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Correlation of Serum Cytokine Levels with Axial Bone Mineral Density

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ABSTRACT

Cytokine has been postulated to play a role in the pathogenesis of post-menopausal osteoporosis. To test this hypothesis we measured circulating levels of IL-1, IL-6,IL-8 and TNF-α in 98 post-menopausal women (30 age matched normal and 68 osteoporotic) with no vertebral fractures. Although the cytokine levels of patients were found in normal cut off values, the difference in cytokine levels between patients and controls was statistically significant for IL-1 and IL-8 ($p<0.01$). In osteoporotic patients, none of the cytokines correlated with lumbar, femoral (neck) and total hip bone mineral densities and also with body mass index ($p>0.01$).

In conclusion, we were unable to demonstrate abnormalities of cytokines affecting bone resorption in peripheral serum of women with post-menopausal osteoporosis. However increased production of these cytokines may occur in the local environment of bone.

Keywords: Osteoporosis, cytokine

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INTRODUCTION

Recent studies have suggested that bone loss in women at post-menopausal period is mediated by increased production of cytokines such as IL-1, IL-6 , IL-8 and TNF[1]. In vivo, IL-1 and TNF are powerful stimulators of bone resorption[1]. IL-6 is also important in osteoclast biology. It stimulates osteoclast progenitors and estrogen inhibits released IL-6. Ovariectomised rats overproduce IL-6 and bone loss can be prevented by administering estrogen[1,2]. IL-6 also potentiates bone resorption by parathyroid hormone related protein[1]. However, ovariectomy in women is related to increased serum soluble IL-6 receptors but not any alteration in serum IL-6 level[2].

TNF and IL-1 are produced in increased amounts by peripheral blood monocyties of ovariectomised rats. IL-1 and TNF also enhance osteoclast survival by preventing apoptotic cell death. Although these results are supported with animal studies, the studies in human cell are controversial[1,2].

We undertake this prospective study to investigate whether circulating cytokine levels are higher in osteoporotic patients than normals and correlate with bone mineral density of the axial skeleton and interrelation of body mass index with serum cytokine levels in postmenopausal women.

SUBJECTS AND METHODS

Sixty-eight post-menopausal women with osteoporosis (according to WHO criteria, as a T score <2.5 SD at any site)[3] were entered into the study. Patients with secondary osteoporosis were excluded by means of laboratory and radiological findings. Bone mineral density (BMD) was measured by DEXA (LUNAR - DPX-L, Madison, WI) at lumbar (L2-L4) , femoral neck and total hip regions. All scans and analyses were performed by the same technician. Thirty (30) age and sex matched controls with normal bone mineral density (according to WHO criteria, as a T score ≥1) were also recruited to compare with osteoporotic patients. They were clinically healthy, non-smokers and no medications known to affect bone metabolism and had no any inflammatory disease that could result in elevation of cytokine levels or chronic infections. Patients had no vertebral, hip and distal forearm fractures as a result of osteoporosis. They had no history of hysterectomy or any operation. We measured circulating levels of IL-1, IL-6, IL-8 and TNF-α in the serum of 68 osteoporotic and 30 healthy post-menopausal women. All blood samples were taken in the morning after an overnight fast. Subjects were in a supine resting position for at least 30 min. Serum was stored at -70 degrees until the time of analysis.

Body mass index was measured as (Kg/m^2). It has been used because of its correlation with total fat mass. Body height and weight were recorded in light clothing.

Serum IL-6 was measured with ELISA (Cytimmune, Cytelisa, C 6001NCD). This assay has a sensitivity of 10 pg/ml. Serum IL-1-β, was measured by ELISA

(I BOO2-NCD). The sensitivity was 8 pg/ml, Serum IL-8 was measured by ELISA (Pelikine compact Tm human IL-8, U 1918). The sensitivity was 10 pg/ml. TNF-α was measured by ELISA (CLB, Pelikine Tm humanTNF-α, U1920) . The sensitivity was 10 pg/ml.

All analyses were performed with SPSS 9.0, All data were expressed as mean ±SD. Data analysis was performed using t test between patients and controls. The significance was set at $p<0.01$. Pearson correlation coefficients were used to evaluate the association among cytokines, BMI, age, duration of menopause and bone mineral density. Correlation analyses between cytokine and BMI were demonstrated by linear regression lines .

RESULTS

Table I shows the clinical characteristics of the subjects. Normal and osteoporotic subjects did not differ with respect to age, BMI and duration of menopause ($p>0.01$).

Table II shows the circulating cytokine levels in the normal and osteoporotic women in post-menopausal period. Although, serum levels of IL-1 and IL-8 were higher in osteoporotic patients than controls and the difference was statistically significant ($p<0.01$), their ranges were not high to confirm the hypotese that osteoporotic patients have higher serum cytokine levels than normal subjects. The serum levels of IL-6 and TNF were not higher than controls. Therefore, the difference was statistically insignificant($p>0.01$). There was no significant correlation between serum IL-1, IL-6, IL-8 and TNF levels and bone mineral density of lumbar, femoral (neck) and total hip regions in osteoporotic patients. ($r=0.747$, $r= 0.367$, $r= 0.809$, $r=0.931$, $p>0.01$, for correlation between lumbar bone mineral density and IL-1, IL-6, IL-8 and TNF). ($r= 0.212$, $r=0.742$, $r=0.748$, $r=0.783$, $p>0.01$, respectively for correlation between femoral neck bone mineral density and IL-1, IL-6, IL-8 and TNF). ($r= 0.756$, $r= 0.783$, $r=0.953$ and $r= 0.892$, $p>0.01$, respectively for correlation of total hip bone mineral density and IL-1, IL-6, IL-8, TNF). We also found no correlation between cytokine levels and bone mineral density at the lumbar, femoral neck and total hip in controls. There was no correlation between cytokines and age, BMI, menopause duration in osteoporotic patients ($p>0.01$).

