Assessment Of Paraoxonase 1 Activity And Malondialdehyde Levels In Patients With Osteoporosis

Osteoporozlu hastalarda paraoksonaz 1 aktivitesi ve malondialdehid düzeylerinin değerlendirilmesi

Gülden Başkol,
Assistant Prof. MD.,
Department of Biochemistry
Erciyes University Medical Faculty,
gbaskol@erciyes.edu.tr

Hüseyin Demir,
Prof. MD.,
Department of Physical Medicine
Erciyes University Medical Faculty
demirh@erciyes.edu.tr

Banu Çavdaroğlu,
Dr., MD.,
Department of Physical Medicine
Erciyes University Medical Faculty

Mevlüt Başkol,
Assistant, Prof., MD.,
Department of Gastroenterolog
Erciyes University Medical Faculty
mbaskol@erciyes.edu.tr

Derya Koçer,
Dr., MD.,
Department of Biochemistry
Erciyes University Medical Faculty,
deryakocer@yahoo.com

Abstract
Purpose: Oxidative stress may change cellular function in multiple pathological conditions, including osteoporosis. We aimed to determine serum paraoxonase 1 (PON1) activities as known lipid antioxidant and malondialdehyde (MDA) levels, end products of lipid peroxidation, induced by reactive oxygen species (ROS) for evaluating oxidative stress in osteoporotic patients.
Material and Methods:Twenty-six osteoporotic patients were included in the study and compared with healthy controls (n=20). Serum PON1 activity and MDA levels were measured according to an enzymatic spectrophotometric method.
Results:The serum MDA level was higher in the patient group (3.8?1.4 nmol/mL) than controls (1.4?0.4 nmol/mL; p<0.001). PON1 activity was found to be lower in the patients group (141.8?88.4 U/L) than the control group (263.4?99.8 U/L; p<0.001). There was a negative correlation between MDA levels and PON1 activities (r=-0.495 , p?0.001).
Conclusion:Increased ROS levels in osteoporotic patients may result in a pro-oxidation environment, which in turn could result in decreased antioxidant PON1 activity and increased MDA levels. As a result, lipid peroxidation may have a role in the pathogenesis of the osteoporotic patients. Since PON1 is also an antioxidant agent, effective antioxidant therapy to inhibit lipid peroxidation is necessary and agents to increase PON1 activity may be a therapeutic option in osteoporotic patients.
Key Word: **Lipid Peroxidation, Malondialdehyde, Oxidative stress, Paraoxonase.**

Özet:
Amaç: Oksidatif stres, osteoporozu da içeren pek çok patolojik durumda hücresel fonksiyonları değiştirebilir. Biz, osteoporozlu hastalarda, oksidatif stresi değerlendirmek için, reaktif oksijen türleri (ROS) ile indüklenen, lipid peroksidasyonu son ürünü malondialdehit (MDA) düzeylerini ve lipid antioksidanı olarak bilinen serum paraoksonaz 1 (PON1) aktivitesini belirlemeyi amaçladık.
Gereç ve Yöntemler: Osteoporozlu 26 hasta çalışmamıza dahil edildi ve sonuçlar sağlıklı kontrol grubu ile karşılaştırıldı (n=20). Serum PON 1 aktivitesi ve MDA düzeyleri enzimatik spektrofotometrik metot ile çalışıldı.
Bulgular:Serum MDA düzeyleri hasta grubunda (3.8±1.4 nmol/mL), kontrol grubuna göre (1.4±0.4 nmol/mL; p<0.001) daha yüksek bulundu. PON1 aktivitesi ise hasta grubunda (141.8±88.4 U/L), kontrol grubuna göre (263.4±99.8 U/L; p<0.001) daha düşüktü. MDA düzeyleri ile PON1 aktivitesi arasında istatistiksel olarak anlamlı negatif korelasyon (r=-0.495) bulundu (Pearson's korelasyon analizi; p<0.001).
Sonuç: Osteoporozlu hastalarda artmış ROS düzeyleri, pro-oksidan bir durum oluşturarak, PON1 aktivitesinin azalıp, MDA düzeylerinin yükselmesine neden olur. Sonuç olarak, osteoporoz patogenezinde lipid peroksidayonu rol oynayabilir. Bu hastalarda, lipid peroksidasyonunu önleyen, etkili bir antioksidan tedavi gereklidir. PON1 antioksidan bir enzim olduğundan, serum PON1 aktivitesini artıran ajanlar, osteoporozlu hastalar için tedavi seçeneği olabilir.
Anahtar Kelimeler: **Lipid peroksidasyonu, malondialdehit, oksidatif stres, paraoksonaz.**

