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C-1562T polymorphism of *matrix metalloproteinase-9* (MMP-9) gene associated with elevated level of plasma MMP-9 concentration in patient with acute myocardial infarction (AMI) in Denpasar-Bali



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ABSTRACT

Background: Acute Myocardial Infarction (AMI) is an emergency medical condition which still has high mortality and morbidity. In recent decades, its prevalence has a tendency to increase, parallel with other chronic diseases. One of suspected contributing factor in AMI is the presence of C1562T polymorphism on MMP-9 gene which increases plasma MMP-9 concentration and destabilizes the plaque. However, this notion needs further confirmatory studies, as there are several contradictory reports regarding their association. This Study aimed to determine the relationship between C1562T polymorphism with the increase of MMP-9 concentration in AMI.

Method: A cross-sectional was conducted in Cardiovascular Centre of Sanglah General Hospital. The peripheral blood samples were obtained from subjects, and the DNA and blood plasma was isolated.

The polymorphism was detected using PCR and RFLP while MMP-9 concentration was determined by ELISA.

Results: The average concentration of plasma MMP-9 was found to be at 8.33 ng/mL which ranged from 0.74 ng/mL until 31.93ng/mL. The proportion of MMP-9 C1562T polymorphism was 20% of all subjects. The average concentration of MMP-9 was higher than *cut-off* standard which is 0.6 ng/mL. Analysis within the group revealed that CT-genotype had significantly higher average MMP-9 compared with CC phenotype (11.27 ng/mL vs. 7.65 ng/mL).

Conclusion: C1562T polymorphism appears to be significantly associated with AMI by increasing the concentration of MMP-9 in blood plasma.

Keywords: C1562T Polymorphism, MMP-9, Acute Myocardial Infarction

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BACKGROUND

Acute Myocardial Infarction (AMI) is the major cause of cardiovascular disease related mortality and morbidity worldwide (WHO). Its principal pathophysiologic mechanism is coronary vascular stenosis or blockage due to atherosclerotic plaque rupture. Most of the risk factors of AMI are modifiable risk factors which include lifestyle, smoking, alcohol, and stress management. However, there are also non-modifiable risk factors which include genetic predisposition (BLA).¹

Left ventricular remodeling is a major determinant of prognosis in patients with acute AMI. This process was associated with post-AMI heart failure and life-threatening arrhythmias (Pfeffer, 1990).² Furthermore, the remodeling still occurs even in a patient who already treated with percutaneous coronary intervention (PCI). Thus, reperfusion is thought to be the inducing factor of ventricular remodeling (Bolognesa, 2002).³ In addition, Reactive Oxygen Species (ROS) produced in the areas of myocardial ischemia, especially after

reperfusion therapy. ROS could cause damage to cardiomyocyte membrane, leading to cell injury and apoptosis. ROS also induce signal transduction leading to increased expression of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 and -6 (IL-1 and -6) in ischemic area and its surrounding. This cytokine stimulates apoptosis through the TNF- α receptor/caspase pathway which results in accumulation of intracellular Ca²⁺ and, eventually, necrosis of myocardial cells. Finally, ROS and proinflammatory cytokine activate matrix metalloproteinase that will hydrolyze extracellular matrix tissue that potentially results in left ventricular dilatation (Hori, 2008).⁴

Matrix metalloproteinase (MMPs) is a hydrolytic enzyme that degrades extracellular matrix and, hence, has a potential role in decreasing plaque stabilization (Peterson, 2000).⁵ In animal models, increased level of myocardial MMP has been associated with dilatation and dysfunction of the left ventricle (Apple and Mair, *et al.*).⁶

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Table 1 Subject baseline characteristics

Variables	Value
Age	Mean: 50.04 (\pm 7.877) years
Plasma MMP-9 Concentration	Mean: 19.48 (\pm 7.18) ng/ml
Frequency of MMP-9 Polymorphism by Genotype	
CC	N: 58
CT	N: 13

