Original Article:

CD40L-CD40 interaction avidity as coronary artery disease predictive factor

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Abstract

Background: Coronary artery disease (CAD) can occur due to atherosclerosis in coronary arteries. Platelet agreement plays an important role in the pathophysiology of CAD. CD40L is a surface antigen on activated platelets. CD40L will bind to CD40 which is expressed by macrophages and endothelials then this activation results in reduced thickness and stability of atherosclerotic plaque and then thrombus appears. Thrombus ultimately inhibits blood flow to the coronary arteries. Proper measurement of these activities can describe the occurrence of CADs driven by platelets. ***Objectives:*** Measuring the avidity of CD40L-CD40 interactions so that the relationship between CD40L activity and CAD events can be seen. ***Materials and Methods:*** This research is non-experimental in nature and uses Case control designs. The subjects of the study were CAD patients at RSUD Dr. Soedirman Kebumen. Patients who met the inclusion and exclusion criteria were then examined and healthy patients were matched and CAD patients matched. The subject's blood is then drawn and sent to Clinical Pathology Laboratory, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Blood samples were then examined using flow cytometry to determine CD40L levels. The results obtained were then analyzed by logistic regression test with Medcalc software. ***Results and discussion:*** Twenty-six subjects participated in this study, with 13 subjects were CAD patients and 13 healthy control subjects.The percentage of platelets expressing CD62P+ CD40L+ in the CAD group was higher than in the control group ($p = 0.0015$). Statistical analysis with T test showed that there were significant differences in CD40-expressing platelets between the CAD group and the control group ($p = 0.0029$).The study conclude that the avidity of CD40L-CD40 interactions as indicated by CD40 expression is related to CAD events. CD40 expression was higher in subjects with CAD compared to controls.

Keywords: CAD; CD40L; atherosclerosis

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Introduction

The prevalence of coronary artery disease based on symptom interviews is estimated at 1.5% of the total population of Indonesia. The proportion of deaths from cardiovascular disease is estimated at 4.6% of the total causes of death of all ages. Despite high mortality, only 0.5% of CAD diagnosed by doctors.[1]

Coronary Artery Disease (CAD) could occur due to atherosclerosis of the coronary arteries. Atherosclerosis is a chronic inflammatory disease in the arterial wall due to humoral and specific immune responses.[2,3]

Fatty streak is an early lesion of atherosclerosis. This lesion could occur because of an increase in lipoprotein content in the endothelial intima layer. Lipoprotein enters the intima layer because it binds to proteoglycan molecules in the extracellular matrix which increases with the amount of LDL

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in the blood. Lipoproteins then undergo chemical modification through oxidase and non-enzymatic glycation processes.[2,3,4]

The process then followed with leukocyte recruitment. Leukocytes normally cannot be attached to the endothelium. Modification of lipoprotein causes the release of cytokines that play a role in the regulation of leukocyte recruitment and activation. [2,3,4]

Leukocytes that play a role in this process are mononuclear cells: monocytes and lymphocytes. Mononuclear phagocytes then differentiate into macrophages. Macrophages will eat lipids and become foam cells through the process of endocytosis. Activated lymphocytes will release pro-inflammatory cytokines such as interferon and tumor necrosis factor (TNF) which will stimulate macrophages. Lipid plaques turn into atheroma plaques with macrophages in them.[2,3,4]

CD40 is a type I transmembrane protein receptor and part of tumor necrosis factor. CD40 is mainly expressed by B cells but can also be found in immune cells, epithelial cells, fibroblasts, vascular walls such as endothelial cells and muscles as well as platelets. Platelets can excrete CD40L after being stimulated by activators such as thrombin.[5]

CD40 / CD40L expression is increased in atheroma plaques by an unknown mechanism. These expressions cause various processes in atherosclerosis. Endothelial surface CD40 / CD40L interactions cause matrix activation and expression of adhesion molecules, which are the first step of atherogenesis CD40 / CD40L also play a role in lymphocyte and dendritic T cell interactions in the vascular wall. CD40L also causes interference with the vascular redox system and endothelial relaxation through the intracellular sensitive redox pathway. CD40L (and possibly CD40L) plays an important role in atherothrombotic. CD40L will connect between platelets, inflammation, thrombosis, and atherogenesis.[5]

This study aimed to measure the avidity of CD40L-CD40 interactions to understand the relation of CD40L activity and CAD events

MATERIALS AND METHODS

Study design and subjects

This was a case control study. The data were obtained from May to December 2017 in Soedirman hospital, Kebumen regency. Case group were patients diagnosed with CAD through electrocardiography (ECG) examination, with the age of 35-70 years old, chest pain onset ≤ 24 hours, and newly diagnosed with CAD. Control group were subject with matched body mass index (BMI), age, and sex with case group. Subjects were excluded from the study if they refuse to participate and if also diagnosed with other disease (chronic kidney disease on dialysis therapy, NYHA functional class II or higher class heart failure, hepatic cirrhosis, cardiac valve disease, acute cerebrovascular event, diabetes mellitus, malignancy, and sepsis), or pregnant patients. An oral and written informed consent were obtained from the subjects.

