Correlation between Receptor Activator of Nuclear Factor-κβ Ligand (RANKL), and Osteoprotegerin (OPG) with Cartilage Degradation in Rheumatoid Arthritis Patients

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ABSTRAK

Tujuan: *untuk menganalisis hubungan antara kadar Receptor activator of nuclear factor κβ ligand (RANKL) dan Osteoprotegrin (OPG) serum terhadap degradasi kartilago berdasarkan kadar cartilage oligomeric matrix protein (COMP) pada pasien arthritis reumatoid.* ***Metode:*** *studi potong lintang dilakukan pada pasien artritis reumatoid yang berobat ke Poliklinik Reumatologi Rumah Sakit Ciptomangunkusumo. Pasien artritis reumatoid baru dan lama yang didiagnosis berdasarkan kriteria yang direvisi oleh American College of Rheumatology (ACR) 1987. Seluruh data numerik, baik data primer maupun data transformasi terdistribusi tidak normal, sehingga analisis bivariat menggunakan uji korelasi Spearman.* ***Hasil:*** *kami mengumpulkan data 60 pasien RA, sebagian besar dengan aktivitas penyakit yang aktif (78,3%). Metotrexate adalah obat golongan disease modifiying anti-rheumatoid drug (DMARD) yang paling banyak digunakan, baik sebagai obat tunggal (51,7%) maupun obat yang dikombinasikan dengan DMARD lain (25,1%). Pada analisis bivariat didapatkan bahwa kadar RANKL serum (r=0,151, p=0,518), OPG serum (r=-0,085, p=0,248), dan rasio OPG/RANKL serum (r= 0,171, p = 0,191) tidak memiliki korelasi yang signifikan dengan kadar COMP serum.* ***Kesimpulan:*** *tidak terdapat korelasi yang signifikan antara kadar serum RANKL dan OPG dengan degradasi kartilago pada pasien artritis reumatoid.*

Kata kunci: *RANKL, OPG, COMP, reumatoid arthritis.*

ABSTRACT

Aim: *to analyze the correlation between Receptor activator of nuclear factor-κβ ligand (RANKL), Osteoprotegrin (OPG) serum level with cartilage oligomeric matrix protein (COMP) serum level as a marker of cartilage degradation in rheumatoid arthritis patients.* ***Methods:*** *a cross-sectional study was conducted on the subjects who came to the outpatient clinic of rheumatology in Cipto Mangunkusumo Hospital. Patients were diagnosed based on the American College of Rheumatology (ACR) 1987 revised criteria. All numerical data, both primary data and data transformation were not normally distributed, so we did bivariate analysis with Spearman correlation test.* ***Results:*** *we collected the data of 60 RA patients with majority of the subject had active disease activity (78.3%). Methotrexate was the most widely disease modifying anti-rheumatoid drug (DMARD) used, either as a single drug (51.7%) or in combination with another DMARD (25.1%). Bivariate analysis was revealed that RANKL, OPG, and OPG/RANKL serum level have no significantly correlation with COMP serum level (p=0.52; p=0.25; p=0.2, respectively).* ***Conclusion:*** *RANKL and OPG serum level, had no correlation with cartilage degradation in rheumatoid arthritis patients.*

Key words: *RANKL, OPG, COMP, rheumatoid arthritis.*

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that primarily affected the synovial tissue, accompanied by progressive joint destruction, bone erosion, and cartilage degradation.[1] Bone erosion occurs mainly due to the inflammatory process with the effect of various mediators such as IL-1 (interleukin-1), IL-6 (interleukin-6) and TNF-α (tumor necrosis factor-α) as the major mediator. Various recent studies show evidence of the role of RANK (receptor activator of nuclear factor-κβ), RANKL (receptor activator of nuclear factor-κβ ligand) and OPG (osteoprotegerin) in the process of bone erosion.[1-6] While the cartilage degradation process has been proven as a result of inflammatory cells and mediators that will spur the production of a variety of degradative enzymes such as MMPs (matrix metalloproteinases) and cathepsin.[1] On the other hand, there are several other studies showed that the inflammatory process is not entirely correlated with joint destruction, including cartilage degradation,[1,7] and maybe thought that the two process is a completely separated mechanisms.[6,8]

Osteoprotegerin/receptor activator of nuclear factor-κβ/receptor activator of nuclear factor-κβ ligand system is also thought to have an essential role in the process of cartilage degradation, for several reasons, especially due to the expression of RANK, RANKL and OPG in chondrocyte.[2,9,10] There are some studies that show a correlation between RANKL, OPG and the cartilage degradation processes,[2,8,11] although the mechanism of how OPG and RANKL affect the cartilage degradation process is still unclear. One of the theories said that OPG has a role to protect chondrocytes by binding to TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), which has the effect of triggering chondrocytes apoptosis. Low OPG/ RANKL ratio will increase the chondrocytes apoptosis process.[12] Study by Fujisaki, et al.[13] showed that the presence of RANKL under the influence of IL-1 will increases the expression of Cathepsin-K and MMP-9 which affects the cartilage degradation process.[13] Other studies have been also conducted to examine the role of the OPG/RANK/RANKL and the cartilage degradation process, but they still give contradictory results, and all of them use experimental animals or cultured chondrocytes (in vitro studies), so it is necessary to conduct a study in humans, especially in RA patients.

