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Correlation of Interleukin-6 Serum Levels with Bone Density in Postmenopausal Women

N.G. PAPADOPOULOS, K. GEORGANAS, V. SKOUTELLAS, E. KONSTANTELLOS*, G.P. LYRITIS

Summary **In order to identify possible correlations between interleukin-6 (IL-6) and hormonal and biochemical parameters of bone metabolism, or bone density, 24 postmenopausal women were studied. Serum IL-6, estradiol, calcium, phosphorus, osteocalcin, alkaline phosphatase, the urinary secretion of calcium, phosphorus and hydroxyproline, and bone density of the lumbar spine, femur and radius were measured. No significant correlation was found between IL-6 and the biochemical parameters. A negative correlation was found between IL-6 and serum estradiol, as well as between IL-6 and bone density in 5 out of 6 sites studied. It is possible that women with high IL-6 levels, may develop lower bone mass.**

Key words **Bone Density, Bone Markers, Osteoporosis, Cytokines, Interleukin-6.**

INTRODUCTION

Women after menopause develop accelerated bone turnover. In most of them, the annual bone loss rate remains relatively low, while in others this rate is higher, leading to osteoporosis and subsequently osteoporotic fractures (1).

Assessment of bone loss is performed by sequential bone density measurements. Furthermore, information about the metabolic state of the skeleton can be obtained by measurements of the so-called biochemical bone markers, such as osteocalcin, alkaline phosphatase, urine calcium to creatinine etc., all of which are indicative of bone cell function but not exclusively bone-related (2).

Interleukin-6 (IL-6) is a pluripotent protein, produced by and acting on various cell types including osteoblasts and osteoclasts (3). The role of IL-6 in the pathogenesis of osteoporosis is currently under scrutiny. Is is produced mainly by the osteoblasts, but also by the osteoclasts. It increases early osteoclast formation, and there is evidence that it is required for the normal mature osteoclasts to form resorption lacunae (4,5). IL-6 has been implicated in the increased osteoclastic activity seen in conditions like Paget's disease of bone, multiple myeloma and hypercalcemia caused by solid tumours (5).

Attempts to correlate serum levels of IL-6 with bone density or its markers have so far been unsuccessful (6,7). We report herein the results from a series of 24 postmenopausal women, on which bone density and biochemical bone markers were compared with IL-6 serum levels. A significant correlation with bone density, but not with biochemical bone markers, was observed and possible explanations for these findings are discussed.

MATERIALS AND METHODS

Twenty-four healthy postmenopausal women that consecutively consulted our center for preventive control, were studied. Their age was 48.9 ± 2.7 years (range 44-54) and the interval since their last menses 2.7 ± 1.4 years, ranging from 8 months to 5 years. In 9 of them menopause followed bilateral oophorectomy, which was done in order to treat benign disease. Women suffering from conditions that can be related to secondary osteoporosis (e.g., liver on renal disease, gastrectomy, enterectomy, thyroid or parathyroid disease, prolonged immobilisation), or from any other metabolic or congenital bone disease, and/or with previous or current treatment with bone acting drugs, and women with diseases associated with an acute phase response (infection, connective tissue or malignant disease) were excluded from the study.

Careful history and clinical examination as well as laboratory tests were performed. These included X-rays of the chest and the lumbar and thoracic spine, complete blood count, erythrocyte sedimentation rate, blood glu-

Th. Garofalidis Research Centre, University of Athens and *Hormonological Laboratory, District General Hospital of Nikea, Greece.

cose, urea, creatinine, transaminases, alkaline phosphatase, γGt, proteins, protein electrophoresis, calcium, phosphorus, T3, T4, TSH, and urinalysis. Venipunctures were performed during early morning consultations. Six women with abnormal clinical or laboratory findings were excluded from the study.

Bone density measurement was done by the method of Dual Energy X-Ray Absorptiometry with a LUNAR DPX (Madison, Wisconsin, USA) at the sites: L2-L4 vertebrae, femur (neck, trochanter, Ward's triangle) and radius of the nondominant side (shaft, ultradistal). The age matched Z-score of these measurements is reported.