Ethical consideration

This study is approved by Ethics Committee of the Faculty of Medicine, Islamic University of Indonesia with Letter No. 05/Ka.Kom.Et/70/KE/VII/2017.

Blood sample analysis

Blood samples were obtained in Soedirman hospital with vacutainer and inserted to EDTA anticoagulant tube. The samples were stored in the temperature of

Table 1. Subject characteristics

	CAD N=13	Case N=13	P
Age (years)	50.333 ± 25.3246	47.5 ± 0.7071	0.7064
Sex			0.8494
Male	10 (38.47 %)	8 (30.77 %)	
Female	3 (11.54 %)	5 (19.24 %)	
BMI	23.74 ± 4.0649	20.22 ± 2.6596	0.2818
Systolic Blood Pressure (mmHg)	130.769 ± 54.9942	136.667 ± 40.4145	0.8650
Diastolic Blood Pressure (mmHg)	75.692 ± 27.0413	86.667 ± 25.1661	0.5327
Heart rate (/ minute)	94.769 ± 13.2737	104.667 ± 40.464	0.7162
Respiratory rate (/minute)	21.154 ± 2.4443	20.667 ± 1.1547	0.7463
Body temperature (°C)	37.415 ± 1.5137	37.53 ± 1.36	0.9036

The mean of CAD onset was 3 ± 2.65 hours. A total of 53.85% of subjects were diagnosed with NSTEMI. While subjects diagnosed with Anterior and Inferior STEMI were 23.08%, respectively. The most common co-morbid factor is Congestive Heart Failure (CHF) with 53.85% of subjects had a history of CHF. Other comorbid factors such as history of Diabetes Mellitus (DM), history of hypertension and history of smoking were found in only 46.16% of study subjects. (Table 2)

2-8°C. All samples were analyzed with Flowcytometry to measure CD40-expressing platelets.

Statistical analysis

The independent variable in this study was CD40L level in numeric scale. While the dependent variable is a CAD event. Logistic regression was done to find the correlation between dependent and independent variable. Data were analyzed using Medcalc software.

RESULTS

Twenty-six subjects participated in this study, with 13 subjects were CAD patients and 13 healthy control subjects. The majority of subjects were dominated by men (69.24%) and there was no difference in the proportion of sex between the CAD and control groups.The mean BMI in the CAD group was higher than in the control group even though there were no significant differences. (Table 1)

Table 2. CAD group characteristics

	N (%)	Mean ± SD
Onset (hours)		3 ± 2.65
Diagnosis		
Anterior STEMI	3 (23.08 %)	
Inferior STEMI	3 (23.08 %)	
NSTEMI	7 (53.85 %)	
CHF history		
Yes	7 (53.85 %)	
No	6 (46.16 %)	
DM history		
Yes	6 (46.16 %)	
No	7 (53.85 %)	
Hypertension history		
Yes	6 (46.16 %)	
No	7 (53.85 %)	
Smoking		
Yes	6 (46.16 %)	
No	7 (53.85 %)	

Routine blood analysis showed the average platelet rate in subjects with CAD was 245,308 ± 98,965x10^3 / µL. The mean platelet count in the CAD group was higher than in the control group (245,308 ± 98,965 vs 204.6 ± 88.7767 x10^3 / µL). However, there was no significant difference in platelet count mean between CAD and control (p = 0.4346). Other routine blood parameters such as hemoglobin, leukocytes, erythrocytes and hematocrit rates were higher in the control group but there were no significant differences between groups (p> 0.05). (Table 3)

Table 3. Blood sample analysis

	CAD	Control	P
Hemoglobin (g/dL)	13.115 ± 2.1733	14.36 ± 1.8174	0.2745
Leukocyte count (×10^9/L)	7.509 ± 2.22	11.09 ± 5.3235	0.2073
Platelet count (×10^3/µL)	245.308 ± 98.965	204.6 ± 88.7767	0.4346
Red blood cell count (×10^9/L)	4.338 ± 1.451	5.036 ± 0.5364	0.3171
Hematocrit (%)	40.054 ± 7.0463	42.82 ± 5.2361	0.4402

Flow cytometry analysis to determine the number of CD40-expressing platelets could be seen in Figure 1. Positive expression was taken from FSC x SSC gating that leads to platelets. The results of the gating are then plotted to see CD62P + and CD40L +.

