C-1562T POLYMORPHISM OF *MATRIX METALLOPROTEINASE-9* (MMP-9) GENE ASSOCIATED WITH ELEVATED LEVEL OF PLASMA MMP-9 CONCENTRATION IN PATIENT WITH ACUTE MYOCARDIAL INFRACTION (AMI) IN DENPASAR, BALI

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BACKGROUND

- Acute Myocardial Infarction (AMI) is emergency medical condition which still has high mortality and morbidity.
- In recent decades, it prevalence has a tendency to increase, parallel with other chronic diseases.
- One of suspected contributing factor in AMI is the presence of C-1562T polymorphism on MMP-9 gene which increase plasma MMP-9 concentration and destabilize the plaque.
- However, this notion needs further confirmatory studies, as there are several contradictive reports regarding their association.

AIM OF THIS STUDY

- To determine the relationship between C-1562T polymorphism with the increase of MMP-9 concentration in AMI.

METHOD

- Cross-sectional was conducted in Cardiovascular Centre of 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.

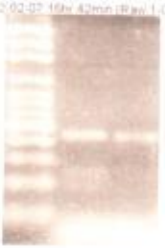
- Samples were collected consecutively from the patient of Sanglah Hospital Denpasar Bali Indonesia.
- Samples whole blood (from peripheral blood) were obtained from subjects.
- All samples that agree to participate were asked to fill the inform consent.

- 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'-GCCTGGCACATAGTAGGCCC-3') and reverse primer (5'-CTTCCTAGCCAGCCGGCATC-3').
- The PCR kit was purchased from Qiagen

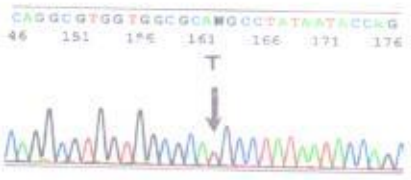
- MMP-9 concentration evaluation was conducted using Enzyme Linked Immunosorbent Assay (ELISA) with reagents/ELISA kit from R & D Quantikine DPM900

RESULTS

- The total DNA from all samples were successfully isolated and subsequently amplified using PCR.
- PCR products were treated by using *Pae-I*, restriction enzyme to identify the C-1562T MMP-9 polymorphism and sequencing.
- The result of the restriction process is described in figure bellow.



- Size of the fragments: 247 bp and 188 bp (Pae-I enzyme restriction). Original fragment: 435 bp



- Presence of such fragment indicates the C-1562T polymorphism within the MMP-9 gene (heterozygote model).

- *Pae-I* cuts the original 435 bpamplicon 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 aheterozygote.
- Overall, we found 14 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.

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

SUBJECT BASELINE CHARACTERISTICS

Variables	Value
Age	Mean: 50.04 (±7.877) years
Plasma MMP-9 Concentration	Mean: 19.48 (±7.18) ng/ml
Frequency of MMP-9 Polymorphism by Genotype	
CC	N: 57
CT	N: 14

- 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 average concentration of MMP-9 was higher than *cut-off* standard which is 0.6 ng/mL.
- The proportion of MMP-9 C-1562T polymorphism was 20% from all subjects.
- 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

- 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 with adverse outcome in patient with AMI.