Submitted : October 13, 2006
Revised : January 24, 2007
Accepted : February 24, 2007

Corresponding Author:
Gülden Başkol,
Department of Biochemistry
Erciyes University Medical Faculty
Kayseri, Turkey

Telephone : +91 -xxx
E-mail : gbaskol@erciyes.edu.tr

Introduction

In recent years, reactive oxygen species (ROS) are considered to be responsible for ageing process and in a number of pathological condition such as atherosclerosis, carcinogenesis and infarction (1, 2). The role of ROS in bone metabolism is unique and dual considering their effect under physiological and pathological conditions (3). Under physiological conditions, the production of ROS by osteoclasts assists in accelerating destruction of calcified tissue and hence assists in bone remodeling (4, 5). Not only might osteoclastic differentiation of bone precursors be modulated by ROS, but osteoblastic differentiation as well. Mody et al. (6) have shown that oxidative stress is able to inhibit bone cell differentiation of a preosteoblastic cell line and of a marrow stromal cell line that undergoes osteoblastic differentiation. Enhanced osteoclastic and depressed osteoblastic activity is attributable to primary estrogen deficiency characteristic of osteoporosis (7).

When bone fractures occur, a remarkably high yield of radicals is generated. It is suggested that as a "break in the bone" is generated, the minimal crystallites separate at grain boundaries with no major chemical changes, but the tightly bound collagen strands running through the mineral phase are forced to break homolytically. However, though enhanced osteoclastic activity and increased production of ROS are linked in many skeletal pathologies, it remains to be studied whether increased ROS production overwhelms the antioxidant defenses, subjecting the individual to hyperoxidant stress (7, 8).

ROS are formed in oxidative processes that normally occur at relatively low levels in all cells and tissues. ROS are highly reactive molecules that, when present in excess, overwhelm the protective systems and results in cell damage and lipid peroxidation (8, 9).

Lipid peroxidation is a well-established mechanism of cellular injury in human, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, which is the most abundant malondialdehyde (MDA). Therefore, measurement of MDA is widely used as an indicator of lipid peroxidation (10, 11). Increased levels of lipid peroxidation products have been associated with a variety of diseases in both humans and model systems (12).

Recently, it has been demonstrated that oxidized lipids might exert their adverse effects on bone by targeting osteoclastic cells as well as osteoblastic cells. Osteoporotic bone loss is in part due to enhanced bone resorption as a result of increased osteoclastic activity (13). Morever, in bone and bone osteoblasts, osteoblastic differentiation is inhibited by oxidized lipids (14, 15).

There are various known antioxidant systems against oxidative stress including paraoxonase (PON1). PON1 is an antioxidant enzyme on high-density lipoprotein (HDL) that hydrolyses lipid peroxides in oxidized lipoproteins (16). PON1 protects low density lipoprotein (LDL) and HDL from oxidation induced by either copper ion, or free radical generator (17). PON1 activity has been suggested to be inversely associated with oxidative stress in serum and macrophages (18). In addition, we have previously shown that PON1 activity was decreased in some diseases due to ROS pathogenesis under oxidative stress and inflammation condition, such as rheumatoid arthritis, Age-related macular degeneration, Ulcerative colitis, Steatohepatitis and Behcet's disease (19-23). MDA has not been searched together with the antioxidant enzyme PON1 in osteoporotic patients. In the present study, we aimed to determine serum PON1 activities as known lipid antioxidant and MDA levels, end products of lipid peroxidation, induced by ROS for evaluating oxidative stress in osteoporotic patients.

