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Original Article

Interleukin 33 in premenopausal women with rheumatoid arthritis: is it related to generalized bone loss?

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Abstract

Aim

The aim was to study the role of interleukin (IL)-33 in rheumatoid arthritis (RA)-induced generalized bone loss in premenopausal women and its relation to general disease activity and various laboratory markers.

Patients and methods

A total of 60 premenopausal women with RA were enrolled in this work as a study group and 30 age-matched healthy women as a control group. The participants were subjected to full clinical, radiological, and laboratory assessments. The following were assessed in patients and controls by enzyme-linked immunosorbent assay technique: serum level of IL-33, 25-hydroxyvitamin D3 [25(OH) D3], and bone turnover markers, osteocalcin and C-terminal telopeptide of type I collagen. Bone mineral density was assessed using dual-energy X-ray absorptiometry.

Results

IL-33 was higher in patients with RA than controls (33.445 ± 10.07 vs 13.713 ± 0.70 pg/ml, $P < 0.001$). In patients with RA, serum level of IL-33 was found to be positively correlated with clinical and laboratory makers of disease activity (disease activity score 28 and C-reactive protein), anticyclic citrullinated peptide antibodies titer ($P < 0.001$), and bone erosions using Larsen's score ($P = 0.026$), in addition to serum level of C-terminal telopeptide of type I collagen ($P = 0.023$). No correlations were detected between serum level of IL-33 and osteocalcin, 25(OH) D3, or bone mineral density.

Conclusion

High serum level of IL-33 in patients with RA was found to be positively correlated with disease activity, bone erosions, and marker of bone resorption, suggesting its possible role in local and generalized bone loss in this disease, but larger studies are needed to ensure this effect.

Keywords: Interleukin-33, osteoporosis, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease affecting predominantly peripheral synovial joints with subsequent development of joint deformities and multiple extra-articular manifestations [1].

Several data suggested an increase rate of local and generalized bone loss in RA and increase liability to fractures relative to healthy persons. This is mediated by different mechanisms, including pain-related diminished physical activity, chronic use of steroid therapy, and cytokines-mediated effects on bone metabolism [2].

Pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-17, IL-6, and IL-1, have negative effects on bone metabolism in patients with RA. RANK-L (receptor activator of nuclear factor κ-B ligand) pathway, which is involved in activation of osteoclasts with subsequent bone loss, was upregulated by these cytokines. Moreover, they stimulate different matrix metalloproteinases causing joint destruction and deformities [3].

IL-1 family include many cytokines, and among them are IL-18, IL-1α, IL-1β, IL-1Ra, and IL-33 [4].

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IL-33 signaling is mediated through binding to ST2 (suppression of tumorigenicity 2) receptor (a member of Toll-like receptor/IL-1R superfamily), which activates several signal transduction molecules that finally converge to activation of NFK-B and activation of several genes expression that play an important role in immune response and bone homeostasis [5].

Several studies declared that IL-33 may have a role in pathogenesis of RA [6–8]. Synovial fibroblasts are considered to be the main source of IL-33 [9], and upregulation of this cytokine in patients with RA promotes proinflammatory and prodestructive function of TNF-α [10], and this was confirmed by other works that linked IL-33 to bone erosions and joint damage [6,11,12].

Regarding the role of IL-33 in bone metabolism, several studies were conducted, and they showed conflicting results. Some of them showed that IL-33 stimulates formation of functional osteoclasts from human CD14 (+) monocytes; hence, it may contribute to osteoporosis [13].

However, others confirmed the antiosteoclastogenic role of IL-33 that is mediated through inhibition of osteoclastogenesis by two different ways: first, it upregulates B lymphocytes-induced maturation protein 1 and interferon regulatory factor 8 expression that inhibit RANKL expression [14], and the other way is through activation of BAX (B-cell lymphoma protein 2 (Bcl-2)-associated X), Fas (Apoptosis-stimulating fragment), and FasL (Fas ligand) that induce osteoclastic apoptosis [15].

