Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2024; 41(1): 60-64
doi: 10.52142/omujecm.41.1.10

Correlation of tumor necrosis factor-α and sclerostin in bone turnover process of premenopausal women with rheumatoid arthritis

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Received: 07.02.2024 • **Accepted/Published Online:** 18.03.2024 • **Final Version:** 29.03.2024

Abstract

Rheumatoid arthritis is associated with systemic bone mass loss due to stimulation of osteoclastogenesis and suppression of osteoblastogenesis through inhibition of Wingless (Wnt)/β catenin canonical and bone morphogenetic proteins (BMP) pathways by sclerostin. Our study assessed the correlation between tumor necrosis factor (TNF)-α and sclerostin with bone resorption markers C-terminal telopeptides of type 1 collagen (CTX) and procollagen type 1 N-terminal propeptide (P1NP) in pre-menopausal rheumatoid arthritis patients. This cross-sectional study involved 38 premenopausal women with RA. Serum TNF-α, sclerostin, P1NP, and CTX level were examined by ELISA method. This study revealed high level of serum CTX (mean 2.74 ng/mL) and a low level of P1NP (median 34.04 pg/mL). There was a significant negative correlation (r = -0.388) between TNF-α and sclerostin levels (p = 0.016), as well as a significant positive correlation (r = 0.362) between TNF-α and P1NP levels (p = 0.026). There was a significant negative correlation between TNF-α and sclerostin, and also a significant positive correlation between TNF-α and P1NP in our study.

Keywords: bone remodeling, premenopause, rheumatoid arthritis, tumor necrosis factor-alpha, Wnt signaling pathway

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease manifested by symmetric, progressive, and chronic polyarthritis (1). It is an autoimmune disorder which affects approximately 1% of the population worldwide, occurs more often in women than men. RA possesses many complications, ranging from musculoskeletal, lung, and even mental health problems (2). One of the most common complications is bone mass loss in RA patients that would increase risk of fracture. British General Practice Database with 430 thousand RA patients reported that the risk of hip fracture increases by 2 times and vertebral fracture by 2.4 times when compared with non-RA controls (3).

Inflammation itself is the main factor that causes loss of bone masses in RA. This is mediated by cytokines which activate osteoclast and at the same time, inhibit the functions of osteoblast. TNF-α is the main pro-inflammatory cytokine which promotes inflammation in RA joints. It is responsible for local joint damage as well as systemic bone loss, either by directly activating the differentiation of osteoclast or indirectly by stimulating the expression of RANKL by synovial fibroblasts and T cells. TNF-α also stimulates fibroblast-like synoviocytes (FLS) and osteocytes to express sclerostin, Dickkopf-1 (DKK-1) and secreted frizzled-related protein (SFRP) which suppress the differentiation of osteoblast (4).

Sclerostin is produced by osteocytes and plays a role in suppressing osteoblast differentiation induced by bone morphogenetic protein (BMP). Moreover, it is also an antagonist of Wnt canonical signaling by binding to co-receptor Wnt, low-density receptor-related protein (LRP) 5 and 6; thus, inhibiting bone formation. The inhibition of Wnt canonical pathway by sclerostin will further decrease beta canenin, thus affecting osteoblastogenesis as well as reducing the production of osteoprotegerin (OPG). A decrease in OPG will cause an increase in RANKL/OPG ratio and osteoclastogenesis (5).

The increase of bone turnover process in RA patients can be identified by measuring bone turnover markers. These markers can be classified into two main categories: marker of bone formation, and of bone resorption. Procollagen type 1 N-terminal propeptide (P1NP) is a marker of bone formation, whilst C-terminal telopeptides of type 1 collagen (CTX) is a marker of bone resorption (6,7).

