

Correlation between Interleukin-6 and E-Selectin as a Marker of Endothelial Dysfunction in Rheumatoid Arthritis Patient without Traditional Cardiovascular Risk Factor

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Abstract

Background: Rheumatoid arthritis (RA) is an autoimmune disease which has recently been recognized to manifest as not only intraarticular but also extraarticular symptoms. Cardiovascular events, presented either subclinically or clinically, were discovered more in RA patients. Atherogenic inflammatory mediator in RA including interleukin-6 (IL-6) was thought to be one of nontraditional cardiovascular risk factor contributing to increase the endothelial dysfunction biomarker such as *E-Selectin*. This study was purposed to determine the correlation between inflammatory mediator and endothelial dysfunction event, especially between IL-6 and *E-Selectin*, in RA patient without traditional cardiovascular risk factor. **Method:** A cross-sectional study was performed to 40 RA patients of Rheumatology Clinic of Cipto Mangunkusumo National General Hospital, Indonesia from September to November 2017. Measurement of the level of IL-6 and *E-Selectin* were performed using enzyme-linked immunosorbent assay (ELISA). Bivariate correlation analysis was performed to determine the correlation between those two biomarkers.

Result: The mean age of this study subjects was 44.9 (13.1) years and median of disease duration was 36 months. This study showed weak correlation between IL-6 and *E-Selectin* level, but not statistically significant. $p=0.149$.

Conclusion: There is weak correlation between IL-6 and *sE-Selectin* in rheumatoid arthritis patient without traditional risk factor cardiovascular

Keywords: traditional risk factor cardiovascular, *E-Selectin*, interleukin-6, pro inflammatory mediator, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease which is characterized by chronic inflammation and causes progressive disability, early mortality, and socioeconomic burden culminating in clinical deterioration and worsening prognosis. This disease has a prevalence of 0.5-1%, can occur in every ethnic, and occasionally attacks young-aged women. The major manifestation of RA is inflammatory arthritis affecting diarthrodial joints, particularly small joints, with a tendency presenta-

tion of symmetric polyarthritis. Most of RA cases have chronic progressive nature. Untreated or inadequately treated disease will cause articular cartilage and juxtaarticular bone destruction, which results in permanent joint damage. In consequence, comprehensive management is required to manage articular symptoms as well as to prevent the potential systemic complications in RA.¹

Rheumatoid arthritis may present as intraarticular manifestations of joints damage; however, it may be accompanied with extraarticular complications, the condition of which becomes a major problem. One possible systemic extraarticular complication in RA is cardiovascular disease. In the last half century, increasing cardiovascular events have been reported on inflammatory rheumatic patients, particularly in RA. RA patients have twice the mortality rate compared to the general populations. As much as 30-50% of the mortality is related to cardiovascular events, the most common of which is ischemic heart disease and cerebrovascular disease.²

Cardiovascular events in RA occur due to traditional and nontraditional risk factors related to the chronic inflammation. Chronic inflammation results in endothelial dysfunction, which eventually triggers an early atherosclerosis. However, to date, there are still no theories which are capable to explain the exact pathogenesis of endothelial dysfunction in RA.

Some cytokines are well-known for contributing in RA pathogenesis, including tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-17 (IL-17). Among those proinflammatory cytokines, IL-6 has been widely studied as one of the most important cytokine in the pathogenesis of RA. This cytokine has pleiotropic effect in influencing systemic inflammation via the hematopoietic and immune system as well as in inducing the production of acute phase reactant - such as C-reactive protein (CRP) - from hepatocyte, influencing the maturity of B cells and inducing the production of autoantibodies, having a role in endothelial activation, and stimulating osteoclast maturation resulting in bone erosion. In the metabolism of lipid, IL-6 has been recognized to stimulate the synthesis of hepatic fatty acid and the lipolysis of adipose tissue

as well as to promote the synthesis of and reduce the secretion of cholesterol.³ Those conditions were thought to contribute in the occurrence of atherosclerosis in AR. Increased IL-6 and CRP levels may be attributed to the increased risk of cardio-vascular events in both male⁴ and female,⁵ as well as to the increase in mortality due to acute coronary syndrome. Those risk is not dependent on other traditional risk factors, including dyslipidemia. The role of IL-6 in endothelial dysfunction has been studied considerably in many populations. Esteve, et al. had reported that in healthy male, IL-6 had a correlation with endothelial dysfunction measured by endothelial-independent vasodilatation test of brachial artery.⁶

