklu lipoprotein (HDL), düşük yoğunluklu lipoprotein (LDL) düzeyleri otoanalizör ile ölçüldü. Serum TAS, TOS seviyeleri ve PON1, ARE enzim aktiviteleri kalorimetrik yöntem ile ölçüldü.

Bulgular: Menopoz süresi, yaş ve PON1 aktivitelerinin gruplar arasında farklı olduğu saptandı (p<0.05). Diğer parametrelerin ise gruplar arasında farklı olmadığı saptandı (p>0.05). Buna göre; osteopeni ve osteoporoz grupları ile karşılaştırıldığında, kontrol grubunda serum PON1 aktivitesinin anlamlı olarak arttığı saptandı (p<0.05).

Sonuç: Çalışmanın sonuçları, artan yaş, menopoz süresi ve oksidatif stresin yanı sıra azalmış PON1 (bir antioksidan enzim) aktivitesinin, lipid profili ve serum açlık kan şekerinden bağımsız olarak artmış osteoporoza yol açtığını göstermektedir.

Anahtar Sözcükler: Postmenopozal osteoporoz, total antioksidan kapasite, total oksidan kapasite, paraoksonaz 1, arilesteraz

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INTRODUCTION

Osteoporosis, which is caused by the deterioration of the bone microstructure, leads to an increase in the risk of low bone mass and bone fracture (1) and is significantly responsible for postmenopausal morbidity and mortality. In developed countries, almost 30% of all post-menopausal women have osteoporosis, and at least 40% of these women are expected to have one or more osteoporotic fractures following diagnosis (2). Many studies have shown that oxidative stress plays a central role in the onset and development of postmenopausal osteoporosis (PO), and decreased bone mineral density is observed due to the increase in oxidative stress (1-3). The mechanisms related to the deterioration of bone formation due to oxidative stress are complicated. Oxidative stress leads to DNA damage, lipid peroxidation (LP), protein carbonylation, and osteoblast apoptosis (4).

Under normal conditions, the levels of reactive oxygen species (ROS) are kept at low by antioxidant systems (enzymes and chemical compounds) found in biological systems. In case of an imbalance between free radicals and antioxidants, which is called oxidative stress, sex hormones (oestrogens and androgens) can affect skeletal growth and structure (5). Due to their short half-life, it is difficult to measure ROS levels. However, determining the activity of antioxidant enzyme status to be informative about antioxidant and vitamin levels (6).

Paraoxonase (PON) is a glycoprotein enzyme that is a calcium-dependent ester hydrolase enzyme and also has arylesterase (ARE) activity. The enzyme, which was first discovered by Abraham Mazur in 1946, took this name because it can hydrolyze the toxic metabolite paraoxon (organophosphate substrate) of the highly toxic organophosphate pesticide parathion (7). The PON gene family is located in the long arm of chromosome 7 (q21.22) in humans and has three members, PON1, PON2, and PON3. PON1 and PON3 are expressed in the liver and can be detected in serum due to high-density lipoprotein (HDL). PON2 cannot be detected in serum but is found in various tissues such as the kidney, testis, liver, and brain (8, 9). It has been shown that PON1 protects low-density lipoprotein (LDL) and HDL against oxidation and reduces oxidative stress (10). It has been reported that PON1, an antioxidant, can prevent oxidative stress effects on bone (11, 12).

ARE enzyme can detoxify organophosphates such as PON1 but does not show genetic polymorphism. Although the native substrate of both enzymes is different, the enzyme PON1 is capable of hydrolyzing the substrate of the ARE, phenyl acetate, thereby displaying both ARE and PON activity. Furthermore, PON1, an antioxidant enzyme bound to plasma HDL, has been shown to protect LDL and HDL against oxidation by free radicals and reduce oxidative stress (13, 14). Though HDL was proposed a multifunctional role of HDL in numerous other biological processes including, inflammation, oxidative stress, nitric oxide production, and regulation of plasma glucose homeostasis (15). Recently, identified a novel role of HDL in the pathogenesis of degenerative and metabolic bone disease using experimental mouse models, suggesting that low and dysfunctional HDL may contribute to an increased prevalence of these diseases by influencing molecular processes associated with bone synthesis and catabolism (16).