In this study, COMP is used as a marker of cartilage degradation, since COMP is one component of the non-collagenous extracellular matrix and non-proteoglycans, which will be detected in serum if cartilage degradation happened and it has been known that the increasing of its level are not influenced by the level of inflammation.[7,14-17] This study is conducted to assess the correlation between OPG, RANKL, OPG/ RANKL ratio, and the cartilage degradation process with serum level of COMP as marker, in RA patients.

METHODS

This is a cross sectional study, conducted at the outpatient clinic of rheumatology in Cipto Mangunkusumo Hospital. Patients were diagnosed based on the American College of Rheumatology (ACR) 1987 revised criteria and eligible with inclusion and exclusion criteria. The inclusion criteria were man or non-menopause woman (menstruating regularly), aged over 16 years old, and willing to participate in the study. Exclusion criteria were patients who have infectious disease or any inflammatory disease, has a history of smoking or alcohol consumption, under treatment with osteoporosis drugs or steroids, has a primary bone malignancy or metastasis, or has a chronic liver or kidney disease.

The subjects were chosen by consecutive sampling techniques, and the collected data were processed using SPSS 11.5 for windows. The result showed that all numerical data, both primary data and data transformation were not normally distributed, so we did bivariate analysis with Spearman correlation test. The study has obtained approval from the Ethics Committee of the Faculty of Medicine, University of Indonesia.

RESULTS

We collected data from 60 patients who were willing and eligible as study subjects. The entire study subjects were women with rheumatoid arthritis who have not reached menopause

and had regular menstrual cycles. Most of the subjects were in the age group 25-34 years (45%). The clinical characteristics of the subjects consisted of 78.3% of the seropositive subjects either by rheumatoid factor and/or anti-CCP examination, and the rest were seronegative. The disease activity has been assessed by DAS28-LED score, and most of them had active disease activity (78.3%). Methotrexate was the most widely disease modifying anti-rheumatoid drugs (DMARD) used, either as a single drug (51.7%) or in combination with another drugs (25.1%) (**Table 1**).

Table 1. Characteristics of the study subjects

Characteristics	n (%)	Median (range)*
Age		
- 16-24 years	3 (5.0)	
- 25-34 years	27 (45.0)	
- 35-44 years	22 (36.7)	
- 45-54 years	8 (13.3)	
Serology (rheumatoid factor/anti-CCP)		
- Seropositive	47 (78.3)	
- Seronegative	13 (21.7)	
Disease activity (DAS28-ESR)		
- Not active/Remision	13 (21.7)	
- Active:	47 (78.3)	
- Low	7 (11.7)	
- Moderate	23 (38.3)	
- High	17 (28.3)	
Period of illness (months)		35 (2-144)
- <24 months	15 (25)	
- ≥24 months	45 (75)	
DMARD treatment		
- MTX	31 (51.7)	
- SSZ	10 (16.7)	
- MTX+SSZ	11 (18.3)	
- MTX+Chloroquine	1 (1.7)	
- MTX+Leflunomide	1 (1.7)	
- MTX+Azathioprine	1 (1.7)	
- SSZ+ Chloroquine	3 (5.0)	
- Chloroquine	1 (1.7)	
- MTX+SSZ+ Chloroquine	1 (1.7)	
ESR (mm/hour)		29.5 (10-100)
OPG serum level (pmol/l)		2.26 (0.14-7.91)
RANKL serum levels (pmol/l)		0.1 (0.02-1.27)
OPG/RANKL ratio		17.12(1.05-395.5)
COMP serum level (U/L)		8.3 (7.06-11.43)

Anti-CCP = anti-cyclic citrullinated peptide; DAS28-ESR = Disease Activity Score-Erythrocyte Sedimentation Rate; DMARD = disease modifying anti-rheumatoid drug; MTX = methotrexate; SSZ = sulfasalazine; ESR = erythrocyte sedimentation rate; OPG = osteoprotegerin; RANKL = Receptor Activator of Nuclear Factor-κβ Ligand; COMP = cartilage oligomeric matrix protein
*= not normally distributed

Bivariate analysis was done to see the effect of confounding variables (ESR) to the independent variables (OPG, RANKL, OPG/RANKL ratio), and the dependent variable (COMP), and the results on all variables showed a very weak correlation, and not statistically significant. Based on this analysis, we considered that ESR likely not affected the other variables (**Table 2**). Bivariate analysis was also done to examine the correlations between independent variables (OPG, RANKL, OPG / RANKL serum level ratio) and dependent variable (COMP serum level). This analysis revealed a very weak correlation, which was not statistically significant (**Table 3**). Evaluation of the power of this study showed a yield of about 80%.