Materials and Methods

Subjects

Twenty-six patients with Osteoporosis were included to the study. Mean (SD) age was 57.613.8 years. The osteoporosis was diagnosed as recommended by the WHO (24). The patients were compared with 20 normal healthy controls, who were matched for age (mean age 55.512) and sex. Informed consent was obtained from patients and controls prior to the study. The study protocol and the procedures were approved by Erciyes University Ethical committee and were in accordance with the Helsinki Declaration of 1975.

Chemicals

All reagents were purchased from Sigma (Sigma-Aldrich Corp, St. Louis, MO, USA) and Merck (Merck KgaA, Darmstadt, Germany). Blood samples were obtained after an overnight fast and serum immediately separated. Serum samples were stored –70 C until analysis.

Measurement

Measurement of Serum PON1 Activity

Serum PON1 activity was measured according to a method described elsewhere (25). The rate of hydrolysis of paraoxon was assessed by monitoring the increase of absorbance at 405 nm and at 25C. The basal assay mixture included 1.0 mM paraoxon and 1.0 mM $CaCl_2$ in 0.05 M glycine buffer pH 10.5. One unit (IU) of PON1 activity is defined as 1mol of p-nitrophenol formed per minute, and activity was expressed as U/L of serum.

Measurement of Serum MDA Levels

Serum MDA levels were measured according to a method described elsewhere (26). The principle of the method is based on the spectrophotometric measurement of the color produced during the reaction of thiobarbituric acid (TBA) with MDA. Concentration of TBA reactive substances was calculated by the absorbance coefficient of malondialdehyde-thiobarbituric acid complex and expressed in nmol/ml. MDA bis (dimethyl acethal)-TBA complex was used as standart for the evaluation of results.

Statistical Analyses

Statistical evaluation was carried out with the SPSS® 11.0 (Statistical Packages for Social Sciences; SPSS Inc, Chicago, Illinois, USA). Data obtained from the study groups were compared by Student's-t test. Correlation analyses between variables were made by Pearson's test; p value less than 0.05 was considered as statistically significant. All the results were expressed as "mean with their standard deviation" (mean ± SD).

Results

All data were subjected to the Kolmogrov-Smirnov test for normality and are presented as mean ± SD. There was no statistically significant difference of age and sex distribution between patients with OP (4 men, 22 women mean age ± SD: 57.6±13.8 years) and control (4 men, 16 women mean age±SD: 55.5 ± 12.0 years) groups (p0.05).

Serum MDA level was higher in the patient group (3.81.4 nmol/mL) than controls (1.40.4 nmol/mL; p<0.001). PON1 activity was found to be lower in the patients group (141.888.4 U/L) than the control group (263.499.8 U/L; p<0.001). There was a negative correlation between MDA level and PON1 (r=-0.495) activity with a statistical significance (Pearson's correlation analysis; p0.001). The results are presented in table I and figure 1.

Discussion

ROS molecules are highly reactive and can attack almost every cell component, causing further damage to the surrounding tissues. When free radicals are produced in excess to the capacity of the body to neutralize them, a condition of oxidative stress takes place. Oxidative stress, defined as an imbalance between antioxidants and prooxidants in favor of the former, potentially leading to damage, generally implies that antioxidants are low and markers of oxidative damage are increased (27, 28).

ROS play a crucial and possibly causative role in the pathogenesis of a number of acute and chronic diseases, such as inflammation, cancer, liver injury, atherosclerosis and osteoporosis (1, 2).

In this study we measured serum MDA as a marker of free radical-mediated lipid peroxidation and found significantly elevated levels of MDA in the sera of osteoporotic patients were observed in the present study. Similar results have been reported (29). In this study, MDA in addition to serving as an index of lipid peroxidation has also served as a measure of osteoclastic activity.

The original observation suggesting that lipid peroxidation might play a role in bone cell function was the inhibition

Table I. Serum MDA levels and PON1 activity in patients with osteoporosis and control.