Serum calcium, phosphorus, alkaline phosphatase, osteocalcin (EIA-DRG Instruments GmbH), estradiol (coated tube RIA kit / DPC), and the 24-hour urine excretion of calcium were measured. The calcium/creatinine, phosphorus/creatinine and OH-proline/creatinine ratios of fasting 2-hour urine collections were also assessed after a 24-hour diet without gelatine.

IL-6 serum levels were evaluated with a bioassay based on the proliferative effect of this cytokine on the IL-6 dependent murine B-cell hybridoma 7TD1 (kindly provided by Dr. J. Van Snick, Ludwig Institute for Cancer Research, Brussels), as previously described (8). Briefly, samples and cytokine standards were added in triplicate wells of 96-well plates, together with 2000 hybridoma cells and incubated for 3 days in a humidified 5% CO_2 atmosphere at 37°C. Four hours before the end of the culture 1μCi per well of tritiated thymidine was added. Thymidine incorporation was measured in a Packard Scintillation Counter (Packard Instruments, Dowers Grove, IL). The inter- and intra-assay variability was always less than 10% (8).

To evaluate the relation between serum IL-6 and the biochemical, hormonal and densitometrical paramteres we used a nonparametric approach and the Kendall tau as the rank correlation coefficient (9). The Statistica for Windows Rel 4.5 software program was used for the calculations.

RESULTS

No correlation was observed between serum IL-6 and the demographic characteristics (age, height, weight, years since menopause, previous oophorectomy number of births) of our subjects. Likewise there was no statistically significant correlation between serum IL-6 and osteocalcin, alkaline phophatase, calcium, phosphorus, nor with the fasting 2-hour urine calcium/ creatinine, phosphorus/ creatinine, OH-proline/ creatinine ratios, and the 24-hour urine excretion of calcium. Estradiol correlated negatively with IL-6 (Kendall tau = –0.344, p = 0.018). Mean values of these parameters and correlation coefficients are shown in Table I.

Table I: *Mean values of demographic characteristics and bone markers and correlation with interleukin-6*

	Mean ± SD	Range	Kendall Tau
Age (years)	48.9 ± 2.9	44-54	–0,05
Height (cm)	156 ± 5.9	146-169	–0,02
Weight (kg)	68.4 ± 12.1	54-102	–0,15
Years since menopause	2.7 ± 1.5	0.6-5	0
Previous oophorectomy	–	–	–0,02
Number of births	1.5 ± 0.9	0-3	–0,06
Osteocalcin (ng/ml)	13.2 ± 2.9	8.7-20	0,02
Alk. Phosphatase (U/dl)	28.7 ± 9.6	16-48	–0,16
Calcium (mg/dl)	9 ± 0.5	8.1-10.6	–0,06
Phosphorus (mg/dl)	3.7 ± 0.3	3-4.1	–0,2
Urine Ca/creatinine	0.08 ± 0.04	0.02-0.17	–0,09
Urine OHproline/creatinine	0.01 ± 0.002	0.007-0.02	0,15
24h urine Ca (mg)	135.4 ± 85.8	32-434	–0,12
Estradiol	12.1 ± 7.3	3.7-34	–0.34*

* p = 0.018

Five out of 6 bone density measurements (lumbar spine, radius, shaft, radius ultradistal, femoral neck and Ward's triangle) negatively correlated with IL-6 serum levels, while trochanter density did not (Fig. 1).

