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Serum levels of soluble receptor activator of NF-κβ ligand are under dual, immune and neural effects in postmenopausal women

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ABSTRACT

Background: The receptor activator of NF-κβ (RANK)/RANK ligand/ osteoprotegerin system is essential for osteoclast maturation and activation to induce bone loss, which plays a role in postmenopausal osteoporosis. Estrogen deficiency in postmenopausal women is associated with T-cell mediated inflammation with increased proinflammatory cytokines, such as IL-17A, IL-1, IL-6 and tumor necrosis factor alpha. Bone repair and remodeling processes highlight the role of nerve growth factor β (NGFβ), whose non-neural production is associated with T-cell mediated inflammation.

Aims: To investigate the involvement of IL-17A and NGFβ cytokines in the elevation of serum soluble RANK ligand levels in postmenopausal and control women.

Methods: Fifty-two postmenopausal and 37 control women were studied for age, body mass index, homeostasis model assessment (HOMA) index, serum levels of IL-17A, NGFβ, soluble RANK ligand (measured by indirect enzyme-linked immunosorbent assay) and estrogen (measured by chemiluminescence assay). Two- and three-way ANOVA (expressed as median with Q1 to Q3) and linear regression analysis were used to evaluate the strength of relationship between soluble RANK ligand levels as dependent and age, serum IL-17A and NGFβ levels as independent variables.

Results: A significant difference in age, serum NGFβ and estrogen levels could be demonstrated between postmenopausal and control women (with borderline significance in HOMA indices). The differences in serum IL-17A and NGFβ levels were relevant between categories for body mass index in postmenopausal and control women, but in soluble RANK ligand levels only in postmenopausal women. The increase in soluble RANK ligand levels was greater in elevated IL-17A [101(291.75 to 39.92) vs. 45.24(95.56 to 25.34) ng/ml, $p<0.015$] and elevated NGFβ [222.2(142.75 to 1086.87) vs. 52.44(25.87 to 76.88) ng/ml, $p<0.0001$] categories compared to low categories in postmenopause, but not in controls. Both elevated IL-17A and NGFβ levels resulted in the greatest increase in serum levels of soluble RANK ligand [252.27(159.9 to 1099.64) vs. 41.4(35.91 to 59.94) ng/ml, $p<0.0001$] compared to elevated IL-17A levels alone.

Conclusion: Both IL-17A and NGFβ cytokines were involved in the increased serum levels of soluble RANK ligand in postmenopausal women. Their joint involvement in postmenopausal osteoporosis highlighted that neurogenic inflammation may play a critical role in bone loss via greater elevated soluble RANK ligand levels induced by concomitant elevated IL-17A and NGFβ levels.

Introduction

Postmenopause represents a new endocrine, immune, cardiovascular and metabolic state for women. Estrogen deficiency can be considered the main causative factor associated with increased inflammatory responses and metabolic alterations in skeletal-bone-neural-vascular-adipose tissues, as well as with the tendency to obesity and atherosclerosis[1]. Estrogen deficiency alters the cytokine profile toward a proinflammatory dominance, increasing serum levels of tumor necrosis factor alpha (TNFα), IL-1, IL-6, and IL-17 with increased numbers of T and B lymphocytes, neutrophils, monocytes, eosinophils and mast cells[2, 3].

In bone remodeling, estrogen deficiency shifts the balance between bone-forming osteoblasts and bone-resorbing osteoclasts toward bone resorption and skeletal fragility. Receptor activator of NF-κβ ligand (RANKL) plays a critical role in osteoclast formation and activation, and is a part of the RANK/RANKL/OPG (osteoprotegerin) system[4]. RANKL is expressed by osteoblasts, osteocytes, chondrocytes, mesenchymal cells, megakaryocytes, and immune cells (T and B cells), as well as extraskeletal tissues such as lung, mammary gland, heart, brain, skeletal muscle, skin, and kidney. The RANK receptor is expressed by osteoclasts, immune cells, dendritic cells and microglia[5,6]. Binding between osteoclast RANK receptor and osteoblast RANKL induces osteoclastogenesis leading to bone loss. OPG referred to as a decoy receptor, plays an inhibitory role in osteoclast maturation and osteoclastogenesis[7]. OPG is produced by various cell types such as osteoblasts, B cells, heart, kidney, liver, intestine, stomach and spleen.

