EFFECT OF GM-CSF LEVELS ON OSTEOPOROSIS IN POSTMENOPAUSAL PERIOD

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ABSTRACT

Objectives*: Cytokines and hematopoietic growth factors may play a role in the pathogenesis of osteoporosis. The aim of this study was to assess the relationship between osteoporosis (OP) and granulocyte-macrophage colony stimulating factor (GM-CSF) in postmenopausal period.*

Materials and methods*: Total number of 80 female patients between the ages of 45-75 and who had entered in menopause from at least one year and diagnosed with OP were enrolled in this study. As a control group, 80 healthy volunteer female patients who had entered in menopause from at least one year and had no diagnosis of OP were selected. The age, height, weight, body mass index, duration of menopause, exercise habits of the patients and controls were recorded and bone mineral density was measured for the diagnosis of OP. The serum GM-CSF concentrations of individuals were quantified using a specific enzyme immunoassay kit.*

Results*: There was no significant relationship between the mean age, duration of menopause and body mass index of patients and controls (p>0.05). Serum GM-CSF levels of patients and control groups did not differ significantly (p> 0.05).*

Conclusion*: The results of our study showed that there was no significant correlation between osteoporosis and serum GM-CSF levels. Osteoporosis might be correlating with the levels of GM-CSF in bone microenvironment rather than serum levels.*

Key words*: Osteoporosis, granulocyte-macrophage colony stimulating factor, menopause.*

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Introduction

Osteoporosis (OP) is a metabolic bone disease which causes an increase in the risk of bone fragility and fracture due to low bone mass and deterioration of bone micro-architecture[(1)]. Estrogen deficiency is the most important mechanism determining both directly and indirectly OP. Estrogen shows its direct effects via estrogen receptors on osteoblasts and osteoclasts and indirectly through the stromal cells of the immune system[(2)].

In osteoporosis, bone resorption increases with rise of osteoclastogenesis and reduction of osteoclasts' apoptosis. In bone resorption, some proinflammatory cytokines are effective by increasing the proosteoclast pool in bone marrow. In addition, osteoclastogenesis is regulated by modulation of growth factors[(3,4,5)]. Granulocyte-macrophage colony stimulating factor (GM-CSF) is a hematopoietic growth factor which have a glycoprotein structure and regulates the formation of cells of myeloid origin and their functional activation[(6)]. The cytokines that mediate the immune response and inflammation are produced by activation of T cells, macrophages, fibroblasts and endothelial cells[(7)].

The goal of this study was to compare GM-CSF levels between postmenopausal osteoporotic patients and control group. Thus, we aim to demonstrate the effect of GM-CSF on OP.

Materials and methods

In this study, 80 female patients between the ages of 45-75 and who had entered in menopause from at least one year and diagnosed with OP were enrolled; contemporary, 80 healthy volunteer fema-

le patients who had entered menopause from at least one year and had no diagnosis of OP, were enrolled as control group. Individuals with disease which can affect bone metabolism including chronic liver disease, chronic renal failure, hyperparathyroidism, malabsorption, osteomalacia, inflammatory rheumatic disease and who use drug that can affect bone tissue including steroids, diuretics and anticonvulsants, as well as immobilized patients, or the ones suffering from diseases which may affect the level of cytokines (major depression, myeloma) and smokers were excluded from the study.

The age, height, weight, body mass index (BMI), duration of menopause, exercise habits of the patients and controls were recorded. OP patients were diagnosed by bone mineral density (BMD) measurements. BMD was measured by dual energy X-ray absorptiometry (DXA) method (Hologic, QDR-4500 Elite, USA) both at the L2-L4 lumbar spine (anterior-posterior projection of L2-L4) and at the proximal left femur. According to World Health Organization criteria lumbar and total hip T-score -2.5 and those below were diagnosed as OP[8]. The serum GM-CSF concentrations were quantified using a specific enzyme immunoassay kit (eBioscience, San Diego, USA). The detection limit was 2.9 pg/ml.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows 18.0 (Statistical Package for Social Sciences) package software. Continuous variables were examined by Kolmogorov-Smirnov test to check normal distribution. Students's t-test and the Mann-Whitney U test were used to compare the groups. $p < 0.05$ was considered as significant for all statistical data.

Results

80 patients with OP and 80 controls were enrolled into our study. The mean age was 61.4±4.8 years while mean duration of menopause was 14.5±0.4 years in the patients group, while the mean age was 61.8±5.3 years and mean duration of menopause was 14.6±0.5 years in control one. There was no significant association between the mean age, duration of menopause and BMI of patients and controls (Table 1). Comparison of demographic data between the patient and control groups is presented in Table 1.

	Patient (n=80) Mean±SD	Control (n=80) Mean±SD	P
Age (year)	61.4±4.8	61.8±5.3	>0.05
Duration of menopause (year)	14.5±0.4	14.6±0.5	>0.05
BMI (kg/m^2)	25.3±0.7	25.5±0.5	>0.05

Table 1: Comparison of demographic data between the patient and control groups.
BMI: Body mass index. p<0.05 statistically significant.

The serum GM-CSF levels of patients and control groups were compared and no significant difference was found. Comparison of serum GM-CSF levels between patients and controls are shown in Figure 1. Lumbar spine and femur BMD values were statistically higher in the control group. Table 2 summarizes the comparison of BMD and serum GM-CSF levels between patients and controls.