The decrease in osteoblastogenesis and increase in osteoclastogenesis place RA patients at risk of osteoporosis and fracture due to loss of bone mass, thus raising the need for a comprehensive assessment of bone metabolism. This study aimed to assess the correlation between sclerostin and tumor necrosis factor (TNF)-α with bone turnover markers C-

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terminal telopeptides of type 1 collagen (CTX) and procollagen type 1 N-terminal propeptide (P1NP) in pre-menopausal rheumatoid arthritis patients. To our knowledge, there are no studies reporting correlation between TNF-α and sclerostin levels with both bone resorption marker (represented by CTX) and bone formation marker (represented by P1NP). Thus, by assessing such correlation, this study would gain a better understanding regarding the pathogenesis of bone mass loss in pre-menopausal women with RA.

2. Materials and Methods

2.1. Design and patients

This study was a cross-sectional study conducted in the Rheumatology Clinic of Cipto Mangunkusumo Hospital (RSCM), Jakarta, Indonesia. Samples were premenopausal female RA patients visiting the Rheumatology Clinic between April 2019 to May 2019, which were then screened according to the inclusion and exclusion criteria. The disease activity was evaluated with the disease activity score for 28 joints (DAS28), which is a clinical assessment of tender and swollen joint counts and patient assessment of disease activity, combined with laboratory evaluation of CRP. The level of disease activity can be interpreted as remission (DAS28 < 2.6), low (2.6 ≤ DAS28 < 3.2), moderate (3.2 ≤ DAS28 ≤ 5.1), or high (DAS28 > 5.1).

Blood samples were obtained from patients immediately following the completion of their self-report questionnaire. Peripheral blood samples were collected in the morning, specifically between 07:30 and 10:00 a.m., after an 8-hour fasting period to account for the diurnal fluctuations of CTx serum levels. The laboratory parameters evaluated included serum levels of IL-6, IL-17, and TNF-α, which were measured using the enzyme-linked immunosorbent assay (ELISA) technique.

2.2. Inclusion and exclusion criteria

The inclusion criteria were pre-menopausal women with RA that fulfilled ACR/EULAR 2010 classification criteria and agreed to participate in this study. Premenopausal women were chosen as the appropriate participants in order to minimise the effect of lack of estrogen on bone metabolism in menopausal patients. Patients were excluded if they were taking steroid equivalent to prednisone of >7.5 mg per day, had bone metabolism disorders (such as hyperparathyroidism, Paget's disease, osteomalacia, osteogenesis imperfecta, and diabetes), had autoimmune disease other than RA, taking drugs that affect bone metabolism process (such as bisphosphonate, hormone therapy, antipsychotic drugs, anti-seizure drugs, heparin, hydrochlorothiazide, and furosemide), had end stage chronic kidney disease, had chronic liver disease, or was suffering from acute infections such as fever and pneumonia.

2.3. Statistical analysis

The study data underwent analysis through SPSS 20.0 for Mac. Descriptive statistics were reported as the mean (SD) for normally distributed data and as the median (min–max) for data that did not follow a normal distribution. Pearson (r) correlation was employed to assess the correlation in cases of normal data distribution, while Spearman (rho) correlation coefficient was used for instances with non-normal data distribution. P values < 0.05 were considered statistically significant.

3. Results

The descriptive analyses of patient characteristics were presented in Table 1. A total of 38 subjects were included and analyzed in this study. Mean age, disease duration, and body mass index of the subjects were 38.82 years, 5 years, and 21.82 kg/m2 respectively. The most widely used glucocorticoid dose by the subjects was methylprednisolone 4 mg (65.8%). The disease-modifying antirheumatic drugs (DMARDs) used in this study were methotrexate (MTX), sulfasalazine and leflunomide. No patients was under biologic DMARDs. Twenty one subjects (55.3%) used MTX monotherapy, 9 subjects (23.7%) used combination of 2 DMARDs, 1 subject (2.6%) used combination of 3 DMARDS, and 7 subjects (18.4%) used DMARD monotherapy other than MTX for their RA treatment. Based on the results of the DAS28-CRP, most of the subjects (44.8%) were in remission state.

