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Original Article

Evaluation of the serum level of osteoprotegerin and bone mineral density in postmenopausal women

Hossein Abdollahi Veshnavei

School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

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Abstract: Objectives: Women might face different issues after menopause. Reduction in bone mineral density (BMD) is one of these problems that put a heavy burden on the healthcare system, especially in developing countries. Studies assume that along with increased age, lack of physical activity and hormonal issues, some other factors might take part in this process. Osteoprotegerin (OPG) is one of the assumed factors. Here we aimed to assess the relation between serum levels of OPG and BMD in postmenopausal women. Methods: In this prospective cross-sectional study, 90 postmenopausal women were entered. Our cases were divided based on former medical documents into two groups of osteoporotic women (n=45) and healthy women (n=45). All cases were then analyzed using Dual-energy X-ray absorptiometry (DXA) and BMD and T-score were assessed for each case in different sites. Serum levels of OPG were also assessed using Enzyme-linked immunosorbent assay (ELISA). Data were then analyzed using SPSS software. Results: There were higher OPG levels in osteoporotic women compared with healthy women (P<0.001). We also indicated a significant difference in BMD between two groups of postmenopausal women in different sites (i.e. lumbar vertebrae L2-L4, trochanters, femoral neck and hip). We showed there is a reverse relation between serum OPG levels and BMD in lumbar vertebrae (r=-0.4, P=0.002), hip (r=-0.3, P=0.03) and femoral neck (r=-0.3, P=0.02) in both groups. There is a reverse relation between BMD and serum levels of OPG in postmenopausal women. Conclusion: OPG levels are higher in osteoporotic women and have a reverse relation with BMD.

Keywords: Osteoprotegerin, bone mineral density, post menopause

Introduction

Osteoporosis is a systemic skeletal disease and a prevalent problem associated with decreased bone density and bone tissue [1]. Different lines of evidence declared that osteoporosis could be related to thinning of bones in the lumbar vertebrae and femoral neck and increased fractures in these sites [2, 3]. Osteoporosis is more prevalent among elderly people and especially women and puts a heavy burden on governments [2]. Studies indicate that osteoporosis has a higher prevalence among women older than 50 years of age especially postmenopausal women [4, 5]. Data also show that one in every 4 women and 8 men older than 50 years might deal with osteoporosis. It has been also declared that almost 50% of women and 30% of men would experience at least one episode of osteoporosis-related bone fracture in their life [6]. The chance of further fractures is higher in patients with a former fracture due to osteoporosis [7].

Osteoporosis is known to be a skeletal disease associated with decreased bone density and a higher probability of fractures. The economic and healthcare burden of osteoporosis is as high as myocardial infarction, stroke, and cancer [8, 9]. Epidemiologic studies showed that there are differences among the prevalence of osteoporosis among different societies. In postmenopausal women, 30% of osteoporosis occurs in the hip, lumbosacral vertebrae or distal of the radius [2, 10].

Bone density in women in almost every age group is significantly lower than men of the same age and race. The decrease in bone density after 40 years of age is less than 1% each year and in postmenopausal women is 2%. This rate would be 3-9% after almost 6 years after menopause, 50% of trabecular bones and 30% of cortical bones would be affected [11].

As spoken above, reduced bone density is an important factor for osteoporosis [12]. Different

lines of evidence declared that genetic factors play pivotal roles in the pathogenesis of osteoporosis. Osteoprotegerin (OPG), vitamin D receptors, type 1 collagen (COL1A1) and transforming growth factor-beta 1 (TGFB1) are known as influencing genes on reduction of bone density and as a result, osteoporosis [13-15]. OPG is a subgroup of tumor necrosis factor-alpha (TNF-a) family which produced by osteoblasts, basic mesenchyme cells, endothelial cells, adipocytes and mesenchyme bone marrow cells [16]. The effects of OPG are mediated through reduced production and differentiation of osteoclasts followed by reduced bone resorption [17]. OPG acts in competition with Receptor activator of nuclear factor kappa B (RANK), a receptor expressed on osteoclasts and dendritic cells, and with its own receptor which is receptor activator of nuclear factor kappa-B ligand (RANKL) [16, 18, 19].

OPG could be a marker that is increased following osteoporosis and injuries in vessels [20]. There have also been studies that declared a significant positive relationship between age and OPG levels which could be assessed as a compensatory mechanism against bone resorption. Some studies on animals declared that lack of OPG is associated with increased calcification in media of aorta and also osteoporosis in rats. There are also some paradoxical reports on the association of OPG and osteoporosis. As a result, we aimed to investigate this relation in the present study.

