



OXIDATIVE STRESS AND SOME ANTIOXIDANT PARAMETERS IN POSTMENOPAUSAL OSTEOPOROTIC WOMEN WITH FRACTURES: A CASE CONTROL STUDY

ABSTRACT

Introduction: Oxidative stress plays an important role in the pathogenesis of postmenopausal osteoporosis (PMO). Antioxidants have the ability to mitigate the damaging effects of reactive oxygen species (ROS).

Materials and Method: This study evaluated the plasma levels of two selected antioxidant defenses: vitamin C and superoxide dismutase (SOD) and Malondialdehyde (MDA) the lipid peroxidation byproduct and oxidative stress indicator in >45 years old postmenopausal osteoporotic women (n=40) as compared to non-osteoporotic controls (n=20). Subjects underwent a full history taking, clinical examination and bone mineral density (BMD) of the proximal femur and measurement of plasma vitamin C, SOD and MDA.

Results: The study showed that women with PMO had significantly lower levels of plasma vitamin C and SOD and higher MDA levels as compared to the controls (p<0.05). A significant positive correlation (p<0.01) was found between plasma vitamin C, SOD and Femoral neck BMD while a significant but negative correlation was found between MDA and femoral neck BMD.

Conclusion: Oxidative stress and decreased antioxidant defenses have an important role in the pathogenesis of PMO and MDA may be an important indicator for bone loss in postmenopausal women, necessitating further research.

Key words: Postmenopausal osteoporosis, Oxidative stress, Antioxidant, Vitamin C, Superoxide dismutase, Malondialdehyde.

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KIRIĞI OLAN POSTMENOPOZAL OSTEOPOROTİK KADINLARDA OKSİDATİF STRES VE BAZI ANTİOKSİDAN PARAMETRELER: BİR OLGU KONTROL ÇALIŞMASI

Öz

Giriş: Postmenopozal osteoporoz (PMO) patogeneğinde oksidatif stres önemli rol oynar. Antioksidanlar serbest oksijen radikallerinin yıkıcı etkilerini yok etme özelliğine sahiptir.

Gereç ve Yöntem: Bu çalışma ile 45 yaşından büyük postmenopozal osteoporotik kadınlar (n=40) ve osteoporotik olmayan kontrollerde (n=20) vitamin C ve superoksid dismutaz (SOD) gibi iki antioksidanın ve lipid peroksidasyon ve oksidatif stres göstergelerinden malondialdehid (MDA) düzeylerinin araştırılması amaçlandı. Tüm olgulardan anamnez alındı fizik muayeneleri yapıldı, proksimal femurdan kemik mineral dansiteleri (BMD) ve plazma vitamin C, SOD ve MDA düzeyleri ölçüldü.

Bulgular: Bu çalışma PMO olan kadınların kontrollere göre daha düşük plazma vitamin C ve SOD düzeylerine, buna karşın artmış MDA düzeylerine sahip olduğunu gösterdi (p<0.05). Plazma vitamin C düzeyleri ile femur boynu BMD'si arasında pozitif korelasyon (p<0.01) saptanırken MDA ve femoral BMD arasında negatif korelasyon izlendi.

Sonuç: Oksidatif stres ve azalmış antioksidan savunma mekanizmaları PMO'nun patogeneğinde önemli rol oynar ve MDA postmenopozal kadınlarda kemik kaybının önemli bir göstergesi olabilir.

Anahtar sözcükler: Osteoporoz, Oksidatif stres, Menopoz, Antioksidanlar, Vitamin C, SOD, MDA.

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Geliş Tarihi: 25/05/2008
(Received)

Kabul Tarihi: 03/07/2008
(Accepted)

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INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by low bone density and micro architectural deterioration of bone tissue with a consequent increase in bone fragility (1). Early osteoporosis not usually diagnosed, remains asymptomatic and does not become clinically evident until fractures occur (1, 2). The latter imposes a considerable medical, social and economic burden (3,4).

