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Evaluation of oxidative stress factors in patients with osteoporosis

Haci Ahmet Deveci[1], Gokhan Nur[2], Hulya Cicek[3], Mahmut Karapehlivan[4]

[1]*Gaziantep University, Vocational School of Higher Education in Islahiye, Department of Health Services and Techniques, Gaziantep, Turkey*
[2]*Gaziantep University, Vocational School of Higher Education in Islahiye, Department of Veterinary, Gaziantep, Turkey*
[3]*Gaziantep University, Medical Faculty, Department of Biochemistry, Gaziantep, Turkey*
[4]*Kafkas University, Medical Faculty, Department of Biochemistry, Kars, Turkey*

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Abstract
Osteoporosis is an important disease with an increasing incidence that leads to serious losses in physical function and adversely affecting the life quality of women especially in postmenopausal period. Age, genetic structure, vitamin D and K abnormalities, estrogen deficiency, chronic inflammation and oxidative stress are considered among the most important risk factors for osteoporosis. Therefore, we aimed to investigate the plasma paraoxonase (PON1) activity, high-density lipoprotein (HDL), total sialic acid (TSA) and total oxidant/antioxidant status (TOS/TAS) of patients with osteoporosis in this study. Comparisons of 25 female patients diagnosed with osteoporosis aged between 51-67 and 10 healthy women between the ages ranged 50-68 were made in this study. PON1 activity, HDL, TSA, TOS and TAS levels were measured by spectrophotometrical method in plasma samples that obtained from blood samples of patients and healthy individuals. Results were expressed as mean ± standard deviation. Plasma PON1 activity, TAS and HDL levels were significantly lower in patiens with osteoporosis than the healty group ($p<0.001$, $p<0.001$ and $p<0.01$ respectively). Also, plasma TSA and TOS levels were higher in patients with osteoporosis compared to the healthy group were found statistically significant ($p<0.01$). Increased reactive oxygen species in patients with osteoporosis may play an important role by causing oxidative stres in the pathogenesis of osteoporosis. Based on oxidative stres, increasing levels of oxidant and reducted levels of antioxidant molecules indicates that may be an important indicator of bone loss in women. Also, higher plasma sialic acid levels in the patients with osteoporosis may be caused by increasing in of sialic acid secretion from the cell membrane surface that based on oxidative tissue damage.

Keywords: Osteoporosis, paraoxonase activity, total sialic acid, total oxidant status, total antioxidant status

Introduction

Osteoporosis (OP) is a disease characterized by the increased brittleness of bone and risk of fractures as a result of low bone density and a deformation in bone microstructure. Osteoporosis has an increasing prevalence in women especially following menopausal period and negatively influences life quality by leading serious losses in physical functions [1-3].

Bone tissue is a complex tissue consisting of various cells such as osteoblast and osteoclast. Osteoblastic and osteoclastic cells are primary actors for reconstruction of bone and repair process. In this process, there is an equilibrium between activities of these two types of cell and this equilibrium is regulated by various hormones and cytokines [4-6]. Today, osteoporosis is one of the most prevalent reasons of fractures in elderly population. While lifelong osteoporotic fracture risk is approximately 35-40% in women older than fifty, this rate is about 15% in men. The effects of free radicals on bone metabolism under physiological and pathological circumstances have been the source of inspiration for new research subjects. Free radicals which are physiologically produced by osteoclasts accelerate deconstruction of calcified tissues and thus are effective on reconstruction process of bone [7]. Age, genetic structure, abnormalities of vitamins D and K, estrogen deficiency, chronic inflammation, oxidative stress are regarded among important risk factors for osteoporosis [8]. There is an equilibrium between oxidant and antioxidant molecules in the living organism and impairment of this balance on behalf of oxidants is defined as oxidative stress. Oxidative stress may cause cellular and tissue damage in the organism. [9]. Previous studies have a correlation between bone mineral density and oxidative stress and oxidative stress was reported to play an important role in development of osteoporosis [10-12].

In this study, plasma paraoxonase (PON1) activity, high density lipoprotein (HDL), total sialic acid (TSA), and total oxidant/antioxidant (TOS/TAS) status in patients with osteoporosis were researched.