The early sign of atherosclerosis is endothelial dysfunction. The process of endothelial dysfunction involves various factors, including genetics, traditional cardiovascular risk factors, and systemic inflammation. Traditional cardiovascular risk factors, including smoking, hypertension, dyslipidemia, obesity, and diabetes, play a greatly important role in the development of endothelial dysfunction. Currently, many biomarkers of endothelial dysfunction have been developed.⁷ The most commonly observed adhesion molecule in various studies are soluble E-selectin (sE-selectin), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1).

Many previous studies have proved the correlation between those adhesion molecules and the development of endothelial dysfunction, including in RA. E-selectin, being the most specific adhesion molecule synthesized by endothelial cells, has also been proven to be correlated with endothelial dysfunction. The expression of E-selectin begins with the activation and production of IL-6, TNF- α , and CRP.^{8,9} Hwang, et al. described that *sE-selectin* can be used as a biomarker of atherosclerosis and the development of coronary heart disease.¹⁰

A few studies have described the correlation between IL-6 and E-selectin, including Dessein, et al. in 2006 ($r=0.43$, $p<0.0005$)¹¹ and in 2013 ($r=0.25$, $p<0.0001$).¹² Nevertheless, those studies include subjects with traditional cardiovascular risk factors, such as hypertension, diabetes, smoking, and dyslipidemia. To date, no studies have measured the correlation between those variables independently. This study was specifically aimed to determine the correlation between IL-6 and E-selectin as the biomarker of endothelial dysfunction in RA patients without any traditional cardiovascular risk factors.

Method

This cross-sectional study was performed in Cipto Mangunkusumo National General Hospital - a national referral hospital in Indonesia - after receiving the approval from FKUI Research Ethical Committee. Some subjects in this study were retrospectively obtained from a previous study "Role of Hydroxychloroquine to Improve Endothelial Dysfunction in Patients with Rheumatoid Arthritis," the recruitment period of which was in August 2016, whereas the other subjects were recruited consecutively from Rheumatology Polyclinic of Cipto Mangunkusumo National General hospital, the recruitment period of which was in October to December 2017.

Subjects included in this study were those diagnosed as RA based on ACR/EULAR 2010 criteria and aged more than 18 years old. Routine anamnesis and physical examination were performed to review the exclusion criteria prior to asking the informed consent to participate in this study. The exclusion criteria include pregnancy, having other autoimmune disease besides RA, suffering from acute severe infection or acute cardiovascular event such as coronary heart disease, heart failure, or stroke, having neoplasm or chronic inflammatory disease such as tuberculosis, smoking or history of smoking within the last five years, dyslipidemia, hypertension, diabetes mellitus, and refusing to participate in this study.

Subjects fulfilling the study criteria underwent further anamnesis and physical examination regarding age, sex, disease duration, and ongoing therapy. Data might be obtained retrospectively in the medical record as well. Laboratory tests of inflammatory marker ESR and CRP were performed in accordance to the routine procedure in Cipto Mangunkusumo National General Hospital. The tests for IL-6 and sE-selectin were performed using 5 mL venous blood sample, which was centrifuged for 15 minutes with the speed of 3,000 rpm. The tests were carried out on the serum using enzyme-linked immunosorbent assay (ELISA).

Each variable was presented descriptively and analyzed bivariately using SPSS 20.0. Pearson correlation test was performed if the data was normally distributed, whereas Spearman correlation test was performed if the data was not distributed normally.

