

CORRELATIONS BETWEEN SERUM LEVEL OF MATRIX METALLOPROTEINASE-9 (MMP-9) AND SERUM LEVEL OF TROPONIN-I IN PATIENT WITH ACUTE CORONARY SYNDROME (ACS)

Ruchanihadi¹, Budi Yuli Setianto², Hariadi Hariawan^{2*}

1. Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta

2. Subdivision of Cardio Vascular, Department of Internal Medicine, Dr. Sardjito Hospital, Yogyakarta

*Corresponding Author: hariadi_yk@yahoo.com

ABSTRACT

Background: The pathophysiology of acute coronary syndromes (ACS) is now accepted as the rupture or erosion of an atherosclerotic plaque, which initially occurs at the shoulder of the plaque and is followed by intra-plaque thrombosis then spread to the vascular lumen and cause total partial vascular occlusion. MMP-9 is an extracellular matrix degrading enzyme that plays a crucial role in the breakdown of the fibrous cap of plaque and subsequent rupture in the pathogenesis of ACS. Cardiac troponins are currently the most sensitive and specific biochemical markers of myocytes necrosis.

Objective: To know the correlation between serum level MMP-9 and serum level of Troponin-I in patients with ACS

Method: Study design was cross sectional. Data were collected by consecutive sampling from patients in ICCU ward of RSUP Dr. Sardjito General Hospital, Yogyakarta, from June 2008-August 2010. A questionnaire was used to collect information from patient. After admission, peripheral venous blood was drawn once and measured concentration of serum level of MMP-9 and Troponin-I before definitive thrombolysis. Data were expressed as means \pm standard deviation (SD). Correlation between serum level of MMP-9 and serum level of Troponin-I were assessed using Spearman's rank correlations test. A value of $p < 0.05$ was considered statistically significant.

Result: There were 139 patients with ACS and comprising 63 patients with STEMI, 27 patients with NSTEMI, and 49 patients with UAP. Means \pm SD of Troponin-I from all of samples was 9.49 ± 10.47 ng/dL. Mean \pm SD of MMP-9 from all of samples was 1296.06 ± 729.97 ng/dL. There were significant

correlations between MMP-9 and Troponin-I in patients with ACS ($r=0.34$, $p=0.000$).

Conclusion: There were significant correlations between MMP-9 and Troponin-I in ACS patients in ICCU ward, RSUP Dr. Sardjito General Hospital, Yogyakarta, from June 2008-August 2010.

Keywords: ACS, MMP-9, Troponin-I

INTRODUCTION

Pathophysiology of ACS is rupture or erosion of atherosclerotic plaque. First rupture occurred in the shoulder of plaque, which followed by thrombosis in the plaque¹. Thrombogenesis initiated by tissue factor released by monocytes, macrophages, endothelial cells, and smooth muscle cells, and other clotting factors will lead to a thrombosis². Thrombosis within the plaque, will spread into the blood vessels by causing platelet aggregation that leads to a thrombus. Thrombus can cause a total or a part occlusion blockage¹.

The main factor which is estimated to cause rupture of atherosclerotic plaques is inflammation and accelerated degradation of collagen and matrix components. Atherosclerotic has been accepted as a chronic inflammation disease. Previous studies showed that ACS occurs through several steps: triggered by proinflammatory cytokines such as interleukin-6 (IL-6) and chemoattractant which will attract leucocytes to the endothelium, and together with CD40-ligand (CD40L), will enable the atherosclerotic plaque macrophages. Macrophages will produce matrix metalloproteinase (MMPs), which will cause damage to the plaque extracellular matrix, so that the plaque becomes unstable³. This instability will cause plaque rupture, ischemia,

necrosis, and impaired muscle function⁴.

Matrix metalloproteinase is endopeptidase proteins that regulate extracellular matrix. Matrix metalloproteinase-9 (MMP-9) believed to play important role in the pathophysiology of ACS. MMP-9 is an extracellular matrix destructive enzyme, which plays a role in atherosclerotic plaque and cause instability of plaque and cause plaque rupture leading to ACS. Plasma level of MMP-9 in peripheral blood vessels is increased in patients with ACS, and is associated with severe coronary artery stenosis and sudden death. Elevated levels of MMP-9 in coronary blood vessels on ACS, showed the production of MMP-9 have effect on the pathophysiology ACS⁵. The increased levels of MMP-9 are also associated with the size infarct⁶.

