SERUM TOTAL OXIDATIVE AND ANTIOXIDATIVE STATUS IN PATIENTS WITH POSTMENOPAUSAL OSTEOPOROSIS

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ABSTRACT

Objective: *The aim of this study was to investigate and compare the relationship between serum oxidative/anti-oxidative capacity, oxidative stress and bone mineral density in patients with postmenopausal osteoporosis and in healthy controls.*

Material and Method: *119 female cases (60 patients and 59 controls) were included in this study. Oxidative status were measured by Erel's method.*

Results: *There was no statistically significant difference between two groups for total oxidative capacity (p>0.05); however, total anti-oxidative capacity was significantly lower in the patient group than that in controls (p<0.001).*

Conclusion: *Total antioxidant capacity is negatively affected in patients with postmenopausal osteoporosis. Antioxidant treatment with diet can contribute to support osteoporosis.*

Key Words: *Osteoporosis, antioxidants, oxidants, oxidative stress*

POSTMENOPOZAL OSTEOPOROZLU HASTALARDA SERUM TOTAL OKSİDATİF VE ANTİOKSIDAN DURUMU

ÖZET

Amaç: Bu çalışmanın amacı, postmenopozal osteoporozlu hastalarda ve sağlıklı kontrol grubunda serum oksidatif/anti-oksidatif kapasite, oksidatif stres ve kemik mineral yoğunluğu arasındaki ilişkiyi araştırmak ve karşılaştırmaktır.

Materyal ve Metot: Bu çalışmaya 119 kadın olgu (60 hasta ve 59 kontrol) alındı. Oksidatif durumları Erel metodu ile değerlendirildi.

Bulgular: Total oksidatif kapasitede iki grup arasında istatistiki olarak anlamlı bir fark yoktu (p>0,05); ancak total anti-oksidatif kapasitesi hasta grubunda kontrol grubuna göre anlamlı olarak daha düşüktü (p<0,001).

Sonuç: Postmenopozal osteoporozlu hastalarda, total antioksidan kapasite olumsuz olarak etkilenir. Antioksidan zengin bir diyet desteği osteoporoz tedavisine katkı sağlayabilir.

Anahtar Kelimeler: Osteoporoz, antioksidan, oksidan, oksidatif stres

INTRODUCTION

Osteoporosis (OP) is a skeletal system disease characterized by increase in bone fragility and fracture risk as a result of decreased bone mass and deterioration in bone microarchitecture.[1] Bone is in a state of continuous turnover via two ongoing processes called modeling and remodeling.[2] OP occurs as a result of decrease in new bone formation or increase in bone resorption.[3]

The role of oxidative stress in the pathogenesis of postmenopausal osteoporosis (PMO) has been emphasized in recent studies. Free oxygen radicals are continuously produced in the organism during normal physiological events; however, these are neutralized by internal and external antioxidants. Free radicals prevent or delay the oxidation of substances found in living cells such as proteins, lipids, carbohydrates and deoxyribonucleic acid (DNA) are called antioxidants and this process is called antioxidant defense.[4]

The state when the equilibrium between oxidants and antioxidants, which are normally found in certain proportions in the serum, change in the favor of oxidants is known as oxidative stress.[4] Oxidative stress has been suggested to play role in the pathogenesis of various disorders such as rheumatoid arthritis (RA), fibromyalgia, Alzheimer's disease, diabetes mellitus, atherosclerosis and Behcet's disease.[5] An increase in the amount of free oxygen radicals which cannot be neutralized by antioxidants has been demonstrated also in osteoporosis. Thus, significant role of oxidative stress has been considered in the pathophysiology of osteoporosis.[3,5]

The aim of this study was to investigate and compare the relationship between serum oxidative/anti-oxidative capacity, oxidative stress and bone mineral density in patients with postmenopausal osteoporosis and healthy controls.

MATERIAL and METHOD

Patient selection

60 patients with PMO (57.4±8.1) were enrolled in this study. Control group was consisted of 59 healthy individuals (56.3±7.2). The controls were recruited from the family of those in the patient group. Routine hematological and biochemical parameters were determined in controls.

