Introduction

Osteoporosis is a progressive systemic skeletal disorder characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue that reduces bone strength and increases the risk of fractures [1].

Postmenopausal osteoporosis is mainly caused by increased bone remodeling resulting from estrogen deficiency with induced imbalance between bone formation and resorption such that resorption is favored over formation [2].

Bone biopsies from postmenopausal osteoporotic patients were characterized by a reduction in sinusoidal and arterial capillaries in the bone marrow (BM) and reduced bone perfusion, suggesting the role of a vascular component in the pathogenesis of this disease and confirming what was reported previously, that is, coupling between angiogenesis and osteogenesis was essential for normal bone formation [3].

Vascular endothelial growth factors (VEGFs) and their corresponding receptors are key regulators in a cascade of molecular and cellular events that ultimately lead to angiogenesis [4]. Although the main effects of VEGFs are on endothelial cells, they also bind to VEGF receptors expressed on monocytes, neurons, chondrocytes, and osteoblasts [5].

Role of vascular endothelial growth factor expression in pathogenesis of postmenopausal osteoporosis

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Background

Vascular endothelial growth factor (VEGF), an angiogenic growth factor, has been proved to play a significant role in bone remodeling. It may be involved in the molecular pathogenesis of postmenopausal osteoporosis.

Aim

The aim of this study was to investigate the expression of VEGF in bone biopsies of postmenopausal osteoporotic patients, assess the relation between the expression of VEGF and bone mineral density (BMD), and to evaluate the association between VEGF, serum estradiol, and bone estrogen receptor-α (ER-α) expression.

Patients and methods

This study was carried out on 30 female patients who were further subdivided into three groups: premenopausal, perimenopausal, and postmenopausal. All of them were subjected to full assessment of history, thorough clinical examination, and routine laboratory investigations. Serum estradiol levels were measured using ELISA. BMD was detected using DEXA. Bone biopsies were taken and three sections were obtained from each specimen. One was stained with hematoxylin and eosin stain for bone histomorphometrical assessment. The other two sections were stained immunohistochemically for the detection of VEGF and estrogen receptor-α (ER-α) expression.

Results

A highly statistically significant difference was found in VEGF expression between the premenopausal, perimenopausal, and postmenopausal women and also between osteoporotic and nonosteoporotic women. A highly statistically positive correlation was found between VEGF and each of the following: BMD, bone anabolic histomorphometrical parameters E2, and ER-α. However, a highly statistically negative correlation was observed between VEGF and bone histomorphometrical resorption parameters.

Conclusion

VEGF expression is decreased in bone of postmenopausal osteoporotic patients and is correlated to BMD. Its release is dependent on E2 and mediated through ER-α. These suggest that bone alterations induced by reduced estrogen in postmenopausal osteoporosis may be partly through decreased VEGF release. This makes it one of the possible targets in the treatment of postmenopausal osteoporosis.

Keywords:
bone mineral density, postmenopausal osteoporosis, vascular endothelial growth factor
VEGF is known to recruit endothelial cells and the endothelial cells themselves organize bone remodeling. Once recruited, endothelial cells release a potent mitogen for osteoblasts. However, endothelial cells could also inhibit and regulate osteoclast activity [6]. A previous study [7] reported that VEGF exerted a direct effect on osteoprogenitor cells by promoting their differentiation into osteoblasts. It could also promote mineralization of the bone and increase the bone density. Later, it was observed that osteoblasts themselves released VEGF in an autocrine manner to regulate their own activity. Furthermore, this expression of VEGF in osteoblasts was regulated by estrogens such as 17 b-estradiol [8].

Activated estrogen receptors (ERs) have been reported to induce hypoxia inducible factor-1 α (HIF-1α) activation, which stimulates VEGF-mediated angiogenesis in bone [9]. Vitamin D3 was proved to increase VEGF expression, suggesting that the anabolic effects of vitamin D on bone tissue may be partly mediated by VEGF [10].

A possible association between VEGF activity and the pathophysiology of osteoporosis was suggested by Pufe et al. [11], who reported that the decrease in VEGF levels played a role in glucocorticoid-induced osteoporosis in experimental animals. A few years later, it was documented that the reduction in the BMD in the lumbar spines of ovariectomized experimental animals correlated with the decrease in VEGF levels [6].

