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Research Brief

# Matrix metalloproteinases and their gene polymorphism in young ST-segment elevation myocardial infarction

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ABSTRACT

*Background:* Genetic polymorphism in MMPs are associated with multiple adverse CV events. There is little evidence regarding role of MMPs and their genetic polymorphisms in young (<50 years) ST-segment elevation myocardial infarction (STEMI) patients.
*Methods:* This study included 100 young (18–50 years) STEMI patients and 100 healthy controls. Serum levels of MMP-3, MMP-9 and TIMP were estimated for both patients as well as controls. Additionally, genetic polymorphisms in the MMP-9 gene (−1562 C/T and R279Q) & MMP-3 gene (5A/6A-1612) was evaluated. All these patients were followed up for one year and major adverse cardiac events (MACE) were determined.
*Results:* Serum levels of MMP-3 (128.16 ± 115.81 vs 102.3 ± 57.28 ng/mL; P = 0.04), MMP-9 (469.63 ± 238.4 vs 188.88 ± 94.08 pg/mL; P < 0.0001) and TIMP (5.84 ± 1.93 vs 2.28 ± 1.42 ng/mL; P < 0.0001) were significantly higher in patients as compared to controls. Additionally, patients with genetic polymorphisms in the MMP genes (5A/5A, 6A/6A and the AG genotypes) had an increased risk of STEMI. Patients with MACE had significantly higher levels of MMP-9 (581.73 ± 260.93 vs 438.01 ± 223.38 pg/mL; P = 0.012). A cutoff value of 375.5 pg/mL of MMP-9 was best able to discriminate patients with STEMI and MACE with sensitivity of 77.3% and specificity of 57%.
*Conclusion:* Novel biomarkers such as MMP-3, MMP-9 and TIMP and their genetic polymorphism are associated with the susceptibility for STEMI in young individuals. Higher MMP-9 levels in STEMI patients with MACE suggests its potential role in predicting cardiac remodeling and left ventricular dysfunction.


## 1. Introduction

The burden of atherosclerotic cardiovascular diseases (ASCVD) continues to be significant in low- and middle-income countries. The prevalence of acute coronary syndrome (ACS) in developing countries has been on a rise especially among individuals <50 years of age.[1] A majority of these ACS events occur due to the rupture of an atheromatous plaque. Vulnerable plaques which are prone to rupture are characterized by the presence of a lipid rich necrotic core, a thin fibrous cap covering the core along with inflammatory cell infiltrates and reduced collagen content in the fibrous cap.[2] Matrix metalloproteinases (MMPs) which belongs to the family of zinc dependent protease are a group of proteolytic enzymes. These are produced by the inflammatory cells in the atheromatous plaque leading to the degradation of the extracellular matrix, weakening of the cap and its subsequent rupture. MMPs also enable the easy migration of the inflammatory cells across the tissues thereby increasing the risk for development of atheromatous plaques.[3] Two important members of this family include MMP-3 and MMP-9 both of which play an important role in plaque formation, smooth muscle cell migration and proliferation.[3] The activity of MMPs is tightly controlled by the tissue inhibitor of metalloproteinase's (TIMPs). These TIMPs regulate the connective tissue metabolism

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through formation of irreversible complexes with the MMPs rendering them inactive. In the inflammatory milieu within an atheromatous plaque, there occurs an imbalance between the MMPs and TIMPs leading to plaque destabilization and prone to rupture.[4]

Coronary artery disease (CAD) is polygenic in nature with multiple genes linked to its occurrence. Recent evidences have highlighted that MMP gene polymorphism are associated with adverse cardiovascular (CV) events.[5] Levels of different MMPs are affected at transcription levels by various genetic polymorphisms. Studies have reported that MMP-3 and MMP-9 gene polymorphisms are associated with CAD and stroke events.[6,7] However, there is little evidence regarding the role of MMPs and their genetic polymorphisms in the occurrence of STEMI in young (<50 years) individuals. This study aimed to determine the levels of novel biomarkers such as MMP-3, MMP-9 and TIMP in young patients with STEMI within three days of symptom onset. Additionally, the distributions of respective gene polymorphism in the MMP-9 gene (−1562 C/T and R279Q) & MMP-3 gene (5A/6A-1612) too was evaluated.

