

MMP- 3 5A/6A PROMOTER GENE POLYMORPHISM AND RISK OF MYOCARDIAL INFARCTION IN SOUTH INDIAN POPULATION.

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ABSTRACT

Introduction: Myocardial infarction (MI) is a multifactorial disease influenced by environmental and genetic factors. Matrix metallo proteinase-3 (MMPs) plays a pivotal role in the development of atherosclerosis and MI.

Objective: The objective of the present study was designed to investigate the association of matrix metallo proteinase- 3 -1612 promoter 5A/6A gene polymorphism.

Methods: In the present study was carried out with 250 myocardial infarction patients and 260 controls. Clinical and demographic characteristics were collected. DNA was isolated and MMP-3 -1612 promoter 5A/6A gene polymorphism was investigated using polymerization reaction followed by restriction digestion for all samples.

Results: The percentage of classical risk factors like body mass index, hypertension, smoking, alcohol and diabetes was high in patients when compared to controls. The matrix metallo proteinase - 3 -1612 promoter 5A gene polymorphism was not associated with myocardial infarction.

Conclusion: The MMP-3 promoter 5A/6A gene polymorphism is not a risk factor for myocardial infarction patients in a South Indian population

INTRODUCTION.

In India, cardiovascular diseases (CVD) are projected to be the largest cause of death and disability by 2020, with 2.6 million Indians predicted to die due to coronary heart disease predominantly with MI (1). The important cause of concern in developing countries such as India is improper detection, treatment and control of risk factors for MI (2). Indians are more prone to cardiovascular diseases including MI at younger age as compared to other populations (3).

Matrix metallo proteases are zinc-dependent endopeptidases also called as stromelysin or transin. It is involved in degradation of matrix proteins (collagen, elastin and proteoglycan) (4). Atheroma mainly contains these matrix proteins, smooth muscle cells, macrophages and lipids, which is a greater risk factor for myocardial infarction (MI). Studies have shown that MMP3 levels are more in the smooth muscle cells and macrophages that are present in atherosclerotic plaque in humans (5).

MMP3 gene is located on the 11q22.2- 22.3. The single nucleotide polymorphism was identified in the promoter region of mmp3, located at -1612 base pair. It is present in the upstream of the transcription start site, here one allele containing with the run of five adenosines (5A), where as another allele containing with the run of six adenosines (6A). Previous studies have shown that mmp3 has more promoter activity with 5A allele compared to 6A allele (6)

The present study has focused on the investigation of the risk of 5A/6A allele gene polymorphism in South Indian MI patients.

MATERIALS AND METHODS.

The study was carried out on 250 MI patients admitted at Osmania General Hospital, ICCU, cardiology division. Hyderabad, Andhra Pradesh. The patients were between 53-62 years of age. All the patients were admitted with acute coronary syndrome underwent coronary angiogram. On the basis of electro cardiogram (ECG) changes, elevated cardiac markers and clinical history they were confirmed as myocardial infarction cases by the cardiologists. All these patients were without previous history of coronary artery disease. Along with 260 healthy age and sex matched controls (between the age of 52- 61 years) were included in the study. Most of the patients and controls were males included under study. All controls were blood donors of the same hospital. The study as approved by the hospital Ethical Committee and written informed consent was obtained from all the patients and controls.

Data Collection

Information on height, weight, body mass index, cigarette smoking, alcohol consumption, hypertension, diabetes, etc, was collected by using a structured questionnaire. Patients with kidney, neurological and cancer problems are excluded from our study.

DNA isolation and genotyping -1612 MMP- 3 5A/6A Gene polymorphism

Venous blood (2 ml) was collected in an EDTA tube for DNA extraction. DNA was isolated by the salting-out method (7). The 5A/6A nucleotide polymorphism was amplified by polymerase chain reaction (Thermal Cycler, MJ Research, USA), using the protocol and primers (forward 5'-GGTTCTCCATTCCTTTGATGGGGGAAAGA-3' and reverse 5'-CTTCCTGGAATTCACATCACTGCCACCACT-3') published previously(8) after amplification polymerase chain reaction the product (130 bp) was subjected to digest with 1 U of psy I enzyme for 15 hours at 37^o c. The un cut 130 bp was identified as homozygous 6A/6A, 130 and 110 bp was identified as heterozygous 5A/6A and only 110 bp was identified as homozygous 5A/5A on separation of 2% agarose gel.

Statistical Analysis.

The Hardy-Weinberg law of equilibrium was tested for the mmp3 gene polymorphism in controls, and MI patients. The frequencies for all groups were in agreement with the law. The association between genotypes of MI patients and controls was examined by using the odds ratio (OR) with 95% confidence interval (CI) and chi-square analysis. For all cases, p<0.05 was considered significant.

RESULTS

The present study group included 250 MI patients and 260 controls. The demographic and clinical characteristics are represented in table 1. The mean age of patients and controls was 61.8 ± 4.2 and 60.3 ± 3.6 years respectively. The BMI was high in patients (28.1 ± 2.4) when compared to controls (23.1 ± 1.6). The patient group had a high prevalence of diabetes (12.4%), hypertension (28.8%) when compared to controls (4.6%, 3.8) respectively. The percentages of smokers and alcoholics were also high in patients (23.6%, 25.6%) when compared to controls. (8.8%, 5.7%).