It has been reported that after menopause, there is a decrease in the levels of VEGF [12]. It is also worth mentioning that hormone replacement therapy increased VEGF in postmenopausal women [6]. To our knowledge, the expression of VEGF in bone biopsies of postmenopausal osteoporotic patients and its correlation with their BMD have not been studied before.

Aim of the work
The aim of this work was to investigate the expression of VEGF in bone biopsies of postmenopausal osteoporotic patients, assess the relation between the expression of VEGF and BMD in these patients, and to evaluate the association between VEGF, serum estradiol, and bone ER-α in order to detect a role of VEGF in the pathogenic pathway of postmenopausal osteoporosis, which, if proved, could potentially be a therapeutic target.

Patients and methods
This study included 30 female patients who attended the Orthopedic and/or the Physical Medicine, Rheumatology, and Rehabilitation Departments of Ain Shams University Hospitals. All were candidates for operations during which an iliac crest bone biopsy was taken from each patient after obtaining an informed written consent and after the study had already been approved by the Ethics Committee of the Faculty of Medicine, Ain Shams University. Patients were divided into three groups according to menopausal status: Premenopausal, perimenopausal, and postmenopausal after the study by Schmidt and Rubinow [13], who defined perimenopausal women as those who had menstrual irregularities and menopause as the permanent cessation of menstruation for 12 months.

Exclusion criteria
Patients with secondary osteoporosis because of any cause such as rheumatological, connective tissue, endocrinical, chronic renal failure, immobilization, and drug-induced osteoporosis were excluded as well as patients receiving medical treatment for osteoporosis or hormone replacement therapy.

All patients were subjected to the following:
1. Full assessment of history, with a special focus on menstrual history, drug intake, history of generalized bone aches, or nontraumatic fractures.
2. Thorough clinical examination that included height and weight measurements to calculate BMI. General, spine, neurological, and joint assessment was performed.
3. Laboratory investigations included the following:
   a. Complete blood count using a Coulter counter.
   b. Erythrocyte sedimentation rate (ESR) using the Westergren method.
   c. Fasting blood sugar.
   d. Liver function tests: Aspartate aminotransferase and alanine aminotransferase.
   e. Renal function tests: Serum creatinine and urea.
   f. Serum calcium, phosphorous, and alkaline phosphatase.
   g. Serum estradiol (E2) using the enzyme linked immunosorbant assay (ELISA) technique.
4. Radiological investigations included the following:
   a. Plain radiograph: Anteroposterior and lateral views of the dorso-lumbar spine to detect any vertebral deformities or fractures.
   b. Dual-energy X-ray absorptiometry (DEXA): BMD was measured at three sites, the femoral neck, lumbar spine, and distal radius, using the LUNAR (DPX-MD+) device (GE Medical System).
5. Bone sample: The bone specimens from the iliac crest graft were fixed using 10% neutral-buffered formalin (NBF). Then, they were decalcified using a Ca-chelating agent EDTA. The time required for decalcifying bone was 6–8 weeks. The fluid was changed every 4 days. The specimens were then dehydrated sequentially in ascending concentrations of ethanol (70, 90, and...
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This was detected by incubation with a biotinylated secondary anti-rabbit antibody. The complexes were visualized after the addition of DAB.

Statistical analysis
Statistical analysis was carried out using the statistical package for social sciences software (SPSS 15.0.1; SPSS Inc.). Quantitative variables (clinical, laboratory, radiological, histomorphometrical parameters of bone, and immunohistochemistry) were presented as mean±SD. The analysis of variance (ANOVA) test was used to compare the means of more than two studied groups. Relationships between parameters were analyzed using Pearson's correlation coefficient. A P value less than 0.05 was considered significant.

Results
This study was carried out on 30 female patients who were divided according to their menopausal status into three groups, each including 10 patients: Premenopausal women were included in group I, perimenopausal women were included in group II, and postmenopausal women were included in group III. Comparison between descriptive and laboratory data of the three groups using the ANOVA test is shown in [Table 1].

Comparison of the three groups in serum E2 using the post-hoc test showed a highly statistically significant difference between group I and group II, group I and group III, and group II and group III as shown in [Fig. 1].

