Smoking-Induced Bone Defects May Be Due to Oxidative Damage in Postmenopausal Women

Postmenopozal Kadınlarda Sigaranın Yol Açtığı Kemik Defektleri Oksidatif Hasar Sonucu Olabilir

Aysun ÇETİN, MD,[a]
Sabahattin MUHTAROĞLU, MD,[a]
Recep SARAYMEN,[a]
Ahmet ÖZTÜRK,[b]
İpek MUDERRİS, MD[c]

Departments of [a]Biochemistry and Clinical Biochemistry,
[b]Biostatistic,
[c]Obstetrics and Gynecology,
Erciyes University Facult of Medicine, Kayseri

Geliş Tarihi/*Received:* 10.06.2008
Kabul Tarihi/*Accepted:* 09.01.2009

Yazışma Adresi/*Correspondence:*
Aysun ÇETİN, MD
Erciyes University Faculty of Medicine, Department of Biochemistry and Clinical Biochemistry, Kayseri, TÜRKİYE/TURKEY
aysuncetin@yahoo.com

ABSTRACT Objective: Smoking has been implicated as a risk factor for bone defects in postmenopausal women (PMW). The exact mechanism by which it exerts its negative effects on bone is not yet fully known. The purpose of this study was to investigate the impact of smoking on the oxidative status in PMW) and to assess the relationship between bone mineral density (BMD) and these oxidant/antioxidant parameters. **Material and Methods:** The female subjects were randomly selected with simple sampling method according to smoking habits among those presenting to outpatient menopause clinics for menauposal symptoms [smokers (n= 30), mean age was 49.7 ± 3.5 and non-snonsmokers (n= 30), mean age 51.2 ± 3.4]. Antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and paraoxonase (PON), oxidative stress biomarkers such as malondialdehyde (MDA) and nitric oxide (NO) levels, hormonal status, bone turnover markers and BMD were evaluated. **Results:** Femoral bone and lumbar spine dual-energy X-ray absorptiometry (DEXA) results revealed that the rate of osteopenia and osteporosis in smokers and non-smokers were 75% and 52.5%, respectively. The T-scores were lower in smokers than non-smokers (median -2.7 [percentile, (-3.8%)-(-2.3%)] and median -1.4 [percentile, (-1.9%)-(-0.9%)]; p< 0.001). The activities of antioxidant enzymes SOD, GSH-Px and PON were lower and the levels of oxidative stress products MDA and NO were higher in smokers than in non-smokers (p< 0.001). We found a significant correlation between decreased T-score and oxidative stress parameters in the smoking group. A positive correlation was found between the T-score and SOD (r= 0.39, p= 0.035), GSH-Px (r= 0.37, p= 0.046) and PON (r= 0.48, p= 0.008). There was a negative correlation between the T-score and the NO level (r= -0.39, p= 0.032), but no significant correlation was found between MDA levels and the T-score (r= -0.15, p= 0.430). **Conclusion:** This increased oxidative stress may represent a risk factor for the progress of osteoporosis in smoking PMW. Further studies are needed to clarify the role of smoking on oxidative bone damage and the underlying mechanisms must be addressed.