DISCUSSION

The involvement of cytokines and growth factors in bone metabolism has been a subject of major interest during recent years (10). Among cytokines, IL-6, a pluripotent protein involved in a variety of physiological and pathophysiological procedures, has been established as an important bone-resorbing substance (5). Initially, the direct effect of IL-6 on osteocytes and their precursors has been demonstrated using in vitro and ex vivo models (11). Subsequently, IL-6 modulation of and by bone acting hormones such as parathormone or oestrogens was established (10,12). Even though it seems natural to postulate that IL-6 would play an important role in human disease states such as post-menopausal osteoporosis (10), definite proof has not been provided. Several reasons account for this difficulty. Firstly, cytokines have an autocrine/paracrine action, so that it is difficult to evaluate their actual in vivo effects from peripheral blood samples (13), which reflect the overall production (and consumption) of the cytokine in the organism. Secondly, cytokines are tightly integrated into a network therefore an imbalance rather than a direct effect of one of them is most likely to result in disease (5). Furthermore, not enough data are available on normal values or fluctuations due to age, sex, diet or a multitude of other pa-

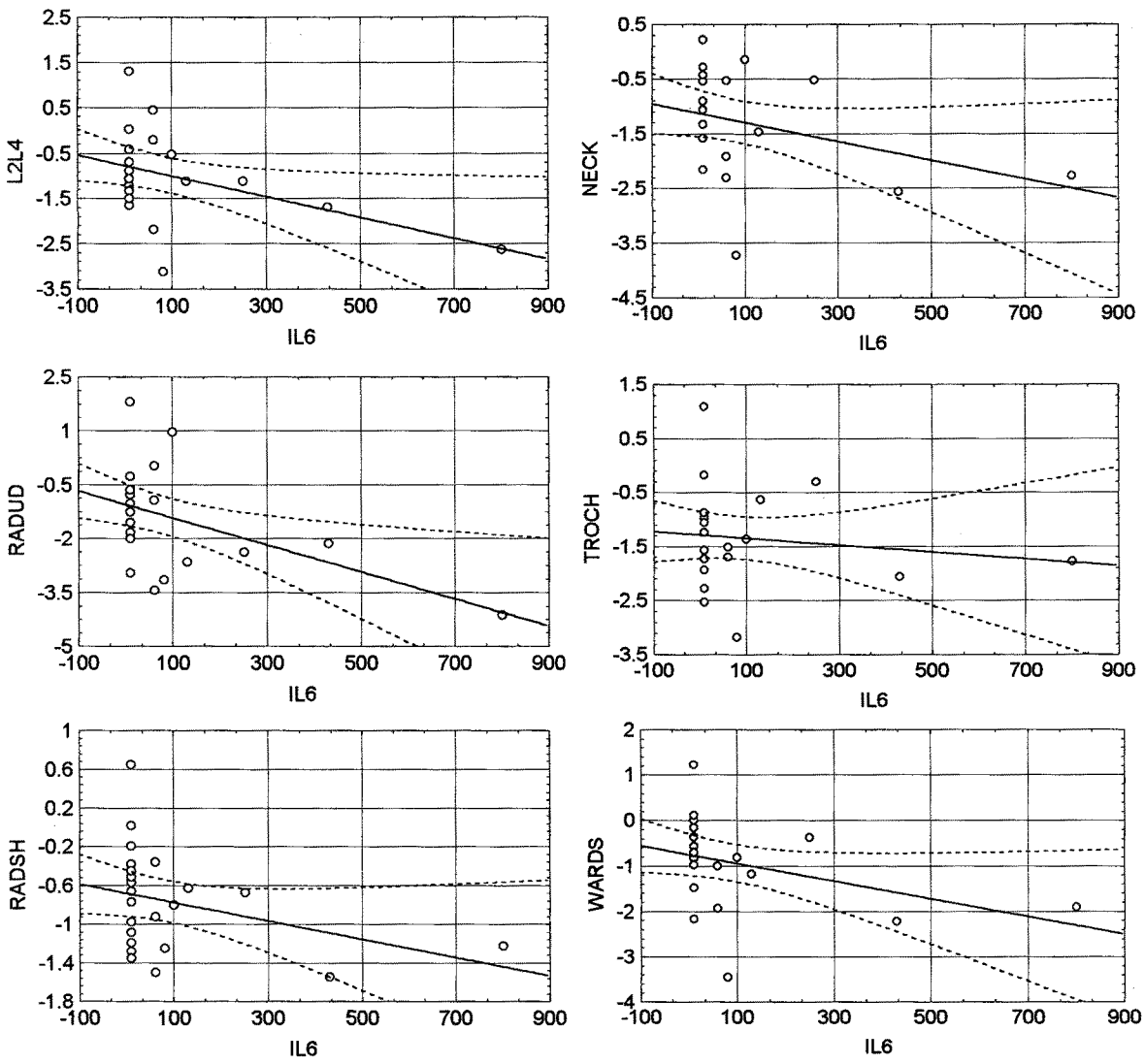

Fig. 1: Correlation plots of IL-6 against bone density measurements. The Kendall tau rank correlation coefficient is statistically significant for wards triangle (WARDS, –0.42, $p < 0.01$) and radius ultradistal (RADUD, –0.30, $p < 0.05$), marginal for radius shaft, femoral neck and lumbar spine (RADSH, –0.26, $p = 0.07$, NECK, –0.25, $p = 0.08$ and L2L4, –0.26, $p = 0.07$ respectively) and nonsignificant for trochanter measurements (TROCH, –0.12).

rameters implicated in osteoporosis (8,14). Despite these inherent difficulties, several groups have attempted to correlate serum IL-6 levels with markers of bone turnover in either normal, or osteoporotic women. Unfortunately, inconclusive results have been produced. In two reports involving healthy women (6,7), IL-6 was shown to correlate positively with age, a fact that adds rather than reduces uncertainty to the possible role of the cytokine after menopause. As a matter of fact, in one, IL-6 levels correlated well with menopause status, while in the other no such effect was noted, after correction for age. In the latter study, an observed negative correlation of IL-6 with estradiol was likewise attributed to age. In our study, all subjects were postmenopausal and their age range (44-54 years) was not wide enough for an age correlation to be established. Nevertheless, we also observed a negative correlation of estradiol and IL-6 serum levels.

