Eklem Hastalıkları ve Cerrahisi
Joint Diseases and Related Surgery

Clinical Study / *Klinik Çalışma*

Eklem Hastalık Cerrahisi
2010;21(2):91-97

Plasma vascular endothelial growth factor levels are similar in subjects with and without osteoporosis

Osteoporozu olan ve olmayan olgularda serum vasküler endotelyal büyüme faktörü düzeyleri aynıdır

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Objectives: The relation between serum vascular endothelial growth factor (VEGF) level and bone mineral density (BMD) value was evaluated to investigate the role of VEGF at etiopathogenesis of the osteoporosis.

Patients and methods: Bone scanning with dual energy X-ray absorptiometry (DEXA) was performed on a total of 276 patients more than 40 years of age between September 2007 and January 2008 in our hospital's radiology department. A total of 88 patients (44 females; mean age 62.8±12.2 year, 44 males; mean age 58.7±12.1 year) meeting the study criteria were included. These patients formed four groups; osteoporotic male patients (group MO, n=22, BMD<–2.5), normal males (group MN, n=22, BMD>–1), osteoporotic female patients (group FO, n=22, BMD<–2.5), and normal females (group FN, n=22, BMD>–1). Bone mineral density measurements were performed with DEXA. Serum VEGF level was determined by the endogenous human ELISA kit. The relationships between body mass index (BMI), age, BMD and serum VEGF levels were analyzed.

Results: The difference between male and female participants in terms of serum VEGF levels was not statistically significant (p>0.05). The differences in terms of mean VEGF values between the MO and MN groups and the FO and FN groups were not statistically significant (p>0.05). In MN cases, BMD was negatively correlated with VEGF levels (p<0.05). In MO group, the correlation between BMD and serum VEGF levels was not statistically significant (p>0.05).

Conclusion: Although the plasma levels of osteoporotic subjects are relatively higher than in the normal groups, this was not statistically significant in either male or female subjects. The small sample size could be a reason for this insignificance. The negative correlation between serum VEGF and BMD levels in the MN group was not present in the MO group. When the various effects of serum VEGF on bone metabolism are taken into account, to clarify the pathophysiology of male osteoporosis, this association between BMD values and VEGF in male population must be investigated in further studies.

Key words: Bone mineral density; osteoporosis; vascular endothelial growth factors.

Amaç: Serum vasküler endotelial büyüme faktör (VEBF) düzeyi ile kemik mineral yoğunluğu (KMY) değerlerinin ilişkisi değerlendirilerek, VEBF'nin osteoporozun etyopatogenezindeki rolü araştırıldı.

Hastalar ve yöntemler: Eylül 2007 - Ocak 2008 tarihleri arasında hastanemizin radyoloji bölümünde, 40 yaş üzerindeki 276 hastaya, çift enerjili X-ray absorpsiometrisi (DEXA) ile kemik mineral yoğunluğu taraması yapıldı. Çalışma kriterlerini karşılayan 88 hasta (44 kadın; ort. yaş 62.8±12.2 yıl, 44 erkek; ort. yaş 58.7±12.1 yıl) çalışmaya dahil edildi. Hastalar, osteoporotik erkek hastalar (grup OE, n=22, KMY<–2.5), normal erkekler (grup NE, n=22, KMY>–1), osteoporotik kadın hastalar (grup OK, n=22, KMY<–2.5) ve normal kadınlar (grup NK, n=22, KMY>–1) olmak üzere dört gruba ayrıldı. Kemik mineral yoğunluğu ölçümleri DEXA yöntemiyle yapıldı. Serum VEBF düzeyleri endojen insan ELISA kiti ile ölçüldü. Vücut kütle indeksi (VKİ), yaş, KMY ve serum VEBF düzeyleri arasındaki ilişki analiz edildi.