Key Words: Smoking; oxidative stress; bone density; postmenopause

ÖZET Amaç: Postmenopozal kadınlar (PMK)'de sigara içmek, kemik defektlerinde bir risk faktörü olarak gösterilmiştir. Sigaranın kemik üzerine olumsuz etkisinin mekanizması henüz tam anlamıyla bilinmemektedir. Bu çalışmanın amacı, PMK'de sigaranın oksidatif stres üzerine etkisini araştırmak ve oksidan/antioksidan parametreler ile kemik mineral yoğunluğu (KMY) arasındaki ilişkiyi değerlendirmektir. **Gereç ve Yöntemler:** Olgular menopoz polikliniğine menopozal semptomlarla başvuran kadınlar arasından, sigara alışkanlığına göre, basit örnekleme yöntemi ile rastgele seçildi [sigara içenler (n= 30), ortalama yaş 49.7 ± 3.5 yıl; sigara içmeyenler (n= 30), ortalama yaş 51.2 ± 3.4 yıl]. Antioksidan enzimler; süperoksid dismutaz (SOD), glutatyon peroksidaz (GSH-Px) ve paraoksonaz (PON), oksidatif stres belirteçleri; malondialdehid (MDA) ve nitrik oksit (NO), hormonal durum, kemik döngüsü belirteçleri ve KMY değerlendirildi. **Bulgular:** Femoral kemik ve lumbal vertebra çift X-ışınlı absorpsiyometri (DEXA) ölçümleri sonucu, osteopenik ve osteoporotik olguların oranı sigara içenlerde %75 iken, sigara içmeyenlerde %52.5 idi. T-skorları sigara içen olgularda içmeyenlere göre anlamlı ölçüde düşük saptandı. (ortanca -2.7 [persentil, (%-3.8)-(%-2.3)] ve ortanca -1.4 [persentil, (%-1.9)-(%-0.9)]; p< 0.001). Sigara içenlerde antioksidan enzimlerden SOD, GSH-Px ve PON aktiviteleri düşük ve oksidatif stress ürünleri MDA ve NO düzeyleri ise yüksek saptandı (p< 0.001). Sigara içen grupta azalmış T-skoru ile oksidatif stres parametreleri arasında anlamlı ilişki bulundu. T-skoru ile SOD (r= 0.39, p= 0.035), GSH-Px (r= 0.37, p= 0.046) ve PON (r= 0.48, p= 0.008) arasında pozitif korelasyon vardı. T-skoru ile NO düzeyleri (r= -0.39, p= 0.032) arasında negatif korelasyon saptanırken, MDA düzeyleri (r= -0.15, p= 0.430) ile ilişki yoktu. **Sonuç:** Sigara alışkanlığı olan PMK'lerdeki yüksek osteoporoz riski artmış oksidatif stresin bir sonucu olabilir. Sigaranın oksidatif kemik hasarındaki rolünü göstermede ve etki mekanizmasını tanımlamada ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Sigara; oksidatif stres; kemik yoğunluğu; postmenopoz

Turkiye Klinikleri J Med Sci 2009;29(4):851-8

Smoking is frequently considered a risk factor for low BMD. In several studies, an increase in the incidence of forearm, vertebral and hip osteoporotic fractures has been observed among postmenopausal smokers.[1,2] This increased bone fragility in smokers has been attributed to a reduced BMD, greater bone loss at various skeletal sites, decreased bone formation and increased bone resorption.[3] In contrast, a few investigators have found no significant relationship between smoking and osteoporosis.[4-6] Nevertheless, a recent meta-analysis has concluded that cigarette smoking has a major deleterious effect on bone mass, including increased bone loss and a risk of hip fracture in postmenopausal smokers.[7,8]

Tobacco smoke is a rich source of oxidants and reactive oxygen species (ROS). Reports suggested that the increased production of ROS associated with smoking might exceed the capacity of the oxidant defense system, resulting in oxidative damage to certain proteins, lipids and DNA.[9,10] MDA is the end product of lipid peroxidation and therefore is used as a biomarker for oxidative stress.[11] NO can also contribute to tissue injury when excessive amounts react with the superoxide to produce the damaging free radical, peroxynitrite.[9] The potential damage that can be caused by ROS is normally minimized by a combination of biological antioxidant systems. Important antioxidant enzymes include SOD, GSH-Px and PON.[12]

Several findings support the concept that smoking is associated with increased oxidative stress that can affect the serum mineral concentrations and bone formation.[11,12] Increased concentrations of oxygen-derived free radicals and decreased activities of antioxidant enzymes in smokers may be involved in stimulating the bone resorption process.[13] Results of previous animal studies have revealed that osteoclastic differentiation and functions were stimulated by ROS, particularly hydrogen peroxide and the superoxide anion.[14] Maggio et al investigated the possible link between plasma antioxidants and BMD in a group of 75 elderly osteoporotic women and compared them with age-matched non-porotic controls.[15] They investigated the levels of plasma vitamin C, E, A, uric acid and MDA, as well as the activities of erythrocyte and plasma SOD and plasma GSH-Px. The osteoporotic elderly group had significantly reduced vitamin levels, SOD and GSHPx activity but similar MDA levels compared to the non-porotic controls. They also reported that the levels, of vitamin A, C and activities of GSH-Px were significantly related to femoral neck BMD, in osteoporotic elderly women.

In the light of these findings, the present study was conducted to obtain information on the effects of cigarette smoking on selected markers of oxidant/antioxidant parameters, hormonal status and serum mineral concentrations in PMW.