Parameters	Patients with Osteoporosis	Control	P
N	26	20	
MDA (nmol/mL)	3.8±1.4	1.4±0.4	0.001*
PON 1 (U/L)	141.8±88.4	263.4±99.8	0.001*
Age (years)	57.6±13.8	55.5±12	0.595

*Serum MDA level was higher (p=0.001) whereas PON1 activity was lower (p=0.001) in the patient group than the controls.

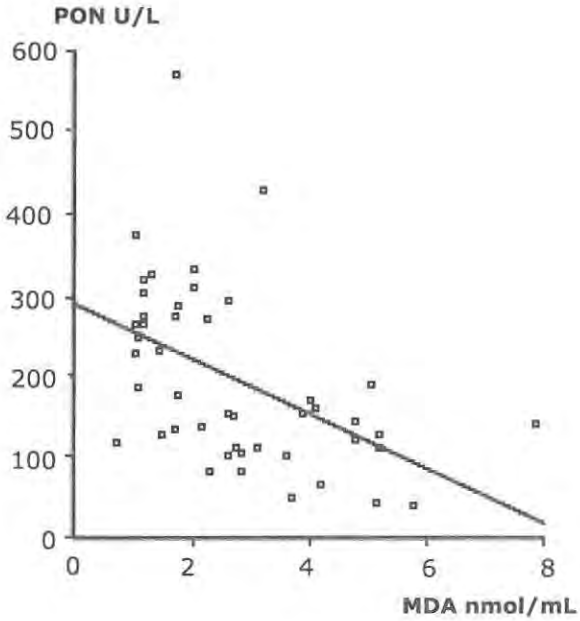

Figure 1. Correlation between MDA levels and PON1 activities.

of osteoblastic differentiation upon treatment with certain oxidized lipid and lipoproteins (30-32).

Lipids have been shown to accumulate in bones of mice and around bone vessels in patients with osteoporosis (33). Because the immature osteoblasts are located immediately adjacent to the subendothelial matrix of bone vessels, lipid accumulation in subendothelial matrix would be expected to inhibit differentiation of the bone-forming cells. In addition, because oxidized lipids induce endothelial expression of monocyte chemotactic factors a potent inducer of osteoclastic differentiation, oxidized lipids would be expected to promote bone resorption by recruitment and differentiation of osteoclast precursor cells (34).

Osteoporosis is in part a result of diminished bone formation by osteoblasts with aging. Thus, similar to the artery wall, lipoproteins and lipids accumulate in bone and undergo oxidation (35) and they may affect the cellular constituents of bone including the osteoblastic cells and inhibit their proper bone-forming activity. This seems quite plausible since bone contains a significant number of blood vessels, with cellular constituents of bone located in close proximity to the interwoven vascular beds, and accumulation of lipids in the osteons of human osteoporotic bone has been demonstrated (33-35).

We found decreased serum PON1 activity in osteoporotic patients in comparison to healthy controls. The mechanism of the observed decrease in serum PON1 activity in osteoporotic patients remains unclear.

This decrease could be related to enhanced lipid peroxidation, since oxidized lipids are reported to inhibit paraoxonase activity. Increased inactivation of PON1 according to increased generation of ROS in osteoporotic patients can explain the decrease in serum PON1 activity (16).

In addition, we have previously shown that PON1 activity was decreased in some diseases due to ROS pathogenesis under oxidative stress and inflammation condition (19-23).

We used MDA as an indicator of oxidative stress; observed increase in MDA may be related to decreased PON1. As to our knowledge, this is the first study, which determines together with MDA level and PON1 activity in osteoporotic patients. In the present study, we found a negative correlation between PON1 activity and MDA levels. This evidence also supports our hypothesis.

It was reported that protection against LDL oxidation is accomplished by PON1 inactivation and the authors attributed this to the interaction between the free sulfhydryl group of PON1 and specific oxidized lipids in oxidized LDL (16).

The effects of oxidized lipids on bone cells may be caused through direct interactions with the cells via receptor-mediated responses and/or through generation of other inflammatory factors such as cytokines that may then produce the observed effects of the oxidized lipids on bone cells. Oxidized lipids induce the expression of cytokines both in vitro and in vivo (30, 36-38).

