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Correlation between Interleukin-1, Interleukin-6 with RANKL/OPG Ratio on Bone Remodelling in SLE Patient

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A B S T R A C T

Background: Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease characterized by the presence of pathogenic autoantibodies that attack many organ systems, giving rise to various clinical manifestations. Osteoporosis is one of the complications of SLE. Interleukin 1 and Interleukin 6, which are the main inflammatory cytokines in SLE, play a role in enhancing osteoclast activity through the regulation of Receptor Activator of Nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG), thereby influencing bone resorption. This study was conducted to analyze whether the inflammatory processes reflected by IL-1 and IL-6 levels in SLE patients are associated with alterations in the RANKL/OPG ratio, thereby providing insight into the link between inflammation and bone resorption and the risk of osteoporosis in SLE patients. **Methods:** This was an analytical observational study with a cross-sectional design involving 30 patients with SLE. Serum IL-1, IL-6 and RANKL/OPG ratio levels were measured using ELISA. **Results:** There is a strong correlation between IL-1 and RANKL/OPG ratio (r=0.771) with p value <0.001 and weak degree of correlation between IL-6 and RANKL/OPG ratio (r=0.37) with p-value 0.022. **Conclusion:** Increased levels of IL-1 and IL-6 followed by an increase in the RANKL/OPG ratio.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a complex systemic autoimmune disease characterized by the presence of pathogenic autoantibodies against cell nuclei that attack many organ systems, giving rise to various clinical manifestations. Tian J et al (2022) showed a global SLE incidence of 5.14 with a prevalence of 43.7 per 100,000 population. At the regional level, the prevalence of SLE varies from 15.9 in southern Asia to 110.85 of 100,000 population in Latin America. The top four countries with the highest prevalence are the UAE 166.92 per 100,000 , Barbados 163.31 per 100,000 , Cuba 149.9 per 100,000, and Brazil 147.37 per 100,000 population. Hamijoyo (2019) found the prevalence of SLE in Indonesia was 0.5% of the total population with a tendency to increase in the number of incidents each year. Dr. M. Djamil Padang Hospital recorded that 357 SLE patients made outpatient visits in the period 2019 to 2021, and 59 people were hospitalized.[1,2]

Osteoporosis is one complication of SLE. Carrasco (2009) showed that osteoporosis in SLE was 42% and osteopenia was 74%.[3] Medical discoveries regarding the involvement of the immune system in the bone remodeling process resulted in a new field of research called osteoimmunology. This field of research explains the role of chronic inflammation that triggers the emergence of inflammatory cytokines related to the

bone remodeling process in SLE.[4] Wu and Xu (2023) regarding the relationship between SLE and osteoporosis provide evidence of a potential causal relationship between SLE and an increased risk of osteoporosis.[5]

The RANK/RANKL/OPG pathway plays the most important role in osteoclast maturation. RANKL binds to RANK as its receptor, causing the maturation of osteoclast precursors. Osteoprotegerin is a blocking receptor for RANKL that prevents RANKL-RANK binding. Increased regulation of RANKL or decreased regulation of OPG causes bone loss. The balance of RANKL and OPG is an important indicator in assessing osteoclast activity, so the RANKL/OPG ratio is used as the main determinant in bone resorption. Chi et al (2023) showed a significant correlation between the RANKL/OPG ratio with osteoporosis.[6,7]

Expression of IL-1 and IL-6 found in 99% of SLE patients. Interleukin-1 is an inflammatory cytokine that plays a role as a key factor in the differentiation and formation of osteoclasts whose mechanism is related to the RANK/RANKL/OPG pathway. Interleukin-6 is also an inflammatory cytokine that plays a crucial role in the pathogenesis of SLE. Interleukin-6 produces stromal cells and monocytes and increases RANKL production. Yang and Liu (2021) reported that IL-1 is a key inflammatory cytokine that enhances osteoblast activity in producing RANKL, thereby promoting osteoclast activation and differentiation, which leads to bone resorption.[8] Furthermore, Cheng et al (2020) demonstrated that IL-1 influences the RANK/RANKL/OPG system and closely associated with bone metabolism. In addition to stimulating osteoclastogenesis, IL-1 also regulates the production of OPG, a decoy receptor for RANKL that inhibits the binding of RANKL to RANK.[9]

In Indonesia, research on the relationship between inflammatory cytokines and BTM in SLE is also still very limited. Until now, there has been no research on the correlation of serum IL-1 and IL-6 levels which are the main inflammatory cytokines of SLE with the RANKL/OPG ratio. This study aims to investigate the relationship between IL-1 and IL-6 levels with RANKL/OPG ratio in order to determine whether the degree of inflammation in SLE can predict the extent of bone damage, thereby serving as a basis for routine screening in this population to prevent osteoporosis in SLE patients.