Bulgular: Erkek ve kadın hastaların serum VEBF seviyeleri arasında fark istatistiksel olarak anlamlı bulunmadı (p>0.05). Normal erkekler ile OE ve NK ile OK grupları arasında ortalama serum VEBF düzeyleri açısından fark saptanmadı (p>0.05). Normal erkek olgularda, KMY değerleri ile VEBF düzeyleri arasında negatif ilişki vardı (p<0.05). Osteoporotik erkek grupta ise, KMY ile VEBF arasında istatistiksel anlamlı ilişki yoktu (p>0.05).

Sonuç: Çalışmamızda osteoporotik hastalardaki serum VEBF düzeyleri, kontrol grubuna göre göreceli olarak fazla çıksa da, ne erkek ne de kadın grubunda bu fark istatistiksel olarak anlamlı değildi. Hasta sayımızın azlığı bu sonuçta etkili olmuş olabilir. Grup NE'de serum VEBF düzeyleri ile KMY arasında saptanan negatif ilişki, grup OE'de saptanmadı. Serum vasküler endotelial büyüme faktörünün kemik metabolizması üzerindeki farklı etkileri göz önüne alındığında, erkek osteoporozunun patofizyolojisini açığa çıkarabilmek için, erkeklerde VEBF ve KMY arasındaki bu ilişkinin yeni çalışmalarla analiz edilmeye değer olduğunu düşünüyoruz.

Anahtar sözcükler: Kemik mineral yoğunluğu; osteoporoz; damar endoteli büyüme faktörleri.

• *Received: September 7, 2009 Accepted: April 26, 2010*

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• XXI. Ulusal Türk Ortopedi ve Travmatoloji Kongresi'nde sunulmuştur 3-8 Kasım 2009 Çeşme, İzmir *(Presented at the 21th National Congress of Turkish Orthopaedic and Traumatology, November 3-8, 2009, Çeşme, Antalya, Turkey).*

Osteoporosis is a skeletal disease characterized by low bone mass, changes in bone micro-architecture, and a resultant increase in bone fragility and fracture risk. It develops as a discontinuation in new bone formation or an increase in bone resorption. The community of cells present in the development of osteoporosis is called the bone multi-cellular unit (BMU). The collaboration of bone destroying (osteoclast) and forming cells (osteoblast) is called the coupling event.[1,2] Osteoblasts are immune-positive for vascular endothelial growth factor (VEGF) and the vascular endothelial growth factor receptor-2 (VEGFR-2). The cross-role of angiogenic factors like VEGF in vascularization was explained by Parfitt[2] via the coupling model. Parfitt[2] stated that the osteoblasts and osteoclasts in the BMU formed the coupling event and the capillary endothelium cells that are right at the center of BMU form the heart of this orchestra. The author also reported that the decrease in VEGF level can lead to postponement in bone remodeling.

Although the role of VEGF in bone metabolism was clarified via clinical and laboratory studies,[3-10] there are few studies analyzing the association between this growth factor and osteoporosis.[11-15] The aim of the present study is to examine the role of serum VEGF levels in the etiopathogenesis of both male and female osteoporosis.

PATIENTS AND METHODS

Bone scanning with dual energy X-ray absorptiometry (DEXA) was performed on a total of 276 patients more than 40 years of age in our hospital's radiology department. Patients with systemic diseases (diabetes, hypertension, renal disease, and atherosclerosis) or abnormal findings in laboratory tests (routine complete blood counts and blood biochemistry) were excluded. Other exclusion criteria were: previous osteoporosis treatment and having undergone drug treatment in the last three months, osteopenic DEXA results, and having used tobacco or alcohol in the last 48 hours preceding venous sampling.

The study group was selected in a cross-sectional fashion. A total of 16 patients that refused to participate were excluded from the study. A total of 48 patients because of systemic diseases, 26 cases because of positive history of osteoporosis medication in the last three months, and 52 patients because of osteopenic DEXA results were not included in the study. The blood pressures of the remaining 134 cases were measured and 24 of them were excluded because of high blood pressure, while another 22 patients were excluded because of abnormal results on complete blood counts and blood biochemistry.