In this work, we aimed to study the role of IL-33 as a proinflammatory cytokine in RA-induced generalized bone loss in premenopausal women and its relation to general disease activity and various laboratory markers.

Patients and methods

According to the Helsinki Declaration [16], and after approval from Ethics Committee of General Organization of Teaching Hospitals and Institutes, 90 adult premenopausal women were enrolled in this cross-sectional observational study. A total of 60 of them were patients with RA fulfilling American College of Rheumatology and European League Against Rheumatism revised criteria [17]. They were considered as a study group. Moreover, 30 age-matched healthy women served as a control group. All study details were explained to the both groups, and written consent was received from them.

The following patients were excluded from this study: postmenopausal women; patients with low BMI less than 17 kg/m^2; patients with the following disorders: other autoimmune diseases, thyroid or parathyroid diseases, type I diabetes mellitus, malabsorption syndrome, hypogonadism, chronic renal or hepatic diseases, and malignant tumors; patients with severe vitamin D3 deficiency (25-hydroxy vitamin D3 < 10 ng/ml); physically debilitated patients with poor functional class (American College of Rheumatology class IV) [18]; and patients on drugs affecting bone metabolism such as steroid therapy greater than or equal to 7.5 mg of prednisolone or equivalent per day for greater than 3 months, anticonvulsant drugs, bisphosphonates, and chemotherapy.

All patients were subjected to extensive clinical assessment, general examination (including anthropometric measures such as weight, height, and BMI), and musculoskeletal examination. Disease activity was assessed by DAS28 (disease activity score using 28 joint count) [19]. Routine laboratory and radiological investigations (including plain radiograph on both hands and feet) were also done. Modified Larsen score [20]. was used for radiological assessment.

Laboratory investigations

25-hydroxyvitamin D3 [25(OH) D3] and bone turnover markers were assessed in patients and controls. Serum osteocalcin was used in this study as a marker of bone formation, whereas serum C-terminal cross-linked telopeptide of type I collagen (CTX-I) was used as a marker of bone resorption. Fasting (no food or beverage but plain water after 12:00 a.m.) blood samples were retrieved before 9:00 a.m. They were all detected by enzyme-linked immunosorbent assay using commercially available kits Bioassay Technology Laboratory, Shanghai Korain Biotech Co. Ltd (Shanghai, China) for CTX1, BioscienceRffymetrix (California, USA) for osteocalcin, and Wkea med supplies CORP (Jilin, China) for 25(OH)D3.

Assessment of IL-33

Serum IL-33 was measured by indirect enzyme-linked immunosorbent assay (by a commercially available from Bioscience Rffymetrix (California, USA)). Anti-human IL-33 coating antibodies were adsorbed onto microwells. Human IL-33 present in the samples or standards binds to those antibodies. Following incubation and washing, a biotin-conjugated anti-human IL-33 antibody was added and bound to human IL-33 captured by the first antibody. Following incubation and wash step, streptavidin-horseradish peroxidase (HRP) was added and bound to the biotin-conjugated antihuman IL-33 antibody complex. Following incubation, unbound streptavidin-HRP was removed during wash step, and a substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of human IL-33 present in the sample or standard. The reaction was terminated by addition of acid, and absorbance is measured at 450 nm [21].

Bone mineral density assessment

Bone mineral density (BMD) was assessed using dual-energy X-ray absorptiometry (DXA) (lunar prodigy primo DXA system, analysis version: 12.3 manufactured by GE Healthcare, Chicago, Illinois, USA). The scan involved total lumbar spines (correct region-of-interest (ROI) was L1–L4), nondominant hip (the ROI for the proximal femur was the femoral neck to the trochanter), and nondominant forearm (the ROI for the distal forearm was the distal one-third of the radius). It is expressed as the amount of bone mineral content of the scanned bone measured by grams divided by the surface area of the bone measured by square centimeter. From BMD,

both *T*-score and Z-score were obtained. T-score represents the number of standard deviations that the patient's BMD differs from that of young adults (peak BMD). According to the WHO criteria [22,23], the score indicated the following:

(1) Normal BMD: T-score greater than or equal to − 1.
(2) Osteopenia: T-score between − 1 and − 2.5.
(3) Osteoporosis: T-score less than or equal to − 2.5.
(4) Established osteoporosis: T-score less than or equal to − 2.5 in the presence of one or more of fragility fractures.