2. Methods

Research Design

This study is an analytical observational study with a cross-sectional approach conducted in the outpatient clinics of rheumatology of internal medicine at M. Djamil Padang Hospital. Samples were collected from December 2024 to March 2025. First, we conducted anamnesis and physical examinations to determine the inclusion and exclusion criteria, then blood tests were performed to SLE patients.

Research Subject

In this study, the sample selection was carried out using consecutive sampling. The sample size was determined using a formula with a desired absolute accuracy level of 0.05, an optimum sample of 30 people was obtained. The subjects taken as samples were subjects who met the inclusion criteria and were not included in the exclusion criteria. The inclusion criteria were SLE patients who met the diagnostic criteria according to the 1997 ACR criteria, and aged ≥ 18 years. While the exclusion criteria were menopause, DM, malignancy, coronary heart disease, chronic kidney disease, chronic liver disease, chronic lung disease, obesity, acute infection, other autoimmune diseases such as MCTD, systemic sclerosis, rheumatoid arthritis, prednisone consumption >7.5 mg per day or equivalent, antiresorptive or anabolic agent therapy.

Laboratory Procedures

Testing of IL-1, IL-6 and RANKL/OPG ratio was carried out using ELISA method using the Bioassay Technology Laboratory (BT LAB) reagent which was carried out in the Biomedical Laboratory of the Faculty of Medicine, Andalas University, Padang.

Statistical Analysis

The relationship between serum IL-1 and IL-6 levels with RANKL/OPG ratio was the outcome

assessed in this study. Predictor variables included gender, age, BMI, and GFR. We performed a normality test to determine the next correlation test used in this study. Based on the Kolmogorov- Smirnov test, the data of IL-1, IL-6, RANKL and OPG were not normally distributed. Therefore, the Spearman test was used and a p value of less than 0.05 was considered statistically significant.

Ethical Considerations

This study was approved by the Health Research Ethics Committee of Dr. M. Djamil Padang Hospital Number: DP.04.03/D.XVI.XI/411/2024.

3. Results

Characteristics of the Study Subjects

This study involved 30 SLE patients. all SLE patients were women with a percentage of 100%. The average age in this study was 31.07 (8.45) years. The body mass index in this study was normoweight in 17 people (56.7%) and overweight in 13 people (43.3%) with an average BMI of 22.03 (3.50). While the average GFR in this study was 111.5 (23.10) with details of 6 people (20%) having GFR 60 - 89 mL/min/1.73m^2 while 24 people (80%) had GFR >90 mL/min/1.73m^2.

Table 1. Characteristics of the Study Subjects

Characteristics	n (%)	Mean (SD)
Gender		
Male	0 (0)	
Female	30 (100)	
Age (years)		31.07 (8.45)
Body Mass Index (kg/m^2)		22.03 (3.50)
Normoweight (18.5-22.9)	17 (56.7)	
Overweight (23-24.9)	13 (43.3)	
Glomerular Filtration Rate (ml/min/1,73m^2)		111.5 (23.10)
60 – 89	6 (20)	
≥ 90	24 (80)	

IL-1, IL-6, RANKL, OPG levels and RANKL/OPG ratio

In this study, the results of serum IL-1 levels were not normally distributed with a median 37.733 (30.58 - 134.41) pg/mL. The results of serum IL-6 levels in this study were also not normally distributed with a median 123.495 (38 - 366.81) pg/mL. In this study, the results of RANKL and OPG were not normally distributed with a median of 0.056 (0.048 - 0.229) ng / mL and 1.67 (1.386 - 5.828) ng/mL. While the RANKL / OPG ratio in this study was normally distributed with a mean of 0.034 (0.0035).

