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SHORT COMMUNICATION

IL-17A-mediated sRANK ligand elevation involved in postmenopausal osteoporosis

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Abstract
Summary The role of proinflammatory IL-17 cytokine was studied in postmenopausal bone loss between 31 osteopenic and 41 osteoporotic women. The effect of serum IL-17A, soluble receptor activator of NF-κB (sRANK) ligand, and osteoprotegerin (OPG) levels on lumbar bone mineral densities was measured. The results demonstrated an increased IL-17A-mediated sRANK ligand elevation in postmenopausal osteoporotic bone loss.
Introduction IL-17 proinflammatory cytokine is a new inducer of bone loss. Postmenopausal osteoporosis represents a cross talk between estrogen deprivation and increased immune reactivity. The role of IL-17 was studied in the bone loss of postmenopausal osteoporosis.
Methods Serum IL-17A, sRANK ligand, and OPG levels were investigated on bone mineral densities (BMDs) in the total lumbar (L1–L4) region in 18 pre- and 72 postmenopausal women. IL-17A, sRANK ligand, OPG levels, and BMDs were measured with enzyme-linked immunosorbent assay (ELISA) and dual-energy X-ray absorptiometry (DXA).
Results Increased serum IL-17A, sRANK ligand, and OPG levels were demonstrated in postmenopausal osteoporotic women compared to osteopenic women (3.65±0.61 vs 3.31±0.43 ng/ml for IL-17A, $P<0.007$; 2.88±0.84 vs 2.49±0.61 ng/ml for sRANK ligand, $P<0.027$; and 1.43±0.07 vs 1.39±0.07 ng/ml for OPG, $P<0.038$). In postmenopausal women, IL-17A levels correlated inversely with total lumbar BMDs ($P<0.008$, $r=-0.279$) and positively with sRANK ligand levels ($P<0.0001$, $r=0.387$) or the ratio of sRANK ligand and OPG ($P<0.013$, $r=0.261$), but did not with OPG levels alone.
Conclusion Increased IL-17A levels are involved in postmenopausal osteoporosis, playing a role in the bone-resorpting processes.

Keywords Bone mineral density · Interleukin-17 · Osteoporosis · Postmenopause · sRANK ligand

Introduction

Postmenopausal osteoporosis is characterized by estrogen-deficiency-induced osteoclastogenesis. Estrogen is a regulator of bone remodeling and inhibits cytokine secretions (IL-6, IL-1, TNF-α, M-CSF, and G-CSF) which participate in bone resorption [1]. Estrogen deficiency enhances osteoclast formation via the production of proinflammatory cytokines and reduces apoptosis of secreting cells [2]. The stimulation of receptor activator of NF-κB (RANK) ligand expression on osteoblast cells initiates the osteoclast differentiation and activation. In inflammatory arthritis, RANK ligand is expressed by activated synovial fibroblasts and T lymphocytes [3]. Osteoprotegerin (OPG) is a soluble decoy RANK receptor produced by osteoblasts which inhibits osteoclastogenesis via binding to RANK ligand [4].

Th17 cells produce IL-17 (called IL-17A), IL-17F, IL-22, and IL-21 cytokines which participate in inflammatory and autoimmune events [5]. Chronic inflammatory diseases like psoriasis, Crohn's disease, rheumatoid arthritis, and allergic asthma are associated with the accumulation of IL-17 secreting CD4+ T cells causing local tissue injury [6]. The role of

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activated human T cells via RANK ligand expression is well known in osteoclastogenesis [7]. In rheumatoid arthritis, the IL-17 production is derived from juxta-articular bone marrow cells and synovium [8].

A functional and anatomical cross talk can be established between the immune system and bone at vascular, cellular, and molecular levels [9]. The immune cells, such as T and B lymphocytes, dendritic cells, fibroblasts, and macrophages directly or indirectly regulate osteoblast and osteoclast activity by secreting proinflammatory cytokines (TNF-α, IL-1, IL-6, IL-17, IFNγ, IL-15, IL-18) and by the RANK ligand/RANK/OPG pathway. Estrogen deficiency connecting to the increased secretion of proinflammatory cytokines leads to bone loss. The role of IL-17 in bone wasting is confirmed in autoimmune rheumatoid arthritis but was not investigated in postmenopausal bone loss.

