CYTOKINES IN THE PATHOGENESIS OF POSTMENOPAUSAL OSTEOPOROSIS AND RELATIONSHIP BETWEEN CYTOKINES AND OSTEOCALCIN

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SUMMARY

It is suggested that interleukins (IL) and Tumor necrosis-alfa (TNF-α) play a role in the pathogenesis of postmenopausal osteoporosis as activating osteoclastic bone resorption. The aim of this study is to search the probable relationships between the serum levels of IL-1β, IL-2, IL-2r, IL-6, IL-6r, IL-8, IL-10, TNF-α and the biochemical parameters of bone metabolism, and also to compare with the serum cytokine levels in the healthy women. Serum osteocalcin, alkaline phosphatase, calcium and phosphor, urine calcium and phosphor levels were measured in 76 postmenopausal osteoporotic women. Postmenopausal 30 healthy women are accepted as controls.

The mean age of postmenopausal osteoporotic and control groups are 59.30 7.39 and 57.63 6.21, respectively. There was significant increase serum IL-8 and urine calcium between patient group than control group (p<0.01). But there was a significant decrease in the serum levels of IL-10 in patient group (p<0.01). There was no significant differences between two groups in respect to others parameters. There was a significant correlation between serum levels of IL-10, IL-1, IL-2, IL-6 and TNF-α (p<0.01). Also, There was a significant correlation between osteocalcin and IL-10 (p<0.05).

Our findings suggest that the serum levels of IL-8 which activate osteoclastic bone resorption were found significantly elevated levels in women with postmenopausal osteoporosis when compare to postmenopausal healthy women, and the serum levels of IL-10 correlated with that of osteocalcin.

Key Words: Postmenopausal osteoporosis, interleukins, Tumor necrosis factor-alpha, osteocalcin.

INTRODUCTION AND OBJECTIVE

Postmenopausal osteoporosis is a disorder characterized by a progressive loss of bone tissue that begins after natural or surgical menopause and leads to the occurrence of spontaneous fractures. Although it is well established that estrogen deficiency plays a causal role in this condition, an understanding of the mechanism by which estrogen prevent bone loss is still not

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Recent evidence suggests that estrogen may modulate the secretion of factors that are produced in the bone microenvironment that, in turn, influence bone remodeling (1,2). These factors include IL-1, a cytokine recognized for its potent effects on bone remodeling (3). IL-1 promotes bone resorption in vitro and in vivo by stimulating the activity of mature osteoclasts and the differentiation of osteoblast precursors (4). Studies have also shown that IL-1 inhibits bone formation in vitro and in vivo, and induces bone cells to secrete several other cytokines that potentiate the effects of IL-1 in bone, such as IL-6, IL-11, and M-CSF(4).

Recent studies have suggested that the increase in bone resorption induced by estrogen deficiency in postmenopausal osteoporotic women is, at least partly, mediated by increased paracrine production of bone resorbing cytokines (2). IL-1 is one of the most potent stimulators of bone resorption and IL-6 appears to be a potent osteotropic factor that may play an important role in diseases characterized with increased bone resorption (5,6) TNF-α, as IL-1, enhances bone resorption by stimulating development of osteoblast progenitors and increasing the activity of mature cells (7). Therefore, it is generally accepted that one of the mechanisms through which estrogen prevents bone loss was a modulation on secretion or release of these various cytokines that are known to influence bone remodeling (8,9), even if some recent data have challenged this hypothesis (10). However, in established osteoporosis, the possibility that enhanced cytokines activity may account for the progression of this disease remains unclear and controversial. More recently, circulating levels of IL-1, IL-1 and IL-6 have been found to be not significantly higher in osteoporotic women than in the normal women (11) and these values did not correlate with markers of bone turnover in healthy postmenopausal women (10).

The aim of this study is to search the probable relationships between the serum levels of IL-1β, IL-2, IL-2r, IL-6, IL-6r, IL-8, IL-10, TNF-α, and the biochemical parameters of bone metabolism, and also to compare with the serum cytokine levels in the healthy women.