Bone is innervated by primary afferent sympathetic and sensory neurons, mainly in the periosteum and to a lesser extent in the bone marrow and mineralized bone[8, 9]. This neuronal microenvironment plays a critical role in osteoclastic activity and repair mechanisms[10, 11]. Nerve growth factor beta (NGFβ) is a neurotrophin involved in the growth, survival, and regeneration of sympathetic and sensory neurons[12,13]. The action of NGFβ is manifested by binding to its two receptors, the high-affinity TrkA (tyrosine kinase A) and the low-affinity p75 receptors, which are expressed on mesenchymal stem cells, osteoblasts, immune cells, mast cells, bone marrow cells, endothelium and various parenchymal cells that are also capable of secreting NGFβ[14-17]. Thus, NGFβ has both neural and non-neural activities. The modulatory role of NGFβ on sensory nociceptive nerves is related to hyperalgic and neuropathic pain phenomena[18,19]. Remarkable production of NGFβ can be observed in immune and inflammatory processes, in which it plays as an accelerating factor.

The proinflammatory cytokine IL-17 can directly induce osteoclastogenesis and express RANKL on osteoblasts and T-cells, as well as accelerate the release of other (IL-1, IL-6, TNFα) proinflammatory cytokines and growth factors [FGF2 (fibroblast growth factor 2), TGFβ (transforming growth factor β), NGFβ][20,21]. These properties make IL-17 a critical factor in postmenopausal osteoporosis[22,23]. In our previous work, we demonstrated a strong association between elevated serum IL-17A levels and estrogen deficiency in postmenopausal women[24]. The low estrogen levels, below 50 pmol/l, were also associated with increased serum OPG and soluble RANKL (sRANKL) levels in postmenopausal women[25]. In another of our previous publications, a positive effect of elevated serum IL-17A levels on elevated serum sRANKL levels was demonstrated[26]. In our last paper, the association of elevated serum NGFβ levels with postmenopausal osteoporosis was shown to be restricted to the lumbar spine region[27].

Therefore, the joint role of serum IL-17A and NGFβ was now investigated with respect to their effects on serum sRANKL levels in postmenopausal women.

Patients and Methods

PATIENTS

Postmenopausal women (n=52, mean age 65±10 years, mean body mass index (BMI) 29±5 kg/m^2) and controls, healthy premenopausal women (n=37, mean age 31±9 years, mean BMI 28±7 kg/m^2)

formed the patient groups. Exclusion criteria were autoimmune, tumor, endocrine (except type 2 diabetes mellitus) and acute diseases. The following biochemical parameters were measured: homeostasis model assessment (HOMA) index was calculated from glucose and insulin levels [HOMA index = (fasting glucose * insulin levels)/22.5], serum IL-17A, NGFβ, sRANKL and estradiol levels.

METHODS

DETECTION OF SERUM IL-17A, NERVE GROWTH FACTOR β AND SOLUBLE RECEPTOR ACTIVATOR OF NF-κβ LIGAND LEVELS

Serum levels of IL-17A, NGFβ and sRANKL were measured by enzyme-linked immunosorbent assay (ELISA) using kits from PeproTech (USA). The detailed method has been described in our previous work[26,27]. Patient sera were used at 1:30 dilution. Plates were previously coated with capture antibodies: 0.05 µg/100 µl per well antihIL-17A, 1 µg/100 µl per well antihsRANKL and 0.05 µg/100 µl per well antihNGFβ antibodies overnight at room temperature. The plates were washed three times before blocking buffer [0.05% Tween-20 and 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS)] was added to the wells at room temperature for 1 h. After rewashing, 100 µl/well standards and patient sera were added to the wells at room temperature for 2 h. The standard concentrations were as follows: 500, 250, 125, 62.5, 31.25 and 16.63 pg/ml for IL-17A; 1000, 500, 250, 125, 62.5, 31.25, 15.6 and 7.8 pg/ml for sRANKL; and 500, 61.25, 30.63 and 3.06 pg/ml for NGFβ. After three washes, 100 µl of biotinylated goat anti-IL-17A and biotinylated rabbit anti-sRANKL and anti-NGFβ detector antibodies were added to the wells at room temperature for 2 h. The bound biotinylated antibodies were labeled with 100 µl of avidin-HRP (horseradish peroxidase) conjugate at a dilution of 1:2000 at room temperature for 30 min. For color development, 100 µl/well of liquid substrate solution [2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS)] was used. The optical density (OD) of the wells was measured using an ELISA reader at 405 nm with a wavelength correction set at 650 nm in 5-minute intervals for approximately 20 minutes. The appropriate serum concentrations were calculated from the corresponding standard curve.

Measurement of serum estradiol levels

Serum estradiol levels were measured by chemiluminescence assay (CIA) in a fully automated method using a commercial kit (Immulite 2000, Siemens, Germany) provided by Prolabor Kft.