MATERIAL AND METHODS

SUBJECTS

Case information was collected in the framework of a cross-sectional study conducted on women presenting to the Erciyes University outpatient menopause clinic. PMW were defined as those whose menstrual cycles had stopped for at least 1 year and not more than 3 years before the interview. Late PMW were excluded from the study, due to the known adverse effects on BMD. A trained interviewer provided a questionnaire for each subject, consisting of the study variables such as age, body mass index (BMI), smoking habits, comorbid diseases, performed a brief physical examination and obtained informed consents. The women admitted for menauposal symptoms to the Erciyes University outpatient menopause clinics, were divided into two groups according to their smoking habits. Subsequently, the subjects were randomly selected for this study using a simple sampling method [smokers (n= 30), mean age 49.70 ± 3.46 and non-smokers (n= 30), mean age 51.17 ± 3.40]. Smokers were defined as those smoking at least 10 cigarettes per day for the past ten years.[16] The non-smoking group included those who had never smoked and ex-smokers. The exclusion criteria for both groups were: alcohol consumption of greater than two drinks per day, passive smokers and use of other tobacco products, unstable psychiatric condition, medications that could affect bone metabolism (i.e., steroid use, an-

ticonvulsants, methotrexate, bisphosphonates, calcitonin), known diseases that could affect the bone (untreated thyroid or hyperparathyroid disease) and chronic liver or kidney disease. Estrogen replacement therapy was an exclusion criterion because of the known effects on bone turnover. The local ethics committee approved the study protocol.

Table 1 shows the demographic characteristics of the smoking and the non-smoking group. The smoking and non-smoking women groups were comparable with regard to the number of years postmenopause; however, the smoking group had passed to menopause 2 years earlier than the non-smoking group. This age difference was not significant. High urinary cotinine concentrations were determined in smokers as an evidence of their habits [(599.0(ranges, 266-1841) ng/mL and 204 (ranges, 60-370) ng/mL)].[17]

ASSAY MEASUREMENTS

Blood samples were collected after an overnight fast. Heparinized blood obtained from patients and controls were centrifuged at 2000 rpm for 15 minutes at 4°C. After seperating the plasma, erythrocytes were washed three times with cold isotonic saline and centrifuged. The packed cells, subdivided into aliquots as well as plasma samples were stored until analyses. The also collected the second voided urine of the day was also collected on the same day of blood collection for measurement of deoxypyridinoline and cotinine. All specimens were subsequently stored at -80°C and batched for analysis.

TABLE 1: Demographic characteristics of the smoking and the non-smoking group.

Characteristics (Values)	Smoking Group n= 30 $\bar{x}$ ± SD/M (25%-75%)	Non-smoking Group n= 30 $\bar{x}$ ± SD/M (25%-75%)	P*
Age (year)	50.0 (48-53)	51.0 (49-54)	0.136$^{\phi}$
Height (cm)	158.4 ± 4.6	158.2 ± 5.4	0.880$^{\theta}$
Weight (kg)	69.9 ± 10.9	69.9 ± 7.6	0.990$^{\theta}$
BMI (kg/m^2)	27.9 ± 4.4	27.8 ± 2.6	0.862$^{\theta}$
Years postmenopause	1.4 (0.8-1.8)	1.3 (0.7-2.0)	0.390$^{\phi}$
Urinary cotinine (ng/mL)	599.0 (425-1164)	204 (137-256)	<0.001$^{\phi}$

* All values reported as mean ± SD or median (percentile, 25%-75%) BMI,body mass index.
ϕ Mann-Whitney U test
θ Unpaired t test
BMI: Body mass index

The following measurements were done: [alkaline phosphatase (ALP), calcium, phosphorus and osteocalcin (OC)], hormonal concentrations [estradiol (E2), total testosterone (TT), parathyroid hormone (PTH), follicle stimulating hormone (FSH), luteinizing hormone (LH), sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS) and androstenedione]. Biochemical markers; ALP, calcium and phosphorus were determined by using an automated KoneLab spectrophotometer (Thermo Clinical Labsystems Ltd, Finland). Sex hormones; E2, TT, FSH, LH, DHEAS and androstenedione were measured by radioimmuno assay using a commercial kit (ICN Biomedicals, Inc., USA) and SHBG, PTH and calcitonin by a chemiluminescence assay (Advia Centaur, Bayer, Germany), using kits from diagnostic systems Biosource, Belgium. All the mentioned hormones were analyzed at the Nuclear Medicine Laboratory. Bone turnover markers were analyzed at the Core Biochemistry Laboratory. Osteocalcin was measured by a sandwich ELISA using two antibodies (Biosource, Europe SA). Deoxypyridinoline was detected in urine using High Performance Liquid Chromatography (HPLC, Thermo Finnigan). Urinary cotinine concentrations were measured using kits from Konelab (Thermo Clinical Labsystems Ltd, Finland) with automated procedures.