Then the number of existing VEGF ELISA kits was taken into account and it was decided that 44 females (22 osteoporotic, 22 normal) and 44 males (22 osteoporotic, 22 normal), a total of 88 patients (44 females; mean age 62.8±12.2 years, 44 males; mean age 58.7±12.1 years), would be included in the study. Thus, patient admission was stopped after reaching necessary numbers in accordance with the inclusion criteria.

Bone mineral density (BMD) measurements were performed with DEXA at lumbar (L) 1-4 regions, femur neck, trochanter, and Ward's triangle as gr/cm^2. Venous sampling was performed following eight hours of fasting and 10 cc of blood was taken in K2 EDTA tubes and in the following 30 minutes, all blood samples were centrifuged at 1500/sec for 15 minutes at the hematology laboratory to acquire serum, which was then stored at –30 °C until the day they were analyzed. Serum VEGF level was determined by the endogenous Human VEGF ELISA kit.

All patients were given detailed information and written informed consent was obtained. Institutional review board approval was obtained for all patients. The patients were formed into four groups; osteoporotic males (group MO, n=22, BMD< –2.5), normal males (group MN, n=22, BMD> –1), osteoporotic females (group FO, n=22, BMD< –2.5), and normal females (group FN, n=22, BMD> –1). The weights and heights of all participants were measured and body mass indexes (BMI) were calculated by dividing weight in kilograms by the square of height in meters (kg/m^2).

Statistical analysis of data was performed with the Statistical Program for Social Sciences (SPSS) for Windows version 11.5 (SPSS Inc., Chicago, Illinois, USA). Parametric or non-parametric (Pearson and Spearman) correlation analyses were performed in order to determine the relation between serum VEGF levels, BMD, BMI, and age. Parametric tests like the Mann-Whitney U-test and Kruskall Wallis variance analysis were used for group comparisons of data that were not normally distributed. One

TABLE I

General features of participating groups

	MO	MN	*p*	FO	FN	*p*
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
Age	60.7±12.8	56.6±11.3	>0.05	66.9±8.2	58.5±14.1	>0.05
Weight	65.2±14.9	80.1±10.8	>0.05	68.5±16.9	79.3±13.6	>0.05
Height	164.6±9.8	170.4±10.8	>0.05	151.1±6.4	155.8±6.7	>0.05
BMI	24.0±4.8	27.6±3.2	>0.05	29.6±5.8	32.6±4.7	>0.05
BMD	-3.6±0.7	0.1±1.0	<0.001	-3.6±0.8	0.1±0.8	<0.001
	Median= -3.5	Median= 0.0		Median= -3.5	Median= 0.15	
VEGF	134.7±46.7	106.1±105.9	>0.05	94.2±54.8	84.6±67.0	>0.05
	Median= 74.6	Median= 67.7		Median= 78.3	Median= 60.05	

MO; Male osteoporotic; MN: Male normal; FO: Female osteoporotic; FN: Female normal. SD: Standard deviation; BMI: Body mass index; BMD: Bone mineral density; VEGF: Vascular endothelial growth factor.

way analysis of variance and the Bonferroni test were used for the analysis of normally distributed data. Data were presented as mean ± standard deviation. P values smaller than 0.05 was considered statistically significant.

RESULTS

The mean BMD value was –1.7±2.2 (median= –1.8) gr/cm^2 for males and –1.7±2.1 (median= –1.7) gr/cm^2 for females. The difference between male and female participant groups in terms of mean BMD values was not statistically significant (p=0.85). When the VEGF values were also analyzed, the serum VEGF level was 120.4±127.2 (Median=73.6) pg/ml for males and 89.4±60.6 (Median=74.1) pg/ml for females. The difference between these two participant groups in terms of serum VEGF levels was not statistically significant (p=0.12).

