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Is there a role of free oxygen radicals in primary male osteoporosis ?

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ABSTRACT

Objective. *There is not enough evidence about the relationship between free radicals and male osteoporosis. In this study we investigated the role of free oxygen radicals and antioxidants on male osteoporosis in 31 male patients with primary osteoporosis and 21 subjects as controls.*

Methods. *Bone mineral densities (BMD) of the lumbar and femoral neck region were evaluated using dual energy X-ray absorbsiometry. Serum malondialdehyde (MDA) and nitric oxide (NO) levels and superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were measured by analytical methods. In addition, serum osteocalcine and C telopeptide levels were determined to evaluate bone turnover. MDA and NO levels and SOD activity were significantly increased ($p < 0.05$) in osteoporotic males.*

Results. *There was a negative correlation between SOD and lumbar BMD levels ($r = -0.328$; $p = 0.021$). The same trend was observed between NO and lumbar BMD ($r = -0.473$; $p = 0.001$) and femoral neck BMD values ($r = –0.540$; $p = 0.000$). There was no significant correlation between free radical levels and bone turnover markers.*

Conclusion. *The data indicate an increase in free oxygen radical levels. As a result, antioxidant defenses would compromise in primary male osteoporotic patients. Therefore, it may be suggested that oxidative stress plays an important role in the pathophysiology of primary male osteoporosis.*

Introduction

Osteoporosis in men is now recognized as an increasingly important public health issue (1, 2). One out of five osteoporosis patients is male and 30% of hip fractures occur in men (3). The ethiopathogenesis of osteoporosis in men is poorly understood. Although aging and genetic factors are important, many factors such as smoking, immobilization, inefficient calcium intake, thyroid and parathyroid function disorders, gastrointestinal and kidney diseases cause osteoporosis and 30-60% percent of cases are associated with one or more of the secondary risk factors (2, 4-6) in male osteoporosis. Kelepouris reported that approximately one third of osteoporotic men have an idiopathic disease (7).

Free oxygen radicals or reactive oxygen species (ROS) include hydroxyl radicals (OH·), superoxide anion radical ($O \cdot 2^-$), hydrogen peroxide (H_2O_2) and nitric oxide (NO) and lead to the specific oxidation of some enzymes, protein oxidation and degradation (8). Their effects are eliminated by enzymatic antioxidant mechanisms such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (9). Oxidative stress is an imbalance between the free radicals and antioxidant mechanism in biological systems and damages cellular macromolecules and functions. It is responsible for the pathophysiology of the aging process and many diseases such as atherosclerosis, carcinogenesis, myocardial infarction and muscle diseases (10, 11).

The role of free radicals and oxidative stress in male osteoporosis is unknown. There is evidence that NO modulates bone remodeling and bone loss *in vitro* and *in vivo* (12). Several studies have demonstrated that bone cells can produce NO and express NOS (nitric oxide synthase) enzymes. Also, NO plays an important role as the paracrine and autocrine mediator of bone cells in response to diverse stimuli such as proinflammatory cytokines (13,14), mechanical strains (15) and sex hormones (16). However, there is not enough evidence about the role of MDA(malondialdehyde) and antioxidant enzymes (SOD, GPx) on bone tissue and the effect of oxidative stress regarding the development of osteoporosis.

The aim of the study was to investigate the role of free oxygen radicals and antioxidants on primary osteoporosis in men. For this study, NO serum and MDA levels, SOD and GPx activities were measured and the relationship between free oxygen radicals and bone turnover markers was investigated.

Materials and methods

Thirty-one male patients attending our outpatient clinic who were diagnosed as primary osteoporosis according to World Health Organization criteria

(WHO) (17) were selected as subjects in this study. Twenty-one healthy volunteer men with matched age, weight and height were evaluated as the control group. The history of smoking was also recorded. None of them had secondary osteoporosis, hypogonadism, diabetes mellitus, renal disease, hepatic disease, thyroid-parathyroid function disorders, inflammatory disease, nor were taking any medication that could effect bone metabolism. All subjects were informed about the content of the study prior to tests and their written consents were obtained. The study protocol was approved by the ethical committee of the Mersin University Hospital.

