

# Circulating vascular endothelial growth factor concentrations in patients with postmenopausal osteoporosis

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## Abstract

**Introduction:** The role of vascular endothelial growth factor (VEGF) in osteoporosis has not yet been clearly established. Vascular endothelial growth factor is an important part of bone formation. In the literature, although the effects of VEGF on bone metabolism were investigated by different studies, there are very rare studies analysing the association between osteoporosis and VEGF. In the present study, our objective was to investigate serum VEGF concentrations in patients with postmenopausal osteoporosis (PMO) and the correlation of serum VEGF levels and bone mineral density (BMD).

**Material and methods:** This study was performed on 35 PMO patients, and 30 age-matched healthy controls. Serum VEGF concentrations were measured using a quantitative sandwich enzyme immunoassay technique according to the manufacturer's instructions. Bone mineral density values were determined by dual energy X-ray absorptiometry (DEXA).

**Results:** Serum VEGF concentrations were statistically significantly lower in PMO patients than in controls ( $150 \pm 65$  pg/ml,  $260 \pm 135$  pg/ml respectively;  $p = 0.005$ ). A positive correlation was found between serum VEGF concentrations and BMD values ( $r = 0.63$ ,  $p = 0.001$ ).

**Conclusions:** Vascular endothelial growth factor concentrations were decreased in PMO patients and VEGF may play an important role in bone health.

**Key words:** post-menopausal osteoporosis, vascular endothelial growth factor.

## Introduction

The role of vascular endothelial growth factor (VEGF) in osteoporosis (OP) has not yet been clearly established [1–3]. Vascular endothelial growth factor is an important part of bone formation. Also, angiogenesis is a crucial part of bone formation [4]. Recent studies have suggested that VEGF is a potent angiogenic stimulator, and it may play an important role during endochondral bone formation in hypertrophic cartilage remodelling, ossification, and angiogenesis. Hypertrophic chondrocytes express VEGF

*in vivo* and osteoblastic cells express VEGF and its receptors *in vitro*. However, VEGF can induce migration and differentiation of osteoblastic cells [4–6]. In addition, VEGF was shown to stimulate the formation, survival and resorption activity of osteoclasts *in vitro* [4, 7].

Vascular endothelial growth factor was first described as tumour vascular permeability factor (VPF) in 1983 but was finally discovered and characterized in 1989 [8]. Vascular endothelial growth factor is a homodimeric, heavily glycosylated protein of 46–48 kDa [2]. There are several isoforms of human VEGF [2, 8].

The role of VEGF in various diseases including diabetic retinopathy, end stage renal disease, rheumatoid arthritis, giant cell arteritis, and breast cancer has been investigated in several studies [9–11]. In the present study, serum VEGF levels in patients with postmenopausal osteoporosis (PMO) were determined and the relationship between VEGF and bone mineral density (BMD) was evaluated. We discuss the possible role of VEGF in PMO patients.

## Material and methods

Thirty-five post-menopausal women fulfilling OP diagnostic criteria of the World Health Organization (WHO) and 30 post-menopausal healthy women without OP were included in this study. Patients with secondary osteoporosis and systemic diseases were excluded by means of laboratory and radiological findings. Bone mineral density values were measured by dual energy X-ray absorptiometry (DEXA) at lumbar, femoral neck and total hip regions. All scans and analyses were performed by

the same technician. The 30 post-menopausal healthy women without OP were taking no medications known to affect bone metabolism and had no inflammatory or infectious diseases included as exclusion criteria for the control and patient group. Patients had no vertebral, hip or distal forearm fractures as a result of osteoporosis.

We measured circulating levels of VEGF in the serum of 35 osteoporotic and 30 healthy post-menopausal women. All blood samples were taken from PMO patients and control subjects in the morning after an overnight fast. Sera were collected by centrifugation of 1500/s for 15 min and stored at  $-70^{\circ}\text{C}$  until the time of analysis. Serum VEGF levels were determined by using a quantitative sandwich enzyme immunoassay technique according to the manufacturer's instructions. The demographic data, the clinical characteristics, the examination findings and DEXA results were recorded in PMO patients and control subjects.

## Statistical analysis

Statistical analyses were performed with the SPSS statistics package. All data were expressed as mean  $\pm$  SD. Data analysis was performed using Mann-Whitney *U* test. Pearson correlation test was used to evaluate the correlations between VEGF levels and BMD results. Values of *p* less than 0.05 were accepted as significant.

## Results

Table I shows the demographic data and clinical characteristics of the patients and controls. There was no statistically significant difference between the groups with respect to age, age of menarche, number of pregnancies, duration of menopause, and body mass index (BMI) results (*p* > 0.05).

Vascular endothelial growth factor serum levels were statistically significantly lower (*p* = 0.005) in patients with PMO ( $150 \pm 65$  pg/ml) than in controls ( $260 \pm 135$  pg/ml). A positive correlation was found between serum VEGF concentrations and BMD T scores ( $r = 0.63$ , *p* = 0.001) and Z scores ( $r = 0.54$ , *p* < 0.01). There was no correlation between VEGF and age, BMI, or menopause duration in PMO patients.