Table I. Clinical characteristics of patients and controls.

	Controls (n=30)	Osteoporotic (n=68)	p value
Age (year)	59.6 ± 5.3	56.9 ± 6.4	0.02
BMI (Kg/m)	28.9 ± 4.0	30.4 ± 4.0	0.176
Duration of menopause	12.5 ± 6.6	10.5 ± 4.7	0.02
Lumbar BMD	1.155 ± 0.11	0.783 ± 0.11	0.00
Femoral neck BMD	1.032 ± 8.5	0.691 ± 7.4	0.00
Total hip BMD	1.031 ± 7.3	0.781 ± 9.9	0.00

Table II. Serum cytokine levels.

Pg/ml	Patients	Controls	p value
IL-1	5.1 ± 2.7	3.5 ± 1.1	0.01
IL-6	3.8 ± 2.0	3.5 ± 1.5	0.58
IL-8	5.1 ± 2.2	6.6 ± 2.2	0.01
TNF-α	5.1 ± 2.5	5.6 ± 2.4	0.457

DISCUSSION

Osteoporosis is the most common cause of morbidity among older people. Loss of ovarian function increases the rate of bone remodelling and resulting in bone loss. Many local factors such as IL-1, IL-6, IL-8 and TNF have been implicated in the pathogenesis of osteoporosis[1]. IL-1 and TNF-a are potent stimulators of bone resorption and can also inhibit bone formation. Estrogen may affect bone remodelling by influencing IL-1 mediated regulation of IL-8. Animal studies also corroborate this hypotese. IL-1 receptors (IL-1R1), rather than IL-1 itself are altered in estrogen deficiency. Animals lacking IL-1 receptors or high serum concentration of soluble TNF receptors do not lose bone, after overiectomy. In estrogen deficient humans and animals, IL-1 activity is increased in cultures and IL-1 mRNA is increased in bone, but concentration of immunoreactive IL-1 is not increased in peripheral blood and marrow cell cultures[1,2].

IL-6 stimulates bone resorption. Estrogen deficiency is associated with increased production of IL-6 in marrow cultures. The bioactivity is enhanced in the presence of the soluble IL-6 receptor. In rodent models, IL-6 mediated stimulation of osteoclast and regulation in the presence of estrogen depletion has been well reported[1,2]. However their role is controversial in human studies[1]. Girasole and coworkers[4] found an increase in serum sIL-6R levels upon ovariectomy. Another study found that sIL-6R, but not IL-6 itself correlated with bone mineral density in postmenopausal women[5].

McKane et al[6] suggested that there was no relation between IL-6 and bone mineral density. Kania et al[7] also found that there was no relationship between plasma IL-6 level ,osteocalcin, and bone density. In animals, inhibition of IL-1 and TNF alpha by the IL-1 receptor antagonist(IL-1RA) and a TNF soluble binding protein limits the bone loss. Bajnok et al[8] observed no significant association between

IL-1 receptor antagonist protein gene polymorphism and bone mineral density. Murray et al[9] also suggested that IL-6 polymorphism may be associated with peak bone mass rather than bone loss. Khosla et al[10] indicated that women with high turnover osteoporosis did not have increased circulating levels of IL-1 or IL-6 in contrast to Pacifici et al[11].

In contrast to cytokines, bone resorption is inhibited and bone stimulation is increased by local growth factors such as TGF-beta. Protective effect of estrogen against bone loss could be mediated by TGF-beta induced apoptosis of osteoclasts. Ovariectomy in rodents is associated with a decrease in the TGF-beta m RNA in bone matrix. Unfortunately TGF-beta was not measured[12].

In the present study, we attempted to correlate plasma IL-1, IL-6, IL-8 and TNF with bone mineral density and observed no correlation between them and bone mineral density at lumbar, femoral (neck) and total hip.

This lack of correlation of bone density and plasma cytokine may indicate that serum cytokines were less important in humans than in rodents. Alternatively, sample size may be inadequate or these results may not reflect the bone microenvironment and small increment of cytokine in the bone has no effect on peripheral circulation.

At the same time, the number of patients was low and the study was preliminary.

There was no correlation between age, BMI, menopause duration and cytokine levels. However, Straub et al[13] found that there was correlation between BMI and IL-6 because of increased fat mass during ageing. The cause of increased plasma IL-6 in the elderly subjects remains unknown. On the other hand, Van duijn et al[14] did not find higher levels of serum IL-6 in the patients with Alzheimer disease than in age and sex matched healthy subjects. However, Jun Wei et al[15] found that IL-6 was higher in older subjects and significantly in the male group. Although our study group consisted of elderly subjects, they were all female. Clark et al[16] reported that spontaneous secretion of IL-8 in elderly males was lower than that of both elderly females and young subjects. O-Mahony et al[17] also suggested that flow cytometry detected significant increases in intracellular T cell TNF, IL-6, IL-1. So these studies were in contrast with our result and controversial with each other.

In conclusion, we suggest that there was no correlation between plasma cytokine levels and axial bone mineral density in the present study. However cytokine may show a correlation with bone mineral density in bone tissue samples.

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