This paper reveals a relationship between postmenopausal osteoporosis and increased IL-17A serum levels.

Patients and methods

Patients

Ninety women were investigated, 72 postmenopausal (mean age 65±9 years, range 48–89 years) and 18 premenopausal (mean age 38±9 years, range 24–50 years) women. Bone mineral densities (BMDs) with the T-score calculations were measured with dual-energy X-ray absorptiometry (DXA) in the total lumbar (L1–L4) region using Hologic Discovery Wi. The evaluation of T-score values was based on the World Health Organization (WHO) criteria: values below −2.5 represented osteoporotic; from −1 to −2.5, osteopenic; and above −1, normal BMD.

Detection of serum IL-17A, soluble RANK ligand, and OPG levels

Enzyme-linked immunosorbent assay (ELISA) was used to measure IL-17A, soluble RANK (sRANK) ligand, and OPG serum levels (PeproTech, USA). Ninety-six-well plates were coated with 0.05 μg/100 μl per well capture anti-hIL-17A antibody, as well as with 1 μg/100 μl per well capture anti-sRANK ligand, or 1 μg/100 μl per well capture anti-hOPG antibodies. Sera and standards (500, 250, 125, 62.5, 31.25, and 16.63 pg/ml for IL-17A; 1,000, 500, 250, 125, 62.5, 31.25, 15.6, and 7.8 pg/ml for sRANK ligand; and 624, 312, 156, 78, 39, 19.5, and 9.75 pg/ml for OPG) were added in duplicates and dilution of 1:100 to the plates. For the detection, 100 μl per well biotinylated goat anti-IL-17A antibody, as well as biotinylated rabbit anti-sRANK ligand and anti-OPG antibodies, was applied. In all measurements, 100 μl avidin–horseradish peroxidase (HRP) conjugate in dilution of 1:2,000 was added for labeling. For the color development, 100 μl 2,2′-azinobis[3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt (ABTS) liquid substrate solution was used and measured with ELISA reader at 405 nm with wavelength correction set at 650 nm in 5-min intervals for approximately 20 min.

Statistics

The data were exhibited as mean ± SD. Student's *t* test was applied for comparison of data between pre- and postmenopausal women, as well as between postmenopausal osteopenic and osteoporotic women. Linear regression analysis was used for demonstration of the associations between IL-17A and body mass index (BMI), BMD, sRANK ligand, and OPG or the ratio of sRANK ligand and OPG. *P* values below 0.05 were regarded as significant, and SPSS 15.0 software for Windows® was used for calculation.

Results

The difference in BMI, total lumbar BMD, serum IL-17A, sRANK ligand, and OPG levels and the ratio of sRANK ligand and OPG between pre- and postmenopausal women were as follows: 24.89±5.81 vs 27.51±4.81 kg/m^2 for BMI, $P<0.05$; 0.92±0.08 vs 0.77±0.12 g/cm^2 for total lumbar BMD, $P<0.0001$; 2.89±0.07 vs 3.5±0.56 ng/ml for IL-17A, $P<0.0001$; 2.62±0.61 vs 2.71±0.77 ng/ml for sRANK ligand, not significant (NS); 1.43±0.07 vs 1.41±0.07 ng/ml for OPG, NS; and 1.83±0.37 vs 1.92±0.52 for the ratio of sRANK ligand and OPG, NS (Table 1). The difference in serum IL-17A, sRANK ligand, and OPG levels between osteopenic and osteoporotic postmenopausal women was

Table 1 Studied parameters in pre- ($n=18$) and postmenopausal ($n=72$) women

Parameters	Patient groups	
	Premenopausal women $n=18$	Postmenopausal women $n=72$
BMI (kg/m^2)	24.89±5.81*	27.51±4.81*
Age (years)	38±9**	65±9**
IL-17A (ng/ml)	2.89±0.07***	3.5±0.56***
sRANK ligand (ng/ml)	2.62±0.61	2.71±0.77
OPG (ng/ml)	1.43±0.07	1.41±0.07
Total lumbar BMD (g/cm^2)	0.92±0.08****	0.77±0.12****