Table 2. IL-1, IL-6, RANKL, OPG levels and RANKL/OPG Ratio

Variable	Mean (SD)	Median (Min-Max)
IL-1 (pg/mL)		37.733 (30.58 – 134.41)
IL-6 (pg/mL)		123.495 (38 – 366.81)
RANKL (ng/mL)		0.0560 (0.048 – 0.229)
OPG (ng/mL)		1.67 (1.386 – 5.828)
RANKL/OPG ratio	0.034 (0.0035)	

Correlation between IL-1, IL-6 with RANKL/OPG ratio

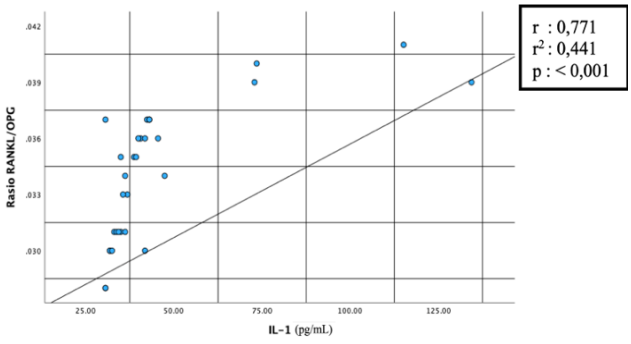

Figure 1. Correlation graph between IL-1 levels and RANKL/OPG ratio

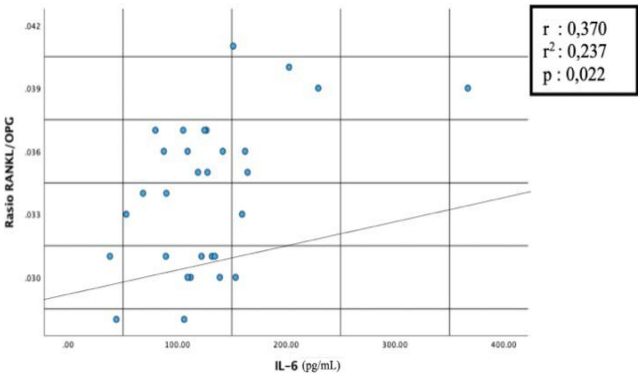

Figure 2. Correlation graph between IL-6 levels and RANKL/OPG ratio

Based on Figure 1, the higher IL-1 level followed by RANKL/OPG ratio. The results of this analysis indicate there is a strong correlation between IL-1 levels and the RANKL/OPG ratio in SLE (r=0.771) with statistically

significant ($p<0.05$) and r^2 of 0.44, means IL-1 levels affect the RANKL/OPG ratio by 44%. Based on Figure 2, the higher IL-6 level, the higher the RANKL/OPG ratio. The results of this analysis indicate that there is a weak correlation between IL-6 levels and RANKL/OPG ratio in SLE ($r=0.37$) with statistically significant ($p<0.05$) and r^2 of 0.237, means that IL-6 levels affect the RANKL/OPG ratio by 23%.