DISCUSSION

In this study, we obtained 60 study subjects, mostly found in the 16-44 years age group. This

Table 2. Correlation between OPG, RANKL, OPG/RANKL ratio, COMP serum level and ESR

Variable 1	Variable 2	r*	p
OPG	ESR	0,159	0,226
RANKL	ESR	-0,057	0,665
OPG/RANKL	ESR	0,092	0,483
COMP	ESR	0,006	0,961

OPG=osteoprotegerin; RANKL=Receptor Activator of Nuclear Factor-κβ Ligand; COMP=cartilage oligomeric matrix protein. *=Spearman correlation test

Table 3. Correlation between OPG, RANKL, OPG/RANKL ratio, and COMP serum level

Variable 1	Variable 2	r*	p
OPG	COMP	0,151	0,248
RANKL	COMP	-0,085	0,518
OPG/RANKL	COMP	0,171	0,191

OPG=osteoprotegerin; RANKL=Receptor Activator of Nuclear Factor-κβ Ligand; COMP=cartilage oligomeric matrix protein. *=Spearman correlation test

data shows a significant difference in the trend of prevalence by age group, compared with the data from epidemiological studies abroad, especially in Europe. Symmons, et al, reported that prevalence of RA patients were significantly higher in the older age group >45 years compared with those aged <45 years.[18] This difference is likely due to variation in study population, while this study subjects were limited to the population of childbearing and who still had period regularly.

Data of the clinical and laboratory characteristics of these subjects showed that the majority of subjects included the RA-seropositive (with rheumatoid factor and/or anti-CCP positive). Assessment of disease activity using DAS28-ESR showed that most of them had active disease activity (DAS28 score >2.6), with domination of moderate disease activity. All subjects in this study had got DMARD therapy, and methotrexate was the most widely used, either as a single drug methotrexate or in combination with another DMARD. According to the 2009 European League Against Rheumatism (EULAR) recommendation, the first choice of conventional DMARD is still methotrexate, either as a single agent or in combination with another DMARD or biological agents,[19] because of efficacy and safety of this drug.[20-24] It should be noted that the majority of subjects still have moderate and severe disease activity, and most of them had a limited access to some conventional DMARD (such as leflunomide), and biological agents (such as infliximab, etanercept, golimumab and tocilizumab).

The result of COMP serum level examination of 60 subjects in this study revealed the median scores was 8.3 (7.06 to 11.43) U/L. We have no data in the normal population that could be used as a comparison, but we found a higher COMP serum level, compared with data from several other studies. Bender et al, and Tampoia, et al, showed that the mean score of COMP serum level in the normal population were 7.3 U/L and 7.4 U/L.[25,26] While Elsammak, et al, found COMP serum level mean in the Greece normal population was 7.49 (4.95 to 9.56) U/L.[27] It is a known fact that the COMP as a marker of cartilage degradation, especially in the case of RA has a very high specificity of 95% (95% CI: 88.7-98.4), although with a lower sensitivity of 47.6% (95% CI: 34.9-60.6).[25] So, based on the data above, we can conclude that increasing of COMP serum level showed that the cartilage degradation is in progress.

Based on bivariate analysis, we found no statistically significant correlation between confounding variables (ESR) and all other variables (COMP, OPG, RANKL, and OPG/RANKL serum level ratio). This is consistent with the results of other studies, such as studies conducted by Hamooda, et al.[28] who concluded that there was no correlation between COMP serum level with ESR and level of CRP (C-Reactive Protein).[28] Study by Lindqvist, et al.[29] also concluded the same thing that COMP serum level did not correlate with any indicators of inflammation.[29] Another study showed a fact that there was a significant correlation relationship between early elevated levels of serum COMP with the progression of joint damage that was detected two years later by the Larsen score, and the independent variable was ESR as the inflammation indicator.[30] The serum levels of OPG and RANKL and OPG/RANKL ratio were not influenced by the ESR. The data found in this study is consistent with the results of other studies that the inflammatory process is not entirely correlated with joint destruction, including cartilage degradation,[1,7] and both processes were most likely due to two separate difference pathological processes.[6,8]

Bivariate analysis were also conducted to examine the correlations between levels of serum OPG, RANKL, and the ratio of OPG/RANKL (as independent variables) with the process of cartilage degradation as assessed by serum COMP level indicator (as the dependent variable). Analysis results obtained showed no statistically significant correlation of each independent variable on the dependent variable.

This study analysis could prove the existence of the cartilage degradation process by increasing the COMP serum level, in most of the RA patients. On the other hand, we did not obtain a statistically significant correlation between the levels of OPG, RANKL, OPG/RANKL serum (as independent variables) and COMP serum level

as cartilage degradation marker (as dependent variable). Based on the results of this study, it appears that cartilage degradation process in RA patients is not caused by an effects of OPG/RANK/RANKL system, but is likely due to the influence of inflammatory pathways. Regarding the study design, we have also evaluated the power of this study about 80%, so the result of this study are not due to a lack of sample size.

CONCLUSION

We could not prove the correlation between RANKL, OPG serum level, and cartilage degradation process which was assessed based on COMP serum level.

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