In agreement with former studies (6,7,15), we found no correlation between IL-6 with markers of bone-turnover. This was not surprising since, as we mentioned before, IL-6 distribution and function are rather too complicated for such a straighforward relationship to be demonstrated. It has been argued that one possibility for erroneous results might be the use of immunoassays that measure the quantity of the cytokine rather than its biological effect (7), so to avoid this complication we have chosen to use a bioassay. Nothwithstanding the theoret-

ical possibility that the bioassay is influenced by other cytokines and/or IL-6 acting substances, this seems not to be the case in disease states examined so far, and the choice of a method for IL-6 quantitation depends more on practical issues (8).

The most interesting finding of this study, to the best of our knowledge not demonstrated before, is the negative correlation between IL-6 serum levels and bone density in five out of six bone sites. We cannot readily explain the mechanism(s) underlying this finding. Bone density values are the expression of cumulative events ranging from skeleton development to progressive skeleton involution. On the other hand, IL-6 is a rapidly metabolised substance, involved in the acute phase response and consequently its serum levels can be highly fluctuating even on a daily basis. It is thus difficult to establish a meaningful correlation between these parameters. Kania et al. (6) whose subjects included both pre- and postmenopausal women, could not establish such a correlation in femoral neck and lumbar spine. A possibility to be considered is that prolonged elevation of serum IL-6, especially after estrogen decline during menopause, can cause bone loss by inducing osteoclastic bone resorption. The "normal" or basal IL-6 levels have not yet been defined in large samples and it is not impossible that otherwise healthy subjects might have elevated IL-6 serum levels, either at the upper limits of the distribution, or because of bone-unrelated causes. The effect of chronically elevated IL-6 could possibly be mild enough not to influence bone markers, but appear cumulatively as bone loss. In support of this is the study of Ershler (16) which found increased serum IL-6 levels in a small number of older women. Moreover, a similar mechanism has been suggested for the bone loss associated with multiple myeloma (17).

Further studies are clearly needed for a better understanding of IL-6 implication in post-menopausal osteoporosis. Sequential IL-6 measurements might help to identify the time-course of this relation and establish a prognostic value for this factor.

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Correspondence to: Prof. G.P. LYRITIS,
"Th. Garofalides" Research Centre, Athinas 10, 14561, Kifisia, GREECE.