Troponin-I (cTn-I) would only be detected if there is a damage to heart muscle trauma, will not increase levels of cTn-I. Troponin-I is released in occlusion of a coronary artery either totally or partially. The negative result of cTn-I, can rule out the heart muscle damage, so that cTn-I can be used as the gold standard to determine muscle infarction of heart¹. Researchers wanted to know the relationship between serum levels of MMP-9 with serum Troponin-I in patients with ACS in the ICCU ward, Dr. Sardjito General Hospital, Yogyakarta.

METHOD

Sample Research and Design Research

The samples were ACS patients treated in the ICCU ward, Dr. Sardjito General Hospital, Yogyakarta, which approved the informed consent. Research was conducted from June 2008 until August 2010, with cross-sectional study methods and consecutive sampling. Data base and demographic patients were collected by questionnaire.

Diagnosis of ACS was based on at least 2 of 3 criteria for myocardial infarction, the typical chest pain, changes in ECG patterns and increased markers of heart muscle damage (cardiac enzymes)^{7,8,9,10}.

Inclusion Criteria

The subjects were patients with onset of ACS <48 hours, male or female with age >18 years and <75 years, written informed consent was obtained from all participant.

Exclusion Criteria

Exclusion criteria in this study were patients who had undergone coronary intervention (primary PTCA, Elective PTCA, CABG), patient with sympathomimetic drug, pulmonary embolus, coronary artery spasm, coronary artery embolisation, coronary artery inflammation with microvascular occlusion, end-stage renal failure, rhythm disorders, acute heart failure, direct coronary trauma, extreme endurance exercise, LVH, stenosis aorta, acute stroke, myocarditis, pericarditis, cardiac trauma, metabolic/toxic (septic/MOF), diabeticketoacidosis, hyperglycemia hyperosmolar state, obstructive lung disease chronic acute exacerbation, pneumonia, sepsis/infection, chronic inflammatory diseases and malignancies and pregnant women^{8,11,1}.

Blood sample Collection and Assay

Blood samples were obtained from venous at initial admission in the hospital and before intravenous thrombolysis or coronary intervention. Serum level of cTn-I was obtained from 5 cc venous blood sample and done in less than 1 hour. Determination of cTn-I serum level were performed by commercially available ELISA kits (VIDAS Troponin-I Ultra, K36-S26 (Dea), Biomerieux SA, Mercy Etoile-France), that could detect from 0.01 to 30 ng/mL. The diagnostic value for STEMI is ≥ 0.6 ng/mL. Determination of MMP-9 serum level were performed from 5 cc venous bloods and allowed to clot for 30 minutes, which then centrifuged at 1000xg for 15 minutes and then stored at -80°C. Measurement of MMP-9 serum level was performed by ELISA kits (Quantikine Human MMP-9 (total) immunoassay DMP900, R&D Systems Inc., Minneapolis, United States of America). The minimum detectable concentration is 156 ng/mL.

Statistical Analysis

Data were expressed as mean \pm SD, unless otherwise indicated. Distribution of normality was determined by Kolmogorov-Smirnov Test because of specific for large samples (>50). Data is said to have a normal distribution if it had a significant value (p)>0.05.

Correlation between level of serum MMP-9 and serum Troponin-I in the ACS group was analyzed using Pearson correlation test if data had

normal distribution or Spearman correlation test, if data distribution was not normal. *P* value of less than 0.05 was considered significant¹². Statistical analysis was performed by SPSS for Windows version 17.0 (SPSS, inc., Chicago, Ill., USA).

RESULT

Baseline Characteristics

There were 139 ACS patients who meet the criteria of the study (table 1).

Table 1. Demographic Data

	Mean±SD	n	%
Mean Age (year)	56.62±9.14		
Male sex		113	81.3
Diabetic Mellitus		34	24.5
Hypertension		83	59.7
Dyslipidemia		74	53.2
Current smoker		49	35.25

Correlation between Serum levels of MMP-9 and serum levels of cTn-I

Means of MMP-9 and cTn-I were presented in table 2. Median of cTn-I was 4.60 ng/mL with minimum value of 0.0 ng/mL and maximum value of 30.0 ng/mL.

Table 2. Correlation between the Mean Serum Levels of MMP-9 and Troponin-I

	Troponin-I (ng/mL)	MMP-9 (ng/mL)	p	r
ACS (139)	9.49±10.47	1,296.06±729.97	0.000	0.34

Cut-off Level of MMP-9

Determination of cut-off levels of MMP-9 in the study was performed by The Receiver Operating Curve (ROC) in figure 1.