Exclusion criteria

1. Smoker or alcohol consumer
2. Used any drug or had any disease or condition known to affect bone
3. Had taken corticosteroid medications during the previous 6 months
4. A history of chronic renal, cardiac, hepatic, gas trointestinal disease or traumatic lumbar com pression fracture
5. Evidence of collapsed or focal vertebral sclerosis
6. Menopause before the age of 40
7. Amenorrhea greater than 6 months
8. Bone diseases, rheumatoid arthritis and malig nancy, treating with fluoride, recent treatment with specific therapy for osteoporosis

All patients were informed of the study. The study protocol was approved by local ethic committee. BMD (bone mineral density) of the lumbar and femoral neck region were measured by using Hologic QDR 4500 Elite device in the Department of Nuclear Medicine in Gaziantep University School of Medicine. All spinal scans were reviewed for evidence of vertebrae with collapse or focal sclerosis by an experienced radiologist. Body mass index (BMI; weight/height^2) was obtained through height and weight measurements by using a wall-mounted ruler and a digital scale.

Blood sampling

After an overnight fasting, venous blood was withdrawn into heparinized tubes and citrated tubes. Remaining blood was centrifuged at 3500 rpm for 10 minutes to separate plasma. The plasma samples were stored at -80°C until analysis of total antioxidant status (TAS), total oxidant status (TOS) were measured from citrated blood.

Total antioxidant capacity measurement

Total antioxidant capacities of the samples were measured by automatic method developed by Erel.[6] Durable and stable ABTS radical caution has been produced in this technique. The characteristic blue-green color of this radical disappears after being reduced by antioxidants. Decolorizing effect of the antioxidants in the sample was regarded as its total antioxidant capacity. Instead of the traditionally used standard vitamin E, its water-soluble analog Trolox has been used. The results have been presented as µmoll trolox equivalent/g.

Total oxidative status (TOC), total antioxidant capacity (TAC) and oxidative stress index (OSI) were measured and calculated by Erel method in the Biochemistry Laboratory.

Total oxidant capacity measurement

Plasma TOS levels were determined using a novel automated measurement method, developed by →

Erel.[7] In this method, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange 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 micromoles hydrogen peroxide equivalent per liter (µmoll H_2O_2 equivalent/l).

Oxidative stress index (OSI)

The percent ratio of the TOS to the TAS gave the oxidative stress index (OSI), an indicator of the degree of oxidative stress. To perform the calculation, the result unit of TAS, µmoll Trolox equivalent/l, was converted to µmoll equivalent/l and the OSI value was calculated as below formula; OSI = [(TOS, µmoll/l) / (TAS, µmoll Trolox equivalent/l) x 100].

Statistical analysis

Statistical evaluation of the results has been performed by statistical package program (SPSS for Windows version 16.0, Chicago, USA). Patient and control groups have been compared by using Student's t-test. Pearson correlation analysis has been used to evaluate the correlation between different variables. The results have been presented as mean ±SD, p value of less than 0.05 was considered as statistically significant.

RESULTS

There were no significant difference between the patient and control groups in terms of mean age, body mass index, age at menarche and menopause, number of pregnancies and lactation duration (p>0.05, Table 1).

There was a statistically significant difference between bone mineral density values of the patient and control groups (p<0.05, Table 2).

There was no significant difference between the patient and control groups in terms of TOC values (p>0.05). TAC was significantly lower and OSI was significantly higher in patients than controls (p<0.05, Table 3).