Loss of bone density and rates of fracture increases markedly with advancing age, giving rise to significant morbidity, mortality and disability (1,3,4). Osteoporosis is three times more common in women than in men, partly because of their lower peak bone mass and the hormonal changes that occur at the menopause in addition to a greater life expectancy among women (1).

Osteoporosis affecting millions of people worldwide [4], is also a major health problem in Egypt (2). National surveys carried out by the National Nutrition Institute (NNI) in 2001 and 2004 reveal that between the ages 40-50 years, 42 percent females had low bone mineral density (BMD). At 60 years of age, about half of the females had osteopenia while a third of the elderly population (65 to over 80 years of age) was osteoporotic (2).

Osteoporosis has multi-factorial pathogenesis but the role of proper nutrition has been duly emphasized. Many nutrients and dietary factors may be important for long-term bone health and the prevention of osteoporosis (4,5).

Increased oxidative stress on the basis of potential biomarkers of oxidative damage has been found to be associated with many chronic human diseases (6). Oxidative stress markers may be an important indicator of bone loss in postmenopausal women (7) and a link of increased oxidative stress with reduced BMD has been documented (8).

Lipid peroxidation is probably the most extensively investigated free radical induced process with consequences in the form of protein oxidation, loss or weakening of cell membrane structure and function and generation of aldehyde products such as Malondialdehyde (MDA) (9). MDA is one of the promising candidates for general biomarkers of oxidative stress (6) and has previously been used as an indicator of oxidative stress among osteoporotic postmenopausal women (7,10).

Oxidative stress related factors partially caused by a low antioxidant status and nutrient deficiency, are a known risk factor for osteoporosis. Epidemiological studies suggest that

antioxidants counteract the effect of stress on bone and may reduce the risk of osteoporosis. Oxidative stress is involved in the activity and function of osteoblast and osteoclasts, the two major bone cells involved in the pathogenesis of osteoporosis (11).

Vitamin C, one of the potentially important micronutrients that play an important role in normal bone metabolism because of its role as a cofactor in collagen formation (3,12) also acts as antioxidant and provides defense against oxidative stress (11,13). It act as a free radical scavenger in reactions involving reactive oxygen species (ROS) such as superoxide, hydroperoxyl, peroxynitrite and siglet oxygen radicals with formation of semi-dehydroascorbate free radical (DHA) which is poorly reactive (14).

Some studies show dietary intake of Vitamin C to be positively correlated with the bone mineral content among post menopausal women while its mean plasma levels were consistently and significantly lower in osteoporotic elderly women as compared to non-osteoporotic ones (10).

Elderly osteoporotic women have also been found to have consistent and significant lower levels of enzymatic antioxidant defenses like superoxide dismutase (SOD), one of the major antioxidant defense systems present in the body when compared with normal age-matched reference population (10,11). It catalyzes the dismutation of superoxide anion into hydrogen peroxide and oxygen. It is not surprising that different SODs were then evolved to deal with the toxicity of the accumulating O_2^- , there are SODs based on Cu^{2+} plus Zn^{2+} , Mn^{3+} , Fe^{3+} and Ni^{2+} (15).

Studies assessing the role of plasma antioxidant defenses in elderly osteoporotic women are scarce (7,10) and absent for Arab populations. Oxidative stress markers as a possible important indicator for bone loss in post menopausal women needs to be further investigated to study the role of antioxidants in regulation of bone mass (7).

A case control study was therefore designed to assess whether some selected plasma antioxidant defenses are decreased in Egyptian postmenopausal osteoporotic women as compared to non osteoporotic controls and determine the MDA levels as an indicator of oxidative stress in both groups.

MATERIALS AND METHOD

Subjects were postmenopausal women recruited from Among the patients admitted to the department of ortho-



paediatric surgery, Minoufiya University Hospital (Egypt) between September and December 2006 for various kinds of fractures. The control group had a femoral neck T-score of -1 or more while it was -2.5 or less for the osteoporotic group. The inclusion criteria for both the groups were a postmenopausal status, age equal or more than 45 years, no previous history of fractures of any kind, independent mobility before incurring fractures and being on a free diet while those with secondary osteoporosis, diseases associated with increased oxidative stress or with previous or current hormone replacement therapy or antioxidant supplementation were excluded from the study.