Statistics

In summary, all data studied were presented as mean±SD. The biochemical data, such as serum levels of estradiol, IL-17A, sRANKL, and NGFβ, were skewed, so their logarithms were used, which showed an approximately normal distribution. Biochemical data calculated by two- or three-way ANOVA were presented as median [interquartile range (IQR):Q1 to Q3] using boxplots in the figures. Receiver operating characteristic (ROC) analysis was used to calculate cutoff values for BMI, IL-17A and NGFβ to transform data into categorical variables. Student's independent *t*-test was used to compare between two different patient groups. Linear regression analysis was used to assess the strength of the relationship between independent and dependent variables. P values less than 0.05 were considered significant. Statistical analyses were performed using Medcalc 17.9.7 and SPSS 26.0.0 softwares.

Results

THE PARAMETERS STUDIED BETWEEN PATIENT GROUPS

Fifty-two postmenopausal and 37 premenopausal control women were examined for age, BMI, HOMA index, serum IL-17A, NGFβ, sRANKL and estrogen levels (Table 1). In the patient groups, a relevant difference was found between age (65±10 vs. 31±9 years, $p<0.001$), NGFβ (14.34±3.72 vs. 21.96±13.49 ng/ml, $p<0.0007$) and estrogen levels (95.32±106.2 vs. 203.16±301.68 pmol/l, $p<0.0001$). No significance

was found between BMI (29±5 vs. 28±7 kg/m^2), serum IL-17A (12.12±2.2 vs. 13.21±3.68 ng/ml) and sRANKL levels (394.76±1398.62 vs. 197.25±534.58 ng/ml), but the difference in HOMA indices was borderline, p<0.0513 (3.29±2.96 vs. 4.68±5.01) between the patient groups.

Table 1 : Studied parameters in the patient groups.

Studied parameters	Postmenopausal women (n=52)	Control women (n=37)	*p*
Age (years)	65±10	31±9	0.0010
BMI* (kg/m^2)	29±5	28±7	0.5185
HOMA index**	25 neg / 7 pos	20 neg / 16 pos	0.0513
IL-17A (ng/ml)	12.12±2.2	13.21±3.68	0.1524
NGFβ (ng/ml)	14.34±3.72	21.96±13.49	0.0007
sRANKL*** (ng/ml)	394.76±1398.62	197.25±534.58	0.5908
Estrogen (pmol/l)	95.32±106.2	203.16±301.68	0.0001

*BMI: body mass index
**HOMA index calculated for insulin resistance:(insulin * fasting blood sugar) /22.5, value above 4 referred to positivity; p was calculated by Chi-squared test
*** sRANKL: soluble receptor activator of NF-κβ ligand.

In the ROC analysis, the cutoff value of BMI was calculated using categorical variables of HOMA indices (values higher than 4 were considered as category 1 and the remaining as category 0). The cutoff value of BMI was above 33 kg/m^2 (p<0.0065, area under the ROC curve (AUC): 0.699 and Younden index: 0.4106). Serum IL-17A, NGFβ and sRANKL levels were presented according to BMI categories and shown as a median(Q1 to Q3) (Figure 1). Serum IL-17A, NGFβ and sRANKL levels were significantly lower in postmenopausal women with higher BMI category compared to those with lower BMI category: 10.79(10.14 to 11.13) vs. 12.06(10.49 to 13.77) ng/ml, p<0.0001 for IL-17A (Figure 1A); 11.85(10.57 to 12.8) vs. 13.25(11.87 to 18.99) ng/ml, p<0.0001 for NGFβ (Figure 1B) and 38.04(27.48 to 51.2) vs. 75.94(35.7 to 174.23) ng/ml p<0.048 for sRANKL (Figure 1C). In controls, the difference in serum IL-17A and NGFβ levels was significant: 12.6(10.11 to 16.69) vs. 11.35 (10.06 to 12.27) ng/ml, p<0.0001 and 16.1(12.25 to 31.3) vs. 13.82(12.95 to 17.95) ng/ml, p<0.0007, respectively. A significant difference in NGFβ levels was found between postmenopausal and control women in BMI categories [in category 0 (p<0.005) and in category 1 (p<0.004)].