The mmp3 5A/6A genotypic and allelic frequencies of patients and controls were presented in table 2. The distribution of genotypes between patients and controls is presented in table 3. The 5A/5A genotype and 5A allele were not significantly associated with MI. 5A/5A vs. 6A/6A was $\chi^2=0.45$, OR was 1.25, CI 95% was 0.64–2.44, p value was 0.50 and the allele 5A vs. 6/A was $\chi^2=0.04$, OR =1.0 CI 95%, p value was 0.82.

1. Demographic and clinical characters of the study population.

| Demographic and clinical characteristics | Controls n = 260 | MI Patients n = 250 |
|--|------------------|---------------------|
| AGE (Mean \pm SD) | 60.3 \pm 3.6 | 61.8 \pm 4.2 |
| BMI(Kg/m ²) (Mean \pm SD) | 23.1 \pm 1.6 | 28.1 \pm 2.4 |
| Smokers n (%) | 23 (8.8) | 59 (23.6) |
| Alcoholics n (%) | 15 (5.7) | 64(25.6) |
| Hypertension n (%) | 10(3.8) | 72(28.8) |
| Diabetes n (%) | 12 (4.6) | 31 (12.4) |

Table 2. Distribution of MMP3 5A/6A genotypes and allelic frequencies of the total study group.

| Study group | MMP3 genotypes | | | Total | Allelic frequencies | | Total |
|----------------|----------------|----------|-----------|-------|---------------------|------------|-------|
| | 5A/5A | 5A/6A | 6A/6A | | 5A | 6A | |
| Controls n (%) | 18(6.9) | 95(36.5) | 147(56.6) | 260 | 131(0.25) | 389 (0.75) | 520 |
| Patients n (%) | 22(8.8) | 85(34) | 143(57.2) | 250 | 129 (0.26) | 371 (0.76) | 500 |

Table 3. Comparison of genotypic and allelic frequencies of MMP3 gene in MI patients and controls.

| Genotypes & Alleles (Patients vs. Controls) | Chi-square (χ^2) | Odds ratio | CI 95% | | p value |
|--|-------------------------|------------|---------|---------|---------|
| | | | L.limit | U.limit | |
| 5A/5A vs. 6A/6A | 0.45 | 1.25 | 0.64 | 2.44 | 0.50 |
| 5A/5A vs. 5A/6A | 0.78 | 1.36 | 0.68 | 2.77 | 0.37 |
| 5A/5A vs. 5A/5A+ 5A/6A | 0.62 | 1.23 | 0.67 | 2.48 | 0.43 |
| 5A vs. 6A | 0.04 | 1.0 | 0.77 | 1.36 | 0.82 |

DISCUSSION

In the present study we observed that the frequencies of alcohol, BMI, Smoking, diabetes were high in MI patients in comparison with controls. Among the traditional risk factors high BMI is one of the major risk factor MI (9). Results of the present study also showed that the mean BMI was high in patients when compared to controls. This could be due to obesity which also plays a major role in developing MI not only in Indians but also among world populations (10)

Earlier studies have suggested that high alcohol consumption was an important risk factor for MI in India (11). According to World Health Report, (1) it was estimated that 2% of CHD among men in developed countries was due to excessive alcohol consumption (1). Rastogi et al., (2005) has reported smoking as one of the independent risk factors for mortality due to MI in urban Indian population. (10). Yusuf et al., 2004 has showed that 22% of heart attacks in Western Europe and South Asia were due to hypertension and they had almost twice the risk for a heart attack (11). In the present study also individuals with hypertension were more among patients.

An association of the MMP3 5A/6A promoter polymorphism and atherosclerosis and MI were first described in 1995, The present study we investigated that the MMP-3 5A/6A gene polymorphism was not associated with MI, because the frequency of 5A allele did not reach statistical significance. The previous Indian study was also revealed that there is no association between 5A/6A gene polymorphism and MI(12), they showed that 5A/5A+5A/6A genotype was significantly more associated with unstable angina(45.7%) as compared to MI (26.7%) group and the frequency of 5A allele in MI(0.14) group was less compared to stable angina(0.24).

However some of the studies showed that positive association between -1612 5A/6A gene polymorphism and risk of MI (13, 14). Few studies are shown that the 6A/6A genepolymorphism was associated with atherosclerosis and MI (6, 14). However ye s et al, 1996, showed that the 5A allele had a higher promoter activity than 6A allele (16).

He identified a polymorphism in the promoter region of the MMP3 gene approximately 1,600 bp upstream from the start of transcription, at position -1171, in which 1 allele has a run of 6 adenosines (6A) while the other has 5 (5A). Pollanen et al, 2002, also showed mmp3 gene polymorphism was not associated with MI and (17). Gao et al, 2004, showed that no association with 5A/6A polymorphism in MMP-3 gene and risk of CHD and AMI, but serum level of MMP-3 is strongly associated with AMI (18). Few studies showed that mmp-3 promoter 5A/6A gene polymorphism was associated with carotid stenosis (19). Recently Seifi et al, 2009, showed no association of mmp3 5A/6A promoter gene polymorphism with coronary atherosclerosis (20).

However to confirm the association to these 5A/6A gene polymorphism there is a need to do meta-analysis studies.

Conclusion

Our investigations support that MMP-3 5A/6A gene polymorphism is not a risk factor for Myocardial infarction in South Indian population. This needs to be confirmed with a large number of samples like meta-analysis studies.

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