The mean age, weight, height, BMI, BMD and VEGF values of the male and female normal [MN], [FN] and osteoporotic [MO], [FO] groups are summarized in table I. When the male and female groups were compared in terms of age, weight, and height, the distributions did not show significant differences (p>0.05; Table I). The differences in terms of mean BMD values between MO and MN groups (p=0.001) and FO and FN groups (p=0.001) were statistically significant (Table I; Figure 1). The differences in terms of mean the VEGF values between the MO and MN groups (p>0.05) and the FO and FN groups (p>0.05) were not statistically significant (Table I; Figure 2).

Correlation studies

There was no significant correlation between BMD and VEGF levels in females and the total participants. In males, the correlation between BMD and VEGF values was statistically significant (p<0.05). In the male groups, the BMD was negatively correlated (r= –0.3) with the VEGF values (Table II).

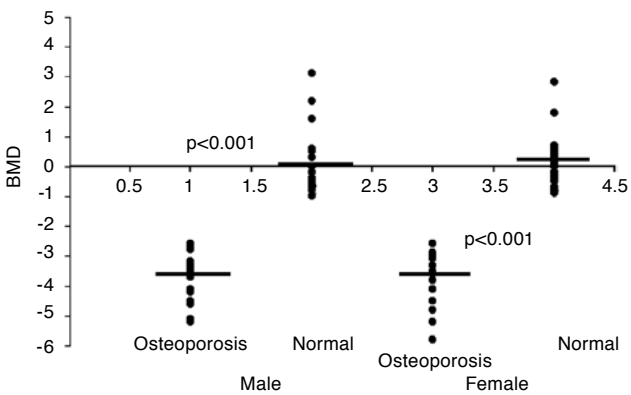

Figure 1. Diagram representing bone mineral density level distributions in osteoporosis and normal groups. BMD: Bone mineral density.

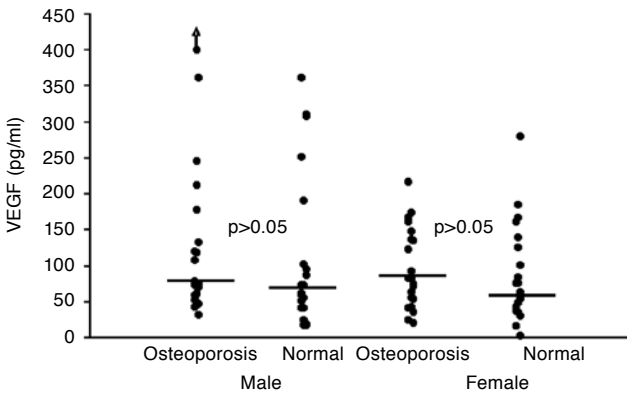

Figure 2. Diagram representing serum vascular endothelial growth factor level distributions in osteoporosis and normal groups. VEGF: Vascular endothelial growth factor.

TABLE II

The correlation between BMD and VEGF levels in male, female and all participants

	VEGF	
	r	*p*
Bone mineral density		
Males	−0.3	p<0.05
Females	0.02	p>0.05
Total	−0.1	p>0.05

BMD: Bone mineral density; VEGF: Vascular endothelial growth factor.

The correlation analysis between serum VEGF levels and age, BMI and BMD values in all groups are summarized in table III. In MN, MO, FN and FO groups; age and BMI were not significantly correlated with serum VEGF levels (p>0.05). In MN group, the correlation between BMD and serum VEGF levels was statistically significant (p<0.05). There was a negative correlation (r= –0.47) between BMD and VEGF.

DISCUSSION

If the etiopathogenesis of osteoporosis can be completely clarified, then better treatment methods in terms of cost and effectiveness can easily be defined. In this study, we aspired to identify the role of VEGF on the pathophysiology of both male and female osteoporosis.