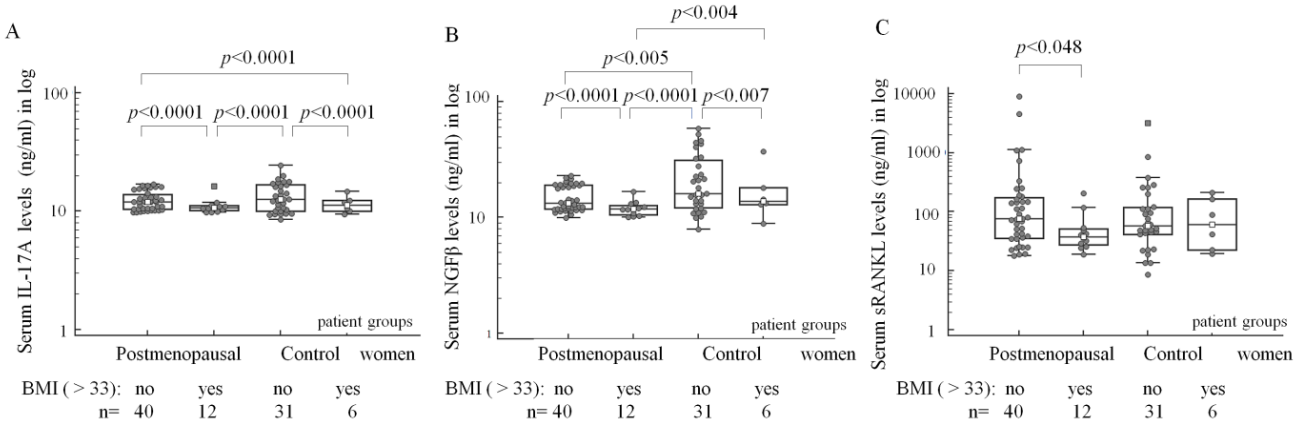


Figure 1: Serum IL-17A, NGFβ, and soluble receptor activator of NF-κβ ligand (sRANKL) levels in relation to body mass index (BMI) categories are shown using a cutoff of 33 kg/m^2 in postmenopausal and control women.

THE INCREASE IN SERUM LEVELS OF SOLUBLE RECEPTOR ACTIVATOR OF NF-κβ LIGAND WAS DEPENDENT ON THE PRESENCE OF ELEVATED SERUM IL-17A AND NERVE GROWTH FACTOR β LEVELS IN POSTMENOPAUSAL AND CONTROL WOMEN

ROC analysis using categorical data of BMI was used to transform serum IL-17A and NGFβ levels into categorical data. The cutoff value was set at ≤11.9 ng/ml for IL-17A levels (p<0.0099, AUC:0.664, Younden index:0.4108) and ≤17.95 ng/ml for NGFβ levels (p<0.006, AUC:0.683, Younden index:0.3529). Serum sRANKL levels were examined according to the categorical variables of IL-17A and NGFβ in postmenopausal and control women (Figure 2). The values higher than 11.9 ng/ml were considered as category 1 for serum IL-17A levels, and the remaining as category 0. Serum NGFβ levels were significantly increased between IL-17A categories in the patient groups [12(11.61 to 13.31) vs. 18.24 (12.38 to 19.6) ng/ml, p<0.013 in postmenopausal women and 11.97(10.68 to 14.02) vs. 25.71(19.27 to 42.04) ng/ml, p<0.0001 in controls] (Figure 2A). The increase was greater in controls than in postmenopausal women (p<0.0001). However, the increase in serum sRANKL levels could only be detected in postmenopausal women and not in control women [45.24(25.34 to 95.56) vs. 101.44(39.92 to 291.75) ng/ml, p<0.015 for postmenopausal women and 66.27(23.29 to 124.99) vs. 53.49(42.45 to 160.97) ng/ml for controls (Figure 2B). The values higher than 17.95 ng/ml were considered as category 1 for serum NGFβ levels and the rest as category 0. The increase in serum IL-17A levels was moderated between NGFβ categories in the patient groups [10.82(10.23 to 12.04) vs. 15.42 (12.33 to 16.02) ng/ml, p<0.0001 in postmenopausal and 10.44(9.54 to 11.89) vs. 16.8(14.8 to 17.89) ng/ml, p<0.0001 in control groups (Figure 2C). The difference in sRANKL levels was significant only in postmenopausal women [42.44(25.87 to 76.88) vs. 222.2(142.75 to 1086.87) ng/ml, p<0.0001] and not in controls [50.49(22.86 to 89.51) vs. 91.43(46.29 to 209.57) ng/ml] (Figure 2D). Elevated serum NGFβ levels were significantly higher in postmenopausal women than in control women (p<0.023). Elevated NGFβ levels were associated with more elevated serum sRANKL levels (2-fold) compared to elevated IL-17A levels in postmenopausal women.

