Association between Soluble Contents CD40 Ligand (sCD40L) and Acute Coronary Syndrome (ACS)

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Abstract

Background: Acute coronary syndrome (ACS) is a manifestation of coronary heart disease (CHD), which the leading cause of death in Indonesia. CD40 ligand (CD40L) stored in alpha platelet granules will be rapidly transferred to the surface when the platelets are activated and subsequently released from the surface as a soluble CD40 ligand (sCD40L). Soluble CD40 ligand (sCD40L) acts as a bridge between the inflammatory process, atherosclerosis, and thrombosis. This study aims to study the relationship between sCD40L levels and the incidence of ACS in patients with chest pain in Dr.Soetomo General Hospital, Surabaya.

Method: Research subjects were 40 patients with chest pain who came to Emergency Room at Dr.Soetomo General Hospital, Surabaya. The patients were grouped based on the diagnosis of ACS and non-ACS with the electrocardiogram and troponin T. Serum levels of patients that examined by sCD40L with enamine-linked immunosorbent assay (ELISA) method from Quantikine®.

Results: Twenty-six (65%) were diagnosed with ACS and 14 (35%) were non ACS. The diagnosis of ACS includes ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI), and unstable angina (UA), with the highest proportion being STEMI at 15 (57%). Spearman correlation test of sCD40L level with ACS incidence got correlation coefficient rho (ρ) = 0.52 (p = 0.001).

Conclusion: There was a moderate positive correlation between sCD40L levels and ACS incidence in patients with chest pain.

Keywords: *CD40L, Acute Coronary Syndrome, Chest Pain, Unstable Angina*

Introduction

Cardiovascular disease is a global health problem responsible for 30% of death globally. Data in 2005 suggested that 17 million deaths from a total of 58 million deaths worldwide were caused by cardiovascular disease [1]. The American Heart Association (AHA) mentions that 71.3 million Americans suffer from the cardiovascular disease in 2003. Cardiovascular disease accounted for nearly 1 million deaths in 2003 with 53% of those caused by coronary heart disease (CHD) [2].

The mortality rate due to cardiovascular disease in Indonesia also increases annually, reaching almost 30% in 2004 compared to only 5% in 1975. Data from the Indonesian National Health Survey conducted by the Ministry of Health of the Republic of Indonesia stated that cardiovascular disease is the leading cause of death in Indonesia with CHD as the main cause by 26.4% [3].

The manifestations of CHD are the occurrence of acute coronary syndromes (ACS), which include unstable angina/UA (angle) and acute myocardial infarction (AMI). The data from the Directorate General of Pharmaceutical and Medical Devices of the Ministry of Health of the Republic of Indonesia in 2006 stated that ACS caused a huge number of hospitalizations and a major and most frequent cause of death for CHD.

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The high rate of ACS death causes the need for a proper understanding of the pathogenesis disease, thus helping to find a quick and accurate examination for diagnosis. Biological markers currently used for diagnosis of molecules released after the occurrence of myocardial necrosis, such as troponin and natriuretic peptides [4]. The markers are clinically useful, but they have not been able to describe the pathogenesis of the early phase of atherothrombosis. An alternate marker is needed that can not only help diagnosis but may also provide an understanding of the pathogenesis of ACS.

The concepts put forward today are that inflammatory markers, endothelial dysfunction, and platelet activation that can be used to identify disease activity even before myocardial necrosis occurs. One of the concepts underlying the mechanism of atherothrombotic pathogenesis involves the interaction between CD40 and CD40 ligand (CD40L). CD40 is a transmembrane protein receptor that belongs to the group of superfamily tumor necrosis factor (TNF). CD40L is stored in the alpha platelet granule and is rapidly transferred to the surface at which platelets are activated. CD40L that has been expressed on the surface will soon be broken down and released from the surface of platelets as soluble CD40 ligand (sCD40L) [5].

Until now the role of sCD40L in the process of ACS has not been fully understood. Several studies have shown that CD40 and CD40L are one of the mediators of vascular inflammation and act as a bridge between the inflammatory process, atherosclerosis, and thrombosis. Research on the sCD40L level and its relation to ACS incidence have not been performed, so that this study aims to know the relationship between sCD40L level with ACS incidence in Dr.Soetomo General Hospital, Surabaya.

Method

This research uses analytic observational research design with the cross-sectional design. The study was conducted at Emergency Room of Dr.Soetomo General Hospital, Surabaya, and Installation of Clinical Pathology Faculty of Medicine Universitas Airlangga/ Dr.Soetomo General Hospital Surabaya. This research implemented in April to June 2013. All patients who become the sample of the study must meet the inclusion criteria, among others; patients with chest pain, aged ≥30 years, have complete medical records. While for exclusion criteria, among others; patients had sepsis, acute stroke, until serum creatinine> 2 mg/dl [6].