Bone mineral density (BMD) was measured at the lumbar vertebrae (L2-4) and femoral neck region using dual energy X-ray absorbsiometry (DEXA) (Norland XR 46). T scores < -2.5 were accepted as the indicator of osteoporosis.

Fasting venous blood samples were collected in test tubes from the subjects and within 30 min they were centrifuged at 1500 x g for 5 min. Serum was separated and analyzed on the same day for enzymatic assays of alkaline phosphatase, SOD, GPx and MDA and NO levels. Serum was kept frozen at – 20 ºC for further analysis. Clinical examination, complete blood cell counts, routine biochemical tests, serum calcium, and phosphorous levels, thyroid function tests, parathormone, sex hormones, prolactin, calcium excretion in 24 hour urine, vitamin D_3, erythrocyte sedimentation rates, C reactive protein and rheumatoid factor levels were determined for osteoporosis and control groups. Height and weight of the patients were measured and body mass index (kg/m^2) was calculated. Osteocalcine and C telopeptide (CTx) levels, as markers of bone formation and bone resorption, were measured in all subjects. Serum osteocalcine was measured by radioimmunassay (RIA-Wallac Gama Counter) and serum C telopeptide by electrochemiluminescence (Elecsysis 2010, Roche Diagnostic).

MDA levels were determined by thiobarbituric acid (TBA) reaction according to the method of Yagi *et al.* (18).

The oxidized end products of NO (nitrite and nitrate) were analyzed by a photometric endpoint determination (nitrite/nitrate, colorimetric method; catalogue no. 1-746-081, Roche Diagnostics GmbH, Mannheim, Germany).

SOD activity was measured based on the inhibition of nitroblue tetrazolium (NBT) reduction due to the generation of O_2^- by the xanthine/xanthine oxidase system (19). One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition in the NBT reduction rate.

GPx activity was measured as described by Paglia and Valentine (20).

Statistical analysis

Statistical analyses were done by SPSS 10.0 (Statistical Package for the Social Sciences Program) statistical program. Levene's test was used to investigate the variance homogeneity between the groups. Student's t-test was applied to the data in order to compare patient and control groups. All data were reported as mean ± standard deviation (SD). Statistical significance was defined as $p < 0.05$. Correlations were expressed using the Pearson's correlation coefficient.

Results

The mean ages of the patients and the control group were 59.31 ± 6.61 and 56.95 ± 6.06, respectively. The age and BMI values were not significantly different between the two groups according to Levene's test. Smoking habits were also similar: 18 among patients and 11 in the control group were smokers. The main clinical and biochemical findings for the patients and the controls are listed in Table I. NO and MDA levels and SOD enzyme activity were significantly higher in patients than in the control group ($p < 0.05$). On the contrary, GPx activities were lower in patients than the controls. However, the difference was not statistically significant. No significant difference in osteocalcine and CTx levels was obtained between patients and control groups. In the entire study population, negative correlations between SOD and lumbar BMD ($r = -0.328$; $p = 0.021$), NO and lumbar BMD ($r = -0.473$; $p = 0.001$) and NO and femoral neck BMD values ($r = -0.540$; $p = 0.000$) were observed (Fig.1). A negative correlation was also found between the SOD/GPx ratio and lumbar BMD ($r = -0.407$, $p = 0.004$) and femoral neck BMD ($r = -0.316$, $p = 0.027$). Free radical levels and bone turnover markers were not significantly correlated.