OXIDATIVE STRESS PARAMETERS

MDA reacts with thiobarbituric acid (TBA) to produce a fluorescence product, which is measured spectrophotometrically. Thus, plasma MDA levels were measured as an index of lipid peroxidation, according to the TBA spectrophotometric method, which was modified from the metods of Satoh and Yagi.[18,19] 1,1,3,3, tetraetoxypropane was used as standard. SOD activity in plasma was assayed by its ability to inhibit the reduction of nitroblue tetrazolium (NBT) with xanthine–xanthine oxidase used as a superoxide anion generator.[20] GSH-*Px* activity in erythrocytes was measured according to the method of Paglia and Valentine.[21] Plasma PON activity was determined according to the method of Phuntuwate

et al.[22] All the reagents of methods were purchased from Sigma (Sigma-Aldrich Corp, St Louis, MO, USA) and Merck (Merck KgaA, Darmstadt, Germany). Plasma NO levels were determined using the colorimetric assay kit (Cayman chemical, Ann Arbor, MI). Total nitrate/nitrite concentration was measured in a simple two step process.The first step was conversion of nitrate to nitrite utilizing nitrate reductase. The second step is the addition of the Griess Reagents which convert nitrite into a deep purple azo compound. Nitrite concentration was determined by photometric measurement of absorbance due to this azo chromophore. SOD, GSH-Px and PON activities and MDA and NO levels were expressed as U/mL, U/gHb, U/L and nmol/mL and mmol/L, respectively.

BONE MINERAL DENSITY

DEXA is considered the gold standard for measuring BMD.[23] In this study, lumbar spine (anterior–posterior) and proximal femoral BMD were measured using DXA on a hologic densitometer (QDR-4500 elite, USA). The results of DXA measurements were expressed as T-score and these values were used to determine the normal (>–1), osteopenia (–1≥, >–2.5), or osteoporosis (≤–2.5) based on the World Health Organization (WHO) criteria for osteoporosis.[24] Correspondingly, women with osteopenia and osteoporosis were defined as low bone density (LBD).

STATISTICAL ANALYSIS

All datasets were subjected to normality tests using the Kolmogorov–Smirnov method and the data were reported as either mean ± standart deviation (x ± SD) (for normally distributed data), or as median with 25%-75% percentile (for skewed data). Comparison of variables between the two groups was made using the Mann-Whitney U test for skewed data and the unpaired t test for normally distributed data. Spearman's correlation analysis was used to assess the associations between the T-score and oxidative parameters. A p value of <0.05 was considered statistically significant. All analyses were performed using the Statistical Package for the Social Sciences version 13.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 2 shows the assay measurements of the smoking and the non-smoking subjects. The slightly higher hemoglobin values in the smoker group were probably due to smoking-induced hypoxemia. Meanwhile, the blood tests and biochemical bone parameters (ie, calcium, phosphorusus and ALP) were similiar in the two groups. The hormone levels in both groups were also compared. The smoking group had lower median levels of estradiol, compared to the non-smoking group [(17.5 (percentile, 14%-24%) pg/mL vs. 25.0 (percentile, 19%-24%) pg/mL; $p<0.01$)]. Other hormone concentrations were similar between the smokers and non-smokers. Urinary deoxypyridinoline, a markedly characterized biochemical bone turnover marker, was detected at a significantly higher level in the smoking group (23.1 ± 9.9 pmol/μmol vs 13.2 ± 5.5 pmol/μmol, $p< 0.001$).

In all cases, the diagnosis of LBD was based on the T-score results on the L2-4 lumbar spine and femoral neck or the femur bone totally. LBD (T-score ≤ -1) in smokers and non-smokers was 75% and 52.5%, respectively. Among the smoking subjects, 42.5% were diagnosed with osteoporosis and 32.5% with osteopenia according to the bone density score. Figure 1 shows the effects of smoking on BMD.