Materials and Methods

This study was conducted on 25 patients aged between 51 and 67 years, who were diagnosed with osteoporosis, applied to Gaziantep University Medical Faculty between

***Coresponding Author:** Gokhan Nur, Gaziantep University, Vocational School of Higher Education in Islahiye, Department of Veterinary, Gaziantep, Turkey
E-mail: gokhannur@gantep.edu.tr

January 2015 and August 2015, and 10 healthy individuals aged between 50 and 68 years. Helsinki declaration was adhered to the criteria of the study and all individuals participating in the study were informed about the study and their patient consents were taken. Participants of the study filled the questionnaire including age, gender, height, weight, and body mass index (BMI). Plasma of blood samples which drawn from the participants into tubes with anticoagulant were separated in Medical Biochemistry Laboratory. The samples were portioned appropriately and kept at – 40 °C until the tests were examined. Paraoxonase (PON1) activity, high density lipoprotein (HDL), total sialic acid (TSA), and total oxidant/antioxidant (TOS/TAS) status were examined when samples reached to adequate number.

Measurement of paraoxonase activity: It was performed according to methods of Eckerson [13] and Gülcü [14]. PON1 activity was determined by spectrophotometric analysis of absorbance of colored product at 25 °C and 412 nanometer, yielded by 4-nitrophenol resulting from enzymatic hydrolysis of paraoxone (Sigma) which is used as substrate. For paraoxonase activity in 1 ml serum transforming 1 nmol paraoxone into 4-nitrophenol in 1 minute was defined as unit and results were given as U/L.

Total antioxidant status (TAS) assay: It was determined by using automatic assay method based on bleaching the characteristic color produced by 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical with antioxidants in sample added into media [15]. The results were given as mmol Trolox equivalent/L.

Total oxidant status (TOS) assay: It was determined by using automatic assay method [16]. Oxidants in sample have functions to transform ferrous ion complex to ferric ion. Ferric ion (Fe^{3+}) emerging as a result of oxidation of iron (Fe^{2+}) into more stabile form (Fe_2O_3) forms color with xylenol orange in acidic media. Density of color measured spectrophotometrically is associated with total amount of oxidant molecules found in the sample. Assay was calibrated with hydrogen peroxide (H_2O_2) and the results were given as micro-molar H_2O_2 equivalent (µmol H_2O_2 equiv./L) per liter.

Total sialic acid assay: It was measured colorimetrically by using spectrophotometer (PowerWave XS, BioTek, USA) according to method by Sydow [17] and values were given as mg/dL.

High density lipoprotein (HDL) assay: It was examined in autoanalyzer (Huma Star 600, Germany) by using Biotrol trade mark kit and were given as mg/dL.

Statistical analyses: Statistical evaluation of the obtained data was conducted by using SPSS 20.0 packaged program. Results were expressed as mean value (X) ±, standard deviation (SD). The difference between mean values of group was determined by using student-t test. In the results, $P<0.05$ was accepted as significant at confidence interval of 95%.

Results

Paraoxonase activity, high density lipoprotein, total sialic acid, and total oxidant/antioxidant status were examined in healthy control and individuals diagnosed with osteoporosis. While PON1 activity, HDL, TSA, TAS and TOS values in healthy individuals were determined as 171.5±22.3, 34±2, 67.5±8.1, 2.5±0.3, and 10.5±1.4, respectively; they were determined as 128.6±32.5, 27±4, 73.6±10.3, 1.7±0.4, and 18.7±2.6 in osteoporosis patients, respectively. While plasma PON1 activity, HDL, and TAS in individuals with osteoporosis was significantly lower statistically when compared to control group ($p<0.001$, $p<0.01$, $p<0.001$), TOS and TSA levels were higher ($p<0.01$, $p<0.01$) (Table 1).

Table 1. Values of PON, HDL, TAS, TOS, TSA, in patient and control groups.