4. Discussion

In this study, all SLE patients were female. The average age was 31.07 (8.457) years. Fatoye (2022) showed the age range of SLE was 25.5 to 45.8 years with the prevalence and incidence of SLE in women higher than men, ranging from 23.8 to 204.3 and 4.72 to 14.1 per 100,000 population, while the prevalence and incidence in men ranged from 0 to 90 and 0.5 to 2.6 per 100,000 population. This is due to the high estrogen hormone in women which is a lymphocyte stimulant. In addition, the clear differences between male and female immunity also contribute to the various responses to SLE predisposition.[10] Serum IL-1 levels in this study obtained a median value of 37.733 (30.58 - 134.41) pg/mL. Italiani (2018) found significantly higher IL-1 levels compared to controls with a median of 219.9 (127- 436.8) pg/mL with a p value = 0.0094.[11] IL-6 levels obtained a median value of 123.495 (38 - 366.81) pg/mL. This is in line with several studies on IL-6 levels in SLE patients which showed higher values than healthy groups. Ding's study (2020) found increased IL-6 levels in SLE patients compared to healthy controls and there was a significant positive correlation between IL-6 levels and SLE disease activity.[12] RANKL levels in this study obtained a median 0.056 (0.048 - 0.229) ng/mL. Ismael (2024) found the average was 1.203 ng/mL for newly diagnosed SLE, 0.921 ng/mL for SLE under therapy, and 0.870 ng/mL for healthy controls.[13] OPG levels in this study had a median value of 1.67 (1.39 - 5.83) ng/mL. Hao's (2022) study found that OPG levels in SLE patients were significantly lower with an average of 0.156 ± 0.057 ng/mL, while the average OPG levels in healthy controls were 0.189 ± 0.068 ng/mL.[14] The average RANKL/OPG ratio in this study was 0.034 (0.0035). Research by Ali (2019) found that the RANKL/OPG ratio levels in patients with SLE were significantly higher with an average of 1.135 ± 0.63 compared to normal controls with an average of 0.79 ± 0.41.55 In Tonny's research (2018), the average RANKL/OPG ratio levels in SLE patients were 0.070 (0.004).[15] This study shows that the higher the serum IL-1 levels, the higher the RANKL/OPG ratio in SLE patients. The results of the analysis showed a strong correlation between serum IL-1 levels and the RANKL/OPG ratio in SLE patients ($r = 0.771$) with a positive correlation direction that was statistically significant ($p <0.05$) and r^2 of 0.44, which means that serum IL-1 levels affect the RANKL/OPG ratio by 44% and the rest is influenced by other factors. A study conducted by Wang (2014) in patients with chondrosarcoma explained that IL-1 was found to significantly increase the RANKL/OPG ratio, known as an indicator of bone loss with a p value <0.05.[16] This is in line with recent research by Sufi (2024) which links several pro-inflammatory cytokines with RANKL, OPG and the RANKL/OPG ratio in Covid-19 patients, and a significant relationship was found between IL-1 and the RANKL/OPG ratio.[17] Bones are very sensitive to IL-1 which has a role in regulating bone formation and resorption. Interleukin-1 is also known as osteoclast activating factor (OAF) which plays a role in various steps of osteoclastogenesis. Interleukin-1 stimulates osteoclast formation indirectly by triggering prostaglandin E2 synthesis in osteoblasts/stromal cells. Interleukin-1 increases RANKL expression in osteoblasts/stromal cells and inhibits OPG expression. Some evidence for IL-1 involvement in bone destruction in pathological conditions has previously been studied in rheumatoid arthritis. This study showed that the higher the serum IL-6 levels, the higher the RANKL/OPG ratio in SLE patients. The results of the analysis showed a weak correlation between serum IL-6 levels and the RANKL/OPG ratio in SLE patients ($r = 0.37$) with a positive correlation direction that was statistically significant ($p <0.05$) and r^2 of 0.237, which means that serum IL-6 levels affect the RANKL/OPG ratio by 23% and the rest is

influenced by other factors.[18]

Omma's (2022) study examined bone metabolism in Graves's disease patients and found a negative correlation between IL-6 and the RANKL/OPG ratio with an r value of -0.363 and a p value of 0.001. The study explains that the use of the RANKL/OPG ratio has been used globally as a marker of bone metabolism disorders, however, changes in its levels in cells do not necessarily cause corresponding changes in serum levels. Because most RANKL is bound to the membrane, studies using serum RANKL sometimes do not accurately describe the levels of RANKL in the bone microenvironment of individuals. Therefore, optimal RANKL detection can be done by in vivo examination through flowcytometry of bone biopsies, but this examination is less practical.[19]

Clinically, these findings may provide a scientific basis for identifying an increase RANKL/OPG ratio as marker of bone turnover through the assessment of IL-1 and IL-6 levels, which are key inflammatory cytokines in SLE, thereby offering insight into the risk of osteoporosis. Furthermore, this may support the need for routine osteoporosis screening in SLE patients and serve as a foundation for patient education regarding the risk of bone loss associated with the disease.

The limitation of this study was that it did not assess other pro-inflammatory cytokines that may influence bone remodeling in SLE.

5. Conclusion

This study showed a significant positive correlation with the strength of the strong correlation between serum IL-1 levels and the RANKL/OPG ratio in the SLE and there is a significant positive correlation with weak correlation strength between serum IL-6 levels and the RANKL/OPG ratio in the SLE.

Conflict of Interest

Competing interests: No relevant disclosures.

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