## 2. Methods

### 2.1. Study design

This was a prospective, single center case−control study in a tertiary care center in Delhi, India over a one-year period. A total of 100 young patients (18−50 years of age) with ST-segment elevation myocardial infarction (STEMI) presenting within 3 days of symptom onset were enrolled. Patients aged <18 years, ST segment elevation due to non-ischemic causes (myocarditis, pericarditis), chronic kidney/liver disease, malignancy and acute as well as chronic inflammatory conditions were excluded. Age and gender matched healthy controls were also included in this study. All patients underwent a detailed clinical evaluation, routine blood investigations, electrocardiography and 2D echocardiography following a written informed consent. Five milliliters of peripheral venous blood were collected of which one ml in EDTA vacutainer for DNA isolation while the remaining blood volume in plain vacutainer was used for serum separation for biomarker analysis at the time of index event.

#### 2.1.1. Biomarker analysis

Serum was separated following clot retraction and centrifugation at 3000 rpm for 10 min. The serum was aliquoted and stored in deep freezer at −20 °C for batch analysis of the ELISA based tests. Analysis of MMP-3, MMP-9 and TIMP levels were done on fully automated analysers based on the principle of chemiluminescence. The concentration of MMP-3, MMP-9 and TIMP level in the samples was then determined by comparing the optical density of the samples to the standard curve.

#### 2.1.2. Genomic analysis

DNA was extracted from the peripheral lymphocytes using commercially available nucleic isolation kit QIA amp DNA Mini and Blood Kit (Qiagen, Chatsworth, CA, USA) and stored at −20 °C. The single nucleotide polymorphisms (SNPs) of R279 Q and −1562 C/T variant of MMP-9 gene and 5A/6A −1612 of MMP 3 were amplified by PCR using specific forward and reverse primers as shown in Supplementary Table 1. The PCR amplified products for each genotype with base pairs 277 bp, 436 bp, 130 bp were analyzed for genotyping by restriction fragment length polymorphism (RFLP) using the restriction enzyme *Sami, SphI* and *PsyI* respectively.The band pattern observed after RFLP for individual genotypes for R279 Q variant of MMP-9 gene, 1562 C/T of MMP-9 gene and 5A/6A-1612 of MMP 3 were separated by gel electrophoresis in 2% agarose gel and visualized in Gel documentation system (Fig. 1A, B and C). The various bands visualized for R279Q variant of MMP-9 gene for homozygous AA was only a single band i.e. 277 bp band app, for homozygous GG two bands i.e. 96 bp band, 181 bp band appeared on the gel and for heterozygous AG all 3 bands i.e. 277 bp,181 bp, 96 bp appeared on the gel. The various bands visualized for −1562 C/T variant of MMP-9 gene for homozygous allele, i.e. TT, a 242bp and 194bp appeared for homozygous CC, a 436bp band appeared on the gel and for heterozygous CT, all 194bp, 242bp, 436bp band appeared on the gel. The band pattern observed after RFLP for individual genotypes for MMP-3 5A/6A −1612 were single band of 130bp 6A/6A, 110-bpband for homozygous 5A/5A (a) and all 2 bands, i.e. 130- bp, & 110-bp, band for heterozygous 5A/6A. Hardy−Weinberg equilibrium was violated for genotype MMP-3 in cases and genotype MMP-3 as well as genotype MMP-9 R279Q in controls.

### 2.2. Follow-up

All enrolled patients were followed-up for a period of one year both telephonically as well as in-person for determination of major adverse cardiovascular events (MACE) which was defined as a composite of total death, myocardial infarction (MI), stroke and hospitalization due to heart failure.