The purpose of our study was to investigate the relationship between the fasting blood glucose, lipid profile, bone mineral density (BMD) values and the total antioxidant status (TAS), total oxidant status (TOS) levels and PON1, ARE enzymes activities in postmenopausal women who underwent bone mineral densitometry measurements.

MATERIALS and METHODS

The participants were divided into three groups, according to World Health Organization criteria for the diagnosis of osteoporosis: group 1 was composed of healthy postmenopausal women, who were randomly selected by a computer program (n=33; T-score>-1), group 2 comprised women diagnosed with osteopenia (n=31; -2.5<T-score≤-1), and group 3 consisted of women who had been diagnosed with postmenopausal osteoporosis (n=31; T-score≤-2.5). To determine the number of patients in the groups; power analysis was performed through the G *Power package program. The number of patients for each group was 35 and the effective width was calculated as 0.475. The power of the study calculated over this effect width was found to be 97.75%. The sample numbers were found to be sufficient.

Two patients with normal BMD, four patients in the osteopenia group, and four patients in the osteoporosis group were excluded from the study because their information was not available. The present study was carried out according to the Yıldırım Beyazıt University Yenimahalle Education and Research Hospital Clinic Research Committee and Human Ethical Committee Regulations (26.02.2019/13).

Written consent was obtained from all the women who participated in this study. Exclusion criteria were secondary osteoporosis, receiving drugs or hormones influencing bone metabolism within the last 6 months, smokers, alcohol users, thyroid disease, diabetes mellitus, hypertension, chronic renal failure, chronic inflammatory disease, and cardiovascular disease. They were not taking any supplements such as vitamins and/or antioxidants and/or aspirin. When the participants were questioned, it was found that they did not exercise regularly.

Biochemical parameters study

A biochemist performed all tests in a standardized biochemistry laboratory that was not affiliated with our institution. In addition, care was taken to utilize standard terminology and current techniques. Venous blood samples were collected after at least 8 hours of fasting, and the samples were maintained at room temperature for 30 minutes to allow clotting, and then centrifuged at 2.400 rpm for 10 minutes at 4°C. The sera were assessed for hemolysis, lipemia, and bilirubinemia, and eligible samples were tested on the same day. Serum samples were stored at -80°C until the analyses are carried out.

Serum fasting blood sugar, cholesterol, triglyceride, HDL, and LDL levels were calculated with an autoanalyzer. Serum TAS, TOS levels, PON1, and ARE enzyme activities were calculated with calorimetric tests.

Biochemical analyses

Serum TAS levels

Serum TAS determinations were done by Rel Assay Diagnostics commercial kit. The principle of the assay is to incubate 2,2′-azinobis (3-ethylbenzothiazoline 6-sulphonate) (ABTS), with the ferryl myoglobin radical formed by the activation of a peroxidase (metmyoglobin) with H_2O_2 to produce the radicalcation ABTS+. This has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree proportional to their concentration and the results are expressed as mmol Trolox Equivalent/L (15).

Serum TOS levels

Serum TOS determinations were done by Rel Assay Diagnostics commercial kit. Oxidants present in the sample oxidize the ferrous ion–chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equivalent/L) (16).

Serum PON1 activities

The serum activity of PON1 was measured by Rel Assay Diagnostics commercial kit. PON1, present in the sample, hydrolyses phenylacetate to its products which are phenol and acetic acid. The produced phenol is colorimetrically measured via oxidative coupling with 4-amino antipyrine and potassium ferricyanide. Nonenzymatic hydrolysis of phenyl acetate was subtracted from the total rate of hydrolysis. The molar absorptivity of colored complex is 4000 M^{-1} cm^{-1} and one unit of ARE activity is equal to 1 mmol of phenyl acetate hydrolyzed per liter per minute at 37°C, and the results are expressed as U/mL (17).