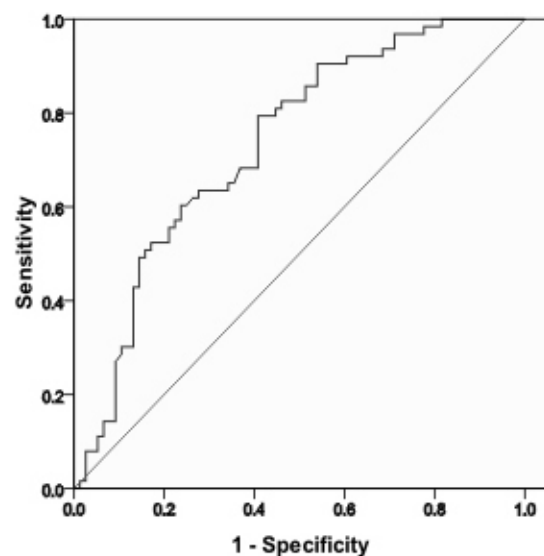


Figure 1. ROC Curve of Cut-Off Levels of MMP-9

A cut-off level of MMP-9 was 1,103 ng/mL. At this level, sensitivity and specificity were 73.5% and 59.2%. Based on this cut-off, subjects were divided into 2 groups, first is the group with MMP-9 higher than the cut-off, 77 subjects (55.4%) and group with MMP-9 lower than the cut-off, 62 subjects (44.6%).

Standard level of cTn-I to diagnosed STEMI was 0.6 ng/mL, subject were divided into 2 groups: the first group with higher level than 0.6 ng/mL (90 subjects/64.7%), and the other group with lower level than 0.6 ng/mL (49 subjects/35.3%).

Correlation between MMP-9 and cTn-I based on the cut-off respectively, were performed by Chi square. Chi square test results showed a significant relationship ($p=0.000$). Prevalence ratio was 1.98 (95% CI=1.45-2.70).

Discussion

Pathophysiology of ACS was sudden decrease of coronary blood flow after the thrombus occlusion, which results imbalance oxygen demand and supplies the heart muscle. In the most cases, infraction occurs when atherosclerotic plaque has fissure, rupture, or ulceration and started the coagulation cascade by exposure of tissue factor (TF) on endothelial cells are damaged, and cause fibrin formation. This process also stimulated aggregation platelets and thrombus formation, so-called red thrombus/fibrin-rich red thrombus. Historically showed coronary plaque prone to rupture if that had a thin fibrous cap, and a rich lipid core (lipid-rich-core)¹³.

Histologically, atherosclerosis appears to be thickening tunica intima with an increased number of smooth muscle cells (VSMC) in the lining of blood vessels and extracellular matrix. The increased of these cells derived from components of hematopoietic stem cells, and then migrate and proliferate in the tunica intima. This was followed by accumulation of intracellular or extracellular lipid, or both, making fatty streak. Fatty streak also contains macrophages and a number of lymphocytes. At an advanced stage, VSMC, macrophages, and cytoplasmic remnants in tunica intima, caused calcification. This process plays a role in changes fatty streaks into atherosclerotic plaques, which occur due to plaque accumulation of lipid rich macrophages, smooth muscle cells, lipids. Fibrous

cap occurs because grown fatty streak with accumulation of connective tissue and increase the number of smooth muscle cells are filled with lipid^{14,2}.

MMP is an endoprotease that contain zinc, with structures that are similar but have differences in the substrate. MMP capable degraded matrix extracellular¹⁵. MMP is classified into 4 groups: (1) Collagenases (MMP-1, MMP-8, MMP-13), able to degrade type I,II,III collagen, (2) gelatinase (MMP-2 and MMP-9), can degrade collagen type IV in basal membrane, (3) Stromelysin (MMP-3, MMP-10, MMP-11), can degrade extracellular matrix components in general, including proteoglycans, laminins, fibronectin, vitronectin, and some types of collagen, (4) Membrane-type MMP (MTI-MMPs) which can degrade extracellular matrix components and may activate the other MMP¹⁵. The sources of MMP-9 were the vascular smooth muscle cell (VSMC), macrophages, T lymphocytes and fibroblast adventitial¹⁵.

Atherosclerotic plaque disruption that lead to thrombosis and coronary artery occlusion can occur in 2 forms, namely: 'frank rupture' and 'superficial Erosion'. Rupture is defined as the outbreak of the fibrous cap and place of exposure to content that is prothrombotic. It identifies the local over-expression of MMP activity and matrix degradation in the shoulder of plaque, as a possible mechanism of the causes. Analysis of plaque atheroma in (unstable angina plaque) UAP and (stable angina plaque) SAP, showed an active synthesis of MMP-9 from macrophages and SMC. MMP-2 and MMP-9 increased in ACS patients, which raise the idea to develop non-invasive method to detect fragility plaque¹⁶.