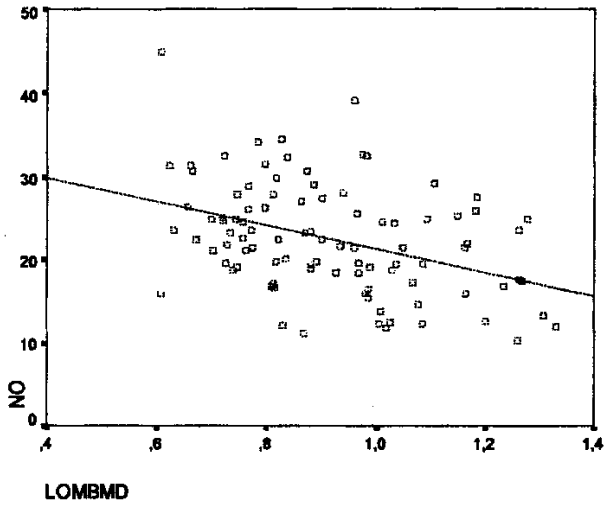
Discussion

In this study, a significant increase in the nitric oxide, malondialdehyde and superoxide dismutase levels of patients with primary male osteoporosis were observed. Similar results have been reported in postmenopausal osteoporotic

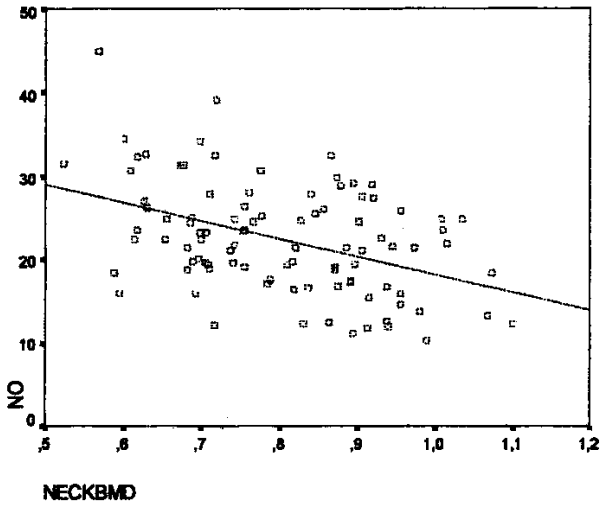
Table I. Clinical and biochemical findings in healthy controls and men with idiopathic osteoporosis (mean ± SD).

	Control (n = 21)	Osteoporosis (n = 31)	P (between groups)
Age (years)	56.95 ± 6.06	59.31 ± 6.61	0.131
BMI (kg/m^2)	25.75 ± 3.24	26.25 ± 2.61	0.356
Lumbar BMD (g/cm^2)	1.13 ± 0.13	0.82 ± 0.13	0.000*
Femoral neck BMD (g/cm^2)	0.93 ± 0.01	0.70 ± 0.01	0.000*
NO (µmol/l)	18.38 ± 6.43	25.27 ± 6.69	0.001*
MDA(µmol/l)	4.88 ± 4.01	9.90 ± 8.54	0.016*
SOD (U/ml)	2.80 ± 1.51	4.77 ± 2.44	0.002*
GPx (IU/ml)	4.10 ± 2.57	2.61 ± 3.07	0.077
Osteocalcine (ng/ml)	27.47 ± 5.25	25.90 ± 9.51	0.551
CTx (ng/ml)	0.32 ± 0.13	0.36 ± 0.19	0.522
Ratio of SOD/GPx	1.5 ± 2.45	174.58 ± 328.18	0.020*

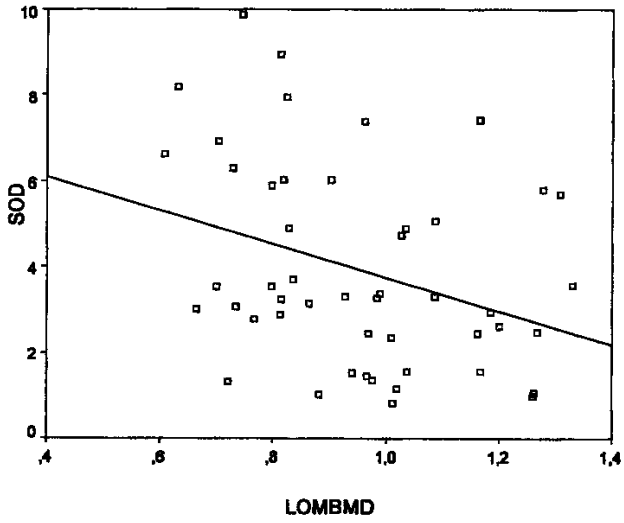
BMI: body mass index; BMD: bone mineral density; NO: nitric oxide; MDA: malondialdehyde; SOD: superoxide dismutase; GPx: glutathione peroxidase; CTx: C telopeptide.