RELATIONSHIP BETWEEN SERUM LEVELS OF SOLUBLE RECEPTOR ACTIVATOR OF NF-κβ AS DEPENDENT VARIABLE AND SERUM LEVELS OF IL-17A AND NERVE GROWTH FACTOR β AND AGE AS INDEPENDENT VARIABLES IN THE PATIENT GROUPS

Linear regression analysis was used to demonstrate the strength of the relationship between the dependent variable logsRANKL and the independent variables logNGFβ, logIL-17A and age in both patient groups (Figure 3). The results showed a strong relationship between logsRANKL and logNGFβ and a moderate relationship between logsRANKL and logIL-17A in postmenopausal women, but not in controls [regression equations: $\log_{(sRANKL)} = (\log_{NGF\beta} * 3.0954) - 1.6189$, r=0.5598, p<0.0001 and $\log_{(sRANKL)} = (\log_{IL\text{-}17A} * 2.4071) - 0.6726$, r=0.3060, p<0.0274 for postmenopausal women and $\log_{(sRANKL)} = (\log_{NGF\beta} * 0.3406) + 1.4211$, r=0.1538, p<0.3634 and $\log_{(sRANKL)} = 1.214 + (\log_{IL\text{-}17A} * 0.5797)$, r=0.1252, p<0.4602 for control women] (Figure 3A and 3B). However, the strength of the relationship between logNGFβ and logIL-17A was greater in controls than in postmenopausal women [regression equations: $\log_{(NGF\beta)} = (\log_{IL\text{-}17A} * 0.7588) - 0.5765$, r=0.8225, p<0.0001 for controls, and $\log_{(NGF\beta)} = 0.3622 + (\log_{IL\text{-}17A} * 0.7588)$, r=0.5333, p<0.0001 for postmenopausal women] (Figure 3C). No relevant relationship between logsRANKL and age was observed in either group (Figure 3D).

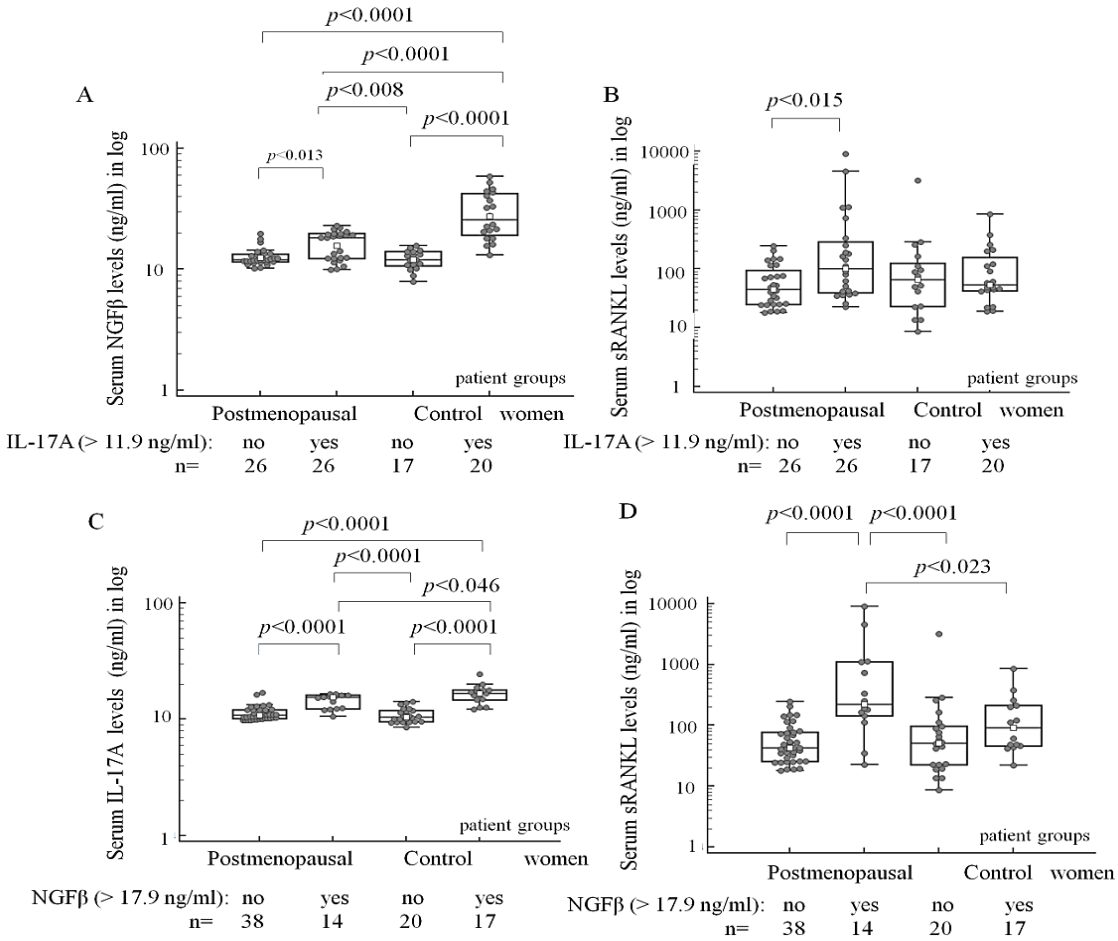


Figure 2: Serum levels of IL-17A, NGFβ, and soluble receptor activator of NF-κβ ligand (sRANKL) in relation to IL-17A or NGFβ categories are shown using the cutoff of 11.9 ng/ml for IL-17 or 17.9 ng/ml for NGFβ in postmenopausal and control women.