BMI body mass index, *BMD* bone mineral density, *sRANK* soluble receptor activator of NF-κB, *OPG* osteoprotegerin

$^*P<0.05$; $^{**}P<0.0001$; $^{***}P<0.0001$; $^{****}P<0.00001$

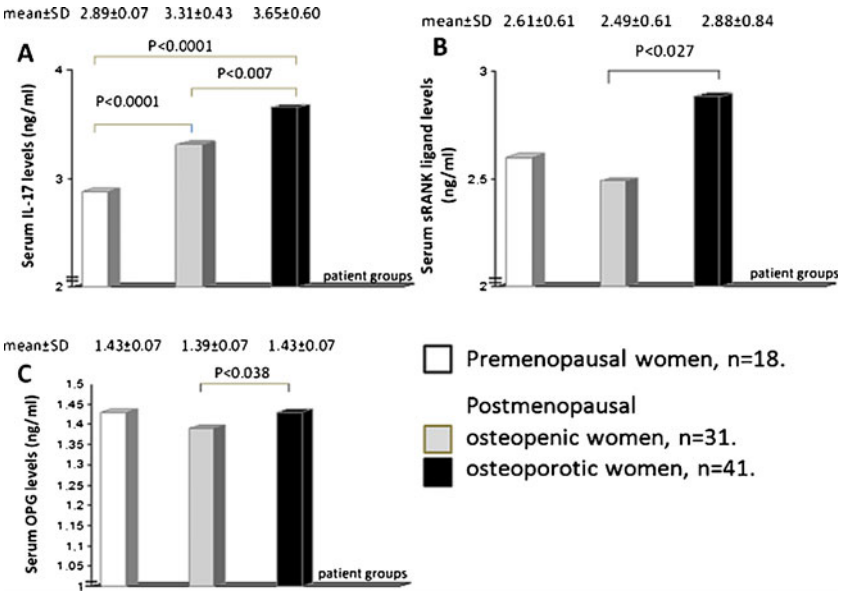

Fig. 1 Serum IL-17A, sRANK ligand, and OPG levels in pre- and postmenopausal women

exhibited in Fig. 1. The serum IL-17A, sRANK ligand, and OPG levels were higher in osteoporotic women compared to those in osteopenic ones (3.65±0.61 vs 3.31±0.43 ng/ml for IL-17A, $P<0.007$; 2.88±0.84 vs 2.49±0.61 ng/ml for sRANK ligand, $P<0.027$; and 1.43±0.07 vs 1.39±0.07 ng/ml for OPG, $P<0.038$). No remarkable difference was found in BMI and in the ratio of sRANK ligand and OPG between osteoporotic and osteopenic women. Only IL-17A levels were significantly higher in women who have osteoporosis compared to those who were in premenopause (3.65±0.61 vs 2.89±0.07 ng/ml, $P<0.0001$).

In postmenopusal women, IL-17A levels positively correlated with sRANK ligand levels ($P<0.0009$, $r=0.3066$) and with the ratio of sRANK ligand and OPG ($P<0.022$, $r=0.270$), but did not correlate with OPG levels ($P>0.07$, $r=0.2145$) or BMI ($P<0.273$, $r=0.130$). Total lumbar BMDs showed an inverse correlation with IL-17A levels ($P<0.022$, $r=-0.270$) (Fig. 2).