Serum ARE activities

The serum activity of ARE was measured by Rel Assay Diagnostics commercial kit. To measure ARE activity, a newly non-salt-stimulated method is used, with phenyl acetate used as substrate. One unit of ARE activity is determined as μmol phenol formed in 1 min and the results are expressed as kU/L (18).

Determination of serum fasting blood sugar, cholesterol, triglyceride, HDL, and LDL levels

Determination of serum fasting blood sugar, cholesterol, triglyceride, HDL, and LDL levels was performed using ROCHOP800 autoanalyzer.

Body mass index measurement

Body weights were measured while the participants were shoeless and wearing only light clothing. To measure height, the participants were placed in a standing position. Body mass index (BMI) was calculated by dividing each participant's body weight in kilograms by her height in meters squared.

Bone mineral densitometry measurements

BMD (g/cm^2) in the anterior-posterior lumbar spine (L1-L4) was determined by utilizing adual-energy X-ray absorptiometry system (Prodigy Advancewith version 11.4 software; GE Lunar, Madison, WI).

Statistical analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS, version 22.0, Chicago, IL, USA). For the three groups that were evaluated according to the BMD values, ANOVA multiple comparison tests were used to determine whether there was a difference between their parameters. The significance levels obtained from both descriptive statistical values and multiple comparison results and the "*p*" values within 95% confidence interval are presented in Table1. After the parameters that showed differences were identified based on a grouping of BMD values, Scheffe's test was performed to determine between which groups these parameters were different. The results are presented below. A value of $p<0.05$ was considered statistically significant.

RESULTS

Based on the BMD values in the multiple comparison test, it was found that duration of menopause ($p=0.002$), age ($p=0.004$), and PON1 activities ($p=0.02$) were different between the control, osteopenia, and osteoporosis groups, whereas other parameters were not different compared to the BMD groups (Table 1). Accordingly, the mean age and duration of menopause in the control group were found to be lower than those in the osteopenia and osteoporosis groups ($p<0.05$).

ANOVA analysis showed that parameters with $p<0.05$ were different between the groups. The parameters from the table above that were different included PON1, duration of menopause, and age. Scheffe's test was used to determine among between groups these parameters were different, and the results are shown in Table 2.

According to Table 2, the comparison of the control group with the osteopenia and osteoporosis groups revealed that serum PON1 activity was significantly higher in the control group ($p<0.05$).

The comparison of the control group with the osteopenia and osteoporosis groups revealed that age and duration of menopause were significantly lower in the control group ($p<0.05$). The comparison of the osteopenia and osteoporosis groups revealed that there was no significant difference in terms of age and duration of menopause ($p>0.05$).

The serum TOS levels were increased in the osteopenia and osteoporosis groups compared when compared to the control group ($p>0.05$). However, this was not a significant difference (Table 1).There were no statistically significant differences for serum fasting blood sugar, cholesterol, triglyceride, HDL, LDL, and TAS levels and ARE activities between the groups ($p>0.05$) (Table 1). The serum PON1 activity is different between the groups, and it was higher in the control group ($p<0.05$) (Figure 1 and Table 1).