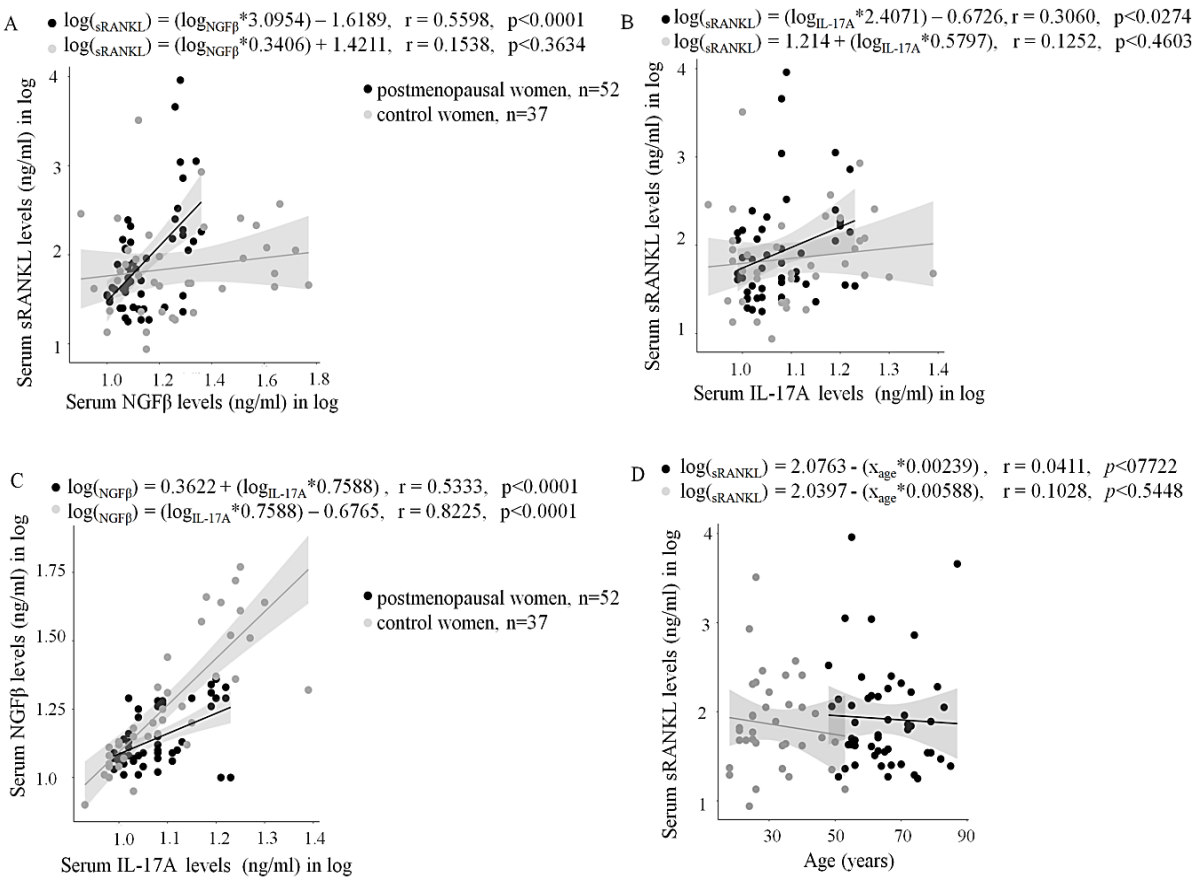
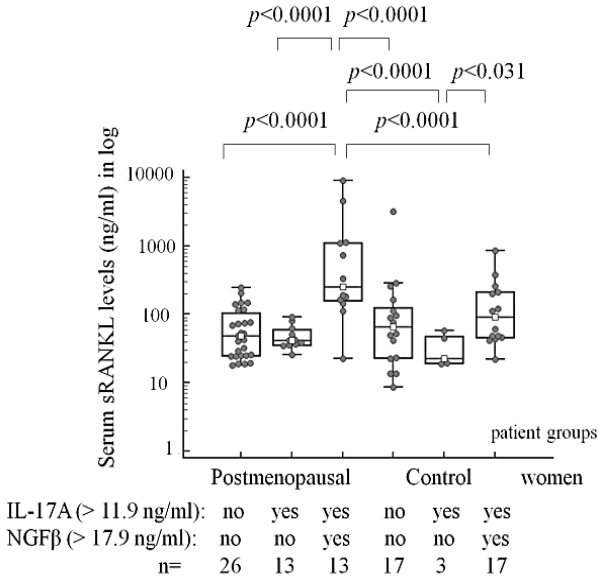


Figure 3: The strength of the relationships among serum soluble receptor activator of NF-κβ ligand (sRANKL), IL-17A, and NGFβ levels and age are shown using linear regression analysis.