Methods and material

Study design

This prospective cross-sectional study was performed in 2019 in Imam Khomeini hospital, Tehran. The study was approved by the ethical committee of Tehran University of Medical Sciences (Ethics code: IR.TUMS.MEDICINE.REC.1392.202).

Inclusion and exclusion criteria

In this study, 90 post-menopausal women were included. These cases were referred to our centers for either a screening of bone mineral density (BMD) or routine clinical checkup. The inclusion criteria were being menopause for at least one year, having access to their clinical documents, absence of any disease which could influence on bone density. The exclusion criteria were histories of cardiovascular diseases, histories of treatments with immunosuppressants, having hyperthyroidism, diabetes mellitus or chronic renal or liver diseases. We should note that informed consent was signed by all patients.

Study population

A total of 90 patients were recruited based on inclusion and exclusion criteria and were divided based on their previous clinical data into two groups of previously diagnosed osteoporotic women (n=45) and healthy women (n=45). It should be noted that we had access to the past medical documents of all these women. Based on their previous bone densitometry documents, patients were assigned to previously diagnosed osteoporotic or normal groups.

Osteoporosis and data assessments

Osteoporosis was considered as T-score ≤-2.50 and normal groups had T-score ≥-1.00. Patients in both groups were matched based on age.

The T-score was assessed with a DXA scanner (model 4500C; Hologic Inc, Bedford, Massachusetts). Scans evaluated overall BMD of the hip, femur and vertebrae and in each dimension, 4 points were assessed to evaluate the T-score. The T-score was obtained for each individual as well as for the region.

At the beginning of our study, patients were examined for measurements for height and weight. Body mass index (BMI) was then measured for every patient. Bone density was then assessed for patients by DXA method using Norland XR-800 apparatus (USA) in lumbar vertebrae (L2-L3 and L4), the neck of femur and left hip.

Bone density and OPG assessments

Bone density assessment results were reported in both T-score and BMD. BMD was also measured and reported based on g/cm^2 unit. Fasting blood samples (8 am) were extracted from all of the patients. Sera of patients were collected in three microtubes of 0.2 ml.

OPG levels were measured using Biospes kits and Sandwich enzyme-linked immunosorbent assay (ELISA) method. The mean OPG levels

Table 1. Demographic data of the post-menopausal women

Variable	Group		*P*-value
	Healthy (n=45)	Osteoporotic (n=45)	
Age (years ± SD)	59.8±2.8	60.3±1.7	0.120
Years after menopause (years ± SD)	6.7±3.2	6.9±4.2	0.100
BMI (kg/m^2)	28.36±1.72	27.14±2.69	0.150

Table 2. Mean BMD in different sites and serum OPG levels

Variable		Group		*P*-value
		Healthy (n=45)	Osteoporotic (n=45)	
Hip BMD	Density (g/cm^2 ± SD)	985.50±121.76	656.40±66.88	P<0.001
	T-score (mean ± SD)	-0.26±0.75	-2.65±0.46	P<0.001
Femoral neck BMD	Density (g/cm^2 ± SD)	894±135.7	618±66.5	P<0.001
	T-score (mean ± SD)	-0.37±1.16	-3.13±0.54	P<0.001
Vertebrae BMD	Density (g/cm^2 ± SD)	1119.2±174.4	706.3±75.1	P<0.001
	T-score (mean ± SD)	-0.123±0.83	-2.61±0.5	P<0.001
OPG levels (Pmol/l ± SD)		111.45±53.43	138.08±82.75	P<0.001

in general populations are 50.83±10.47 pg/ml. The OPG levels increase by age. Increased OPG levels are observed in conditions that induce bone resorptions and could cause osteoporosis such as hemodialysis and Cushing syndrome.

Statistical analysis

The obtained data were entered into the Statistical Package for Social Sciences (SPSS) (version 24, SPSS Inc., Chicago, IL). Quantitative data were reported as mean ± standard deviation and qualitative data as frequency distribution (percentage). Independent t-test, Chi-square was used to analyze the data. *P*-value <0.05 was considered as significance threshold.

Results

Here we investigated 90 post-menopausal women and evaluated serum OPG and BMD. Our data showed that there was no significant relationship between two groups regarding mean age, time of being menopause and BMI (**Table 1**).

Furthermore, we showed that mean BMD in different sites (vertebrae, femoral neck, and hip) is significantly lower in postmenopausal group with osteoporosis compared with non-osteoporotic group (P<0.05). We also showed that a significant difference exist between the two groups regarding OPG measurements. We showed that women with lower BMD had higher OPG levels. These data are summarized in **Table 2**.