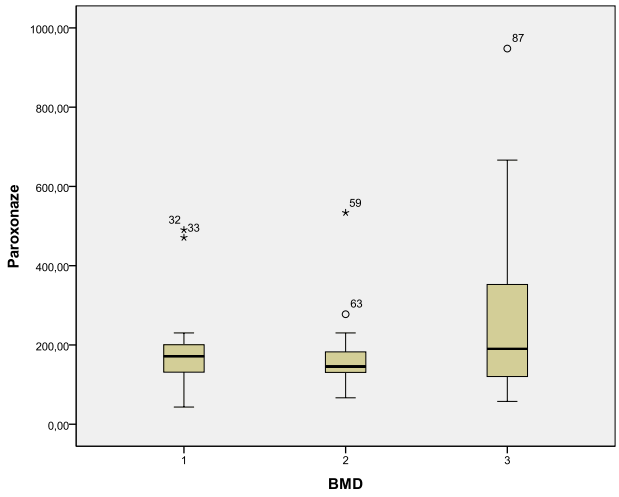

Figure 1. Boxplot graphs of PON1 values based on BMD groups.
1: Osteoporosis; 2: Osteopenia; 3: Control

Table 1. Descriptive statistics of the biochemical parameters and enzyme activities, and descriptive characteristics of the data that were divided into three groups according to the BMD values (mean±SD).

Parameters/BMD	Control (n=33) (Tskor>-1)	Osteopenia (n=31) (-2.5> T skor≥-1)	Osteoporosis (n=31) (T skor≥ -2.5)	*P* value
Age (years)	51.61±5.73 (44-72)	57.61±6.67 (45-72)	56.43±9.75 (42-81)	**0.004**
BMI (kg/m²)	20.22±0.99 (18.8-22.3)	20.36±0.99 (19.2-22.8)	20.43±1.26 (18.8-21.9)	0.759
Menopause time (years)	4.39±3.96 (1-20)	9.23±6.13 (2-25)	9.82±9.04 (1-35)	**0.002**
Fasting blood sugar (mg/dL)	106.21±4.321 (96-115)	106.84±5.490 (98-116)	108.50±10.595 (84-142)	0.446
Cholesterol (mg/dL)	201.36±30.907 (150-300)	195.61±22.263 (148-250)	200.50±31.161 (148-300)	0.690
Triglycerides (mg/dL)	180.52±33.768 (105-230)	181.19±32.276 (105-230)	179.93±37.434 (105-230)	0.99
HDL (mg/dL)	47.03±14.352 (24-85)	46.29±14.476 (24-85)	46.46±14.600 (24-85)	0.97
LDL (mg/dL)	124.24±34.128 (55-190)	125.00±35.054 (55-190)	123.20±34.102 (55-190)	0.834
TOS (µmol/L)	14.73±13.77 (1.32-59.81)	22.81±2.02 (0.19-59.82)	21.58±17.97 (1.51-59.25)	0.140
TAS (mmol/L)	2.34±0.52 (1.29-3.45)	2.17±0.64 (1.10-3.32)	2.29±0.54 (1.12-3.53)	0.47
PON1 (U/mL)	161.72±90.63 (102.86-460.29)	154.55±82.12 (98.99-375.68)	157.93±75.94 (124.76-502.63)	**0.02**
ARE (kU/L)	68.66±19.24 (23.69-115.81)	67.17±12.58 (44.74-96.07)	70.49±36.06 (9.21-180.94)	0.870

Table 2. Evaluation of statistically significant differences parameters between groups.

	BMD		Mean difference (I-J)	SE	*P* value
Different parameters (PON1)	Control	Osteopenia	13.061	34.814	**0.038**
		Osteoporosis	-80.314	35.763	**0.041**
	Osteopenia	Osteoporosis	-93.376*	36.289	0.086
Menopause time (years)	Control	Osteopenia	-4.832*	1.642	**0.016**
		Osteoporosis	-5.427*	1.687	**0.007**
	Osteopenia	Osteoporosis	-0.596	1.712	0.941
Age (years)	Control	Osteopenia	-6.007*	1.867	**0.007**
		Osteoporosis	-4.823*	1.918	**0.047**
	Osteopenia	Osteoporosis	1.184	1.946	0.831

DISCUSSION

Osteoporosis is characterized by a reduction in BMD and a deterioration in the micro-architecture of bone, with a consequent susceptibility to fracture and enhancement in bone fragility (19, 20). It has been shown that osteoporosis was related to a nearly four times higher fracture rate, while osteopenia was associated with a 1.8-fold higher rate compared to that of normal BMD in women (21, 22). It is widely accepted that risk factors for the development of osteoporosis and osteopenia have been recognized by several environmental factors, such as diet and physical exercise, influence BMD, increased oxidative stress, and diminished antioxidant capacity (23).