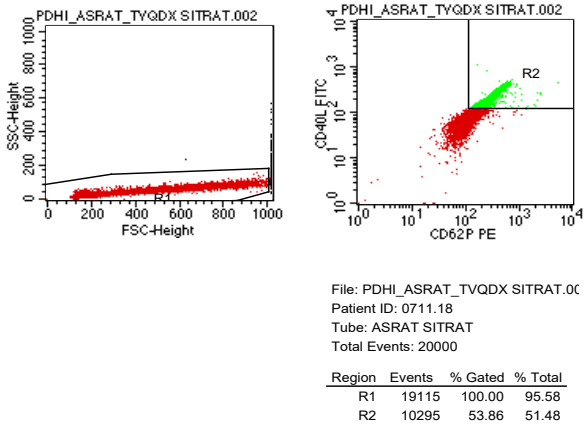

Figure 1. Gating strategy

The number of CD40-expressing platelets on flow cytometry examination in the CAD group was higher. Statistical analysis with T test showed that there were significant differences between the CAD group and the control group (p = 0.0029). (Figure 2)

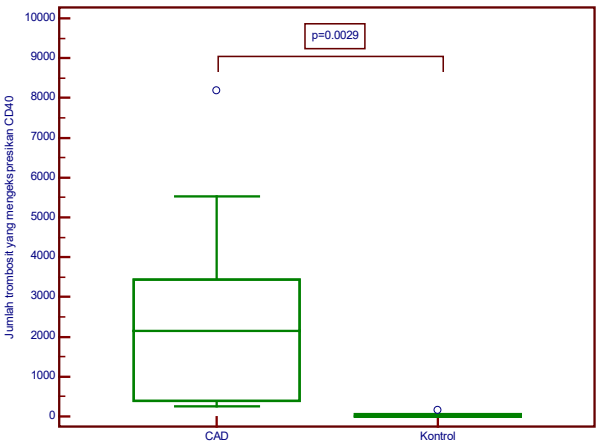

Figure 2. CD40-expressing platelets absolute number

These results are parallel with the percentage of platelets expressing CD62P + CD40L +. The percentage of platelets expressing CD62P + CD40L + in the CAD group was higher than in thecontrol group (p = 0.0015) as shown in figure 3. These results mean that there is a significant difference in the percentage of platelets expressing CD62P + CD40L +.

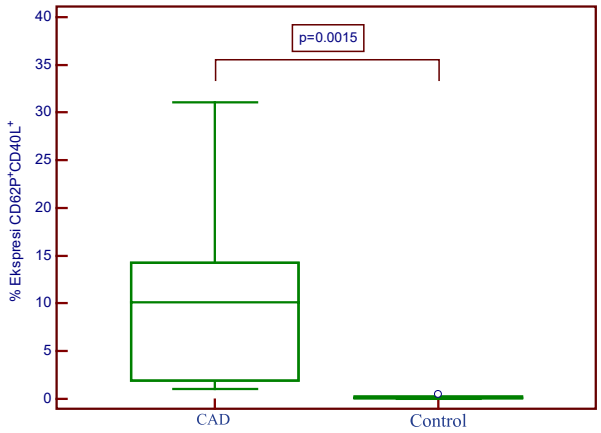

Figure 3. CD40 expression percentage

DISCUSSION

CD40 is a type I transmembrane protein receptor and part of tumor necrosis factor. CD40 is mainly expressed by B cells but can also be identified in immune cells, epithelial cells, fibroblasts, vascular walls such as endothelial cells and muscles as well as platelets. Platelets can excrete CD40L after being stimulated by activators such as thrombin. CD40 / CD40L expression is increased in atheroma plaques by an unknown mechanism. These expressions cause various processes in atherosclerosis.[5,6]

In this study, the number of platelets expressing CD40 on flow cytometry examination in the CAD group was higher. Statistical analysis with t-test showed that there were significant differences between the CAD group and the control group. P-value obtained is 0.0029.

In another study conducted by Plaikner et al (2009), an analysis of sCD40L concentrations was significantly related to C-reactive protein concentration (P 0.012) and platelet count (P 0.001).[7] The study also found a significant relationship between sCD40L and platelet counts.[7] Another study conducted by Napoleao et al (2015) showed different results.[7,8] CD40L platelet expression was the same in stable and control angina patients. However, there was an increase after one month of observation in myocardial infarction patients.[8]

Other studies found sCD40 levels increase in 57.8% of patients with acute coronary syndrome.[9] High CD40L levels in the coronary arteries will increase the odds ratio for cardiovascular events because they show impaired myocardial perfusion and myocardial damage.[10] The sCD40L level is strongly correlated with platelet activation.[7,9] This is because platelets are the main source of sCD40L and are where 95% of sCD40L is circulated.