SUBJECTS AND METHODS

Serum IL-1β, IL-2, IL-2r, IL-6, IL-6r, IL-8, IL-10, TNF-α, osteocalcin, alkaline phosphatase, creatine kinase, calcium and phosphor, urine calcium and phosphor levels were measured in 76 postmenopausal osteoporotic and 30 postmenopausal healthy women.

All 76 patients in the study were postmenopausal osteoporotic women and were selected from the Department of Physical Therapy and Rehabilitation of Dicle University Hospital. Their ages varied from 52 to 69 years for the patients with postmenopausal osteoporosis, and from 50 to 67 years for the controls (the means ages of the groups were not significantly different). Subjects were eligible for our study if they were age 50 years or older and in good general health as determined by a medical history and routine clinical blood analysis (complete blood count and differential count). Subjects were excluded if they had 1) used any drug, or had any disease or condition known to
affect bone or calcium metabolism; 2) had taken corticosteroids medications during the previous 6 months; 3) had a history of chronic renal, hepatic, or gastrointestinal disease or lumbar compression fracture; 4) evidence of collapsed or focal vertebral sclerosis.

All of subjects were free of any disease able to interfere with bone metabolism such as renal, hepatic, inflammatory, malignant or immune disorders. Since their menopause, they never received significant amounts of drugs commonly prescribed for prevention or treatment of osteoporosis, like estrogens, calcitonin, bisphosphonates, fluoride salts, anabolic steroids, vitamin D or drugs known to interact with cytokine production such as corticosteroids or immunomodulators.

We measured bone mineral density (BMD) with posteroanterior projection using standard techniques from dual energy X-ray absorptiometry. The variation coefficient for consecutive determinations on spine and femur images in our laboratory was 1.8% at lumbar spine and 1.5% at the femur region. All spinal scans were reviewed for evidence of vertebrae with collapsed or focal sclerosis by an experienced radiologist.

Blood was obtained after an all-night fast, and precautions were taken to avoid contamination. Freshly drawn blood (15 ml) samples obtained and immediately centrifuged at 200 g (20 min at 24 °C). Then, centrifuged samples were stored at -75 °C until detection at the end of the study. Serum IL-1β, IL-6, IL-8, TNF-α and sIL-2R levels were determined with quantitative ELISA and IMMULITE diagnostic kits (DPC-Diagnostic Products Corporation, USA). IMMULITE diagnostic kits are an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human cytokine in serum. We detected IL-1β (normal range 0-5 pg/ml), IL-6 (normal range 0-5.4 pg/ml), IL-8 (normal range 0-62 pg/ml), TNF-α (normal range 4-8.1 pg/ml) and sIL-2R (normal range 223-710 U/ml). The assays have undergone extensive testing with a multitude of different cytokines to ensure absence of cross-reactivity with other molecules. Serum and urinary chemical estimations were performed using Becman-Synchron CX-5 technology.

Statistical analyses were done by SPSS 8.0 PC program. Results were expressed as means SD. Groups were compared by unpaired student t-test and Pearson correlation tests were computed to measure the association between the variables studied. Results were considered to be significant at p<0.05.

RESULTS

The mean age of postmenopausal osteoporotic and control groups are 59.30 7.39 and 57.63 6.21, respectively. Osteoporotics and controls did not differ with respect to age (p>0.05). There was significant increase serum IL-8 (p<0.001) and osteocalcin (p<0.05) in patients group than control group. But there was a significant decrease serum IL-10 (p<0.003), CK (p<0.001) and urinary Ca (p<0.004) in patients group than control group. There was no significant differences between two groups in respect to others parameters.
There was a significant correlation between serum levels of IL-10 and IL-2 (r=0.26, p<0.05), IL-2r (r=0.43, p<0.01), IL-6 (r=0.37, p<0.01), TNF-α (r=0.37, p<0.01), osteocalcin (r=0.23, p<0.05). Also, there was a significant correlation between IL-1 and IL-6 (r=0.23, p<0.05), TNF-α (r=0.45, p<0.01); between IL-2 and TNF-α (r=0.35, p<0.01); between IL-6 and IL-6r (r=0.34, p<0.01), TNF-α (r=0.27, p<0.05) (Table 2).