Oxidative stress is caused by the inadequate functioning of the endogenous antioxidant defense system against ROS. In addition, excessive ROS can cause oxidative damage in lipids, proteins, and DNA by forming oxidized biomolecule products that are relatively stable (6). Oestrogen depletion in menopause may lead to the development of osteoporosis (24). There is some evidence suggesting a correlation between oxidative stress and the loss of bone density (25). Some studies have shown that oestrogen deprivation in menopause increases oxidative stress and loss of bone density (26). In our study, the mean age in the osteopenia and the osteoporosis groups were higher than the mean age in the control group. In the present study, it was determined that the serum TOS levels were increased in the osteopenia and osteoporosis groups compared when compared to the control group. However, this was not a significant difference. There were not statistically significant differences in serum TAS levels among groups.

It has recently been suggested that ROS may play a role in postmenopausal bone loss by creating a more oxidized bone microenvironment. Oxidative stress has been shown *in vitro* to impair antioxidant enzyme production (27). The mechanisms of action of ROS and the cellular targets that regulate bone mass are not fully understood. It has been shown that osteoclasts are both produced and activated through the action of ROS (28, 29). Oestrogen causes an increase in the level of antioxidants in many cells, including osteoclasts (30). In the past two years, significant progress has been made in our understanding of the mechanisms of bone destruction associated with oestrogen deficiency. Our insight into postmenopausal osteoporosis will undoubtedly continue to evolve in the next decade, and as new information and knowledge are gained on this multi-faceted disease, new radical treatments will develop. Under physiological conditions, the production of ROS by osteoclasts helps accelerate the destruction of calcified tissue, thus assisting in bone remodelling (4).

PON and ARE enzymes are enzymes encoded by the same gene and have similar active sites. Although the natural substrates of both enzymes are different, the PON enzyme can hydrolyze phenyl acetate, a substrate of ARE (31, 32). Factors that increase oxidative stress reduce the activity of certain antioxidant enzymes such as PON (33). These results comply with the results of the present study. The most studied member of the family, serum PON1 is a HDL-associated esterase/lactonase. PONs enzymes are enzymes with antioxidant effects. That increased serum PON1 activity and/or concentration may be useful for the treatment of diseases that increase the oxidative state. Although the main substrate of PON1 in the human body is not known, its direct or indirect antioxidant effects appear to be its primary function. PON1 decreases due to depletion during inflammation and many infections (33).

Studies on different diseases have shown that there may be increases or decreases in PON and ARE enzyme activities. In a study investigating the femoral and lumbar bone mineral density in 97 healthy postmenopausal women, no correlation were found between PON and ARE activities, and BMD (34). In a controlled study on women with perimenopausal symptoms, no correlation was found between perimenopausal symptoms and biochemical parameters of oxidative stress markers (35).

It was demonstrated that serum ARE, PON, lipid hydroperoxide (LPO) levels weren't significantly different between osteoporotic and nonosteoporotic postmenopausal women (36). In our study, there was no significant difference between the groups in terms of ARE enzyme activities relative to BMD.

In a study investigating PON1 polymorphisms in patients with osteoporotic, osteopenic, and control BMD, it was shown that PON1 activity is significantly associated with decreased BMD (37). It has been observed in some animal models that PON1 can prevent the harmful effects of oxidative stress in serum macrophages (38, 39) besides, it reduces cholesterol biosynthesis in macrophages (40). Increased oxidative stress and decreased antioxidant capacity of the PON1 activity have been recently reported in postmenopausal women (41).