Our results revealed that VEGF levels were high in osteoporotic groups, although not statistically significant. In the female groups, no significant correlation between BMD values and plasma VEGF levels could be established. However, in the normal males forming the control group, there was a significant negative correlation between BMD values and VEGF levels. This negative correlation between serum VEGF and BMD levels in the normal male group was not present in osteoporotic male population.

To understand the role of VEGF on the pathophysiology of osteoporosis, as well as the pathophysiology of osteoporosis itself, the impact of this growth factor on bone tissue should also be analyzed. In the literature, there are many studies analyzing the role of VEGF on bone tissue.[4-10] Vascular endothelial growth factor is an angiogenic growth factor binding heparin and has high specificity for vascular endothelium cells.[4,5] Vascular endothelial growth factor is released from very diverse cell types and as a result capillary permeability and endothelial cell proliferation increase.

The number of studies showing VEGF especially promoting fracture healing is striking. Vascular endothelial growth factor-A are released by hypertrophic chondrocytes in the growth plate and play a role in stimulating the growth of vascular structures which is needed for bone formation and cartilage resorption.[4,6] Ferrara[7] stated that VEGF also play a major role in angiogenesis as well as the proliferation and migration of osteoblasts. Zelzer et al.[8] stated that in bone development VEGF played three major roles: the induction of angiogenesis (intramembranous, enchondral), chemotactic migration of osteoclastic cells to the hypertrophic cartilage and osteoblastic activation. Additionally, in a systematic review reported by Keramaris et al,[9] the direct effects of VEGF on osteoprogenitor cells by promoting the differentiaton of osteoblast and increasing mineralization of the regenerated bone were stated.

There are many factors involved in bone formation. Among these, VEGF has a special role because of its neovascularization feature. With the aim of determining the effects of VEGF on bone formation, Street et al.[3] inhibited VEGF in the enchondral ossification and intramembranous ossification of bone healing in mice and it was shown that in mice with femur fractures, a decrease in the

TABLE III

The correlations among VEGF and age, BMI and BMD values in all groups

	Vascular endothelial growth factor							
	MO		MN		FO		FN	
	r	*p*	r	*p*	r	*p*	r	*p*
Age	−0.02	>0.05	−0.32	>0.05	0.33	>0.05	0.22	>0.05
Body mass index	−0.22	>0.05	−0.21	>0.05	0.29	>0.05	0.21	>0.05
Bone mineral density	−0.03	>0.05	−0.47	<0.05*	0.10	>0.05	0.21	>0.05

*: Statistically significant; MO; Male osteoporotic; MN: Male normal; FO: Female osteoporotic; FN: Female normal.

number of VEGF receptors decreased angiogenesis, bone formation, and callus mineralization. Vascular endothelial growth factor inhibition also decreased the healing of tibial cortical bone defects. Thus, these studies have demonstrated that VEGF plays a direct autocrine role in the differentiation of osteoblasts.[3] However, VEGF was found in high concentrations in bone hematomas in the early term.[10]

Although the effects of VEGF on bone metabolism were analyzed by different studies, there are few studies investigating the association between osteoporosis and VEGF. In a recent study conducted by Costa et al.,[11] the association between VEGF and BMD in postmenopausal female participants was analyzed. They reported no significant association between circulating VEGF and BMD in female osteoporotic subjects but stated that further larger confirmatory studies are needed to clarify the role of VEGF in female osteoporosis. There are some studies reporting lower VEGF levels in osteoporosis.[12,13] For example, Pufe et al.[12] investigated the changes in VEGF in the bones of animals under glucocorticoid treatment. One group was given glucocorticoids for osteoporosis and the other group consisted of control cases. The VEGF levels of animals under glucocorticoid treatment were 57% lower when compared with the control group. It was established that the decrease in VEGF levels in osteoporosis secondary to glucocorticoid treatment was parallel to the decrease in BMD values. As a result, the decrease in VEGF levels was demonstrated to play a role in animal osteoporosis secondary to glucocorticoid treatment.