DISCUSSION

PMO is the most frequent form of OP and occurs in women between 51 and 75 years of age following the cessation of ovarian functions. However, bone loss initiates years before menopause and is accelerated in the perimenopausal period. Osteoclastic bone resorption is a complex process that involves the release of mineral from the bone and then degradation of the proteinaceous bone matrix.[8,9]

Table 1. Demographic characteristics of the patient and control groups

	Patient Group (n=60)	Control Group (n=59)	p
Mean age (years)	57.4± 8.1	56.3 ± 7.2	> 0.05
Body mass index (kg/m2)	28.6 ± 5.3	27.5 ± 4.2	> 0.05
Mean age at menarche (years)	13.7 ± 0.8	13.4 ± 0.7	> 0.05
Mean age at menopause (years)	45.3± 7.3	44.9 ± 7.8	> 0.05
Mean lactation duration (years)	9.3 ± 6.1	8.4 ± 5.3	> 0.05

Table 2. Bone mineral density in patients and controls

BMD	Patient Group (Mean ± SD)	Control Group (Mean ± SD)	p
Lumbar total (L1-4) (g/cm2)	0.6 ± 0.1	1.0 ± 0.5	< 0.01
Lumbar T score	-2.8 ± 0.8	-0.6 ± 1.0	< 0.01
Femur total (g/cm2)	0.8 ± 0.1	1.0 ± 0.1	< 0.01
Femur T score	-1.8 ± 1.1	0.5 ± 1.2	< 0.01

BMD: Bone mineral density

Table 3. Total oxidative capacity, antioxidant capacity, oxidative stress index results in patient and control groups

Parameter	Patients (Mean ± SD)	Controls (Mean ± SD)	t value	p value
TOC (µmol, H_2O_2 equivalent/l)	11.7±5.3	11.7±3.4	0.26	0.9
TAC (mmol, Trolox equivalent/l)	1.0±0.5	1.6±0.2	-8.850	<0.001*
OSI	14.2±8.1	7.0±2.3	6.257	<0.001*

TOC: Total oxidant capacity, **TAC**: Total antioxidant capacity, **OSI**: Oxidative stress index, *: Statistical significant

The relation between oxidative stress and BMD was investigated in a various studies. However, it is not clear the role of oxidative stress on etiopathogenesis of the osteoporosis. Wolf et al. investigated the associations between dietary intake, total intake or serum concentrations of antioxidants and BMD in postmenopausal women.[10] They reported that there was no relation between serum antioxidant level and BMD in postmenopausal women. In the present study, we found lower level of total antioxidative status, and higher level of oxidative stress index, in patients with osteoporosis than in healthy controls. Studies demonstrated that generation of oxygen derived free radicals were associated with osteoclastic bone resorption stimulated by parathyroid hormone (PTH), interleukin (IL)-1beta, tumor necrosis factor (TNF) alpha.[11,12]

Karatas et al. have investigated RA patients and have →

found a decrease in antioxidant status and an increase in oxidative stress,[13] similarly, significantly increased plasma oxidants and decreased antioxidants in osteoporotic patients when compared to controls.[14,15] Altindag et al. have reported in knee osteoarthritis patients, there was an increase in oxidant parameters and a decrease in antioxidant parameters.[16] Özgöçmen et al. have found that oxidative stress parameters were increased and the level antioxidant enzymes were decreased in patients with osteoporosis.[17] Özgöçmen and colleagues have been reported that oxidative stress plays an important role in the pathogenesis of PMO and drugs had effects on antioxidant defense system.[18] It has been suggested that plasma ascorbic acid, alpha-tocopherol, total thiol groups and erythrocyte glutathione were reduced and lipid peroxidation was decreased in patients at postmenopausal period.[19] Similarly, decreased level of antioxidant enzymes have been proposed by Sanchez-Rodrigez et al.[20] Deyhim et al. have examined the role of antioxidant diet on bone strength; they have reported that drinking citrus juice for six days positively affects serum antioxidant capacity and bone mineralization.[21]

We have found a significant increase in oxidative stress in patients with PMO. Although there was no difference between TOC values, TAC values were significantly decreased in the patient group. We thought increased osteoclastic activity and decreased osteoblastic activity may be associated with an imbalance between oxidant and antioxidant status in postmenopausal osteoporosis. Therefore, we believe that maintenance of oxidative stress equilibrium is crucial in patients with PMO and supplementation of antioxidant-enriched diet to the therapy might shed light on the development of novel therapeutic strategies for osteoporosis.

* The authors declare that there are no conflicts of interest.

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