Radiological data
On examining the lateral view radiograph of the dorsolumbar spines, no abnormalities were detected in both groups I and II. However, in group III, four patients (40%) showed both kyphosis and compression fractures in the dorsal spine.

DEXA studies
All women in group one showed BMD within normal ranges, whereas in group II, four women had normal

Table 1 Comparison of descriptive and laboratory data of patients of the three groups

<table>
<thead>
<tr>
<th>Item</th>
<th>Group I (n = 10)</th>
<th>Group II (n = 10)</th>
<th>Group III (n = 10)</th>
<th>F</th>
<th>P</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.60 ± 7.38</td>
<td>52.70 ± 2.16</td>
<td>58.70 ± 4.79</td>
<td>43.21</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Menarche (years)</td>
<td>12.30 ± 1.25</td>
<td>12.30 ± 1.25</td>
<td>12.50 ± 1.27</td>
<td>0.08</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>32.35 ± 4.65</td>
<td>32.88 ± 3.36</td>
<td>32.72 ± 5.20</td>
<td>0.04</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Serum calcium (mg/dl)</td>
<td>9.49 ± 0.47</td>
<td>9.27 ± 0.34</td>
<td>9.44 ± 0.54</td>
<td>0.628</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Serum phosphorous (mg/dl)</td>
<td>3.61 ± 0.66</td>
<td>3.74 ± 0.36</td>
<td>3.67 ± 0.38</td>
<td>0.18</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Serum alkaline phosphatase (UI)</td>
<td>63.40 ± 20.91</td>
<td>78.00 ± 24.55</td>
<td>70.40 ± 18.80</td>
<td>1.148</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Serum estradiol (E2) (pg/ml)</td>
<td>183.50 ± 46.63</td>
<td>58.30 ± 13.74</td>
<td>27.20 ± 11.11</td>
<td>82.584</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
</tbody>
</table>

HS, highly significant.
BMD, five women were osteopenic, and only one woman was osteoporotic. In group III, all 10 postmenopausal patients were osteoporotic. Comparison of the three groups indicated a statistically significant difference in the T-score at the femoral neck, lumbar spine, and distal forearm as can be seen in [Table 2] and [Fig. 2].

**Histological results**

Light microscopic examination of trabecular bone of iliac crest biopsies stained with H&E showed that in group I, continuous thick bone trabeculae separated by BM spaces could be observed as shown in [Figs 3 and 4]. In group II, iliac crest bone biopsies showed slightly discontinuous bone trabeculae separated by widened BM spaces. Larger erosion cavities were detected in comparison with premenopausal biopsies as shown in [Fig. 5]. In group III, bone trabeculae appeared significantly discontinuous and thin. They were separated by markedly widened BM spaces containing abundant fat cells. The largest erosion cavities were as frequently detected as shown in [Figs 6 and 7].

**Histomorphometrical assessment of bone biopsies**

Comparison of the three groups in the histomorphometrical parameters of bone that included BV, TbTh, ObS, TS, and ES using the ANOVA test showed a highly statistically significant difference ($P < 0.001$) between them all as shown in [Table 3] and [Figs 8 and 9].

<table>
<thead>
<tr>
<th>Item</th>
<th>Group I (n = 10)</th>
<th>Group II (n = 10)</th>
<th>Group III (n = 10)</th>
<th>F</th>
<th>P</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV (%)</td>
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<td></td>
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<tr>
<td>Min-max</td>
<td>21.60-28.80</td>
<td>14.80-19.90</td>
<td>11.00-17.70</td>
<td>66.81</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>25.32 ± 2.37</td>
<td>17.23 ± 1.74</td>
<td>14.22 ± 2.48</td>
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<td>TbTh (mm)</td>
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<tr>
<td>Min-max</td>
<td>146.80-164.50</td>
<td>99.60-134.90</td>
<td>65.80-92.80</td>
<td>181.13</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>155.66 ± 5.84</td>
<td>117.37 ± 9.99</td>
<td>81.38 ± 9.72</td>
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<td>ObS (%)</td>
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<tr>
<td>Min-max</td>
<td>6.90-8.90</td>
<td>4.50-5.50</td>
<td>2.50-4.30</td>
<td>170.80</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>7.84 ± 0.62</td>
<td>4.89 ± 0.31</td>
<td>3.42 ± 0.64</td>
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<tr>
<td>TS (mm)</td>
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</tr>
<tr>
<td>Min-max</td>
<td>480.30-548.60</td>
<td>590.20-635.80</td>
<td>660.70-782.90</td>
<td>122.71</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>522.13 ± 21.77</td>
<td>612.34 ± 13.78</td>
<td>714.53 ± 40.02</td>
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<td>ES (%)</td>
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</tr>
<tr>
<td>Min-max</td>
<td>2.90-5.20</td>
<td>5.50-7.00</td>
<td>6.60-8.90</td>
<td>66.09</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.37 ± 0.73</td>
<td>6.26 ± 0.47</td>
<td>7.76 ± 0.74</td>
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</tbody>
</table>