The laboratory procedure in this study was started by examining the sCD40L level using the enzyme-linked immunosorbent assay (ELISA) method of Quantikine®. Then the inspection principle of sCD40L was an enzyme-labeled enzyme by the sandwich. The microplate coating was coated with polyclonal antibodies specific to CD40L. A number of samples were dripped with a pipette into the well so that the CD40L present in the sample would bind to the antibody. Washing was done to remove unbound substances, then polyclonal antibodies labeled with specific enzymes for CD40L are added to the well. The washing was performed again to remove the unbound enzyme-labeled antibody reagents .

The substrate was added and the color change occurs that measured after the addition of the reagent to stop the reaction. After that, the sample for serum sCD40L level examination. Serum samples were obtained from 5 ml of venous blood inserted into serum separator tube (SST). The sample was allowed to form the clot for 30 minutes before centrifugation at 1000g for 15 minutes.[7]

The serum transferred into an aliquot tube and stored at a temperature of -20oC until examination. Serum samples were diluted 5 times by adding 50 µl samples with 200 µl diluent calibrator RD5P. Followed by preparing the reagent which the first left for some time at room temperature before use [8]. The inspection procedure for sCD40L levels was expanded when all reagents, standards, and samples have been prepared. The microplate strip to be used as released from the intact microplate. The microplate was placed in a horizontal orbital shaker with a speed of 500 ± 50 rpm and incubated for 2 hours at room temperature [9].

The standard curve was made by inserting the absorbent of each standard solution on the y-axis and the level on the x-axis, then connecting the dots into a straight line. Nonlinear data can use absorbent logs and logs. The levels can be read with standard curves based on each absorbent. SCD40L sample rate was the multiplied reading result level 5 [10].

All data on ACS patient characteristics, distribution, and frequency of ACS are presented descriptively. Diagnostic value of examination of sCD40L as the marker of ACS in dr. Soetomo Surabaya obtained from the calculation of the formula to calculate the sensitivity, specificity, positive predictive value, and negative predictive value, using 2x2 tables. Statistical analysis to

determine the relationship between sCD40L and ACS incidence by using Pearson correlation test [11].

Results

The lowest SCD40L test result of patients with chest pain was 0.07 ng/ml obtained in Stable Angina patients while the highest level was 12.39 ng/ml obtained in patients with NSTEMI diagnosis. The mean sCD40L level of chest pain patients was 4.23 ± 3.49 ng/ml. ACS patients had mean sCD40L levels of 5.45 ± 3.70 ng/ml, while non-ACS patients had mean sCD40L levels of 1.97 ± 1.33 ng/ml. The mean rate of patients with the diagnosis of ACS was significantly higher than the mean rate of patients with non-ACS diagnosis ($p < 0.05$), whereas the mean sCD40L concentration in ACS subgroup did not find the significant difference (Table 1).

The SCD40L levels of ACS patients were 16 (61.53%) above the cut off (> 2.99 ng/ml) while 10 (38.47%) were obtained to be lower than cut off (<2.99 ng/ml). Most non ACS patients, 10 (71.42%) had sCD40L levels below cut off (<2.99 ng/ml), while the remaining 4 (0.28%) had sCD40L levels above cut off (> 2.99 ng/ml) (Table 2). The calculation of the diagnostic value of sCD40L based on 2x2 table showed 61.53% sensivitas, specificity 71.4%, predictive value 80% and 50% negative predictive value (Table 3).

Correlation analysis aims to determine the pattern and closeness of the relationship between two or more variables. Statistical analysis to determine the relationship between sCD40L level and ACS incidence using Spearman test because the sCD40L content data is not normally distributed. Result of statistical analysis got coefficient correlation rho (ρ) = 0.52 with value p = 0.001. This means that the sCD40L level was moderately correlated with the ACS incidence and this correlation was significant. The positive correlation coefficient indicates that the correlation was unidirectional, i.e., higher sCD40L levels will increase the incidence of ACS.