Z-score represents the number of standard deviations that the patient's BMD differs from age-matched and sex-matched healthy controls. Osteoporosis is diagnosed when the score is less than 2 standard deviations less than that of controls [24]. Patients with *Z*-score between −1 and −2 SD were defined to have osteopenia [25].

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 software (SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean ± SD, median, and range. Data were tested for normality using Shapiro–Wilk test, assuming normality at *P* greater than 0.05. Student *t* test was used to analyze difference among two independent groups regarding normally distributed variables, whereas Man-Whitney *U*-test (Z_{MWU}) was used for nonparametric ones. Spearman's correlation coefficient (ρ) was used to assess correlation. The accepted level of significance in this work was stated at 0.05 ($P < 0.05$ was considered significant).

P greater than 0.05 is nonsignificant.

P less than 0.05 is significant.

P less than or equal to 0.001 is highly significant.

Results

Two groups were included in this study. Group I included 60 premenopausal women with RA, and their ages ranged from 26 to 45 (mean ± SD: 35.1 ± 4.9) years. Group II included 30 age-matched healthy women considered as a control group. Demographic, clinical, laboratory, and radiographic characteristics of patients with RA are demonstrated in Table 1. BMD was assessed in patients with RA and showed osteopenia with the lowest score being in the forearm (T score mean ± SD −1.29 ± 0.22, median −1.3; Z score mean ± SD −1.15 ± 0.23, median −1.1) (Table 1).

Comparison between both study groups showed no significant difference regarding their BMI (Table 2). IL-33 was reported to be significantly higher in patients with RA (33.445 ± 10.07 vs 13.713 ± 0.70 pg/ml, $P < 0.001$) than controls (Table 3). Markers of bone metabolism were assessed in both groups. Patients with RA showed elevated serum level of marker of bone resorption, CTX 1 (14.84 ± 18.06 ng/ml), relative to controls (9.13 ± 1.178 ng/ml) ($P = 0.002$), whereas both groups showed no significant differences in the serum level of 25(OH) D3 ($P = 0.26$) and osteocalcin ($P = 0.27$) (Table 4).

Table 1: Demographic, clinical, laboratory, and radiographic characteristics of patients with rheumatoid arthritis group

Variables	No ($n=60$)
Age (years)	
Mean±SD	35.1±4.9
Duration (years)	
Mean±SD	10.7±3.9
DAS28	
Mean±SD	4.1±1.1
CRP (mg/l)	
Mean±SD	94.9±22.2
Anti-CCP Abs titre (Ul/ml)	
Mean±SD	357.4±265.8
Larsen's score	
Mean±SD	30.83±11
Serum calcium (mg/dl)	
Mean±SD	9.56±0.79
BMD spines (L1-L4) (g/cm^2)	
Mean±SD	1.061±0.21
T score spines	
Mean±SD	−0.98±0.23
Median (range)	−1.0 (−1.6 to−0.42)
Z score spines	
Mean±SD	−0.93±1.08
Median (range)	−0.85 (−1.2 to−0.25)
BMD/total femur (g/cm^2)	
Mean±SD	0.8361±0.254
T score/total femur	
Mean±SD	−1.19±0.27
Median (range)	−1.2 (−1.9 to−0.72)
Z score/total femur	
Mean±SD	−0.92±0.21
Median (range)	−0.99 (−1.5 to−0.48)
BMD/forearm (g/cm^2)	
Mean±SD	0.731±0.31
T score forearm	
Mean±SD	−1.29±0.22
Median (range)	−1.3 (−2.0 to−1.0)
Z score forearm	
Mean±SD	−1.15±0.23
Median (range)	−1.1 (−1.9 to−0.89)

Anti-CCP abs, anticyclic citrullinated peptide antibodies; BMD, bone mineral density; CRP, Creactive protein; DAS28, 28-joint disease activity score.