Kumon et al reported that serum paraoxonase activity was down-regulated by interleukins (39). There are also studies indicating that these parameters are increased in sera of osteoporotic patients (36). Consequently, decrease in PON1 in sera of osteoporotic patients may be related to the increase of these cytokines.

PON1 activity may also be altered as part of the inflammatory response. Van Lenten et al. (40) showed that HDL became proinflammatory during the acute phase response, possibly due to loss of PON1 activity from

HDL. Moreover, Feingold et al. (41) showed a decrease in serum PON1 activity and hepatic PON1 mRNA in Syrian hamsters injected with lipopolysaccharide to induce the acute phase response.

In conclusion, increased ROS levels in osteoporotic patients may result in a pro-oxidation environment, which in turn could result in decreased antioxidant PON1 activity and increased MDA levels. As a result, lipid peroxidation may have a role in the pathogenesis of the osteoporotic patients. Since PON1 is also an antioxidant agent, effective antioxidant therapy to inhibit lipid peroxidation is necessary and agents to increase PON1 activity may be a therapeutic option in osteoporotic patients.

References

1-Halliwell B, Gutteridge JM. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. Lancet 1984; 23: 1396–1397.

2-Nohl H. Involvement of free radicals in ageing: a consequence or cause of senescence. Br Med Bull 1993; 49: 653–667.

3-Kasten TP, Collin-Osdoby P, Patel N, et al. Potentiation of osteoclast bone resorptive activity by inhibition of nitric oxide synthase. Proc Natl Acad Sci USA 1994; 91: 3569–3573.

4-Silverton SF, Mesaros S. Osteoclast radical interactions: NADPH causes pulsatile release of NO and stimulates superoxide production. Endocrinology 1995; 136: 5244–5247.

5-Yang S, Ries WL, Key LL. NADP oxidase in the formation of superoxide in osteoclasts. Calcif Tissue Int 1998; 63: 346–350.

6-Mody N, Parhami F, Sarafian TA, Demer LL. Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. Free Radical Biol Med 2001; 31: 509–516.

7-Raisz LG. The osteoporosis revoluation. Ann Intern Med 1997; 126: 458-462.

8-Gutteridge JM. Free radicals in disease processes: a compilation of cause and consequence. Free Radic Res Commun 1993; 19: 141-158.

9-Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet 1994; 344; 721-724.

10-Gambhir JK, Lali P, Jain AK. Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis. Clin Biochem 1997; 30: 351-355.

11-Draper HH, Hadley M. A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. Xenobiotica 1990; 20: 901-907.

12-Romero FJ,Bosch Morell B, Romero MJ, et al. Lipid peroxidation products and antioxidants in human disease. Environ Health Perspect 1998; 106: 1229-1234.

13-Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 2000; 21: 115–137.

14-Parhami F, Morrow AD, Balucan J, et al. Lipid oxidation products have opposite effects on calcifying vascular cell and bone cell differentiation. A possible explanation for the paradox of arterial calcification in osteoporotic patients. Arterioscler Thromb Vasc Biol 1997; 17: 680–687.

15-Parhami F, Jackson SM, Le V, et al. Atherogenic diet and minimally oxidized low density lipoprotein inhibit osteogenic and promote adipogenic differentiation of marrow stromal cells. J Bone Miner Res 1999; 14: 2067–2078.

16-Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its function: a possible peroxidative role for paraoxonase. J Clin Invest 1998; 101: 1581-1590.

17-Gan KN, Smolen A, Eckerson HW, La Du BN. Purification of human serum paraoxonase/arylesterase: evidence for one esterase catalyzing both activities. Drug Metab Dispos 1991; 19: 100-106.

18-Rozenberg O, Rosenblat M, Coleman R, Shih DM, Aviram M. Paraoxonase (PON1) deficiency is associated with increased macrophage oxidative stress: studies in PON1-knockout mice. Free Radic Biol Med 2003; 34: 774-784.

19-Baskol G, Demir H, Baskol M, et al. Assessment of paraoxonase 1 activity and malondialdehyde levels in patients with rheumatoid arthritis. Clin Biochem 2005; 38: 951-955.