Based on these criteria, 60 women who were admitted during September and December 2006 were asked to participate in the study. All 60 women: 40 osteoporotic and 20 controls gave their informed written consent and were enrolled in the study.

A questionnaire administered by a trained interviewer was used to collect information from all subjects related to variables like age, years since menopause, body mass index (BMI), smoking habit and nutritional status (evaluated by means of the Mini Nutritional Assessment questionnaire) (16).

Bone densitometry is the gold standard for the early diagnosis of osteoporosis (17) and dual energy X-ray absorptiometry (DXA) is the preferred technique for its measurement because of it being non-invasive (18), easy to use and associated with a very low radiation exposure. Femoral neck BMD was measured by DXA with a Hologic QRD 1000 scanner (Waltham, MA). The proximal femur has been recommended as the best site for measurement of BMD (17) as compared to other anatomic sites (18).

Measurements for bone-mineral content (BMC) were in g/cm^2 and results were also expressed as T scores for sex-matched young adults. The four general diagnostic categories for women as proposed by a WHO Study Group for measurement of BMD by DXA were applied to define osteoporosis as given below (19).

Normal: Values of BMD within 1 standard deviation of the young adult reference mean (T-score ≥ -1).

Low bone mass (osteopenia): A value of BMD more than 1 standard deviation below the young adult mean, but less than 2 standard deviations below this value (T-score < -1 and > -2.5).

Osteoporosis: A value of BMD, 2.5 standard deviations or more below the young adult mean (T-score ≤ -2.5).

Severe osteoporosis (established osteoporosis): A value of BMD 2.5 standard deviations or more below the young adult mean (T-score ≤ -2.5) in the presence of one or more fragility fractures.

Height and weight for calculation of body mass index (BMI) were taken by a single person using standard techniques for non bedridden patients and for non-ambulatory and/or elderly patients (20) before the start of any medical intervention.

Sample Collection and Assay

All subjects underwent a fasting blood withdrawal on the same day as the bone scan. For plasma, blood was collected in 3 ml heparinized tubes and separated at once and part of the plasma stored at $-70^{\circ}C$. For serum, blood collected in 2 ml plain a tube was let to clot and serum was separated in aliquots after centrifugation and also stored at $-70^{\circ}C$ until analysis of the following:

MDA assay using thiobarbituric acid reactive substances (21) for measuring the peroxidation of fatty acids, membranes and food products. Cu/Zn SOD assay by enzyme linked immunosorbent was used for quantitative detection of human Cu/Zn SOD in plasma (22). Vitamin C was determined by the colorimetric method as redox reaction of ascorbate with 2,6-dichlorophenol indophenol in acid solution involving the reduction of this dye to a colourless leucobase while ascorbate is oxidized to dehydroascorbate (23).

Statistical Analysis

Statistical analysis was carried out using SPSS version 11. All data were reported as mean \pm standard deviation. Variables were compared by independent sample *t* test. Correlation analysis was performed by means of the Pearson's test. Statistical significance was defined as $p < 0.05$.

RESULTS

The study participants were 60 postmenopausal women admitted during September and December 2006 for various kinds of fractures and included 40 subjects in the osteoporotic and 20 in the control group.

Characteristics of cases and controls according to the selected variables are shown in Table 1. The osteoporotic group did not differ significantly ($p < 0.05$) from the control group in terms of variables like age, BMI and years since menopause.