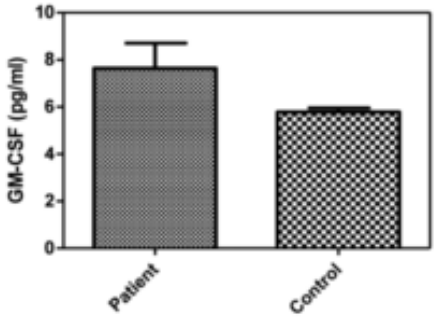

Fig. 1: Comparison of GM-CSF levels between patients and controls

Discussion

The results of our study demonstrated that there was no significant difference in the level of serum GM-CSF between postmenopausal OP patients and control group.

Estrogen deficiency is of great importance in loss of bone tissue in postmenopausal period. In addition, age and low BMI are important risk factors[9,10]. Although estrogen levels of patients and controls were not evaluated in the study, results did not differ significantly in terms of age and BMI.

There is a correlation between OP and variety of inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematous. Inflammation affects bone metabolism increasing the risk of bone

	GM-CSF(pg/ml) *Median (min-max)*	Lumber spine BMD(g/cm²) *Mean±SD*	Lumber spine T score *Mean±SD*	Lumber spine Z score *Mean±SD*	Total femur BMD(g/cm²) *Mean±SD*	Total femur T score *Mean±SD*	Total femur Z score *Mean±SD*
Control	5.51 (0.72-10.06)	1.12±0.2	- 1.6±1.2	-0.3±0.4	1.24±0.3	-1.2±1.4	-0.2±0.5
OP	5.39 (4.48-85.50)	0.88±0.4	- 2.9±0.7	-0.9±0.8	0.91±0.7	-2.5±1.2	-0.8±0.7
P	***> 0.05****	***< 0.05****	***< 0.05****	***< 0.05****	***< 0.05****	***< 0.05****	***< 0.05****
*Mann-Whitney U test **Students's t test							

Table 2: Comparison of BMD and GM-CSF values between patients and controls.
GM-CSF: Granulocyte-macrophage colony stimulating factor, BMD: Bone mineral density, p<0.05 statistically significant.

resorption and bone fracture. In inflammation-related bone resorption, proinflammatory cytokines and hematopoietic growth factors are usually involved[5]. Best of our knowledge in the literature, there were few studies and different results about the relationship between GM-CSF levels and OP.

Kung et al.[11] investigated the effect of a selective estrogen receptor modulator (SERM), SP500263, on osteoclastogenesis. They found that Interleukin-6 (IL-6) and GM-CSF were involved in the formation of osteoclasts. In postmenopausal period, estrogen deficiency is associated with increased synthesis of cytokines by the osteoblasts. They also determined that decreasing the production of these cytokines by SERM and estrogen therapies prevented bone loss.

In another study, levels of GM-CSF, a strong inhibitor of osteoclastogenesis, was identified to increase in the synovial fluid of inflammatory arthritis such as rheumatoid arthritis. In the presence of tumor necrosis factor α (TNF-α), the myeloid cells was differentiated by GM-CSF to osteoclasts and these osteoclast precursor cells had thought to be specific to inflammatory arthritis[12]. Atanga et al.[13] reported that TNF-α (an activator of osteoclastogenesis) blocked the formation of osteoclast in mice, and GM-CSF could be a mediator in TNF-dependent inhibition of osteoclastogenesis.

In another other study, in which were assessed immune changes in postmenopausal OP women, 26 patients and 24 healthy controls were compared and it was found that GM-CSF was secretion by B lymphocytes was higher in the first group. There was a positive relationship between GM-CSF and fracture rate and a negative correlation with BMD[14]. In an experimental research, it was found that GM-CSF caused bone resorption by stimulating the synthesis of prostaglandin E2[15].

Pacifici et al. in their study including 15 patients who entered surgical menopause by oophorectomy and 9 patients received a simple hysterectomy, proved that activity of the GM-CSF, IL-1 and TNF-α were increased in 15 patients after one week of oophorectomy surgery. After 8 weeks, in six out of 15 patients who had not received estrogen therapy, these three cytokines had the highest levels. For the remaining 9 patients estrogen replacement therapy was initiated four weeks after surgery and decreased secretion of cytokines in parallel with a decrease in bone resorption were determined. In 9 patients who had simple hysterectomy, no significant changes were detected in cytokine release and bone turnover markers. These outcomes may be associated with changes in the level of estrogen[4]. Lorenzo et al.[16] investigated the effects GM-CSF and IL-3 on bone resorption and osteoclast formation. They found that GM-CSF and IL-3 had a role in the formation of osteoclast from the precursor cells.

In another study investigating the levels of the cytokine in patients with postmenopausal osteoporosis, IL-1 beta, IL-6 and TNF-α levels were found to be increased whereas there was no change in the GM-CSF levels[17]. We also found that there was no significant difference in terms of serum GM-CSF levels. Another study by Pacifici et al.[18] revealed that cytokine levels returned to premenopausal levels after 8 years of menopause. In our study, there was no significant difference between the groups in aspect of serum GM-CSF levels. This might be explained by over 10 years of menopausal duration of subjects, the small number of participants and different cytokine measurement method. However, mean BMD rate of our patients was not very low. This also can be a factor for the result of our study. Additionally, osteoporosis might be correlating with the levels of GM-CSF in bone micro-

environment rather than serum levels.

Our study had some limitations; the sample size of the study was small, only serum GM-CSF levels were quantified in the cytokines and estrogen levels of subjects were not evaluated in our study.

In conclusion, although there were no significant correlation between osteoporosis and GM-CSF in our study, some cytokines levels may change during the postmenopausal period. Therefore, further prospective studies with larger sample size are needed to better understand the relationship between GM-CSF levels and osteoporosis.

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