20-Baskol G, Karakucuk S, Oner AÖ, et al. Serum paraoxonase 1 activity and lipid peroxidation levels in patients with age related macular degeneration. Ophthalmologica 2006; 220: 12-16.

21-Baskol G, Baskol M, Yurci A, Ozbakır O, Yucesoy M. Serum paraoxonase 1 activity and malondialdehyde levels in patients with ulcerative colitis. Cell Biochem Funct 2006; 24: 283-286.

22-Baskol M, Baskol G, Deniz K, Ozbakır O, Yucesoy M. A new marker for lipid peroxidation: serum paraoxonase activity in non-alcoholic steatohepatitis. Turk J Gastroenterol 2005; 16: 119-123.

23-Karakucuk S, Baskol G, Oner A, Baskol M, Mirza E, Ustdal M. Serum paraoxonase activity is decreased in the active stage of Behcet's disease. Br J Ophthalmol 2004; 88: 1256-1258.

24-World Health Organization Study Group. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis.Technical report series 843. 1994, Geneva, WHO.

25-Eckerson HW, Romson J, Wyte C, La Du BN. The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. Am J Hum Genet 1983; 35: 214-227.

26-Jain SK. Evidence for membrane lipid peroxidation during the in vivo aging of human erythrocytes. Biochim Biophys Acta 1998; 937: 205-210.

27-Sies H. Oxidative stress: introductory remarks. In: Sies H (ed); Oxidative stress. Academic Press, London 1985, 1–8.

28-Polidori MC, Stahl W, Eichler O, Niestroj I, Sies H. Profiles of antioxidants in human plasma. Free Radic Biol Med 2001; 30: 456–462.

29-Maggio D, Barabani M, Pierandrei M, et al. Marked decrease in plasma antioxidants in aged osteoporotic women: results of a cross-sectional study. J Clin Endocrinol Metab 2003; 88:1523-1527.

30-Parhami F. Possible role of oxidized lipids in osteoporosis: could hyperlipidemia be a risk factor? Prostaglandins Leukot Essent Fatty Acids 2003; 68: 373-378.

31-Parhami F, Demer LL. Arterial calcification in face of osteoporosis in ageing: can we blame oxidized lipids?. Curr Opin Lipidol 1997; 8: 312–314.

32-Boukhris R, Becker KL. Calcification of the aorta and osteoporosis. JAMA 1972; 219; 1307–1311.

33-Rajendran KG, Chen SY, Sood A, Spielvogel BF, Hall IH. The anti-osteoporotic activity of amine-carboxyboranes in rodents. Biomed Pharmacother 1995; 49: 131–140.

34-Parhami F, Garfinkel A, Demer LL. Role of lipids in osteoporosis. Arterioscler Thromb Vasc Biol 2000; 20: 2346-2348.

35-Navab M, Fogelman AM, Berliner JA, et al. Pathogenesis of atherosclerosis. Am J Cardiol 1995; 76: 18–23.

36-Manolagas SC, Jilka RL. Bone marrow cytokines and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. N Engl J Med 1995; 332: 305–311.

37-Theill LE, Boyle WJ, Penninger JM. RANK-L and RANK: T cells bone loss and mammalian evolution. Annu Rev Immunol 2002; 20: 795–823.

38-Ershler WB, Harman SM, Keller ET. Immunologic aspects of osteoporosis. Dev Comp Immunol 1997; 21: 487–499.

39-Kumon Y, Nakauchi Y, Suehiro T, et al. Proinflammatory cytokines but not acute phase serum amyloid A or C-reactive protein, down regulate paraoxonase 1 (PON1) expression by Hep G2. Amyloid 2002; 9: 160- 164.

40-Van Lenten BJ, Wagner AC, Nayak DP, Hama S, Navab M, Fogelman AM. High-density lipoprotein loses its anti-inflammatory properties during acute influenza a infection. Circulation 2001; 103: 2283-2288.

41-Feingold KR, Memon RA, Moser AH, Grunfeld C. Paraoxonase activity in the serum and hepatic mRNA levels decrease during the acute phase response. Atherosclerosis 1998; 139: 307-315.