In patients with RA, serum level of IL-33 was found to be positively correlated with clinical and laboratory makers of disease activity [DAS 28 and C-reactive protein], anticyclic citrullinated peptide antibodies titer ($P < 0.001$), and radiographic findings using Larsen's score ($P = 0.026$), in addition to serum level of CTX1 ($P = 0.023$) (Table 5). No correlations were detected between serum level of IL-33 and osteocalcin, 25(OH)D3, or BMD (Table 5).

Discussion

Cytokines-mediated inflammation is the hallmark of joint destruction and bone loss in RA, comprising IL-1, IL-6, IL-8, IL-15, TNF-α, and IL-33 [9,26–28].

In RA, IL-33 is produced by different cells including synovial fibroblasts [4]. It locates to the nucleus, and it is excreted to extracellular environment from necrotic or activated cells under stressful conditions such as inflammation [29].

In this work, we aimed to elucidate the role of IL-33 in RA-associated generalized bone loss in premenopausal women and its relation to various clinical and laboratory markers of disease activity.

The current study reported that serum level of IL-33 was higher in premenopausal female patients with RA than in age-matched healthy controls, and its level was positively correlated with disease activity (DAS28), C-reactive protein, and anticyclic citrullinated peptide antibodies titer.

Similar results were reported in two Egyptian studies conducted in 2017 to evaluate the role of IL-33 in RA; both concluded that IL-33 was considered as an active player in RA pathogenesis and a valuable marker of disease activity and should be a therapeutic target in this disease [6,7]. Older studies demonstrated the effect of IL-33 on experimental collagen-induced arthritis (CIA) in mice, and they showed that the proposed mechanism in arthritis is mediated through mast cell activation with subsequent production of IL6 and IL1β [27]. The crucial role of neutrophils in CIA has been demonstrated in another work. The chemoattractant role of IL-33 was supposed to be mediated by augmented production of proinflammatory cytokines from inflammatory and resident cells beside enhanced expression chemokine receptors that allow neutrophils to gain access to the joint, although direct chemo-attractant role of IL-33 for neutrophils was also reported [30].

IL-33 and TNF-α were linked to the pathogenesis of RA in many studies [28], and it was found that IL-33 play a pivotal role in induction and regulation of TNF-α activity. Positive loop of activation exists between IL-33 and TNF-α, as IL-33 mRNA over-expression promoted TNF-α-induced pro-inflammatory and pro-destructive activity in experimental studies. On silencing IL-33 mRNA, the pro-inflammatory activity of TNF-α was suppressed, whereas pro-destructive properties were less affected. Through p38-mediated signaling pathway, TNF-α induces IL-33 mRNA over-expression. Moreover, the nuclear translocation of IL-33 was enhanced by TNF-α [10]. So IL-33 may be a target of TNF-α blocking agents [30].

In patients who are unresponsive to TNF-α blocking agents, IL-33 was found to be positively correlated with persistent IL-1β signaling, and these findings were associated with poor prognostic value in those patients [31].

On the contrary, patients with RA with good response to anti-TNF-α agents showed lower level of IL-33 in serum and synovial fluid than methotrexate-treated patients or anti-TNF-α nonresponders [30,31]. Moreover, low baseline level of ST2 receptors predicted good clinical response to conventional DMARDs and anti-TNF-α treatment [32].

In addition to the role of IL-33 as a proinflammatory cytokine especially during early stages of the disease, it exerts immunoregulatory functions particularly during disease resolution and this occurs through its putative effects on Th2 [33,34].