Serum sRANKL levels increased more with concomitantly elevated IL17A and NGFβ levels than with lower levels in postmenopausal women [252.27(159.9 to 1099.64) vs. 48.03 (25.24 to 104.9) ng/ml, $p<0.0001$] and not in control women [91.43(46.29 to 209.57) vs. 66.27(23.29 to 123.99) ng/ml] (Figure 4). The difference in elevated sRANKL levels between the patient groups was significant ($p<0.0001$). Elevated IL-17A levels alone were associated with significantly lower sRANKL levels than both elevated IL-17A and NGFβ levels [41.4(35.91 to 59.94) ng/ml vs., $p<0.0001$ in postmenopausal women and 22.86(19.46 to 48.09) ng/ml vs., $p<0.031$ in control women]. Multiple linear regression analysis was used to demonstrate the effect of independent logNGFβ and logL-17A variables and their interactions on dependent logsRANKL variable. The effect of interactions between categories of IL-17A (catIL-17A) and NGFβ (catNGFβ) on dependent sRANKL variable was more significant ($p<0.035$) than catIL-17A alone ($p<0.051$). The model was significant ($p<0.000$, adjusted R^2: 0.288). The results for serum sRANKL levels in postmenopausal women showed a significantly greater increase in patients with concomitant elevated IL-17A and NGFβ levels compared to controls and patients with elevated IL-17A levels alone using three-way ANOVA or multiple linear regression analysis.



Model summary **Adj. R^2:0.288** **$p < 0.000$** **n=89**

Dependent variable: logsRANKL

Independent variables	Type III Sum of squares	df	Mean square	F	Signifi-cance	Partial Eta squared
Intercept	128.137	1	128.137	575.046	.000	.875
Catgroups	.000	0				.000
CatIL-17A	1.373	2	0.686	3.080	0.051	.070
CatNGFβ	1.568	2	0.784	3.519	.034	.079
CatIL-17A* CatNGFβ	1.027	1	1.027	4.611	.035	.053
Error	18.272	82	.223			
Total	346.433	89				

Figure 4: Serum soluble receptor activator of NF-κβ ligand (sRANKL) levels in relation to both categories of IL-17A (cutoff 11.9 ng/ml) and NGFβ (cutoff 17.9 ng/ml) are shown using three-way ANOVA and their interactions in multiple linear regression analysis.

Discussion

Both IL-17A and NGFβ cytokine productions show a strong association with obesity[13,28,29]. Adipocytes and cells that infiltrate adipose tissue (macrophages, T-cells) are capable of secreting IL-17A and NGFβ. Increased IL-17A and concomitantly increased other proinflammatory cytokines (TNFα, IL-1, IL-6) maintain chronic inflammation with chronic sympathetic activity in obesity. Our patients showed elevated BMI and borderline significance in HOMA indices. Therefore, the HOMA index was used to transform BMI values into categorical variables to study the BMI dependence of serum IL-17A, NGFβ and sRANKL levels in the patient groups. The results confirmed a significant difference in all cytokines studied between BMI categories in postmenopausal women and except for sRANKL levels, in control women with decreased cytokine levels with BMI above 33 kg/m^2. Decreased sRANKL levels have been demonstrated in overweight and obese children and in obese postmenopausal women compared to controls[30,31]. In our study, the decrease in IL-17A, NGFβ and sRANKL levels was more dominant in postmenopausal women. Decreased sRANKL levels have been shown to be a risk factor for long-term mortality in critically ill patients[32]. Zang et coworkers

found an inverse correlation between IL-17A and BMI[33]. No correlations between BMI and serum IL-17A, NGFβ and sRANKL levels could be detected in our patient groups. The increased interactions between cytokines and other factors, such as adipokines, may explain why the levels decreased and the fact that increased BMI values were also associated with increased HOMA indices. Five postmenopausal women out of 52 suffered from type 2 diabetes mellitus in our patient groups.