### 2.3. Consent and ethical issues

A written informed consent was obtained from all patients and controls. The present study was approved by the institutional ethics committee [EC number: F.1/IEC/MAMC/(72/07/2020/No 86)] and was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and that are consistent with Good Clinical Practice and all local regulations.

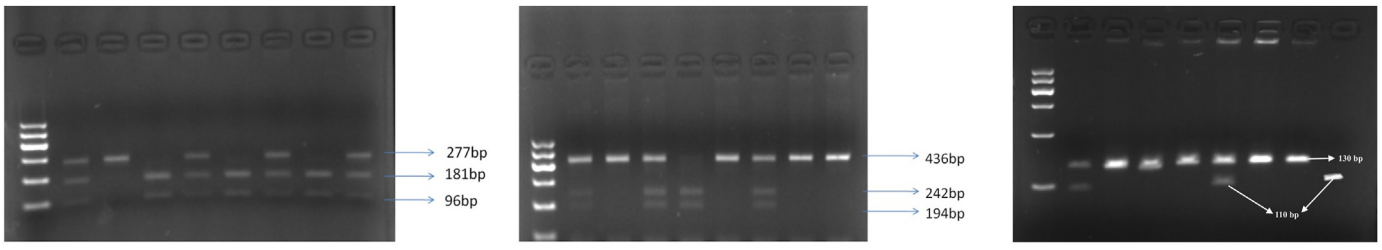


**Fig. 1.** A: Restriction fragment length polymorphism gel picture of MMP-9 R279Q gene polymorphism. B: Restriction fragment length polymorphism gel picture of MMP-9 -1562 C/T gene polymorphism. C: Restriction fragment length polymorphism gel picture of MMP3 -1612 5A/6A gene polymorphism.

### 2.4. Statistical analysis

Continuous data was expressed as mean ± standard deviation (SD) and categorical data was represented as proportions. Normality of distribution of continuous variables were assessed using the Kolmogorov–Smirnov test. Comparison of means of continuous variables was done using Student's t-test or Mann–Whitney U test as appropriate while Fisher exact test or $\chi 2$ test was used for categorical variables. Univariate and multivariate logistic regression analysis were done to determine the independent predictors of STEMI in these patients. Diagnostic sensitivity and specificity of MMP-9 in predicting MACE were calculated by the receiver operating characteristics (ROC) curve. Kaplan-Meir (KM) curve was plotted for survival analysis. A two-sided P value of <0.05 was considered to be statistically significant. SPSS version 24.0 (IBM Corp, Armonk, NY) software were used for statistical analysis.

## 3. Results

A total of 100 patients with STEMI and 100 age and gender matched controls were enrolled in the study. The mean age of the study population was 38.3 ± 6.6 years while that of the control group was 37.6 ± 6.3 years (P = 0.444). Majority of the enrolled subjects were males (93%) with co-morbidities such as hypertension (16%) and diabetes (13%). Among the predisposing risk factors for CAD, tobacco use in form of smoking was significantly higher in STEMI group as compared to controls (70% vs 48.2%; P = 0.002). Patients with STEMI had significantly higher levels of total cholesterol, LDL-C and triglycerides as compared to the control group. Additionally, STEMI had significantly lower levels of HDL-C as compared to healthy controls. The demographic features of the enrolled subjects has been enlisted in Table 1.

### 3.1. Genotypic analysis of MMP-3 (1612 5A/6A) and MMP-9 (R279Q) & (1562 C/T)

MMP 3–5A/5A, 6A/6A genotypes were significantly higher in young STEMI patients as compared to controls (5A/5A: 17% vs 10%; 6A/6A: 78% vs 71%; P = 0.04). The proportion of subjects with genotype MMP 3–5A/6A was significantly lower in young STEMI patients as compared to controls (5A/6A: 5% vs 19%; P = 0.006). The MMP9 279Q- AG genotype was the predominant genotype among enrolled STEMI patients as compared to controls (AG: 42% vs 22%; P = 0.002) while MMP9 279Q-AA and MMP9 279Q-GG genotypes were lower in subjects as compared to controls (AA: 36% vs 46%; P = 0.151; GG: 22% vs 32%, P = 0.111). The genotypes MMP9 1562C/T was comparable between subjects and controls (CC: 66% vs 60%, P = 0.380, CT: 33% vs 37%, P = 0.553; TT: - 1% vs 3%, P = 0.621) (Supplementary Table 2).