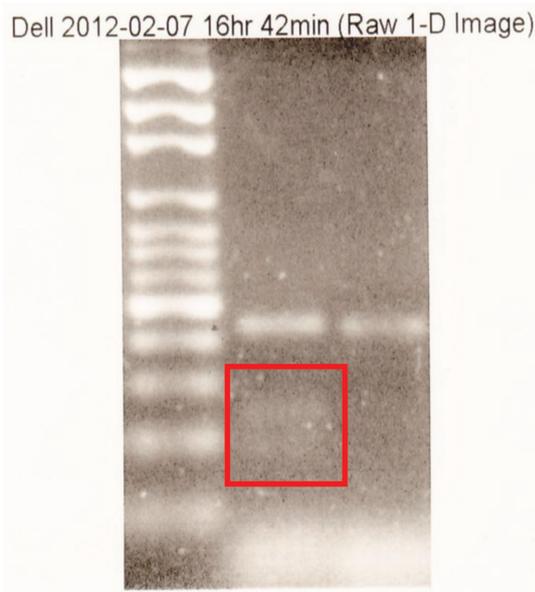


Figure 1 The result of amplicon restriction using *Pae I* (red square). The size of the fragments is 247 bp and 188 bp. Original fragment size is 435 bp.

Furthermore, genetic polymorphism in MMP-9 was known to increase the expression of MMP-9 both in vitro and in vivo studies (Blakenber, 2003 and Medley, 2003).^{7,8} MMP-9 is not only retained in the tissue level but also secreted into the blood plasma. Increase level of MMP-9 is known to be associated with left ventricular remodeling and leukocytosis (Spinale, 2000).⁹

Increased concentration of MMP-9 is related to the degree or severity of remodeling of the myocardium. However, constant plasma level of MMP-9 was related with stable ventricular systolic function. It appears that temporal increase rather than the absolute level of MMP-9 plays an important role in altering the degree of remodeling after MI (Kelly, 2007).¹⁰ In regards to plasma MMP-9, one of a predisposing genetic factor thought to be related to the risk of AMI is C1562T polymorphism on MMP-9 gene (Setianto, *et al.*, 2011).¹¹

In general, the relationship between polymorphisms with increased levels of MMP-9 and

left ventricular remodeling is still contradictory. The importance of knowledge about the measurement of the levels of MMP-9 and its relationship with the C1562T polymorphism of genes encoding MMP-9 would provide important development in a patient with AMI to avoid or inhibit the development of ventricular remodeling. Furthermore, as the study of MMP-9 polymorphism is never conducted in Indonesia, the polymorphism profile of Indonesian is still elusive and, hence, its relationship with AMI and left ventricular remodeling.

MATERIALS AND METHODS

A cross-sectional study evaluating the association between single-nucleotide polymorphisms (SNPs) in the promoter regions of MMP-9 was conducted in Sanglah General Hospital. 70 patients were enrolled and divided into acute myocardial infarction (AMI) with STEMI group, and Non-STEMI group with age ranged from 37 to 75 years old. Samples were collected consecutively from the patient of Sanglah Hospital Denpasar Bali Indonesia from June until December in 2011 which fulfilled the inclusion criteria. All samples that agree to participate were asked to fill the inform consent.

A whole blood sample was obtained for DNA isolation and plasma MMP-9 measurement. Total DNA was isolated using DNA isolation kit from Qiagen. MMP-9 gene was amplified using Polymerase Chain Reaction (PCR) procedure with a forward primer (5'-GCCTGGCACATAGTAGGCC-3') and reverse primer (5'-CTTCCTAGCCAGCCG GCATC-3'). The PCR kit was purchased from Qiagen. The PCR will yield 435 bp DNA products. To identify C1562T polymorphism of MMP-9 gene the products were treated with *PaeI* which resulted in 247 bp and 188 bp DNA fragments. Some of the PCR products were also sequenced to confirm the polymorphism.