Table. 1 SCD40L levels in ACS and non-ACS patients

Groups	**sCD40L Level (ng/ml)**
Non-ACS	1.97 ± 1.33*
ACS	5.45 ± 3.70*
UA	6.02 ± 3.31
NSTEMI	5.60 ± 4.52
STEMI	5.26 ± 3.55

Table. 2 Table 2x2 levels of sCD40L and diagnostic groups

		Groups	
		ACS	Non-ACS
sCD40L(ng/ml)	>2.99	16	4
	<2.99	10	10

Table 3. The diagnostic value of sCD40L for diagnosis of ACS

Diagnostic value	Results
Diagnostic sensitivity	61.53%
Diagnostic specificity	71.42%
Predictive value is positive	80%
Predictive value is negative	50%
Positive ratio is positive	2.15
Positive ratio is negative	0.54

Discussion

The results of mean random blood glucose levels in ACS patients were higher than non-ACS patients. This is in accordance with previous studies that mention that acute hyperglycemia occurs in about 50% of patients with ACS. Cardiovascular stress will trigger the release of catecholamine, cortisol, and glucagon hormones resulting in increased glucose and free fatty acids increased hepatic gluconeogenesis and decreased peripheral glucose uptake. Acute hyperglycemia will worsen the prognosis and improve the mortality of ACS patients with or without previous diabetes history [12].

There was no difference between total cholesterol, triglyceride, and LDL cholesterol in ACS and non-ACS patients. The mean rate of HDL cholesterol in ACS patients was lower compared with non-ACS patients. This is in accordance with studies suggesting that elevated levels of HDL cholesterol are a protective factor, while low HDL cholesterol levels increase the risk of atherogenesis [13].

SCD40L examination results in patients with chest pain have the diagnosis of ACS was significantly higher than that of chest pain patients with a non-ACS diagnosis. It was in accordance with previous studies suggesting that elevated sCD40L may be used as a marker for thrombotic inflammatory activity in ACS patients. However, elevated sCD40L levels cannot be used to distinguish the ST elevation of myocardial infarction (STEMI), Non-ST elevation myocardial infarction *(NSTEM)I*, or Unstable Angina (UA) [14].

Platelet activation is an important key to the development of ACS. Subendothelial collagen exposure to platelets causes the activation and secretion of several thrombotic and proinflammatory molecules during the acute phase of myocardial infarction. sCD40L is one of the proinflammatory molecules secreted by active platelets and involved in the process of plaque destabilization and thrombus formation [15].

Increased sCD40L levels were also one of the causes of ACS, which allows continuous cycles in ACS patients. The occurrence of elevated sCD40L levels is due to plaque rupture. The results of the use of sCD40L levels were lower than previous studies. Previous studies have suggested that the use of sCD40L for diagnosis of ACS in patients with chest pain has a sensitivity of 90% and a specificity of 86%. The reason for the difference between this study and the previous was the number of samples used [16].

Risk factors that may affect the occurrence of ACS were smoking history. Smokers tend to have higher levels of sCD40L compared to nonsmokers. If the patient has a history of previous diseases such as diabetes, hypertension, and dyslipidemia may affect the patient's sCD40L level. Patients with a history of hypertension tend to have the CD40/CD40L system become more reactive so that sCD40L levels become higher. SCD40L levels will also increase in dyslipidemia patients. Other conditions of increased levels of sCD40L were autoimmune diseases, multiple sclerosis, inflammatory bowel disease, and stroke [2].

Several ACS patients in this study had a history of using drugs such as Aspirin and Simvastatin. Studies show that there is no association between aspirin use and serum sCD40L levels. Aspirin is often used in patients

with angina and may inhibit some platelet function, but inhibition of sCD40L release requires a stronger platelet inhibitor, such as GP IIb/IIIa inhibitors. The use of drugs for the treatment of dyslipidemia and hypertension such as Simvastatin, Losartan, or a combination of both for 2 months can lower sCD40L. [1]

The result of correlation of sCD40L with ACS incidence has the moderate correlation, and the higher the sCD40L cause the higher incidence of ACS. Studies suggest that elevated levels of sCD40L are an independent risk factor for the occurrence of death and recurrent myocardial infarction in ACS patients. Other studies suggest that elevated sCD40L levels will increase the risk of follow-up cardiovascular events, such as Acute myocardial infarction (AMI), sudden death, and recurrent angina in CHD patients [8].

Ethical Clearance: This research process involves participants in the survey using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

Conflict of Interest: The author reports no conflict of interest of this work.

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Conclusion: Based on the results of analysis and discussion it can be concluded that sCD40L levels in patients with chest pain have diagnosed of ACS that significantly higher than patients with the non-ACS diagnosis. The use of sCD40L level for diagnosis of ACS in patients with chest pain was 61.53% sensitivity and 71.4% specificity. The correlation results obtained positive moderate correlation between sCD40L level and the incidence of ACS in patients with chest pain.

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