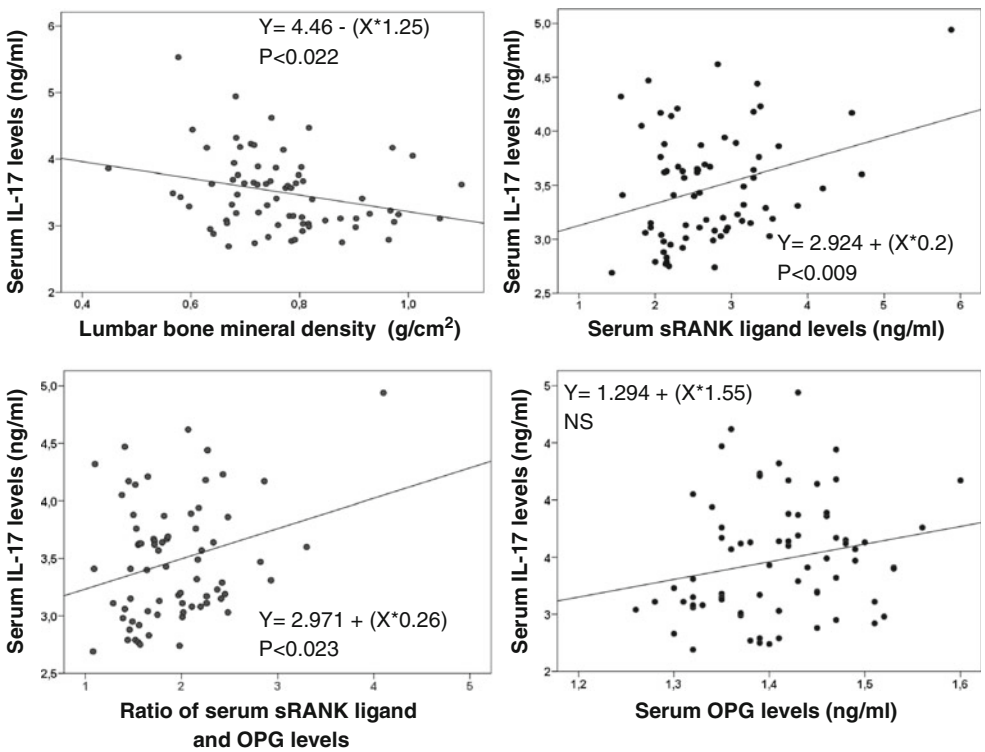

Fig. 2 Serum IL-17A levels correlated inversely with total lumbar bone mineral density and positively with sRANK ligand levels or the ratio of sRANK ligand and OPG, but did not with OPG levels

Discussion

IL-17 is secreted primarily by T cells, and IL-17 receptor is expressed on the surface of fibroblasts, osteoblasts, chondrocytes, macrophages, dendritic, as well as on endothelial, epithelial, mucosal, and most parenchymal cells [10]. IL-17-cytokine-induced bone loss is initiated through RANK ligand-mediated osteoclastogenesis [11]. IL-17 plays a role in the local inflammatory processes via regulating neutrophil production and recruitment [12]. Increased serum IL-17 levels together other proinflammatory cytokines are involved in the osteoclastogenesis of rheumatoid arthritis (RA) [13]. The excess production of RANK ligand due to activated T lymphocytes leads to bone destruction in RA. OPG as a soluble decoy RANK receptor inhibits the binding of RANK ligand to its receptor, preventing the bone loss. Denosumab is a fully human monoclonal antibody against RANK ligand acting as successful antiresorptive drug in the clinical practice [14].

Postmenopausal osteoporosis develops after ovarian failure due to estrogen deprivation accompanied with the production of bone-resorptive inflammatory cytokines [15]. RANK ligand expression enhances on bone marrow stromal cells in postmenopausal women. The cross talk between estrogen and immune system was demonstrated by enhanced immune reactivity in postmenopause. Tyagi et al. demonstrated in mouse collagen-induced arthritis model that estrogen deficiency induces differentiation of Th17 cells with increased secretion of IL-17 together accelerated bone loss [16]. In our earlier report, we demonstrated a strong association of estrogen deficiency with increased IL-17A levels which showed postmenopausal period-related dependence [17]. The prevalence of estrogen deficiency with high IL-17A levels (all women were in postmenopause) was 5.64 times higher than that in women with normal estrogen and high IL-17A levels.

Our results highlighted the role of IL-17A in postmenopausal bone loss in the total lumbar region. IL-17A levels enhanced significantly in postmenopausal osteopenic or osteoporotic women compared to those in premenopausal women. Osteoporotic postmenopausal women showed a remarkable increase in IL-17A and sRANK ligand levels, as well as a weakly increase in OPG levels in comparison with the levels of osteopenic women, highlighting the role of RANK ligand excess in the bone wasting. IL-17A positively correlated with sRANK ligand or the ratio of sRANK ligand and OPG, but did not with OPG levels alone.

In conclusion, increased IL-17A serum levels are associated with postmenopausal osteoporosis, aggravating the bone-resorptive processes.

Conflicts of interest None.

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