Table 1. Patient characteristics

Characteristics	N=38
Age (years), mean (SD)	38.82 (7.25)
Disease duration (years), median (min-max)	5 (1-19)
BMI, median (min-max)	21.82 (14.5-37)
Glucocorticoid dose, n (%)	
None	7 (18.4)
Methylprednisolone <4 mg, n (%)	25 (65.8)
Methylprednisolone 4 mg, n (%)	6 (15.8)
RA treatment, n (%)	
Monotherapy MTX	21 (55.3)
2 DMARDs	9 (23.7)
3 DMARDs	1 (2.6)
DMARD monotherapy other than MTX	7 (18.4)
C-reactive protein (mg/dL), median (min-max)	4.20 (0.6-88.4)
DAS28-CRP score, n (%)	
Remission	17 (44.8)
Low	6 (15.8)
Moderate	14 (36.8)
High	1 (2.6)
TNF-α (pg/mL), median (min-max)	10.58 (7-17.7)
Serum sclerostin (pg/mL), median (min-max)	101.72 (7-17.7)
Serum CTX (ng/mL), mean (SD)	2.74 (1.37)
Serum P1NP (pg/mL), median (min-max)	34.04 (7-17.7)

BMI, body mass index; RA, rheumatoid arthritis; MTX, methotrexate; DMARDs, disease-modifying antirheumatic drugs; DAS28-CRP, Disease activity score 28- joint count C-reactive protein; CTX, C-terminal telopeptides of type 1 collagen; P1NP, procollagen type 1 N-terminal propeptide. Data were reported as mean (SD) if normally distributed and as median (min–max) if not normally distributed.

Fig. 1 showed that there were significant negative correlation between TNF-α and sclerostin (r=-0.388, p=0.016) and significant positive correlation between TNF-α and P1NP (r=-0.388, p=0.026). Fig. 2 showed that there were no significant correlation between sclerostin and CTx and P1NP.

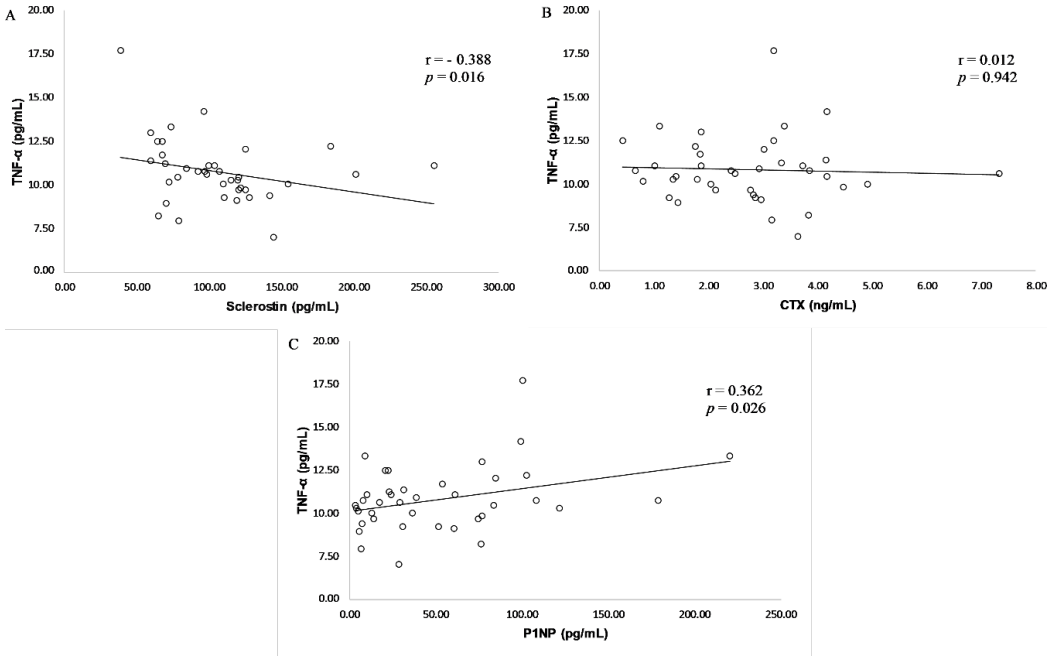

Fig. 1. Correlation of TNF-α with (A) sclerostin, (B) CTX, and (C) P1NP levels in RA patients. TNF, tumor necrosis factor-α; CTX, C-terminal telopeptides of type 1 collagen; P1NP, procollagen type 1 N-terminal propeptide. Pearson (r) correlation was employed to assess the correlation; P values < 0.05 were considered statistically significant