It was demonstrated that plasma PON1 activity of osteoporotic and osteopenic patients was significantly lower than in the control group (37). In our study, there was a significant decrease in PON activity in the osteopenia and osteoporosis groups compared to the control group. The antioxidant capacity of PON1 may prevent the effects of oxidative stress on bone development. Therefore, PON1 may be a relationship in bone metabolism and as a result of osteoporosis.

High levels of total cholesterol, triglyceride, LDL, and HDL are the most frequent forms of dyslipidemia, reported in postmenopausal women (42). The lower HDL level is a major risk factor for cardiovascular events and is the most important lipoprotein predictor of coronary heart disease in women. PON1 is found in serum associated with HDL. PON1 protects HDL against LP (34).

Some studies have also been made about the relationship between antioxidants and osteoporosis but the results were conflicting (43, 44). It has been reported that antioxidants were markedly decreased in elderly postmenopausal osteoporotic women. Higher dietary antioxidant intake has been suggested to have a protective role on bone health (45).

Recent studies have shown that the incidence of osteoporosis and bone fractures is increased in obese individuals compared to individuals within normal weight (46). In this line, a more recent study on healthy premenopausal women showed that central adiposity is strongly associated with reduced bone quality, stiffness, and bone formation rate. Recently, a negative association between BMD and hyperlipidemia was demonstrated; supporting the hypothesis that hyperlipidemia could potentially aggravate bone loss (47).

Recent data propose that there is an association between serum HDL levels and bone mass (48). However, the results that have been generated from epidemiological studies on humans are contradicting. Indeed, even though a considerable volume of these studies have shown that increased HDL is associated with better bone quality and reduced risk of osteoporosis, others support a negative relation between serum HDL levels and bone mass (49). It is believed that factors such as genetic background, age, dietary habits, and metabolic status are responsible for this in consistency.

Oxidized LDL and other bioactive oxidized lipids, which promote atherogenesis, could inhibit osteoblastic differentiation of bone and marrow-derived preosteoblasts in vitro, suggesting that an atherogenic diet could contribute a common risk factor for both diseases (50). In addition, some, but not all investigations have suggested that statin drugs used to treathy perlipoproteinemia may also have a beneWcialeVect on bone. However, Tanko et al. found an indirect association between serum cholesterol and BMD. They suggested that the association was governed by estrogen deficiency and the direct pathogenic factor would be tissue rather than serum cholesterol (44).

Another study by Samelson et al. (45) reported that there were no correlations between serum cholesterol levels and BMD.

It was demonstrated that it wasn't significant differences in terms of age, BMI, ARE, PON1, cholesterol, triglyceride, HDL, LDL, and very-low-density lipoprotein (VLDL) between postmenopausal osteoporotic and non osteoporotic groups. It also found no relationship between serum cholesterol levels and FN BMD and LS BMD in the study (34).

In the present study, it was determined that the serum fasting blood sugar, cholesterol, triglyceride, HDL, and LDL levels were not different between the groups.

It has been determined that low PON1 is associated with an oxidative stress-associated process such as dyslipidemia and hypercholesterolemia, diabetes mellitus, advancing age (51). In addition, reduced PON1 activity has been suggested to be related to the pathogenesis of Alzheimer's disease and vascular damage (50).

All these reports and our results suggest that decreased serum PON1 activity due to menopause may be a contributing factor to unfavorable stages of certain metabolic pathologies, and an increase in bone mineral loss.

CONCLUSION

The results of the study suggest that increased age, duration of menopause, and oxidative stress as well as decreased PON1 (an antioxidant enzyme) activity lead to increased osteoporosis, independently of the lipid profile and serum fasting blood sugar.

The differences we found; further studies are needed to determine whether dietary, parathyroid hormone levels, thyroid hormone levels, vitamin D levels, dietary differences, and whether clinically oxidative stress has an impact on BMD.

Conflict of interest

No conflict of interest was declared by the authors.

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