### 3.2. Biomarkers analysis

Young patients with STEMI had significantly higher levels of TIMP (5.84 ± 1.93 vs 2.28 ± 1.42 ng/mL; P < 0.0001), MMP-3 (128.16 ± 115.81 vs 102.3 ± 57.28 ng/mL; P = 0.04) and MMP-9 (469.63 ± 238.4 vs 188.88 ± 94.08 pg/mL; P < 0.0001) as compared to the control group. In terms of the MMP-3 gene polymorphism, subjects with 5A/5A and 6A/6A genotype had significantly higher levels of MMP-3 as compared to those with 5A/6A genotype (5A/5A: 87.41 ± 75.57 vs 5A/6A: 52 ± 19.43 vs 6A/6A: 115.23 ± 92.04 ng/mL; P < 0.0001). Similarly, subjects with MMP9 279Q- AG and GG genotype had significantly higher levels of MMP-9 as compared to those with AA genotype (AA: 240.24 ± 159.58 vs AG: 307.73 ± 129.51 vs GG: 489.93 ± 314.72; P < 0.0001). However, for the MMP9 1562C/T polymorphism, there was no significant difference between the three phenotypes in terms of MMP-9 levels (CC: 324.34 ± 221.06 vs CT: 343.36 ± 248.83 vs TT: 237.25 ± 62.81 pg/mL; P = 0.618).

### 3.3. Follow-up

Over a period of one year of follow-up, there were 22 MACE (Supplementary Fig. 1) with majority of them being heart failure hospitalizations ($n$ = 18). The mean duration of follow-up was 1.06 ± 0.12 years. STEMI patients with MACE had significantly higher levels of MMP-9 as compared to those without MACE (581.73 ± 260.93 vs 438.01 ± 223.38 pg/mL; P = 0.012). However, there was no significant difference in terms of TIMP (5.65 ± 1.62 vs 5.89 ± 2 ng/mL; P = 0.60) and MMP-3 (122.09 ± 131.15 vs 129.87 ± 111.97 ng/mL; P = 0.78) levels in subjects with or without MACE. The ROC curve analysis revealed that the best cutoff value of 375.5 pg/mL of MMP-9 was able to discriminate patients with STEMI and MACE as compared to those without MACE with a sensitivity of 77.3%, specificity of 57% and an AUC of 0.693 (Fig. 2).

**Table 1**
Demographic characteristics of the two groups.

| | Subjects($n$ = 100) | Controls($n$ = 100) | P |
|---|---|---|---|
| Age (Mean ± SD) | 38.33 ± 6.6 | 37.63 ± 6.29 | 0.444* |
| Male | 93 (93%) | 93 (93%) | 1 |
| Oral tobacco use | 5 (5%) | 1 (1%) | 0.212† |
| Smoking | 70 (70%) | 3 (3%) | <0.0001† |
| Total cholesterol (mg/dL) | 181.03 ± 51.42 | 164.42 ± 42.09 | 0.013* |
| LDL (mg/dL) | 99.1 ± 32.9 | 77.47 ± 21.63 | <0.005* |
| HDL (mg/dl) | 39.62 ± 8.86 | 44.02 ± 11.04 | 0.002* |
| Triglycerides (mg/dL) | 170.97 ± 80.85 | 117.18 ± 43.37 | <0.0001* |
| **Type of STEMI** | | | |
| AWMI | 55 (55%) | – | – |
| IWMI | 38 (38%) | – | – |
| ALWMI | 3 (3%) | – | – |
| LWMI | 4 (4) | – | – |
| Delayed presentation | 10 (10%) | – | – |
| Primary PCI | 65 (65%) | – | – |
| Mean time of sample collection (hours) | 2.25 ± 1.42 | | |

**Abbreviations:** PCI: percutaneous coronary intervention; STEMI: ST-segment myocardial infarction; AWMI: Anterior wall myocardial infarction; IWMI: Inferior wall myocardial infarction; ALWMI: Antero-lateral wall myocardial infarction; LWMI: Lateral wall myocardial infarction; LDL: Low density lipoprotein; HDL: High density lipoprotein; SD: standard deviation.
**Footnote:** * Independent t test† Fisher's exact test, ‡ Chi square test.