**Table 1**: Mean SD of serum cytokines, serum and urinary chemical parameters in control and patients and osteoporotic postmenopausal women.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients (n=76)</th>
<th>Controls (n=30)</th>
<th>t</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>88.51 ± 30.47</td>
<td>98.83 ± 24.48</td>
<td>0.10</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>CK</td>
<td>90.11 ± 54.89</td>
<td>92.9 ± 38.4</td>
<td>3.46</td>
<td>0.001</td>
</tr>
<tr>
<td>Urinary Ca</td>
<td>17.53 ± 10.36</td>
<td>24.28 ± 11.07</td>
<td>2.97</td>
<td>0.004</td>
</tr>
<tr>
<td>Urinary P</td>
<td>34.02 ± 8.10</td>
<td>35.60 ± 6.21</td>
<td>0.96</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>IL-10</td>
<td>4.28 ± 1.56</td>
<td>5.35 ± 1.76</td>
<td>3.07</td>
<td>0.003</td>
</tr>
<tr>
<td>IL-1</td>
<td>5.07 ± 0.34</td>
<td>5.01 ± 0.01</td>
<td>1.18</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>IL-2</td>
<td>15.19 ± 3.90</td>
<td>14.42 ± 2.06</td>
<td>1.02</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>IL-2r</td>
<td>525.22 ± 61.7</td>
<td>482.21 ± 72.4</td>
<td>0.82</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>IL-6</td>
<td>5.78 ± 2.4</td>
<td>6.06 ± 3.29</td>
<td>0.47</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>IL-6r</td>
<td>22.35 ± 9.67</td>
<td>26.32 ± 10.17</td>
<td>1.88</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>IL-8</td>
<td>39.05 ± 28.2</td>
<td>16.26 ± 15.59</td>
<td>3.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>21.1 ± 11.94</td>
<td>15.57 ± 11.92</td>
<td>2.82</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum Ca</td>
<td>9.26 ± 1.11</td>
<td>9.4 ± 0.59</td>
<td>0.63</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Serum P</td>
<td>3.39 ± 0.62</td>
<td>3.25 ± 0.5</td>
<td>1.08</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TNF-α</td>
<td>14.65 ± 12.81</td>
<td>11.75 ± 8.88</td>
<td>1.14</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Age</td>
<td>59.30 ± 7.39</td>
<td>57.63 ± 6.21</td>
<td>1.08</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
**DISCUSSION AND CONCLUSION**

Much interest has been focused on the role of the immune system in bone remodeling and, particularly, on the potential influence of cytokines in the autocrine and paracrine regulation of bone cell activity (12-15). Cytokines in the microenvironment of the bone, such as interleukins 1 and 6 (IL-1 and IL-6), tumor necrosis factor (TNF), interferon-γ (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF), affect the bone-remodeling process by regulating the differentiation as well as the activity of osteoblasts and osteoclasts (13,16-18). Cytokines play an important role in the regulation of bone resorption and formation during pathologic bone remodeling and also play a role during normal bone remodeling (18).

Osteoclast- mediated resorption is influenced by two processes: activation, in which resorptive function of mature osteoclasts is increased, and recruitment, in which osteoclast progenitors are stimulated to generate new osteoclasts. For example, PTH, IL-1 or TNF-α are believed to activate mature osteoclasts in directly via stromal cells or osteoblasts (19).