Table 2 Comparison of the three groups in the T-score at the femoral neck, lumbar spine, and forearm

<table>
<thead>
<tr>
<th>T-score</th>
<th>Group I (n = 10)</th>
<th>Group II (n = 10)</th>
<th>Group III (n = 10)</th>
<th>F</th>
<th>P</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur</td>
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</tr>
<tr>
<td>Min-max</td>
<td>−0.90 to 2.00</td>
<td>−2.00 to 1.50</td>
<td>−3.30 to −0.60</td>
<td>11.73</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.65 ± 1.05</td>
<td>−0.35 ± 1.01</td>
<td>−1.45 ± 0.84</td>
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<tr>
<td>Lumbar</td>
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<tr>
<td>Min-max</td>
<td>−0.90 to 1.70</td>
<td>−3.00 to −0.20</td>
<td>−3.10 to 0.10</td>
<td>14.66</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.09 ± 0.99</td>
<td>−1.44 ± 0.90</td>
<td>−2.16 ± 0.96</td>
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<tr>
<td>Forearm</td>
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</tr>
<tr>
<td>Min-max</td>
<td>−0.80 to 1.30</td>
<td>−1.90 to 1.00</td>
<td>−3.30 to 1.20</td>
<td>4.721</td>
<td>&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.10 ± 0.76</td>
<td>−0.63 ± 1.00</td>
<td>−1.29 ± 1.23</td>
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</tr>
</tbody>
</table>

HS, highly significant; Min, minimum; Max, maximum; S, significant.
A bar chart showing a comparison of the three groups in the T-score at the femoral neck, lumbar spine, and forearm.

Figure 2

A photomicrograph of a section from iliac crest bone of a premenopausal woman showing branching and anatomizing thick trabeculae with bone marrow spaces (BM) in between (H&E stain, ×400).

Figure 3

A photomicrograph of a section from iliac crest bone of a premenopausal woman showing the cancellous bone trabeculae and bone marrow spaces (BM). Bone trabeculae are lined with cuboidal osteoblasts (Ob) with rounded nuclei. Osteocytes (Os) inside their lacunae in between the lamellae can also be seen (H&E stain, ×400).

Figure 4

A photomicrograph of a section from iliac crest bone of a perimenopausal woman showing slightly discontinuous bone trabeculae that appeared relatively thin separated by widened bone marrow spaces (BM) (H&E stain, ×400).

Figure 5

A photomicrograph of a section from iliac crest bone of a postmenopausal woman showing significantly discontinuous thin bone trabeculae separated by widened bone marrow spaces (BM) containing abundant fat cells (H&E stain, ×400).

Figure 6

A photomicrograph of a section from iliac crest bone of a postmenopausal woman showing an irregular eroded surface (ES) of bone trabeculae (H&E stain, ×400).

Figure 7
In postmenopausal patients of group III, VEGF expression was almost absent and was only found in very few and scattered cells (expression percentage ranged from 0.13 to 1.22%, mean 0.64 ± 0.37%) as shown in [Fig. 12].

### Immunohistochemical results for ER-α expression

ER-α expression appeared as both nuclear and cytoplasmic immunolabeling, which consisted of brownish granules or aggregates of variable density.

In perimenopausal patients of group II, there was decreased VEGF expression (ranging from 1.40 to 2.78%, mean 1.98 ± 0.48%) in fewer osteogenic, osteoblasts, and BM stromal cells compared with group I as shown in [Fig. 11].