These data exert a therapeutic challenge during manipulating this cytokine as its function depends on the type and the stage of the disease. These opposing results are not limited to IL-33 but are also observed in other cytokines that were treated successfully in RA such as IL-6 [35,36].

In this study, IL-33 was reported to be correlated with bone erosions evaluated by Larsen's score, and these results were confirmed by other works [6,11,12], which showed that bone erosions in RA could be caused by IL-6 and metalloproteinase

Table 2: BMI among the studied groups

Groups	*n*	BMI (kg/m²)		Student's '*t*' test	*P*
		Mean±SD	Range		
Patients	60	28.9±2.04	25.1-32.2	0.21	0.83 (NS)
Controls	30	29.0±2.04	25.0-32.0		

Table 3: Comparing patients with rheumatoid arthritis and controls regarding serum level of interleukin-33

Groups	*n*	Serum IL-33 (pg/ml)		'*Z*' of MWU	*P*
		Mean±SD	Range		
Patients	60	33.445±10.07	15.6-58.2	7.69	<0.001 (HS)
Controls	30	13.713±0.70	9-15		

HS, highly significant; IL, interleukin; MWU, Mann–Whitney *U*-test.

Table 4: Comparing patients with rheumatoid arthritis and controls regarding bone turnover markers

Variables	Patients (*n*=60)		Controls (*n*=30)		Z_{MWU} test	*P*
	Mean±SD	Range	Mean±SD	Range		
Serum vitamin D 3 [25(OH)D] (ng/ml)	35.1±6.88	15-55	37.8±7.18	20-54	1.04	0.26 (NS)
Serum osteocalcin (ng/ml)	3.23±0.47	2.65-4.24	3.33±0.46	2.58-5.21	1.09	0.27 (NS)
Serum CTX 1 (ng/ml)	14.84±18.06	8.85-70.87	9.13±1.178	4.84-9.94	3.13	0.002 (S)

CTX 1, C-terminal cross-linked telopeptide of type I collagen; MWU, Mann–Whitney *U*-test; S, significant.

Table 5: Correlation between interleukin-33 level and the studied variables in patients with rheumatoid arthritis group

With	Serum IL-33 ($n=60$)	
	ρ	*P*
Age (years)	−0.058	0.66
Duration (years)	−0.014	0.91
BMI	0.043	0.74
DAS28	0.826	<0.001 (HS)
CRP	0.925	<0.001 (HS)
Anti-CCP abs	0.882	<0.001 (HS)
Vitamin D3 (ng/ml)	−0.210	0.107
Osteocalcin (ng/ml)	−0.101	0.44
Larsen's score	0.314	0.026 (S)
CTX 1 (ng/ml)	0.321	0.023 (S)
BMD of spines (L1-L4)	−0.184	0.16
BMD of total femur	−0.149	0.25
BMD of forearm	−0.148	0.26

Anti-CCP abs, anticyclic citrullinated peptide antibodies; BMD, bone mineral density; CRP, C-reactive protein; CTX 1, C-terminal cross-linked telopeptide of type I collagen; HS, highly significant.

3 that were released by synoviocytes under the influence of IL-33.

Patients with RA included in the current work showed osteopenia and high serum level of marker of bone resorption, CTX1, which positively correlated with IL-33.

In agreement of these data, several experimental studies support the role of IL-33 on generalized bone resorption. Mun and colleagues studied the effect of IL-33 on differentiation of osteoclast from its progenitor human CD14+ monocytes, and they found that these cells exhibit IL-33 receptor, ST2, on their surfaces, and when cultivated in the presence of IL-33, they developed to well-differentiated osteoclasts that express tartrate-resistant acid phosphatase (TRAP) even in the presence of OPG (Osteoprotegerin) or anti-RANKL antibody, and this effect was abolished by anti-ST2 receptor antibody, suggesting that IL-33 works through its receptor independent on RANK-L pathway. Several transcription factors involved in mature osteoclastic development from monocytes in RANK-L-dependent differentiation were also activated by IL-33 such as c-Fos, Syk, NFATc1 (nuclear factor of activated T cells, cytoplasmic 1), and TRAF6 (TNF receptor associated factor 6). Additionally, there was a positive feedback mechanism between RANK-L and IL-33, as RANK-L increases the expression of IL-33 receptor ST2 on the cultured cells [13]. They finally concluded that IL-33 enhanced bone resorption.