(p = 0.001, r = -0.473)
(a)



(p = 0.000, r = -0.540)
(b)



(p = 0.021, r = -0.328)
(c)

Fig. 1. Correlation analyses between NO and lumbar BMD (**a**), NO and femoral neck BMD (**b**) and MDAand lumbar BMD (**c**).
NO: nitric oxide; lom BMD: lumbar bone mineral density; neck BMD: femoral neck bone mineral density.

women (21,22) but there is no such evidence for men.

Limited data about the role of free oxygen radicals on bone metabolism are available. Only the effect of nitric oxide has been extensively investigated. NO is widely expressed in bone marrow stromal cells, osteoblasts, osteocytes and osteoclasts, but its role in the regulation of bone formation and resorption yielded conflicting results (12). Some studies reported that NO has biphasic effects on bone. According to these results, low concentration of NO potentiates IL-1 induced bone resorption and stimulates osteoblast growth and differentiation, whereas high concentration of NO inhibits osteoclast formation and activity and also inhibits osteoblast growth and differentiation (12). The relationship between NO levels and BMD is also controversial. Aguire (23) reported that disruption of the endothelial NO gene in mice resulted in a marked reduction in bone volume, bone formation rate and bone mineral density, whereas Wimalawansa (24) found that NO donors prevented postmenopausal bone loss. Armour (25) and Cuzzorea (26) reported that there is a negative correlation between BMD and NO production in inflammation-induced osteoporosis. Similarly, the data obtained in our study indicate an inverse correlation between the NO level and lumbar and femoral neck BMD (Fig. 2). Also, NO levels were higher in osteoporotic male patients than in healthy men.

There are two reports about the MDA levels in postmenopausal osteoporosis. While Sontakke (21) reported increased levels of MDA in osteoporosis, Maggio (22) did not find any difference. In our study MDA levels were higher in osteoporotic patients than in the controls.

The reason for the increase of free radicals in osteoporosis remains unclear. According to Sakurai (27), a moderate level of nitric oxide might stimulate bone resorption through IL-1 and TNF. Sontakke (21), Farrel (29) and Garret (30) suggested that enhanced osteoclastic activity might be responsible for increased production of free oxygen radicals. We could not confirm the above

hypothesis with our patients as our results indicated that bone formation and resorption markers were not related to free oxygen radicals.

SOD and GPx are considered as the markers of antioxidant defense mechanism (30). Sontakke (21) and Maggio (22) found that SOD and GPx levels were lower in osteoporotic patients and they suggested that antioxidant defense mechanism is markedly decreased in osteoporosis. We found that SOD activity was higher and GPx activity was lower in osteoporotic men than in the controls. Generally, decreased GPx activity is associated with increased reactive oxygen radicals (H_2O_2), and probably SOD activity was the first increasing one. Changes in the SOD/GPx ratio have a profound effect on the cellular resistance to oxidant induced damage and cell killing. The imbalance of the SOD/GPx ratio increases the free oxygen radical production (30). A higher SOD/GPx ratio was found in osteoporotic patients than in the control groups. There was no data about this ratio in idiopathic male osteoporosis.

In conclusion, our results indicate that oxidative stress is increased and antioxidant defense is compromised in osteoporotic patients. In addition, the negative correlation between BMD, and NO and MDA values might suggest that NO and MDA contribute to the depression on bone formation. Free radical mediated peroxidative injury due to the inhibited differentiation of bone cells might play a crucial role in the pathophysiology of osteoporosis.

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