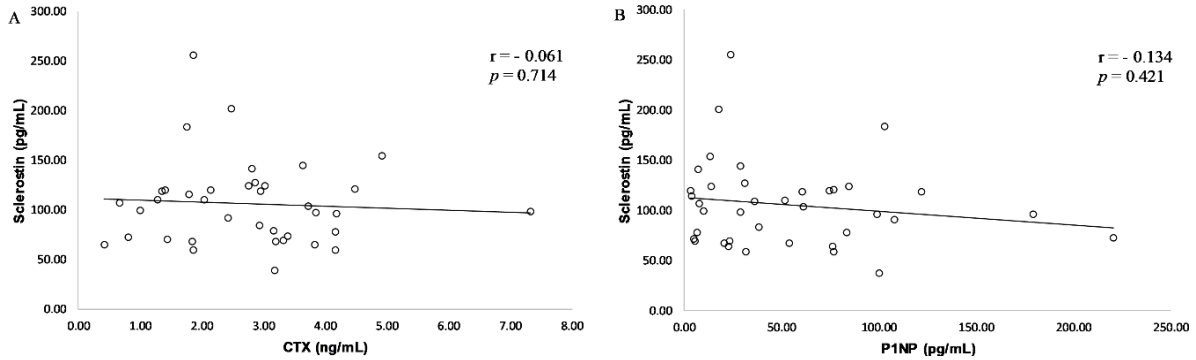

Fig. 2. Correlation of sclerostin with (A) CTX and (B) P1NP levels in RA patients. CTX, C-terminal telopeptides of type 1 collagen; P1NP, procollagen type 1 N-terminal propeptide. Pearson (r) correlation was employed to assess the correlation; P values < 0.05 were considered statistically significant

4. Discussion

As seen in Table 1, our patients had an average age of 38.82 years. This finding was consistent with current understanding that the prevalence of RA tends to peak within the age range of 35 to 50 years. Our study showed that our subjects tended to be in the younger side of the RA patient demographic. A current nationwide epidemiological study in Indonesia found that the mean age of RA patients was 48.7 ± 12.7 years (8).

In this study, median of serum TNF-α level was 10.58 pg/mL. Thilagar et al found that mean of serum TNF-α level are 17.9±2.6 pg/mL in RA patients and 5.5±3.3 pg/mL in healthy patients (9). A study of premenopausal women with RA in Malang discovered mean TNF-α level of 332 pg/mL (10). TNF-α level in this study was lower than that study and might be due to majority of patients in this study being in remission or low disease activity. Previous studies had proven that TNF-α level was correlated with disease activity of RA. Hence, patients with lower disease activity and/or patients in remission would also have lower TNF-α level (11).

Low sclerostin level (101.72 pg/mL) was found in this study, whereas previous studies had reported sclerostin level of 568.18 pg/mL in healthy premenopausal women (12). Other study also revealed higher sclerostin level of 768,75±232,95 pg/mL in RA patients compared to 777.95± 392,95 pg/mL in healthy patients (13). These disparities might arise from different population age, BMI, and/or menopausal status (14,15). Mean population age of 38 years in this study might affect sclerostin level, as Modder et al (16) reported that sclerostin level was positively correlated with age, regardless of menopausal status. Furthermore, Kalem et al (14) found that BMI was correlated with sclerostin level either in osteoporotic or non-osteoporotic postmenopausal population, making low BMI in this study might affect low level of sclerostin. In addition, low disease activity in most of the population in this study might also explain our finding, as Singh et al had reported that sclerostin level was positively correlated with disease activity (17).