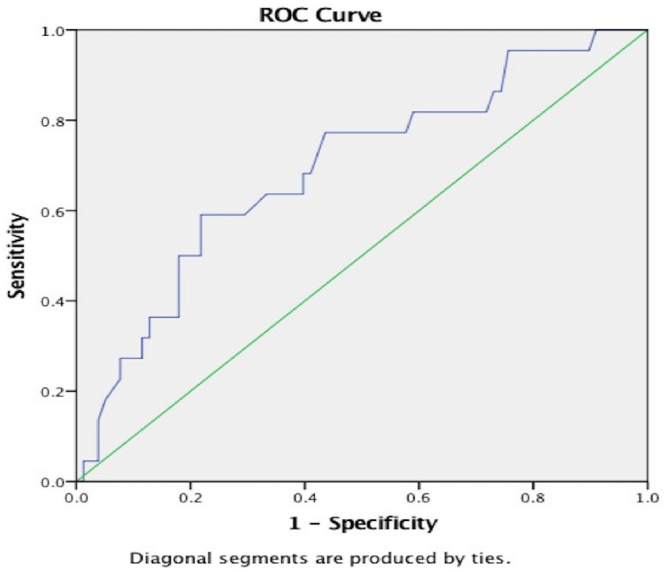


**Fig. 2.** Receiver operating characteristics (ROC) curve analysis to determine sensitivity and specificity of MMP-9 in predicting MACE in young STEMI patients.

### 3.4. Predictors of STEMI

Univariate logistic regression analysis (Supplementary table 3) in the study population revealed that smoking (OR: 75.44), LDL Cholesterol (OR: 1.029), TIMP (OR: 3.147), MMP-3 (OR: 1.003), MMP-9 (OR:1.017) and MMP-9 279Q AG genotype (OR: 2.567) were predictors of STEMI. Multivariate regression model showed that Smoking (OR: 165.214), LDL Cholesterol (OR: 1.060), TIMP (OR: 4.670), MMP-9 (OR: 1.025) and MMP-9 279Q AG genotype (OR: 13.458) were independent predictors of STEMI (Table 2).

### 3.5. Discussion

The present study showed that young patients with STEMI had significantly higher levels of MMP-3, MMP-9 and TIMP as compared to healthy controls. Findings of our study also revealed that increased levels of MMP-3, MMP-9 and TIMP and their genetic polymorphisms i.e. 5A/5A, 6A/6A and the AG genotypes were more common in STEMI patients as compared to controls. Additionally, subjects with MACE especially heart failure hospitalizations had significantly higher levels of MMP-9 thereby suggesting its role in cardiac remodelling and impact on left ventricular (LV) functions.

One of the key pathophysiological mechanisms of ACS include activation of major MMP's such as MMP-3 and MMP-9. Serum levels of MMP's reflect vulnerability of the atheromatous plaque for rupture.[3] In our study, serum levels of MMPs such as MMP-3 and MMP-9 were significantly higher in patients with STEMI as compared to controls. Similar findings were reported in smaller observational studies evaluating role of MMP's in ACS.[6,8] MMPs such as MMP-9 not only plays an important role in development of ACS but also in the healing process post-acute MI. MMP-9 has been implicated in the LV remodelling post-acute MI.[9] Studies have shown a correlation between serum MMP-9 levels and echocardiographic parameters of LV dysfunction post MI.[9] In a study among 75 patients with ACS, MMP-9 levels were significantly higher among those with poor disease outcome in terms of recurrent ischemic attacks, heart failure, or death.[10] Similarly, in the biomarker sub-study of the VIP trial among 225 patients with ACS, MMP-9 levels were the most powerful predictor for MACE.[11] In our study too, MMP-9 levels were an important predictor of MACE with a cut-off value of 375.5 pg/mL being established to differentiate patients with or without MACE. Serum MMP-9 levels can thereby be considered as one of the potential biomarkers of poor outcomes in STEMI.