Result

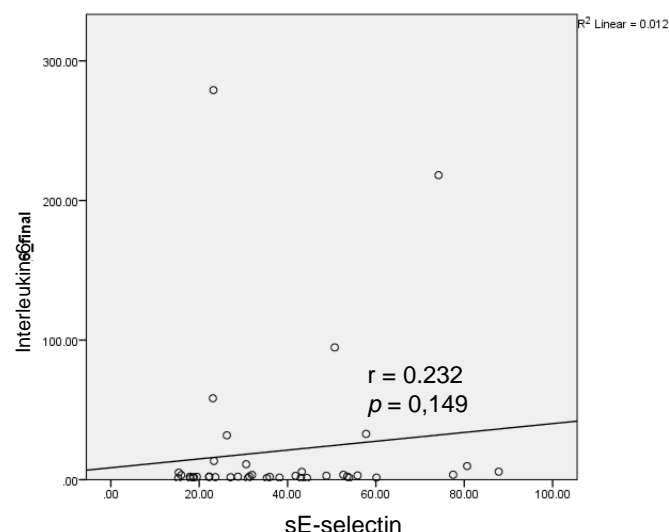
Of 40 subjects participating in this study, 39 subjects (97.5%) were female with the mean age of 44.9 years, the median disease duration of 36 months, and receiving DMARD either as monotherapy or in combination. Based on DAS28 CRP, most subjects were grouped into low (32.5%) and moderate (27.5%) category. The other demographic profiles were described in Table 1.

Table 1. Characteristics of the Study Subjects

Characteristics	Value (n=40)
Sex, n (%)	
Male	1 (2.5)
Female	39 (97.5)
Age (years), mean (SD)	44.9 (13.1)
Disease duration (months), median (min-max)	36 (2-300)
ESR (mm), median (min-max)	43.5 (10-130)
CRP (mg/L), median (min-max)	5.15 (0.3-105.2)
DAS28 CRP, median (min-max)	3.13 (1.32-7.68)
Remission (%)	25
Low (%)	32.5
Moderate (%)	27.5
High (%)	15
Therapy	
Methotrexate (%)	20
Methotrexate + Steroid (%)	72.5
Sulfasalazine + Steroid (%)	2.5
IL-6 (pg/mL), median (min-max)	2.3 (1.04-279.09)
sE-selectin (ng/mL), median (min-max)	31.66 (15.26-87.8)

IL-6 and sE-selectin level in this study were described in table 1. This study did not compare the level of those inflammatory mediator and adhesion molecule to the normal control. Spearman correlation test between serum IL-6 and *sE-selectin* showed weak positive correlation which was not statistically significant ($r=0.232$; $p=0.149$; Figure 1).

Figure 1. Scatterplot showing the correlation between serum IL-6 and *E-Selectin* using Spearman correlation test



Discussion

Rheumatoid arthritis is an inflammatory disease affecting the joints (arthritis), has a chronic nature, and is generally prevalent on young-aged female. This study recruited subjects with similar characteristics; most of the subjects were female (97.5%) with the mean age of 44.9 ± 13.1 years. These characteristics corresponded as well with the systematic review by Cross, et al. that female contributed twice the prevalence of RA compared to male (0.35% vs 0.15%)¹³ and the meta analysis by Rudan, et al. that female contributes five times more than male to the prevalence of RA.¹⁴

Assessing the relationship between inflammation and atherosclerosis in RA, this study was the first study in Indonesia observing the relationship between inflammatory mediator IL-6 and biomarker of endothelial dysfunction E-selectin. This study found a weak positive correlation that was not statistically significant between those variables ($r=0.232$; $p=0.149$). This finding was different to the report by Dessein, et al. in 2005 ($r=0.359$, $p<0.006$)¹¹ and 2013 ($r=0.392$, $p<0.0001$)¹⁵ but similar to the report by Foster, et al. ($r=0.200$, $p=0.155$).¹⁶

Interleukin-6 is one of the inflammatory mediators which is known to contribute in the pathogenesis of RA. This cytokine induced acute phase reactant such as CRP and played a role in endothelial activation as well as increased the synthesis of cholesterol and reduced the secretion of cholesterol; thereby it was atherogenic. This cytokine was thought to be the nontraditional cardiovascular risk factor resulting in the increase of cardiovascular event by triggering atherosclerosis. Atherosclerosis was initially started with endothelial dysfunction showed by increased expression of endothelial adhesion molecule such as E-selectin.^{5,6,8-10} Unfortunately, this study, as

well as other studies, had still not found a consistent relationship between those two variables. The difference among this study compared to the other studies could be explained by the difference in study subject characteristics and the influence of traditional cardiovascular risk factors as well as the difference in the disease severity and the received therapy.