Bone turnover markers in this study were illustrated by CTx

and P1NP levels. Mean CTx level in this study was 2.74 ng/mL, about ten-times fold of healthy population (0.28 ng/mL) as reported by Fassio et al (13) and of healthy premenopausal women (0.2 ng/mL) as reported by Bottela et al (18). This finding was in accordance with current pathogenesis understanding that RA patients had increased bone resorption. On the contrary, P1NP level in this study (median, 34.04 pg/mL) was relatively low compared to previous study by Fassio et al (13) that reported mean P1NP level of 42.49±11.52 ng/mL in healthy patients and 39.19±21.38 ng/mL in RA patients. Moreover, Gutierrez-Buey et al (19) also reported mean P1NP level of 37.0±10.27 ng/mL from 64 healthy premenopausal and perimenopausal women. This showed that bone formation process was low in our patients.

TNF-α was found to be negatively correlated with sclerostin (r=-0.388, p=0.026) as seen in Fig. 1A. Sclerostin might inhibit the increase of TNF-α production via NF-kB pathway in a negative feedback manner (20–22). Moreover, anti-sclerostin antibody therapy might worsen clinical RA in chronic TNF-α dependent inflammatory condition, implying that sclerostin exerted a protective effect in such condition (22).

Positive correlation between TNF-α and P1NP levels were found in this study (Fig. 1C). Theoretically, TNF-α was negatively correlated with P1NP as TNF-α would activate fibroblast like synoviocyte (FLS) which then would antagonize Wnt signaling pathway, resulting in lower stimulation of bone formation and reduced P1NP level (23). Our study indicated that in our patients there was inability of bone formation process to counterbalance the bone resorption. On the other hand, our study showed no correlation between TNF-α and CTx levels (Fig. 1B). This finding might be caused by interference of proinflammatory cytokines other than TNF-α that affected osteoclastogenesis. Previous studies had shown that sclerostin could reduce CTx level by inhibiting TNF pathway (24); however, this study did not exhibit such result (Fig. 2A). This might be due to proinflammatory cytokines other than TNF-α which could increase the level of CTx.

This was the first study investigating the correlation of inflammatory cytokine (i.e. TNF-α), Wnt signaling inhibitor (i.e. sclerostin), and bone turnover markers (i.e. CTx and P1NP) together. Another strength was the use of human serum to assess systemic inflammation process, compared to previous studies which only described local effects.

The limitation of this study was the cross-sectional design which did not assess the causal relationship. Furthermore, the effects of previous treatments towards bone metabolism process were inevitable. Moreover, other Wnt signaling inhibitor such as DKK-1 and SFRP were not examined.

Our study found significant negative correlation between TNF-α and sclerostin levels, and a positive correlation between TNF-α and P1NP levels. Our study also found a bone turnover imbalance in female pre-menopausal RA patients who had received DMARD therapy, as indicated by increased CTx and decreased P1NP levels.

Conflict of interest

The authors declared no conflict of interest.

Funding

This project was funded by Universitas Indonesia. The sponsor of this study had no role in the study design, data collection, data analysis, data interpretation, or writing of this report.

Acknowledgments

The authors wish to thank Harry Isbagio, Zuljasri Albar, Bambang Setyohadi, Suryo Anggoro, and Anna Ariane for their time and tremendous support in the field. This study was supported by Universitas Indonesia PITTA B Grant Scheme (Grant Number PENG- 1/UN2.R3.1/PPM.00/2019).

Authors' contributions

Concept: M.C., F.P., Design: M.A., Data Collection or Processing: M.C., F.P., M.A., Analysis or Interpretation: M.C., F.P., M.A., Literature Search: M.C., F.P., Writing: S., R.H.

Ethical Statement

This study had been approved by the Committee of Medical Research Ethics, Faculty of Medicine, Universitas Indonesia, number KET- 377/UN2.F1/ETIK/PMM.00.02/2019.

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