**Table 2**
Multivariate logistic regression analysis for independent predictors of STEMI.

| | OR | 95% CI | P-value |
|---|---|---|---|
| Smoking | 165.214 | 5.056–5398.450 | **0.004** |
| LDL Cholesterol (mg/dL) | 1.060 | 1.002–1.122 | **0.044** |
| TIMP (ng/ml) | 4.670 | 2.008–10.857 | **<0.0001** |
| MMP-3 (ng/ml) | 1.002 | 0.988–1.017 | 0.757 |
| MMP-9 (pg/ml) | 1.025 | 1.010–1.041 | **<0.0001** |
| MMP-9 279Q genotype (AG) | 13.458 | 1.007–179.917 | **0.049** |

**Abbreviations:** CI: confidence interval; dl: decilitre; ml: millilitre; mg: milligram; MMP: Matrix metalloproteinases; ng: nanogram; OR: odds ratio; pg: picogram; TIMP: tissue inhibitor of metalloproteinase.

In terms of genetic polymorphism in the MMP-3 gene, in our study, the 5A/5A and the 6A/6A genotype was significantly higher in subjects with STEMI as compared to control subjects. The high activity 5A allele has been shown to be associated with an increased predisposition to plaque rupture among Chinese[12] and Japanese[13] young STEMI patients. Similarly, the 6A allele has been associated with increased risk for MI as reported in a cohort of 4152 Japanese subjects with ACS.[14] Other studies have established the role of 5A and 6A alleles in the development of coronary as well as carotid atherosclerosis.[15] Studies have also shown that in patients with 5A/5A and 6A/6A genotypes, there is a heightened MMP-3 expression in serum and tissues thereby increasing predisposition to plaque rupture and ACS.[16] Studies evaluating role of genetic polymorphism in the MMP-9 gene in CAD have revealed contradictory findings. In a meta-analysis from China, the authors concluded that risk of MI was significantly higher in subjects with T allele (TC and TT genotypes) as compared to those with CC genotype of the MMP-9 gene.[17] Moreover, the increased susceptibility was only seen in those with white ethnicity and not the Asian population. Contrarily, a recent meta-analysis concluded that MMP-9 (C1562T) SNP led to greater susceptibility risk for CAD among Asians.[18] Similarly, for the R279Q SNPs of the MMP-9 gene too, there have been contradictory results with published reports from Iran[6] and United States[19] suggesting its role in increasing susceptibility for MI. In our study among the South-East Asian population, R279Q SNPs of the MMP-9 gene was associated with an increased risk for STEMI as compared to controls. However, the 1562C/T SNPs was comparable between subjects and controls. These contrasting results are often due to demographic and ethnic differences, study design, sample size along with other confounding factors.

One of the important limitations is that it is a single centre study with relatively small sample size thereby the findings cannot be generalised to the entire population group. Another limitation is the relatively shorter duration of follow-up. Additionally, a comparative evaluation between young and old STEMI patients was not established in this study. The present study depicted the association between various MMP's and their genetic polymorphisms in ACS patients as well as the utility of MMP-9 in prediction of MACE following STEMI. It is very difficult to say whether the elevated MMP levels in our study population is related to disease process per se or to the genetic polymorphisms. To the best of our knowledge, this is one of the first studies evaluating role of MMP's and their genetic polymorphisms in young patients with STEMI in South-East Asian population group. However, there is a need for large scale, multi-centre randomised clinical studies to evaluate the role of these novel biomarkers and associated genetic polymorphism on cardiovascular disease outcomes.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ihj.2022.11.001.

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