The main mechanism of inducing platelet activation is not yet fully known. CD40L will be expressed immediately after platelet activation. CD40L will cause endothelial cells to express adhesion molecules. Soluble CD40L will also stabilize the atrial thrombus with an integrin-dependent beta-3 mechanism so that platelets are more active. sCD40L also binds to glycoprotein (GP) IIb / IIIa on platelets thereby increasing platelet activity.[5,6,11]

Other results from this study indicate that the percentage of platelets expressing CD62P + CD40L + in the CAD group is higher than in the control group. The P-value obtained from the t-test differs for (p = 0.0015). These results mean that there is a significant difference in the percentage of platelets expressing CD62P + CD40L +.

CD62P or P-selectin is a marker of platelet activation. Increased P-selectin proves that more platelets are activated. Different things were found in other studies that the sCD40L level did not significantly correlate with CD62P expression.[8] The difference may be due to the study sample in the patient taken before PCI while in this study the sample was taken immediately in the emergency room. In that study it was assumed that sCD40L was not related to platelet activation over time. In line with the results of other studies that showed sCD40L levels had no effect on prognosis.[9]

CONCLUSION:

This study conclude that the activity of CD40L-CD40 interaction as indicated by CD40 expression is related to CAD events. CD40 expression was higher in subjects with CAD compared to controls.

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Conflict of Interest : The authors declares that there is no conflict of interest regarding the publication of this paper

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Writing and submitting manuscript: Linda Rosita, Adika ZA, NindaDevita, Erlina Marfianti;

Editing and approval of final draft: Linda Rosita, Erlina Marfianti

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Reference

1. Ministry of Health Republic of Indonesia. 2013. Riset Kesehatan Dasar; RISKESDAS. Jakarta: National Institute of Health Research and Development, Ministry of Health Republic of Indonesia
2. Libby, P., Ridker, P. M. and Maseri, A. 'Inflammation and atherosclerosis', *Circulation,* (2002) ;**105**(9), pp. 1135-1143. doi: 10.1161/hc0902.104353. https://doi.org/10.1161/hc0902.104353
3. Ait-Oufella, H. et al'Recent advances on the role of cytokines in atherosclerosis', Arteriosclerosis, *Thrombosis, and Vascular Biology*, .(2011) ; **31**(5), pp. 969-979. doi: 10.1161/ATVBAHA.110.207415. https://doi.org/10.1161/ATVBAHA.110.207415
4. Lilly, L. S. (2011) Pathophysiology of heart disease. Wolters Kluwer/Lippincott Williams & Wilkins.
5. Antoniades, C. et al. 'The CD40/CD40 Ligand System. Linking Inflammation With Atherothrombosis', Journal of the American College of Cardiology. *American College of Cardiology Foundation*, 2009;**54**(8), pp. 669-677. doi: 10.1016/j.jacc.2009.03.076. https://doi.org/10.1016/j.jacc.2009.03.076
6. Zhang, B. et al'The CD40/CD40L system: A new therapeutic target for disease', *Immunology Letters.* Elsevier B.V., . 2013;**153**(1-2), pp. 58-61. doi: 10.1016/j.imlet.2013.07.005. https://doi.org/10.1016/j.imlet.2013.07.005
7. Plaikner, M. et al. 'Lack of association of soluble CD40 ligand with the presence of acute myocardial infarction or ischemic stroke in the emergency department', *Clinical Chemistry*, 2009;**55**(1), pp. 175-178. doi: 10.1373/clinchem.2008.114231. https://doi.org/10.1373/clinchem.2008.114231
8. Napoleao, P. et al 'Changes of soluble CD40 ligand in the progression of acute myocardial infarction associate to endothelial nitric oxide synthase polymorphisms and vascular endothelial growth factor but not to platelet CD62P expression', *Translational Research*, . 2015;**166**(6), pp. 650-659. doi: 10.1016/j.trsl.2015.07.006. https://doi.org/10.1016/j.trsl.2015.07.006
9. Yan, J. C. et al'The effect of elevated serum soluble CD40 ligand on the prognostic value in patients with acute coronary syndromes', *Clinica Chimica Acta,* . 2004; **343**(1-2), pp. 155-159. doi: 10.1016/j.cccn.2004.01.012. https://doi.org/10.1016/j.cccn.2004.01.012
10. Yoshioka, T. et al. 'Association of CD40 ligand levels in the culprit coronary arteries with subsequent prognosis of acute myocardial infarction', *Atherosclerosis.* Elsevier Ireland Ltd, 2010; **213**(1), pp. 268-272. doi: 10.1016/j.atherosclerosis.2010.07.044. https://doi.org/10.1016/j.atherosclerosis.2010.07.044
11. Sevcik, R. et al. (2007) 'CD40 Soluble Ligand And Acute Coronary Syndromes', pp. 61-63.