In the study of Mao-wei et al.,[14] anti-hyperlipemic medication (fluvastatine) was given in cases of osteoporosis secondary to ovariectomy and it was stated that fluvastatine can be effective in fractures developed due to osteoporosis by increasing VEGF levels.

The physiopathology of postmenopausal osteoporosis is complex, especially when the direct effect of estrogen on osteoblasts is taken into account. Estrogen increases vitamin D receptors on the duodenal mucosa and thus prevents osteoporosis by increasing duodenal calcium absorption. 1-25 dihydroxyvitamin D increases the release of VEGF. This is a way of showing their anabolic effects on the bone.[16,17] However, VEGF stimulates its receptors on the endothelial cells and thus increases the production of osteotrophic growth factors like the insulin-like growth factor-I and ET-1.

There are striking studies regarding the possible association of increased VEGF activity and osteoporosis in the physiopathology of osteoporosis.[18,19] As a matter of fact, it is known that the stimulation of osteoclastic production is one of the mechanisms by which estrogen insufficiency leads to bone loss. Macrophage colony-stimulating factor (M-CSF) is required for the proliferation and differentiation of osteoclastic precursors[18,20] in mice. Vascular endothelial growth factor was demonstrated to be an important regulator of osteoclastic bone resorption. Some findings supported that in mice even when VEGF led to normal M-CSF production, the osteoclastic bone resorption resulting from estrogen insufficiency was regulated. As a result, it was reported that in mice estrogen insufficiency led to osteoclastic bone loss due to an increase in the VEGF production of bone tissue. Additionaly, Niida et al.[19] stated that in op/op mice, VEGF was required for the survival and functionality of mature osteoclasts.

Prostaglandins (PGs) were defined to have stimulant effects on bone metabolism in terms of both resorption and formation.[21] The exact mechanism of their resorption increasing effect is not yet clarified. However, in culture media PGs increase osteoclastic formation, along with increasing the efficiency of other factors that promote osteoclastic formation. Despite the mechanism, PGs are known to regulate the resorptive response of most growth factors.[22] Some of these factors can influence resorption independent from PGs. Harada et al.[23] showed that in human osteoblasts PGE2 increased VEGF mRNA levels.

When the studies supporting the anabolic and bone healing effects of VEGF are taken into account,[3,12-15] VEGF levels of patients with osteoporosis were expected to be low. In the present study, the observation of the association of osteoporosis and increased VEGF activity could be explained as in the studies of Kodama et al.[18] described above, with the regulatory effect of VEGF on osteoclastic bone resorption. Furthermore, when the findings of Harada et al.[23] showing that PGE2 increasing VEGF mRNA levels in human osteoblasts and PGE2 being a potent agent in bone resorption are considered, the high VEGF levels associating osteoporosis in

the present study could be explained in a PGE2-dependent fashion.

Our study has certain limitations. Since osteoporosis is a complex disease, and it is known to be affected by many factors such as age, gender, adiposity, menopause, we need studies with a larger number of participants both male and female. In addition, although we excluded patients with certain systemic diseases or abnormal findings on laboratory tests, any disease that could not be detected with conventional screening methods could cause changes of plasma VEGF levels.

We think that when both the anabolic effects on bone tissue and the regulatory effects on osteoclastic bone resorption of VEGF are taken into account, the real role of VEGF in osteoporosis merits being analyzed via studies with larger series. The studies performed thus far could not clarify the exact mechanisms underlying the physiopathology of male osteoporosis. As shown in the present study, the statistically significant negative correlation between BMD values and VEGF levels in the male normal group suggests that VEGF could play a role in male osteoporosis. To clarify the pathophysiology of male osteoporosis, this association between BMD values and VEGF in male population should be analyzed in further studies.

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