There were increased levels of MMP-1, -2, -3, -7, -9, -11, -12, -13, -14, and -16 in human atherosclerotic plaques, especially in the shoulder-rich plaque macrophages. Much more research that trained correlation with the plaque disruption/rupture. MMP-11 was limited to advanced atherosclerotic plaque, rather than lipid-rich-plaque core. There was no difference in levels of MMP-1, -2, and -3 at the condition of plaque instability, although evidence of plaque rupture, but there was an increase in the levels of MMP-9, two-four times in plaque rupture. In other research, there was an increase of MMP-9 levels in patients with

UAP compared with SAP patients, and there was a clear link between increased levels of MMP-9 and plaque instability¹⁷.

Measurement of MMP-9 in the ACS was first done by Kai *et al.*¹⁸. This research was conducted on 33 ACS (which consisted of 22 patients with acute myocardial infarction (AMI) and 11 patients with UAP), 17 patients with SAP and control patients. Plasma levels of MMP-9 in UAP group was 87 ± 26 ng/mL, and significantly different than control ($p < 0.001$), also significantly different to the SAP (34 ± 11 ng/mL, $p < 0.001$) and different with AMI (49 ± 28 ng/mL, $p < 0.001$). Plasma levels of MMP-9 in the AMI group was also significantly different than control ($p < 0.05$), although there were 6 patients with increased of MMP-9 and 7 patients with similar levels of the control group and SAP. Fukuda *et al.*¹⁹ conducted on 47 patients with AMI, 23 patients with UAP and 19 patients with SAP. MMP-9 levels in AMI was significantly higher (328 ± 209.6 ng/mL, $p = 0.04$) than the SAP group. Levels of MMP-9 in UAP was significantly higher (306.6 ± 209.6 ng/mL, $p = 0.04$) than SAP (199.0 ± 156.8 ng/mL).

Increased levels of MMP-9 also showed in this study. The mean and standard deviation levels of MMP-9 in this study were 1296.06 ± 729.97 ng/mL, which clearly increased than the normal levels. MMP-9 levels increased and changed with time as long as duration of ACS. Kaden *et al.*⁶ study, the highest levels of MMP-9 obtained during hospitalization and then decreased and reached normal after 1 week later. Kai *et al.*¹⁸ study were showed the highest level of MMP-9 at 0-day in patients with UAP (when they come to the hospital) and about 3 times higher than normal controls and 2-fold higher compared to patients with angina, which then decreases approaching control levels within 7 days. However, some patients with UAP showed increased MMP-9 up to day-to-3, and then declined until day 7. In the group of AMI patients, the profile of increase in MMP-9 was also varied. In some patients with AMI, the early level 0-day, which showed similarity to the control group and angina, then increased to peak on day-3. But in others, a significant increase (2-fold compared to control) was happened from 0-day, and remained high on day-3.

This study had successfully demonstrated the correlation between MMP-9 and cTn-I in ACS

patients, although weak relationship ($r = 0.34$) but remained significant ($p < 0.05$)²⁰. This correlation was consistent with Manginas *et al.*²¹, who found a weak correlation ($r = 0.29$). This result was different than Tan *et al.*²², that showed insignificant correlation between MMP-9 and cTn-1.

Plaque atheroma has been known to contain Vascular Smooth Muscle Cell (VSMC) which showed activity as histocompatibility class II antigens, and different with the normal arterial tissue. This antigen will trigger chemotaxis of macrophages, which are very much in place of plaque rupture, triggering CD40sL, and stimulate the expression of collagenase and proteinase (stromelysin). It has been proven that an increasing in expression of MMPs in the shoulder region of atherosclerotic plaque, but not in the arteries that are not involved²³.

This study showed inflammatory markers (MMP-9) involved in the pathophysiology of ACS. Increased levels of MMP-9, resulted an increased collagen and extracellular matrix disruption that lead to atherosclerotic plaque instability and prone to plaque rupture. The impact of increased MMP-9, cared therapeutic implications controlled the levels of MMP-9 for the prevention ACS.

Based on the cut-off levels of MMP-9, the prevalence ratio of elevated levels of cTn-1 more than 0.6ng/mL was 1.98 times. This elevation was found that will found in the ACS patients with serum levels of MMP-9 above the cut off (> 1103 ng/mL), compared with the below cut-off. Correlation in this study analyzed in 1 group of ACS and correlation in sub group of ACS was unknown. Furthermore, the cross sectional method used in this study could not show a direct causal relationship.

Conclusion

Correlation between serum levels of MMP-9 and Troponin-I showed the role of MMP-9 in pathophysiology of ACS and higher levels of MMP-9 prone higher prevalence of heart muscle damage.

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