In premenopausal patients of group I, bone biopsies showed enhanced VEGF expression. Many osteogenic cells, osteoblasts lining trabeculae, and BM stromal cells showed an intensely positive cytoplasmic reaction, with the percentage of VEGF expression ranging from 3.87 to 9.39%, mean of 7.23 ± 1.94%, as shown in [Fig. 10].

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**Figure 8**

A bar chart showing a comparison of the three groups in BV, TbTh, and ObS. BV, bone volume; ObS, osteoblast surface; TbTh, trabecular bone thickness.

**Figure 9**

A bar chart showing comparison of the three groups in TS and ES. ES, eroded surface; TS, trabecular separation.

**Figure 10**

A photomicrograph of a section from iliac crest bone of a premenopausal patient showing cytoplasmic reaction of the vascular endothelial growth factor expression in the osteoblasts (IHC stain, ×400).

**Figure 11**

A photomicrograph of a section from iliac crest bone of a perimenopausal woman shows decreased vascular endothelial growth factor expression in fewer osteoblasts (IHC stain, ×400).
intensity in osteoblasts, osteogenic cells, and BM stromal cells.

In group I, enhanced ER-α expression was observed (ranging from 3.93 to 7.89%, mean 6.00 ± 1.64%) as shown in [Figs 13 and 14].

In group II, decreased ER-α expression (ranging from 2.81 to 3.87%, mean 3.31 ± 0.38%) was detected as compared with group I as shown in [Figs 15 and 16].

In group III, the least ER-α expression (ranging from 1.12 to 2.57%, mean 1.78 ± 0.51%) was observed as shown in [Fig. 17].

Comparison of the three groups in VEGF and ER-α expression using the ANOVA test showed a highly
statistically significant difference ($P < 0.001$) between groups in both markers as shown in [Table 4] and [Fig. 18].

Reconsidering BMD, all patients were further subdivided into 14 normal (46.66%), five osteopenic (16.66%), and 11 osteoporotic (36.66%) patients. Comparison of VEGF and ER-$$\alpha$$ expressions between these groups using the ANOVA test showed a highly statistically significant difference ($P < 0.001$) in both as shown in [Table 5] and [Fig. 19].

**Correlating VEGF and ER-$$\alpha$$ expressions with clinical and laboratory data of the patients**

The expression of both VEGF and ER-$$\alpha$$ showed a highly statistically significant positive correlation ($P < 0.001$) with E2 levels. However, expression of both VEGF and ER-$$\alpha$$ showed a highly statistically significant negative correlation ($P < 0.001$) with age, duration of menstrual irregularities, and menopause.

Correlation of these with age of menarche, BMI, and other laboratory data indicated a nonsignificant correlation ($P > 0.05$) as shown in [Table 6] and [Fig. 20a and b].

Moreover, VEGF expression showed a highly statistically significant positive correlation with ER-$$\alpha$$ expression ($r = 0.968$ and $P < 0.001$) as shown in [Fig. 21].

Correlations between VEGF, ER-$$\alpha$$ expressions, E2 levels, and BMD were all highly statistically significantly positive ($P < 0.001$) as shown in [Table 7] and [Fig. 22].

Correlation of VEGF, ER-$$\alpha$$ expressions, and E2 levels to histomorphometrical bone parameters indicated highly statistically significant positive

### Table 4 Comparison of the three groups in VEGF and ER$$\alpha$$ expressions

<table>
<thead>
<tr>
<th>Expression (%)</th>
<th>Group I ($n = 10$)</th>
<th>Group II ($n = 10$)</th>
<th>Group III ($n = 10$)</th>
<th>$F$</th>
<th>$P$</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Min-max</td>
<td>3.87-9.39</td>
<td>1.40-2.78</td>
<td>0.13-1.22</td>
<td>88.019</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>7.23 ± 1.94</td>
<td>1.98 ± 0.48</td>
<td>0.64 ± 0.37</td>
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<tr>
<td>ER$$\alpha$$</td>
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</tr>
<tr>
<td>Min-max</td>
<td>3.93-7.89</td>
<td>2.81-3.87</td>
<td>1.12-2.57</td>
<td>44.195</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.00 ± 1.64</td>
<td>3.31 ± 0.38</td>
<td>1.78 ± 0.51</td>
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</table>

**Note:** ER$$\alpha$$, estrogen receptor-$$\alpha$$; Min, minimum; Max, maximum; S, significant; VEGF, vascular endothelial growth factor.