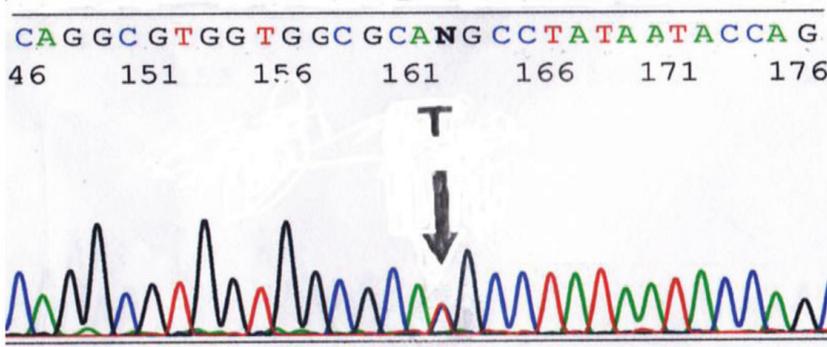
MMP-9 concentration evaluation was conducted using Enzyme Linked Immunosorbent Assay (ELISA) with reagents/ELISA kit from R & D Quantikine DPM900. The sample treatment, standard, and controls were formulated based on manufacturer protocol and measured at a wavelength 450.

Statistical analyses were conducted using SPSS version 17 for windows. Descriptive analysis was used initially to identify the proportion of MMP-9 gene polymorphism and the mean of MMP-9 concentration. Normality test was conducted using Kolmogorov-Smirnov test for numerical data. Independent sample T-test or Mann-Whitney test was used to compare the mean of MMP-9 between polymorphism groups.

Table 2 Mann-Whitney test results in comparing plasma MMP-9 concentration between CC and CT phenotype

MMP-9 Genotype	Mean Plasma MMP-9 Concentration
CC	18.2581 (± 7.057) ng/ml*
CT	24.9369 (± 4.97) ng/ml*

Note: The result is statistically significant ($p < 0.05$) based on Mann-Whitney test

**Figure 2** DNA sequencing heterozygote (CT) model

RESULTS

70 patients with AMI were enrolled in the course of this study. The age of the samples ranged from 37-80 years with most samples ages between 45-56 years (Table 1). The total DNA from all samples were successfully isolated and subsequently amplified using PCR. Then, the PCR products were treated by using *PaeI*, restriction enzyme to identify the C1562T MMP-9 polymorphism. The result of the restriction process is described in Figure 1.

As shown in Figure 1, *Pae I* cuts the original 435 bp amplicon into 247 bp and 188 bp fragments. The presence of such fragment indicates the presence of the C1562T polymorphism within the MMP-9 gene. However, because there is still original fragment within the same lane, the individual is considered as a heterozygote. Overall, we found 13 samples with CT-heterozygote (20%). We also sequenced several samples using BLAST method. The result of the heterozygote samples matched the result of restriction method.

The result of ELISA revealed that the plasma concentration of MMP-9 was varied but ranged from 0.74 ng/ml to 31.93 ng/ml. The average level was 19.48 (± 7.18) ng/ml. Analysis within each group revealed that the mean concentration of MMP-9 was 18.25 ng/ml for CC group and 24.93 for CT group (Table 2). Normality analysis showed that the data of plasma MMP-9 level was not normally distributed and, thus, the bivariate analysis was conducted using Mann-Whitney test instead of independent sample T-test. Bivariate analysis showed a significant association between plasma MMP-9 level with

MMP-9 gene C1562T polymorphism in which the heterozygote CT-genotype tend to has a higher level of MMP-9 compared to the homozygote one.