Over the past decades, numerous reports have been published demonstrating that natural or surgical menopause increases blood, bone marrow, and monocyte levels of IL-1, IL-6, TNF, and the related factors IL-1R (20,21). In spite of these observations, controversy persists concerning the specific contribution of each of these factors to postmenopausal bone loss.
Proinflammatory cytokines secreted by immunocompetent cells have a role in the regulation of the activity of osteoblasts and osteoclasts. The effects of these proinflammatory cytokines include the inhibition of bone formation and an increase in bone resorption. The inflammatory process in rheumatoid arthritis may cause periarticular and systemic bone loss by various cytokine and hormone mediated mechanisms. Concluding from these pathogenetic mechanisms, bisphosphonates and active vitamin D metabolites are likely to be effective therapeutic options in osteoporosis associated with rheumatoid arthritis (22).

One does not typically consider osteoporosis an immunologic disorder. However, recent data from a number of laboratories have indicated a potential critical role for certain proinflammatory cytokines, both in the normal bone remodeling process and in the pathogenesis of perimenopausal and late-life osteoporosis (2,20). It is currently believed that during premenopausal years, estrogen in the bone marrow microenvironment regulates the expression of certain of these cytokines, most notably IL-6. In the presence of estrogen, IL-6 expression is suppressed, but in its absence, levels increase. IL-6 is a potent activator of osteoclasts and bone resorption. Similarly, other cytokines, such as IL-1, IL-11 and TNF influence osteoclast function and an age-associated dysregulation of these may also contribute to the development of bone disease (23).

Our findings suggest that the serum levels of IL-8 which activate osteoclastic bone resorption were found significantly elevated levels in women with postmenopausal osteoporosis when compared to postmenopausal healthy women, and the serum levels of IL-10 correlated with that of osteocalcin.

ÖZET

POSTMENOPOZAL OSTEOPOROZUN PATOGENEZİNDE SİTOKİNLER VE SİTOKİNLERLE OSTEOKALCİN ARASINDAKİ İLİŞKİ

İnterlökınler (IL) ve tümör nekroz faktör-α (TNF-α) ’nin osteoklastik kemik rezorpsiyonunu aktive ederek postmenopozal osteoporozun patogenezinde rol oynadıkları ileri sürülmektedir. Bu çalışmanın amacı postmenopozal osteoporozlu hastalarda IL-1β, IL-2, IL-2r, IL-6, IL-6r, IL-8, IL-10, TNF-α’nın serum düzeyleri ile kemik metabolizmasının biyokimyasal parametreleri arasındaki muhtemel ilişiğini araştırmak ve aynı zamanda serum sitokin düzeylerini sağlıklı bireylerle kıyaslamaktır.

Postmenopozal osteoporotik ve sağlıklı kontrol grubunun yaş ortalamaları sırasıyla 59.30 7.39 ve 57.63 6.21 idi. Hasta grubunda kontrol grubuna göre serum IL-8 ve uriner kalsiyum atılımından anlamılı artış vardır (p<0.01). Fakat hasta grupunun serum IL-10 düzeyleri kontrol grubuna göre anlamılı düzeye daha düşüktü (p<0.01). Diğer parametreler açısından iki grup arasında anlamılı farklılık yoktu (p>0.05). Serum IL-10, IL-1, IL-2, IL-6 ve TNF-α düzeyleri arasında anlamılı korelasyonlar mevcuttu (p<0.01). Aynı zamanda,
serum IL-10 düzeyleri ile serum osteokalsin düzeyleri arasında da anlamli korelasyon vardı (p<0.05).

Bizim bulgularımız postmenopozal osteoporozlu hastalarda postmenopozal sağlıklı kontrollere göre, osteoklastik kemik rezopsiyonunu aktive eden IL-8'in serum düzeylerinin anlamlı düzeyde yokseldigini ve serum IL-10 düzeyleri ile osteokalsin düzeyleri arasında anlamli korelasyon olduğunu ortaya koymaktadır.

Anahtar Kelimeler: Postmenopozal osteoporoz, İnterlokinler, TNF-α, osteocalcin.

REFERENCES