### Table 5 Comparison of the normal, osteopenics, and osteoporotics in VEGF and ER$$\alpha$$ expressions

<table>
<thead>
<tr>
<th>Expression (%)</th>
<th>Normal ($n = 14$)</th>
<th>Osteopenic ($n = 5$)</th>
<th>Osteoporotic ($n = 11$)</th>
<th>$F$</th>
<th>$P$</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>VEGF</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Min-max</td>
<td>1.22-9.39</td>
<td>0.94-2.1</td>
<td>0.13-1.7</td>
<td>20.9</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.80 ± 2.87</td>
<td>1.70 ± 0.48</td>
<td>0.80 ± 0.54</td>
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<tr>
<td>ER$$\alpha$$</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min-max</td>
<td>2.57-7.89</td>
<td>2.42-3.5</td>
<td>1.12-3.12</td>
<td>19.05</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.27 ± 1.83</td>
<td>3.09 ± 0.42</td>
<td>1.96 ± 0.70</td>
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</tr>
</tbody>
</table>

**Note:** ER$$\alpha$$, estrogen receptor-$$\alpha$$; HS, highly significant; Min, minimum; Max, maximum; VEGF, vascular endothelial growth factor.
Discussion

The incidences of osteoporosis and its potentially devastating sequelae as fractures are increasing and are associated with morbidity, disability, and reduced quality of life. Therefore, prevention and treatment of osteoporosis is of major importance [14].

Estrogen deficiency leads to an increase in the rate of bone remodeling, with resorption exceeding formation. Undoubtedly, this is considered one of the most important factors for postmenopausal osteoporosis [15].

Coupling between osteogenesis and angiogenesis was reported to be essential for normal bone formation. It was suggested that impairment of angiogenesis would decrease trabecular bone formation [3].

VEGF is an angiogenic growth factor that couples angiogenesis to osteogenesis [16]. Future therapeutic use of VEGF in the management of bone metabolic pathology therefore depends on a better understanding of the action of VEGF in the bone environment [17].
This study was designed to investigate the expression of VEGF in bone of postmenopausal osteoporotic patients, assess its relation with BMD, and evaluate the association between VEGF expression, serum E2 levels, and ER- α expression in order to detect its role in the pathogenic pathway of postmenopausal osteoporosis and the possibility of its therapeutic use.

In this study, most patients were overweight and their mean BMI was within that of the obese category (30-40 kg/m). This finding was not surprising as a high prevalence of overweight in Egyptian women has been documented. This may have played a role in the high total percentage of those with low BMD, represented by 16 of 30 patients (53%). It has been suggested that obesity may have a deleterious effect on bone by increasing adipocyte differentiation and fat accumulation while decreasing osteoblast differentiation and bone formation [18].

In terms of laboratory data, serum calcium, phosphorous, and alkaline phosphatase were all within

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**Table 6 Correlation of VEGF and ER-α expressions with clinical and laboratory data**

<table>
<thead>
<tr>
<th>Item</th>
<th>VEGF expression (%)</th>
<th>ER-α expression (%)</th>
<th>BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>−0.925</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Age of menarche (years)</td>
<td>−0.103</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of menstrual irregularities or menopause (years)</td>
<td>−0.810</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>−0.011</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Serum calcium (mg/dl)</td>
<td>0.067</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Serum phosphorous (mg/dl)</td>
<td>−0.144</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Serum alkaline phosphatase (U/l)</td>
<td>−0.221</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Serum estradiol levels (pg/ml)</td>
<td>0.997</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
</tbody>
</table>

ER-α, estrogen receptor-α; HS, highly significant; VEGF, vascular endothelial growth factor.