DISCUSSIONS

Cardiovascular disease remains the number one cause of death worldwide despite the development in its diagnosis and treatment.¹ The majority of CVD mortality and morbidity resulting from heart failure.² The main pathogenic mechanism for heart failure is ventricular remodeling in which MMP-9 plays a very important role.⁹ Development of heart failure and ventricular remodeling could significantly decrease cardiac function, decreasing the quality of life and increase the risk of cardiovascular-related death.

Because of the central role of MMP-9, the increasing level of MMP-9 could potentially elevate the risk and the degree of ventricular remodeling. In this study, we found a direct association of a C1562T polymorphism of MMP-9 with increased level of plasma MMP-9. MMP-9 plays an essential role in the pathophysiology of AMI. The basic pathophysiology of AMI is the rupture or erosion of atherosclerotic plaque. Plaque rupture is the result of collagen hydrolysis by a protease, especially MMP-9 (Newby, 2005).¹² The damaged heart muscle release intracellular protein such as troponin I (CTN-I) which is also a biomarker for AMI (Collinson, 2007; Setianto, *et al.* 2011).^{11,13}

According to our result, the plasma MMP-9 concentration was ranged from as low as 0.74 ng/ml to 31.93 ng/ml with an average of 8.33 ng/ml. This is considerably low compared with other studies.^{14,15} For example, Kelly *et al.* reported that the plasma MMP-9 concentration in AMI patients 12-hours after the onset was ranged from 15-376 ng/ml with median concentration at 70.0 ng/dl.¹⁴ Other study conducted by Squire *et al.* also report higher mean MMP-9 level at 49 \pm 11 ng/dl.¹⁵ The difference might result from the difference in subject criteria. Both studies used samples which already had ventricular remodeling. Meanwhile, this study used patients with AMI in which the remodeling status was still unknown. However, this study confirmed that the level of plasma MMP-9 was increased in a patient with AMI (normal plasma level: 140 pg/ml).¹⁶

Regarding the CT polymorphism, our study found that 20% of the subjects had the polymorphism. This finding is a little bit lower compared with Baiping *et al.* (1999) which found 26% proportion.¹⁷ However, Demack *et al.* found that the proportion was actually lower at 15.9%.¹⁸ The difference could result from the differences in the study population as well as the number of samples.

Nevertheless, because our study had fulfilled the minimum number of samples required, the validity of the proportion was not an issue.

Regarding the effect of the polymorphism toward the concentration of the plasma MMP-9, we found that there is a strong association between C-T polymorphism higher concentrations of plasma MMP-9. Our finding is consistent with several other studies despite the difference in the study population. The finding of Wu et.al and Opstad et.al were similar with us in whom they confirmed the relationship between the polymorphism with an elevated MMP-9 concentration in a patient with MI and ventricular remodeling.^{19,20} Opstad et.al also found that the risk was even more prominent in patients with metabolic syndrome.²⁰

The basic mechanism of increased risk of AMI and ventricular remodeling in a patient with C-T polymorphism can be explained as follow. Increased activity and level of MMP-9 increase the degradation rate of elastin, proteoglycans and collagen MEC which constitute the majority of subendothelial connective tissue.²¹ The loss of those components reduces the stability of the fibrous cap of the atheromatous plaque which will easily rupture by shearing stress from blood flow. After AMI had occurred, MMP-9 also had an important role in ventricular remodeling. The presence of increased amount of MMP-9 within myocardium increases the rate of fibrosis within the infarcted and penumbral area. Thus, lowering the contractile ability of ventricle and contribute to the development of heart failure. To conclude, C-T polymorphism act as negative predictor of the outcome of patient with AMI.²²

Nevertheless, this study had a limitation because the MMP-9 measurement was only conducted once and we did not use the peak level. Thus, the level of MMP-9 recorded in this study did not reflect the timely change of plasma MMP-9 in the plasma.

CONCLUSIONS

We demonstrate that the C-T polymorphism of MMP-9 gene is quite prevalent in Balinese population and it significantly associated with elevated level of plasma MMP-9 which could be related to adverse outcome in a patient with AMI.

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