Further analysis also indicated that there is a reverse relation between amounts of OPG and BMD in lumbar vertebrae (r=-0.4, P=0.002) (**Figure 1**). Same reverse relations were found between OPG levels and BMD in hip (r=-0.3, P=0.030) and femoral neck (r=-0.31, P=0.020) (**Figures 2** and **3** respectively). We also showed that the relation between OPG levels and BMD in vertebrae was greater than the relation with BMD in the hip and femoral neck. This issue puts emphasis on the relation between OPG levels and BMD in vertebrae.

Discussion

In this cross-sectional study, we included 90 post-menopausal women and assessed their serum OPG levels and BMD. Patients were entered based on inclusion and exclusion criteria. We showed that patients with lower BMD have higher OPG levels. We also indicated that there is a significant reverse relation between OPG levels and BMD which is most significant in vertebrae. OPG was first described in 1997 as a secretory glycoprotein resulted from a single-copy gene which has 5 exons and 29 pairs of kb [21]. This gene is located in the No.9 chro-

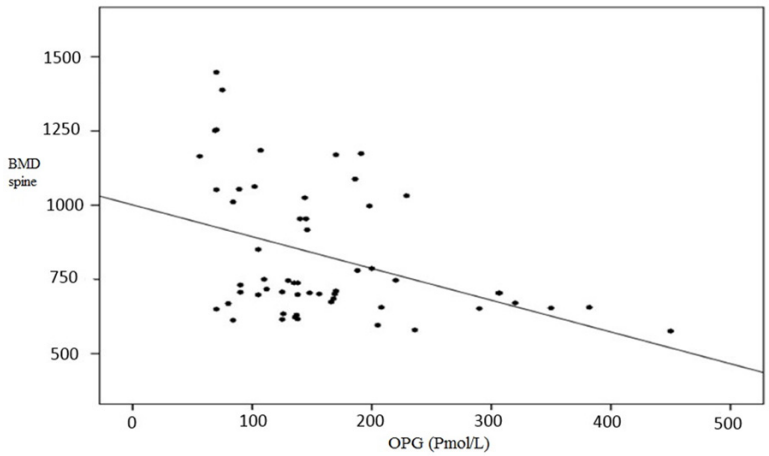

Figure 1. Relation between amounts of OPG and BMD in lumbar vertebrae (r=-0.4, P=0.002).

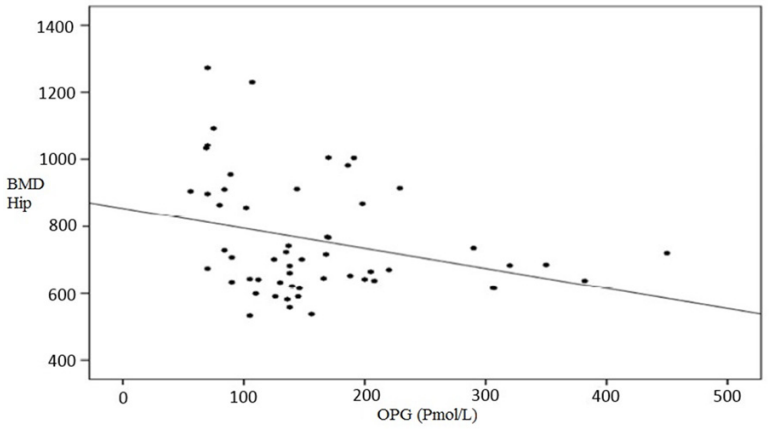

Figure 2. Relation between amounts of OPG and BMD in hip (r=-0.3, P=0.030).

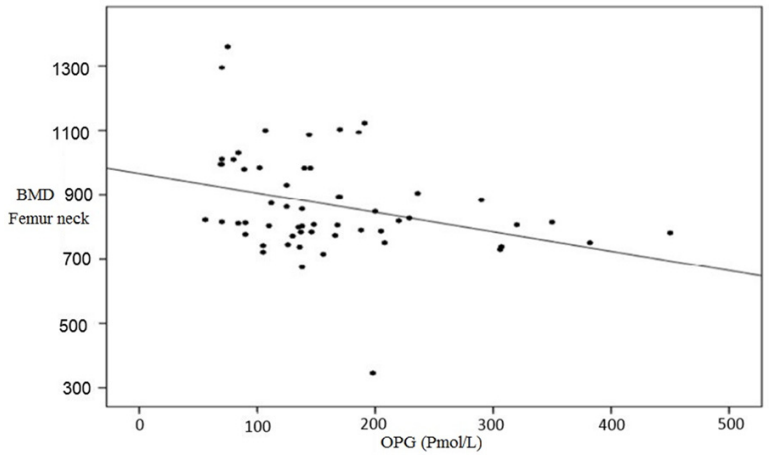

Figure 3. Relation between amounts of OPG and BMD in femoral neck (r=-0.31, P=0.020).