## Discussion

In the literature, although the effects of VEGF on bone metabolism have been investigated by different studies, there are few studies analysing the association between osteoporosis and VEGF [1–3, 12, 13]. The role of VEGF in PMO is controversial [14–16].

There are some studies reporting decreased VEGF levels in osteoporosis [2, 12]. It has been suggested that VEGF is produced in osteoblasts and its concentrations are decreased in patients with

**Table I.** Demographic data, clinical, radiological and laboratory features of the patients and controls

Parameter	Controls (n = 30)	Patients (n = 35)	Value of <i>p</i>
Age [year]	65.6 $\pm$ 6.8	66.2 $\pm$ 7.5	> 0.05
Age of menarche [year]	13.7 $\pm$ 1.3	13.5 $\pm$ 1.2	> 0.05
Number of pregnancies	3.4 $\pm$ 1.8	3.6 $\pm$ 1.7	> 0.05
Duration of menopause [year]	17 $\pm$ 2.6	18 $\pm$ 2.4	> 0.05
BMI [kg/m <sup>2</sup> ]	29.8 $\pm$ 4.9	27.6 $\pm$ 4.3	> 0.05
BMD T scores [g/cm <sup>2</sup> ]	0.2 $\pm$ 1.1	-3.2 $\pm$ 0.7	< 0.01
BMD Z scores [g/cm <sup>2</sup> ]	0.5 $\pm$ 1.2	-2.4 $\pm$ 0.6	< 0.01
VEGF [pg/ml]	260 $\pm$ 135	150 $\pm$ 65	< 0.01

BMI – body mass index, BMD – bone mineral density, VEGF – vascular endothelial growth factor

osteoporosis. The reduction in VEGF levels correlated with the measurement of BMD. Vascular endothelial growth factor concentrations were 60% lower ( $p < 0.001$ ) in osteoporosis patients [2, 12]. Martinez *et al.* observed an age-related decrease in the secreted levels of VEGF in osteoblasts [17]. They suggested that VEGF may be an important modulating factor for bone remodelling in PMO patients.

In the present study, we postulated that VEGF concentrations would be reduced in PMO patients. We investigated the VEGF levels and their association with BMD in PMO patients. Vascular endothelial growth factor levels were found lower in PMO patients than control subjects and positive correlations were determined between VEGF levels and BMD values. In contrast, there are striking studies regarding the possible association of increased VEGF activity and osteoporosis [13, 18]. It was reported that in mice oestrogen insufficiency led to osteoblastic bone loss due to an increase in the VEGF production of bone tissue [13, 18]. Harada *et al.* vascular endothelial growth factor detected expression of VEGF in human osteoblasts and they showed that prostaglandin  $E_2$  increased VEGF mRNA levels [19]. Costa *et al.*, in 252 post-menopausal women, determined BMD, serum oestradiol and VEGF levels. They observed no significant difference in serum VEGF concentrations between cases and controls [3]. Similarity, Cebi *et al.* investigated the relation between serum VEGF levels and BMD values and the role of VEGF in aetiopathogenesis of osteoporosis [1]. Their results showed that plasma VEGF levels of osteoporotic subjects were higher than in the normal group, although the difference was not statistically significant. In the present study, our results are in line with the studies of Pufe *et al.* [2]. We think that the decrease in VEGF levels in PMO patients may also be the results of medications, genetic, environmental and endocrine factors. However, we excluded patients taking medications that may affect bone metabolism and we did not assess the effect of environmental factors and considering genetic factors we did not study the polymorphism. Several hormones have been shown to regulate VEGF production locally [3, 20]. Oestrogen has been shown to regulate VEGF gene transcription [20]. Glucocorticoids inhibit COX-2 and  $PGE_2$  production. Because  $PGE_2$  is able to increase VEGF synthesis, this may be a link between GC and VEGF decrease and  $PGE_2$  is able to increase VEGF synthesis [2]. Oestrogen increases vitamin D receptors on the duodenal mucosa. 1,25 dihydroxyvitamin D increases the release of VEGF. This may explain the anabolic effects of VEGF on bone metabolism [21].

Several studies have shown that two common functional polymorphisms are associated with VEGF production [22, 23]. The 936 T allele has been associated with lower production of VEGF [22]. A sig-

nificant correlation was observed between VEGF protein production and genotype for the +405 polymorphism [23]. Previous studies have shown significant associations between VEGF levels and this polymorphism in healthy males and postmenopausal women [22, 24]. However, previous reports have demonstrated that individuals with a 634 C allele are predisposed to disorders where enhanced VEGF expression may play a role in disease progression such as diabetic retinopathy, giant cell arteritis, and breast cancer [3]. Moreover, a study analysing 252 post-menopausal women reported no significant difference in serum VEGF concentrations between cases and controls [25].

In conclusion, our results demonstrated that VEGF concentrations were decreased in PMO patients compared to controls and VEGF may play an important role in bone metabolism. However, VEGF levels may be influenced by genetic, environmental and endocrine factors. Further studies on the role of VEGF in PMO patients will be helpful to clarify the aetiopathogenesis and treatment of PMO.

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