In concordance to these data, a study conducted on MC3T3-E1 cells (an osteoblast precursor cell line derived from mice) showed that IL-33 stimulates RANK-L expression through ERK and p38 MAPK signaling [37]. Additionally, receptor activator of nuclear factor kappa B ligand (RANK-L) mRNA was suppressed by anti-ST2 antibodies in a study conducted by *Palmer G* and coworkers [9].

Bone loss is the one of the most characteristic feature of chronic inflammatory diseases such as RA and periodontal disease, which occurs locally at inflamed sites as well as systemically in the form of osteopenia and osteoporosis. Chronic inflammation in RA is a consequence of an autoimmune disorder primarily affecting synovial joints, whereas in periodontal disease, the persistent bacterial gingival infection repeatedly triggers immune response; both are considered as an early warning signs of existing osteoporosis risk [3].

In patients with periodontitis, IL-33 was reported to induce RANK-L expression that exaggerates bone resorption induced by infection; it acts as an extracellular alarmin released by necrotic cells to induce proinflammatory reaction, osteonecrosis, and osteoporosis [38].

Opposing to this opinion, there were other studies suggesting a bone-protective effect of IL-33, which is mediated through its suppressive action on osteoclasts. Zaiss *et al.* [39] showed that IL-33 has an antiosteoclastogenic role, especially during early stages by shifting osteoclasts development toward M2 macrophages, thus protecting against TNF-α-induced bone loss.

Moreover, IL-33 inhibits osteoclastogenesis through its suppressive effects on CD11b + monocytes in mice [40]. In addition, IL-33 may enhance osteoclast apoptosis [15].

Moreover, IL-33 has suppressive effect on osteoclastic differentiation owing to its role in osteoblast hypoxia-inducible factor-1α pathway and dectin-1 signaling [41,42].

In the context of RA, the suppressive effect of IL-33 on osteoclastogenesis may be counter-balanced by the influence of other inflammatory cytokines, such as IL6, IL17, TNF-α, IL-8, and IL-23, with the net effect toward bone loss and osteoporosis [43].

A more recent study showed that IL-33 was found to be lower in postmenopausal patients with osteoporosis than in age-matched healthy women, and it was positively correlated with markers of bone formation, whereas negatively correlated with markers of bone resorption, referring to osteoprotective effects of this cytokine [44].

Despite that, this concept cannot be applied on patients with RA owing different cytokines milieu between the two diseases (RA and postmenopausal osteoporosis).

Those contradictory reports emphasized on double-bladed effects of IL-33, as it may have protective role against osteoporosis through its influence on bone metabolism, whereas may exert a proresorptive role through its proinflammatory functions and depending on the specific clinical context and disease stage [45].

These data could explain the results of the current study where IL-33 was correlated with bone erosions and markers of bone resorption rather than general BMD. Despite contradictory data related to the role of IL-33 on bone metabolism, this

study pointed to the probable role of this cytokine in bone resorption in RA. Owing to the pliometric action of IL-33 and cross-sectional design of this work, these results cannot represent a general finding of RA, so larger longitudinal works regarding microenvironment of bone-related cells in the inflammatory conditions are required to confirm these results.

Conclusion

Finally, we concluded that IL-33 was found to be elevated in patients with RA than healthy controls, and this elevation was significantly correlated with disease activity, bone erosions, and marker of bone resorption, suggesting its possible role in local and generalized bone loss in this disease, but other longer studies involving microenvironment are needed to ensure this effect. We recommended regular follow-up of those patients for early detection of progression to osteoporosis and adequate treatment to prevent fracture risk.

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Conflicts of interest

There are no conflicts of interest.

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