mosome in humans. This gene also encodes Core-binding factor alpha-1 (CBFA-1) which increases osteoblasts production and activities [22, 23]. Based on the evidence, higher levels of OPG could be associated with reduced bone resorption and osteoporosis. But here in this report, we showed that OPG levels are increased in patients with osteoporosis [24].

Here we showed that OPG levels are higher in osteoporotic women and have a reverse relation with BMD. This issue could have high clinical and mostly predictive importance. Due to the high prevalence of osteoporosis and the related problems such as fractures, early non-invasive assessment tools might help physicians for early diagnosis of osteoporosis. We also believe that OPG could have higher value in post-menopausal women and larger studies should be conducted in this regard.

The association of OPG and BMD is discussed in various reports but less attention has been given to OPG levels in post-menopausal women. In a study performed in 2011 by Jabbar and colleagues in England, they evaluated 370 post-menopausal women and compared their BMD and OPG levels along with other factors including RANKL, interleukin-6 (IL-6), sex steroids, calciotropic hormones and biochemical markers of bone turnover. In the end, they concluded that OPG levels were higher in women with osteoporosis. They also declared that OPG levels are inversely

related to BMD and contribute to the development of osteoporosis in postmenopausal women [25]. These data are completely in line with our results. Here we indicated higher levels of OPG are detected in post-menopausal women and these levels have significant reverse relation with BMD. As spoken above, we assumed that OPG along with RANKL cause reduced bone resorption but here we showed higher OPG levels in osteoporotic women and a reverse relation between OPG and BMD. We assume that this issue might be due to a reflexive or responsive elevation in OPG levels in patients with reduced BMD. As Ostrowska and others declared, higher OPG levels might compensate for excessive bone resorption. In their study, they evaluated 91 females with anorexia nervosa and 29 healthy controls and indicated higher levels of OPG in patients with anorexia nervosa which was associated with bone markers suppression and possible osteoporosis [26]. Another study by Pobeha and others was performed in 2011 in Slovakia. They evaluated the pathogenesis of osteoporosis in 39 patients with chronic obstructive pulmonary disease (COPD). They declared that OPG expressions are related to osteoporosis in patients with COPD [27]. These results are also in line with our data and together, put emphasis on the increased levels of OPG in chronic diseases which might cause osteoporosis.

Furthermore, Loureiro and others investigated BMD and OPG expressions in children and adolescents with type 1 diabetes and showed that a low BMD in these patients is associated with not only increased OPG expression but also a poor glycemic control compared with controls [28]. In a meta-analysis by in 2010, associations were declared between OPG gene polymorphism and BMD and indicated that different polymorphisms are associated with different BMD in variable sites [29]. We also showed that the reverse relation between OPG levels and BMD in vertebrae was greater than the relations with BMD in the hip and femoral neck. These data are along with our data. The role of OPG in bone physiology has been proven by different studies. Marin and Sims also clarified this role and hoped that more studies should be performed on this issue in order to develop new pharmaceutical agents for bone diseases [30]. Taken together, our results, along with the results of the other studies, put emphasis on the role of OPG in the pathogenesis of osteoporosis in chronic diseases and especially in postmenopausal women. Therefore, physicians should pay more attention to the occurrence of osteoporosis in these populations. We also suggest that more studies should be performed in order to assess possible therapeutic agents which could affect OPG.

Conclusion

Here in this cross-sectional study, we evaluated OPG levels in post-menopausal women and indicated that OPG levels are higher in osteoporotic women. Furthermore, we showed that OPG level has a reverse relation with BMD. We suggest more studies should be performed on possible therapeutic roles of this agent especially in postmenopausal women.

Disclosure of conflict of interest

None.

Address correspondence to: Hossein Abdollahi Veshnavei, School of Medicine, Isfahan University of Medical Sciences, No. 8, Hezar Jarib Blvd., Isfahan, Iran. Tel: +98-9111375056; Fax: +98-3137265007; E-mail: abdollahi.hosseinvesh@gmail.com

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