**Table 1**— Descriptive characteristics of the study subjects (mean + SD)

Variable	Subjects		p value
	Osteoporotic (n = 40)	Control (n = 20)	
	Mean + SD	Mean + SD	
Age (years)	66.2 + 5.4	63.6 + 7.9	> 0.05
BMI*	28.4 + 3.3	26.8 + 2.7	> 0.05
Years since menopause	16.2 + 5.6	14.0 + 4.3	> 0.05
Femoral BMD (g/cm ²)	0.58 + 0.05	0.80 + 0.04	< 0.05
Femoral T score	- 2.8 + 0.15	- 0.9 + 0.12	< 0.05

$$*BMI = \frac{\text{Weight in kilograms}}{(\text{Height in meters})^2}$$

Table 2— Selective antioxidant and MDA plasma levels in study subjects (mean + SD)

Variable	Subjects		p value
	Osteoporotic (n = 40)	Control (n = 20)	
	Mean + SD	Mean + SD	
Plasma vitamin C (mg/L)	1.9 + 0.1	10.1 + 0.7	< 0.05
Plasma SOD (ng/ml)	112.5 + 16.9	143.8 + 7.0	< 0.05
Serum MDA (nmol/L)	6.5 + 0.4	4.5 + 0.4	< 0.05

The subjects differed in their mean femoral BMD and T scores ($p < 0.05$) and also the types of fractures. The control group had significant trauma fractures while the osteoporotic group had fragility (minimal or no identifiable trauma) fractures. The sites of fractures among the two groups are as follows. Osteoporotic group: 7 wrist fractures, 6 fractures of neck of femur, 5 supracondylar fractures of femur, 4 intertrochanteric fractures of femur, 4 hip fractures, 2 both bone leg fractures, 6 humerus fractures and 6 ankle fractures. The control group: 4 elbow fractures, 8 ankle fractures and 8 both bone forearm fractures.

Mean plasma levels of vitamin C and SOD were significantly ($p < 0.05$) lower in osteoporotic than in the control group (Table 2). The osteoporotic group had a mean plasma vitamin C lower than the normal physiological value while it was within the normal range for the control group. MDA level (also in Table 2), a marker of lipid oxidative damage was significantly more in the osteoporotic group as compared to the control group ($p < 0.05$).

Table 3— Correlation between selective plasma antioxidants and serum MDA with femoral neck BMD in studied subjects

Antioxidant and MDA	Femoral neck BMD	
	r *	P
Plasma vitamin C	0.91	< 0.01
Plasma SOD	0.65	< 0.01
Serum MDA	- 0.85	< 0.01

*Pearson's correlation coefficient.

Table 4— Correlation between femoral neck BMD and some related factors in studied subjects

	Femoral neck BMD	
	r *	P
Age (years)	-0.22	> 0.05
Postmenopausal period (years)	-0.20	> 0.05
Weight (Kilograms)	- 0.13	> 0.05
Height (Centimeters)	0.01	> 0.05
BMI	- 0.15	> 0.05

*Pearson's correlation coefficient.

Femoral neck BMD showed a significant positive correlation ($p < 0.01$) with plasma levels of vitamin C and SOD while serum MDA had a significant but negative correlation ($p < 0.01$) with femoral neck BMD as shown in Table 3. Negative but non significant correlation was also found between femoral BMD with age, postmenopausal period, weight and BMI (Table 4).

DISCUSSION

Oxidative stress results from weakening of the antioxidant defense or an excess production of ROS and may also result from normal metabolic activity or environmental factors such as diet. ROS-induced oxidative stress has been associated with the pathogenesis of osteoporosis. The major antioxidant defense systems present in the body are SOD, glutathione-s-transferase (GSTs), glutathione-s-peroxidase (GXP) and catalase (24,25).

The role of antioxidants obtained from diet in protection against disease is a topic of continuing interest as well as some controversy (26). Epidemiological studies suggest that certain antioxidants (like vitamin C, E, and β -carotene) may reduce the risk of osteoporosis [24]. It is already established



that several vitamins influence bone turnover, bone mineral density or even the risk of fracture. Deficiency of vitamins C, K and B₁₂ may also be important modifiable risk factors for osteoporosis and bone fracture (27).

This study investigates selected plasma antioxidants like vitamin C as an example of non-enzymatic and SOD as an example of enzymatic anti-oxidants in postmenopausal osteoporotic and non osteoporotic women.