	Control Group (Mean±SD) (n:10)	Patient Group (Mean±SD) (n:25)	p <
PON (U/L)	171,5±22,3	128.6±32.5	0,001
HDL (mg/dl)	34±2	27±4	0,01
TAS (mmol Trolox eq/L)	2.5±0.3	1.7±0.4	0,001
TOS (µmol H_2O_2 eq/L)	10.5±1.4	18.7±2.6	0,01
TSA (mg/dl)	67.5±8.1	73.6±10.3	0,01

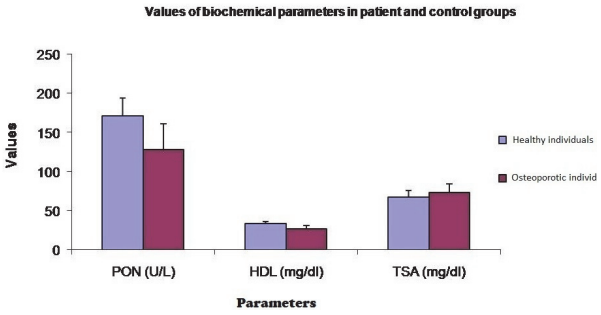

Figure 1. Levels of PON, TSA, and HDL in healthy individuals and individuals with osteoporosis.

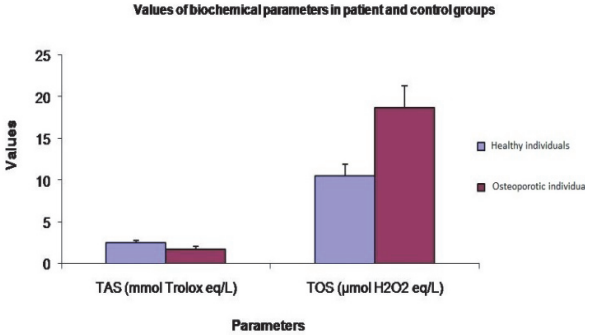

Figure 2. Levels of TAS and TOS in healthy individuals and individuals with osteoporosis.

Discussion

Osteoporosis is a condition that is more prevalent especially in women and in which numerous factors such as life style, feeding style, hormonal balance, and stress are effective. A correlation hasn't been observed between PON1 activity and bone mineral density in 97 healthy postmenopausal Turkish women [18]. A correlation hasn't been determined yet between serum PON1 activity and spinal and femoral bone mineral density in postmenopausal Japanese women [19]. It was determined in a previous study that antioxidant status decreased and oxidative values increased in patients with rheumatoid arthritis [20], in similar studies comparing patients with osteoporosis and healthy individuals, increase of plasma oxidant level was statistically significant; whereas, antioxidant status decreased significantly [10,21]. In the study of Altındağ et al. [22], plasma oxidant and oxidative stress index were notably high but plasma antioxidant level was lower in patients with osteoporosis compared to healthy individuals ($p<0.001$). In the same study, it was found that there was a negative correlation between both the femur bone mineral density and the lumbar bone mineral density by the oxidative stress index separately.

In a study comparing patients with osteoporosis and healthy individuals; it was determined that while total oxidant status in osteoporosis patients was low, oxidative stress index was considerably high [12]. Özgeçmen et al. reported that antioxidant enzyme levels were low in patients with osteoporosis, on the other hand oxidative stress parameters were high [23]. It was declared that glutathione peroxidase (GPx) from antioxidants was considerably low ($p<0.01$), there was no significant difference in superoxide dismutase (SOD) activity, and SOD/GPx ratio increased significantly ($p<0.05$) in elderly Mexicans with osteoporosis [24]. In this study, there was an increase in oxidant parameters and a decrease in antioxidant parameters in knee osteoarthritis patients [25]. In a study conducted on diet and bone durability, they reported that a six-day diet of citrus juice would increase antioxidant capacity and parallelly it might have positive effect on bone mineral density [26].

As parallel to other studies, in our study, based on oxidative stres, consisting of paraoxonase activity in osteoporosis group, total antioxidant capacity and high density lipoprotein were detected significant reductions in levels, while increase in total sialic acid and total oxidant capacity was observed.

Conclusion

Increased reactive oxygen species in patients with osteoporosis may play an important role by causing oxidative stres in the pathogenesis of osteoporosis. Based on oxidative stres, increasing levels of oxidant and reducted levels of antioxidant molecules indicates that may be an important indicator of bone loss in women. Also, higher plasma sialic acid levels in the patients with osteoporosis may be caused by increasing in of sialic acid secretion from the cell membrane surface that based on oxidative tissue damage.

Conflict of interest: The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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