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**Table 7 Correlations between VEGF, ERα expressions, and E2 levels with BMD**

<table>
<thead>
<tr>
<th>BMD</th>
<th>VEGF expression (%)</th>
<th>ER-α expression (%)</th>
<th>E2 levels (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur</td>
<td>0.768 &lt;0.001 HS</td>
<td>0.818 &lt;0.001 HS</td>
<td>0.783 &lt;0.001 HS</td>
</tr>
<tr>
<td>Lumbar</td>
<td>0.839 &lt;0.001 HS</td>
<td>0.879 &lt;0.001 HS</td>
<td>0.843 &lt;0.001 HS</td>
</tr>
<tr>
<td>Forearm</td>
<td>0.654 &lt;0.001 HS</td>
<td>0.641 &lt;0.001 HS</td>
<td>0.677 &lt;0.001 HS</td>
</tr>
</tbody>
</table>

BMD, bone mineral density; HS, highly significant.

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**Table 8 Correlations between VEGF, ER-α expressions, E2 levels, and histomorphometrical parameters of bone**

<table>
<thead>
<tr>
<th>Item</th>
<th>VEGF expression (%)</th>
<th>ER-α expression (%)</th>
<th>E2 levels (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV (%)</td>
<td>0.965 &lt;0.001 HS</td>
<td>0.959 &lt;0.001 HS</td>
<td>0.969 &lt;0.001 HS</td>
</tr>
<tr>
<td>TbTh (mm)</td>
<td>0.914 &lt;0.001 HS</td>
<td>0.920 &lt;0.001 HS</td>
<td>0.916 &lt;0.001 HS</td>
</tr>
<tr>
<td>ObS (%)</td>
<td>0.966 &lt;0.001 HS</td>
<td>0.952 &lt;0.001 HS</td>
<td>0.968 &lt;0.001 HS</td>
</tr>
<tr>
<td>TS (mm)</td>
<td>−0.893 &lt;0.001 HS</td>
<td>−0.918 &lt;0.001 HS</td>
<td>−0.897 &lt;0.001 HS</td>
</tr>
<tr>
<td>ES (%)</td>
<td>−0.929 &lt;0.001 HS</td>
<td>−0.954 &lt;0.001 HS</td>
<td>−0.935 &lt;0.001 HS</td>
</tr>
</tbody>
</table>

BV, bone volume; E2, estradiol; ER-α, estrogen receptor-α; ES, eroded surface; HS, highly significant; ObS, osteoblast surface; TbTh, trabecular bone thickness; TS, trabecular separation; VEGF, vascular endothelial growth factor.

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(a) Positive correlation between E2 levels (pg/ml) and TbTh (mm).
(b) Negative correlation between E2 levels (pg/ml) and ES (%).
E2, estradiol; ES, eroded surface; TbTh, trabecular bone thickness.

---

Figure 23

(a) Positive correlation between E2 levels (pg/ml) and TbTh (mm).
(b) Negative correlation between E2 levels (pg/ml) and ES (%).
E2, estradiol; ES, eroded surface; TbTh, trabecular bone thickness.
normal ranges. This result is consistent with Nordin et al. [19], who reported that Ca absorption remained constant in postmenopausal women till 75 years of age, when it decreased by 30%. Other studies [20,21] have also reported that these biochemical markers were not affected in primary osteoporosis.

The mean serum E2 level was within the normal range for age for each of our three groups, with a highly statistically significant difference between them. These results were in agreement with several previous studies [21–24]. The steady decrease in E2 in the perimenopausal period is because of the depletion of ovarian follicles, whereas in the postmenopausal period, E2 is formed by extragonadal conversion of testosterone [22].

On examining the plain radiograph of dorsolumbar spines, four osteoporotic patients (40%) of group III showed compression fractures in the dorsal spines. This is in agreement with Jergas [25], who observed that vertebral fractures were the hallmarks of osteoporosis.

Comparison of the three groups in our study in terms of BMD showed a highly statistically significant difference (P < 0.001). Similar results were reported by Khaled and Omar [21]. It was found that all premenopausal patients had normal BMD. On the other extreme, all postmenopausal patients were osteoporotic. Such findings were almost identical to those of Hassab-Elnaby et al.’s study [24]. In their study, none of the premenopausal women were osteoporotic and eight of nine postmenopausal patients (88.8%) were osteoporotic. Also, it was observed that osteoporosis was mostly recorded in postmenopausal women [26]. The gradual loss of the positive effect of estrogen on bone during the perimenopausal period and after menopause is surely a well-established cause of this deterioration in bone mass [27].