Postmenopause due to decreased estrogen levels represents an increased T-mediated inflammation with the main regulator being IL-17A[34]. IL-17A has direct and indirect effects on osteoclast activation[35]. The effects of IL-17 on osteoclastogenesis are dose-dependent, with lower IL-17 levels increasing and higher levels inhibiting osteoclast formation. IL-17 levels inhibit the differentiation of osteoblasts derived from bone marrow mesenchymal stem cells. Antibodies against IL-17 have bone-protective properties and inhibit the production of osteoclast-derived cytokines such as TNFα, IL-6 and RANKL. In our previous work, serum IL-17A levels showed an age-related increase in postmenopausal women, and estrogen deficiency was associated with increased IL-17A levels[24]. An inverse relationship between increased serum IL-17A levels and total lumbar bone mineral density and a positive relationship between IL-17A and sRANKL or the ratio of sRANK to OPG levels have been demonstrated[26]. The increase in serum OPG and sRANKL levels was strongly associated with serum IL-6 levels[25]. In bone, especially in the cortical, trabecular, periosteal and the bone marrow regions, sensory and sympathetic neuronal activity is involved in bone remodeling and repair processes[36,37]. TrkA receptors play an important role in neuronal growth and regeneration. Bone marrow-derived mesenchymal stem cells and osteoblasts express TrkA receptors, which are associated with the processes of angiogenesis and regeneration in bone repair. In addition, neuronal TrkA receptors transduce sensations of pain, pressure and heat. The non-neuronal role of NGFβ is also involved in postmenopausal immune inflammation. NGFβ has immunomodulatory activity because it can regulate the differentiation of immune cells that are sources of NGFβ secretion, especially proinflammatory cytokines such as TNFα, IL-1 and IL-6. In another study, increased NGFβ levels were associated with the severity of lumbar spine osteoporosis in postmenopausal women. This fact demonstrated that increased sympathetic and sensory neural activities are present in osteoporosis causing pain and neuropathy[27]. The involvement of both IL-17A and NGFβ in osteoporosis may represent postmenopausal neurogenic inflammation[38,39]. In this study, serum levels of IL-17A and NGFβ alone or together may be associated with increased serum levels of sRANKL. The RANK/sRANKL/OPG system is critical to induce increased bone resorption, with bone loss initiating osteoclast maturation and activation[40]. Elevated sRANKL levels may be implicated as a causative factor in excessive bone loss, particularly in postmenopausal osteoporosis[41]. Our results showed that elevated NGFβ levels alone may be more associated with elevated serum sRANKL levels than elevated IL-17A levels alone. In turn, elevated NGFβ levels together with elevated IL-17A levels were associated with the greatest increase in serum sRANKL levels, highlighting an enhancing effect of NGFβ on sRANKL production in postmenopausal osteoporotic neurogenic inflammation. RANKL is capable of acting directly on neurons to inhibit their growth[42]. NGFβ receptors are upregulated on osteochondral cells induced by TNFα in osteoarthritic joints and are associated with suppressed sRANKL levels. This fact highlighted the role of NGFβ receptors as a limiting factor for repair processes in affected skeletal tissues[43]. NGFβ receptor deficiency is associated with impaired bone formation and increased bone resorption. PTH-induced bone loss is mediated by increased production of sRANKL levels (4-fold) and a dose-dependent increase in IL-17A levels (7-fold)[44]. The role of PTH in the background of increased sRANKL levels was not investigated in this study, but none of the patients had elevated serum PTH levels. The effect of the RANK/sRANKL/OPG system is not limited

to bone, immune diseases, cancer, autoimmune diseases, but also plays a role in the brain[45]. RANKL/RANK signaling is involved in tumorigenesis and metastasis. Their expressions are increased in breast, lung, endometrial, kidney and gastric cancers. Mutations in BRCA1 in breast cancer and KRAS in lung cancer lead to increased RANK expression, resulting in cell proliferation and aberrant DNA repair. Its role is involved in ischemic brain injury by activating microglia to ameloriate inflammatory processes and protect the brain.

Only the interactions of three cytokines (IL-17A, NGFβ and sRANKL) were investigated in postmenopausal and control women, which can be considered a limitation of this study. The transformation of BMI, IL-17A and NGFβ into categorical variables may be another limiting factor, although their metabolic involvement in postmenopause is well known.

Conclusions

The results evaluated by two- or three-way ANOVA and multiple linear regression analysis confirmed that both IL-17A and NGFβ cytokines were involved in increased serum sRANKL levels, especially in postmenopausal women. Their common involvement in postmenopausal osteoporosis highlighted that neurogenic inflammation may play a critical role in bone loss via the greater elevation of sRANKL levels induced by concomitantly increased IL-17A and NGFβ levels compared to those in controls.

Conflicts of Interest Statement:

The authors declare no conflicts of interest.

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