Table 3 shows that the correlation between plasma antioxidant activities and femoral BMD (T-score) in PMW. There was a significant correlation between decreased T-score and oxidative stress parameters in the smoking group. A slightly positive correlation was found between the T-score and SOD ($r= 0.39$, $p= 0.035$), GSH-Px ($r= 0.37$, $p= 0.046$) and PON ($r= .48$, $p= 0.008$) levels. There was a slightly negative correlation between the T-score and the NO level ($r=-0.39$, $p= 0.032$). There was no significant correlation between MDA levels and the T-score ($r= -0.15$, $p= 0.43$). Also, there was no significant correlation between the T-score and oxidative stress parameters in the non-smoking group.

TABLE 2: Assay measurements of the subjects in the smoking and the nonsmoking group.

Characteristics (Normal Values)	Smoking Group n= 30 $\bar{x}$ ± SD/M (25%-75%)	Non-smoking Group n=30 $\bar{x}$ ± SD/M (25%-75%)	P
HGB (12-16 g/dL)	13.0 ± 2.4	12.9 ± 2.1	0.880θ
WBC (4.000–10.000 mm³)	6.5 ± 1.4	7.1 ± 1.3	0.090θ
Calcium (8.8-10.8 mg/dL)	8.6 ± 0.8	8.9 ± 0.9	0.324θ
Phosphorusus (2.5-4.8 mg/dL)	3.6 ± 0.6	3.7 ± 0.71	0.696θ
ALP (100-280 mg/dL)	112.0 (88-131)	119.0 (100-176)	0.111ϕ
Estradiol (0-37 pg/ml)	17.5 (14-24)	25.0 (19-24)	<0.01ϕ
FSH (23-116 mIU/mL)	95.9 ± 26.9	96.1 ± 29.7	0.975θ
LH (16-54 mIU/mL)	33.1 ± 12.1	34.7 ± 11.6	0.588θ
SHBG (20-85nmol/L)	29.5 (25-37)	30.0 (27-41)	0.548ϕ
DHEA-SO4 (70-3840ng/mL)	98.3 ± 34.8	95.0 ± 21.4	0.663θ
Andrestenodione (0.1-2.99 nmol/L)	0.9 (0.6-2.3)	1.9 (0.08-2.7)	0.121ϕ
Total Testosterone (6-50 pg/m))	23.8 ± 12.9	24.2 ± 12.8	0.905θΛ
Calcitonin (0-10 pg/mL)	5.7 ± 2.2	5.78 ± 2.2	0.893θ
PTH (6-29 pg/mL)	15.5±4.9	15.9±5.5	0.750θ
Osteocalcin (5-25 ng/mL)	10.0 (9-16)	14.0 (10-15)	0.274ϕ
Deoxypyridinoline (6-26 pmol/µmol)	23.1 ± 9.9	13.2 ± 5.5	<0.001θ
T-Score	-2.7 [(-3.8)-(-2.3)]	-1.4 [(-1.9)-(-0.9)]	<0.001ϕ

* All values reported as mean ± SD and median (percentile, 25%-75%). HGB, hemoglobin; WBC,white blood count; SHBG, sex hormone-binding globulin; PTH, parathyroid hormone; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosteronesulfate. ALP, alkaline phosphatase.
ϕ Mann-Whitney U test
θ Unpaired t test

OXIDATIVE STRESS PARAMETERS

The mean SOD, GSHPx and PON activities were 3.7 ± 1.3 U/mL; 26.7 U/gHb (percentile, 21%-29%); and 49.5 U/L (percentile, 29%-79%) in smokers and 5.7 ± 2.1 U/ml; 30.1 U/gHb (percentile, 26%-33%); and 95.0 U/L (percentile, 70%-115%) in non-smokers, respectively. According to these results, endogenous antioxidant markers were significantly decreased in smokers compared with non-smokers [SOD ($p< 0.01$), GSH ($p< 0.001$) and PON ($p< 0.001$); (Figure 2). The mean MDA and NO levels were 3.2 ± 0.8 nmol/ml and 42.8 ± 14.5 mmol/L in smokers and 1.9 ± 0.3 nmol/ml and 33.6 ± 11.5 mmol/L in non-smokers, respectively. Oxidative parameters were significantly increased in smokers compared with non-smokers [MDA ($p< 0.001$) and NO ($p< 0.01$); (Figure 2)].

DISCUSSION

In several studies, free radicals were involved in the pathogenesis of bone loss by stimulating osteoclasts differentiation and bone resorption.[14,25,26] Basu *et al* investigated a possible relationship between 8-iso-$PGF_{2\alpha}$ (F_2 isoprostane is a marker of oxidative stress and reflects a non-enzymatic process of lipid peroxidation) and BMD.[27] They concluded that there

TABLE 3: Correlation between plasma antioxidant levels and femoral BMD (T-score) in PMW.