The subjects in this study had younger age (44.9 ± 13.1 years) compared to the ones described by Dessein, et al. ($57 (27-81)$ years in 2005¹¹ and 55.8 ± 10.2 years in 2013¹⁵) and Foster, dkk. (58.5 ± 14 years).¹⁶ Various studies had reported that aging was independently contributed to atherogenesis process by elevating oxidative stress and the level of inflammatory mediators. Higher level of IL-6 reported by Dessein, et al. ($5.4 (0.5-186.3)$ pg/ml)¹⁵ compared to the one in this study ($2.3 (1.04-278.09)$ pg/mL) could be explained by the difference in age, which became a factor in causing the difference among those studies. Therefore, age difference might be a confounding factor in the relationship between IL-6 and E-selectin.

Unlike both studies by Dessein, et al., this study excluded subjects with traditional cardiovascular risk factors, including hypertension, diabetes mellitus, metabolic syndrome, and hypercholesterolemia. Those factors were well-known to play some role in the process of endothelial dysfunction as well as in the increase of either inflammatory mediators or biomarkers of endothelial dysfunction. Those differences might also have a potential in causing the discrepancy of result with this study.

In terms of disease severity, this study had subjects with lower DAS28 CRP value ($3.13 (1.32-7.68)$) compared with the ones reported in both studies by Dessein, et al. (4.2 ± 1.3).^{11,15} This reflected higher inflammation process in the study by Dessein, et al., which might result in higher potential of endothelial dysfunction event in those studies.

The last factor suspected to cause differences between this study and the study conducted by Dessein, et al. was the difference in therapy received by the subjects. It was found that the use of steroid in this study was much higher than the one found in the report by Dessein, et al. (75% vs 15%).¹⁵ Some studies had reported that steroid inhibited the “shedding” process by posttranslational modification of adhesion molecules that resulted in reduced expression of E-selectin. In vitro study by Cronstein, et al.¹⁷ showed that dexamethasone inhibited the mRNA of E-selectin in *human umbilical vein endothelial cells* (HUVECs) stimulated with LPS and IL-1. A study by Ray, et al.¹⁸ had also reported that glucocorticoid inhibited the promoter of NF-KB, which would result in reduced expression of E-selectin.

While the study by Foster, et al. had similar age characteristics compared to the ones by Dessein, et al. and included traditional cardiovascular risk factors in his study as well, Foster, et al. reported different result. This difference was thought due to the difference in therapy between those studies. Unfortunately, Foster, et al did not include the variable of therapy in the demographic characteristics.¹⁶

Measuring the power of this study according to computer-ized calculation using application by computing the data of correlation coefficient, α value, and sample size, the power for correlation coefficient between IL-6 and sE-Selectin in this

study was 30%. This number was considered low comparing to the target power set in the early sample size calculation, which was 80%. Therefore, the correlation coefficient in this study was still deemed unable to explain the exact correlation between those variables. The difference between this study and the report by Dessein, et al.,¹¹ which showed a significant result, was due to differences in subject characteristics. Dessein, et al. included traditional cardiovascular risk factors in the study. Therefore, sample size calculation referring to the correlation coefficient from the study by Dessein, et al. was not applicable to this study, which resulted in more sample size needed in this study.

Conclusions

There was weak positive correlation between IL-6 and E-selectin in RA. However, the result was not statistically significant. This finding was thought to occur due to the younger age of recruited subject with shorter duration of disease, the exclusion of traditional cardiovascular risk factors, the difference in the severity of disease, and the variation of therapy received.

Suggestions

This is the first study in Indonesia evaluating the correlation between IL-6 as inflammatory mediator and E-selectin as a biomarker of endothelial dysfunction in RA. Further studies, including a similar study with larger sample size or without restriction of traditional cardiovascular risk factors, were needed to ensure the relationship between those variables.

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