Comparison of the histomorphometrical bone parameters of the three groups showed highly statistically significant differences in all parameters (P < 0.001). This was more or less in agreement with the results of Hassab-Elnaby et al. [24], who reported highly statistically significant differences between the premenopausal and the postmenopausal considering BV, TbTh, TS, and ES, but they recorded no significant difference in ObS. These findings indicate the gradual microarchitectural deterioration of bone during perimenopausal and postmenopausal periods, which may be attributed to the steady decline in E2 levels. It is worth mentioning that histological examination of postmenopausal bone sections showed widened BM spaces with abundant fat cells. This was similar to the results of Zhao et al. [28], who reported that histological analysis of postovariectomized bone sections indicated more fat cells and reduced amount of erythropoietic marrow. A decrease in osteoblast differentiation was found to be accompanied by an increase in adipocyte differentiation as both are derived from a common multipotent mesenchymal stem cell [18].

Moreover, the highly statistical difference between the three groups in our study in the histomorphometrical parameters is in agreement with BMD differences measured by DEXA. This finding indicates that although histomorphometry remains the gold standard technique for determining the state of bone, the noninvasive DEXA, showing high efficacy and accuracy, can be used instead. It was documented that DEXA could also identify the risk of fracture as for approximately every 1 SD falls in BMD, there was a two-fold increase in the risk of fracture [24].

The immunohistochemical detection of VEGF expression in the bone biopsies showed a highly statistically significant difference between premenopausal, perimenopausal, and postmenopausal women in the VEGF percentage of expression. This was in agreement with Ding et al. [29], who observed decreased expression of VEGF and its association with bone loss in the removed lumbar vertebral fractures of ovariectionized experimental animals. They related this decrease in the level of VEGF to the withdrawal of estrogen, which was found to be associated with altered bone microcirculation, leading to local abnormal bone metabolism. Decreased VEGF may be the link through which reduced estrogen causes bone angiogenic defects both after an ovariection and after menopause. This explanation was indeed confirmed by the highly significant positive correlation between VEGF expression and both plasma E2 levels and ER-α expression in bone biopsies that was found in our study and in a previous study [30].

Comparison of the VEGF expression between those with normal BMD, osteopenic, and osteoporotic patients showed highly statistically significant differences. Furthermore, a highly statistically significant positive correlation was found between VEGF and BMD. These results are in agreement with Costa et al. [30], who reported that serum VEGF levels were lower in osteoporotic than in nonosteoporotic women, and with Zhao et al. [28], who found that in ovariectionized mice, BMD deterioration was correlated to VEGF expression and the associated decreased bone vascularization. However, our results are not in agreement with Cebi et al. [10], who reported that serum VEGF levels were higher in osteoporotic than nonosteoporotic women and reported a
nonsignificant correlation between BMD and serum VEGF in 44 women. However, several previous in vitro and experimental studies supported our finding and reported that VEGF played an anabolic role in bone both by expansion of the vascular bed and by direct bone formation [17].

VEGF expression showed a highly statistically significant positive correlation with BV, TbTh, and ObS. However, a highly statistical negative correlation with TS and ES was detected. These findings are in agreement with those of Hiltunen et al. [31], who found that VEGF gene transfer into femurs of experimental animals significantly increased bone formation parameters such as BV and ObS and decreased bone resorption surface as well. These results could be attributed to what was reported earlier by Athanasopoulos et al. [32] that VEGF increased the activity of osteoblasts both directly by its chemotactic effect on them and indirectly by stimulating endothelial cells to increase their expression of BMP-2 and BMP-4, resulting in osteoblast proliferation and differentiation. Also, it was observed that pretreatment of the osteoblast cultures with exogenous VEGF increased alkaline phosphatase release, upregulated the antiapoptotic gene BCL2 expression, and decreased the rates of programmed cell death [8].

Conclusion

VEGF expression is decreased in bone of postmenopausal osteoporotic patients and is correlated to BMD. Its release is dependent on E2 and mediated through ER-α. These findings suggest that the bone alterations induced by reduced estrogen in postmenopausal osteoporosis may be partly through decreased VEGF release, which makes it one of the possible targets in the treatment of postmenopausal osteoporosis.

Acknowledgements

Conflicts of interest
None declared.

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