Oxidative Parameters	Smoking Group		Non-smoking Group	
	r	p	r	p
SOD (U/mL)	0.39	0.035	-0.17	0.378
GSH-Px (U/gHb)	0.37	0.046	-0.10	0.586
PON (U/L)	0.48	0.008	0.01	0.947
MDA (nmol/mL)	-0.15	0.430	-0,04	0.838
NO (µmol/L)	-0.39	0.032	-0.02	0.930

* BMD, bone mineral density; PMW, postmenopausal women; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase;
PON, paraoxonase; MDA, malondialdehyde; NO, nitric oxide.
Spearman's correlation coefficient

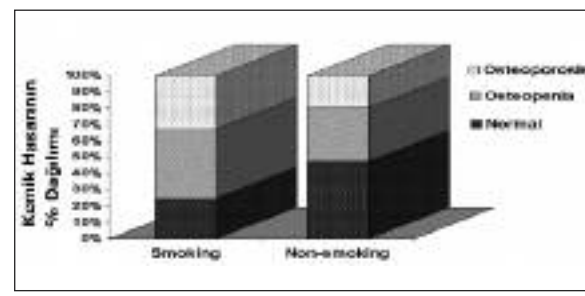

FIGURE 1: The effects of smoking on bone mineral density in postmenapousal women.