In the present study the osteoporotic group had significantly lower levels of plasma vitamin C and SOD as compared to the control group. These findings are in agreement with a previous study carried out by Maggio et al (10) who also reported that antioxidant defences are markedly decreased in osteoporotic women. In contrast a more recent study done by Wolf et al did not support an independent association between serum concentrations of antioxidants and BMD in postmenopausal women (28).

One of the most damaging effects of ROS is lipid peroxidation, the end product of which is MDA [29]. MDA in addition to serving as an index of lipid peroxidation and a general biomarker of oxidative stress has also been shown to serve as a measure of osteoclastic activity (6,25).

Our study results show serum MDA levels to be significantly higher in the osteoporotic group as compared to non osteoporotic control group. This result are in accordance with a recent similar study by Ozgocem et al. (2007) who found significantly higher MDA levels in osteoporotic compared to non osteoporotic controls (7) and also in line with the study done by Sontakke et al. (2002) who reported an increased level of MDA in a limited sample of post menopausal osteoporotic women (25).

However the study done by Maggio et al did not show any significant difference between osteoporotic and control subjects for MDA (10) and reported that the absence of MDA increase in osteoporotic subjects may be explained by the decreased antioxidants levels which reflect an increased production of reactive oxygen species to such an extent that they are unable to generate high levels of MDA.

Osteoporotic women have been found to have consistently lower vitamin C levels in plasma than controls (10). Our study findings also show that mean plasma vitamin C of the osteoporotic group (1.9 ± 0.1 mg/L) was lower and statistically different ($p < 0.05$) from the mean value of the control group (10.1 ± 0.7 mg/L).

Vitamin C concentration in 35-60 year old adults including post-menopausal women has been shown to be primarily

affected by sex, smoking, obesity and dietary intakes (30). However since all our subjects were of the same female gender, were non-smokers and the mean body mass index (BMI) of the two groups were not statistically different ($p > 0.05$) we can rule out the difference in the mean plasma values of the control and test group to be due to these reasons. It can be inferred that the plasma vitamin C in our study appears to be influenced by dietary intakes. Other studies also show that dietary ascorbic acid is the most important predictor of its serum (30,31) and plasma concentrations in elderly populations (32-34).

Dietary vitamin C deficiency is associated with decreased bone density [5] in addition to be documented as a risk factor for hip fractures in the elderly (12). A positive but insignificant ($p > 0.05$) correlation exists for vitamin C intake and bone mineral content (BMC) in a study on post-menopausal women carried out by Freudenheim et al in 1986 (35).

This study reported a positive and highly significant correlation ($r = 0.91$, $p < 0.01$) between the plasma vitamin C and femoral neck BMD. Our results are in concurrence with the study done by Maggio et al, 2003 which shows a significant and positive correlation ($r = 0.26$, $p < 0.05$) between plasma vitamin C and femoral neck BMD (10).

The present study also shows a positive and highly significant correlation between plasma SOD as an example of enzymatic antioxidant ($r = 0.654$, $p < 0.01$) and femoral neck BMD, while the study done by Maggio et al (10) reported a positive but non significant correlation between the two of them. On the other hand, the study done by Wolf et al in 2005 did not show any correlation between serum concentrations of neither enzymatic nor non-enzymatic antioxidants and BMD (28) while the study done by Yalin et al reported a negative and significant correlation between SOD and lumbar BMD levels in male osteoporosis (36).

The present study also revealed a significant but negative correlation ($r = -0.85$, $p < 0.01$) between MDA and femoral neck BMD among the studied subjects. This result also agrees with previous studies which reported higher levels of MDA as an indicator of oxidative stress among osteoporotic women. On the other hand, the study done by Yalin et al in 2005 did not show any correlation between serum concentrations of MDA and bone turnover markers in male osteoporosis (36).

Consequently, it could be concluded that oxidative stress and decreased antioxidant defenses may have an important role in the pathogenesis of postmenopausal osteoporosis and



MDA, the oxidative stress marker may be an important indicator for bone loss in postmenopausal women. Further studies need to be carried out to investigate the exact role of anti-oxidants in osteoporosis and their promising use as chemopreventive agents.

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