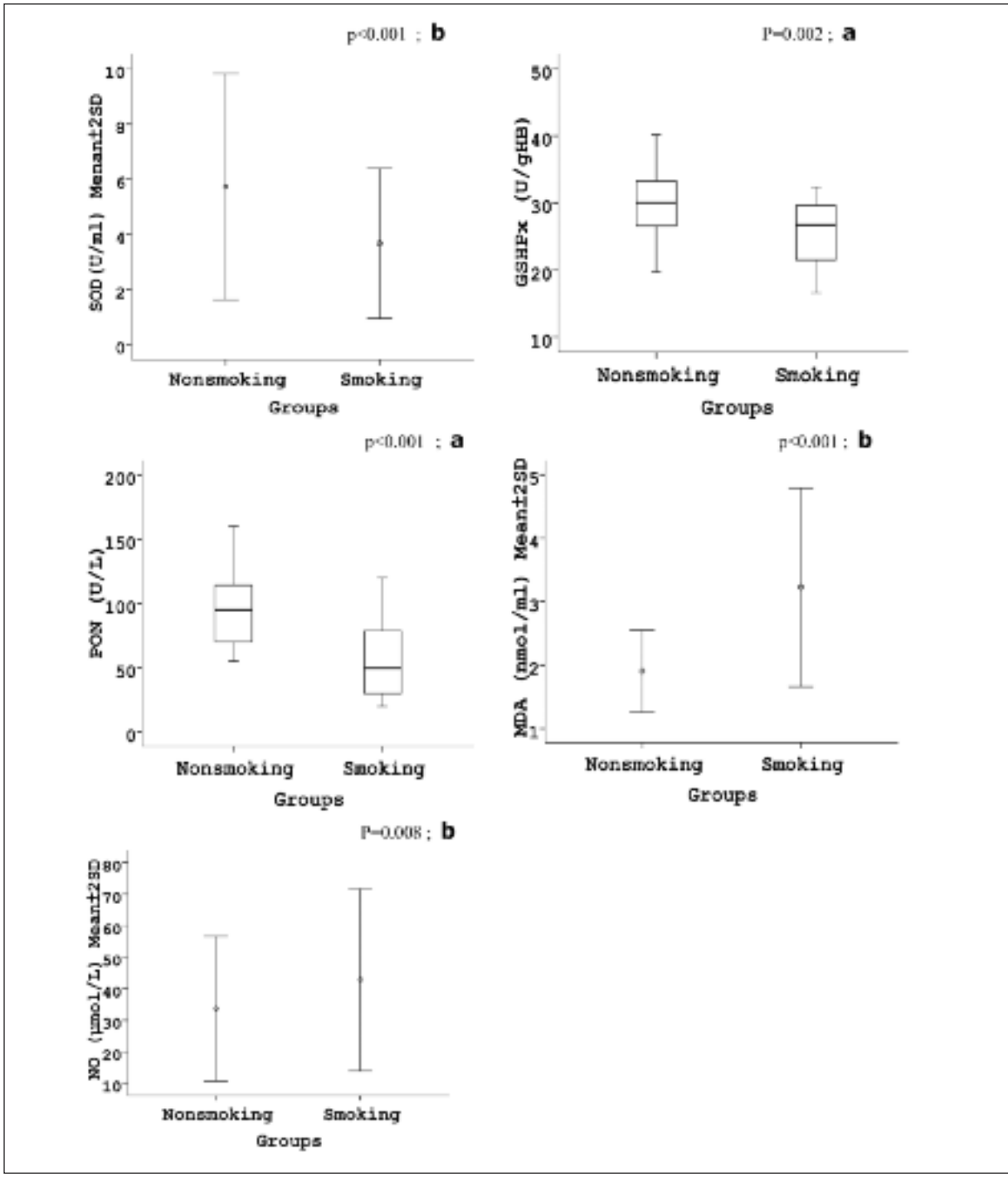

FIGURE 2: Comparison of oxidative stress parameters in non-smoking and smoking subjects. a. Box-and-whisker plot. b. Error bar.

was a link between lipid peroxidation and decreased BMD in the study population. Sontakke et *al* showed elevated MDA levels and reduced SOD and GSH-Px activities in osteoporotic groups in comparison with healthy controls and claimed an unfavorable role of free radicals on bone metabolism.[28] Another controlled study showed that there was a possible link between plasma antioxidants and LBD in elderly osteoporotic women.[15] They also reported that significantly reduced vitamin A and C levels, and SOD and GSH-Px activity was related to reduced BMD. In the present study, smok-

ing women had increased oxidative stress and decreased antioxidant activity compared to the non-smoking women. Smoking women also had LBD compared to the non-smokers.

Authors have suggested that exposure to cigarette smoke leads to stimulation of NO synthesis by the O_2 present in the smoke.[29] High levels of NO induce the inhibition of osteoclast activity and apoptosis of osteoclast progenitors.[30,31] In this study, high NO levels may be responsible for the bone loss in smoking PMW. We found a significant relationship between the decreased T-score and the higher NO levels in smoking women with postmenopausal osteoporosis.

Smoking may enhance oxidative stress not only through the production of reactive oxygen radicals in smoke but also through weakening the antioxidant defense systems. Several studies have shown that extracts of cigarette smoke lead to inhibition of endogenous anti-oxidative mechanisms, such as SOD, GSH-Px and PON.[32-38] Despite abundant experimental studies conducted in animals or cell cultures, in vivo human studies assess the role of oxidative stress or antioxidant systems in osteoporosis are scarce. Melhus et al investigated the potential role of antioxidant vitamins, in preventing hip fracture in smokers.[12]They concluded that a low intake of vitamins E and C increased the relative risk for hip fracture almost 5-fold after adjustment for age, weight and other osteoporosis risk factors and that more adequate intake of these vitamins seemed to be protective against the adverse effects of smoking. There are some reports stating that the inhibition of PON levels is due to cigarette smoking extracts.[39,40] These results indicate that there may be a new association between decreased PON, activity due to smoking and LBD.[40] Our results revealed decreased levels of universal antioxidant parameters SOD, GSH-Px and PON in smoking PMW. There was a significant positive correlation between decreased antioxidant levels and decreased T-scores in smokers. According to the present study, increased bone fractures and decreased bone density in postmenopausal women may be potentialized by stimulation of oxidative stress and inhibition of antioxidant scavering.

Ortego et al suggested that smoking might accelerate the hydroxylation of estrogen in the liver or the inhibition of aromatization of androgens to estrogen in the tissues.[41] Smoking produces significant reductions in estradiol, but no other hormonal concentrations are reduced (i.e. SHBG, testosterone and others). Our findings are concordant with those in Ortego's study, regarding a decrease in estradiol concentrations in smoking subjects. Since smoking may induce the metabolism of estradiol, female smokers may experience menopause earlier than non-smoking women and this may be explained by promoting an effect of smoking on menopause.[42] In this study, menopause age was 2 years earlier in the smokers than in the non-smokers.

In conclusion, the postmenapousal smokers examined in the study displayed evidence of LBD due to oxidative stress and an impaired oxidant defense system. Further studies are needed to clarify the role of smoking on oxidative bone damage and the underlying mechanisms must be addressed.

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