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Research Article

MATRIX METALLOPROTEINASES-9 (MMP-9) GENE POLYMORPHISMS IN PATIENTS WITH OR WITHOUT FAMILY HISTORY OF MYOCARDIAL INFARCTION

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A B S T R A C T

Objectives: The present study aims to evaluate the role of Matrix metalloproteinases-9 (MMP-9) gene polymorphism and serum MMP-9 levels, if any, in patients with or without family history of myocardial infarction (MI).

Materials and Methods: The levels of serum MMP-9, MMP-9 genotypes were evaluated by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism and ELISA method, respectively. Study subjects (N=125) were divided in two groups; Group I: Patients with family history (F/H) of MI (N=52) and Group II: Patients who did not had any family history (without F/H) of MI (N= 73).

Results: There was no significant difference found in the genotypes (CC Vs CT+TT; p=0.40) and alleles (p=0.28) for MMP-9 C-1562T polymorphism between patients with F/H group and in patients without F/H group. Similarly, we have not observed any significant difference in the genotypes (AA Vs AG+GG; p=0.16) and alleles (p=0.18) for MMP-9 R279Q polymorphism between both the study groups. There were no statistically significant results found for the intergenotypic association of MMP-9 C-1562T and MMP-9 R279Q polymorphisms with MMP-9 levels in patients with F/H group and in patients without F/H group, respectively. Serum MMP-9 levels were non-significantly (p=0.053) higher in patients with F/H group (54.4±17.4) as compared to patients without F/H group (52.2±17.9).

Conclusions: Our findings suggest that MMP-9 (C-1562T and R279Q) gene polymorphisms and serum MMP-9 levels may not contribute to the development of myocardial infarction in patients having no family history as compared to patients with family history.

INTRODUCTION

Coronary artery disease is one of the major cardiovascular diseases (CVD), which cause morbidity and mortality in developed countries as well as in low- and middle-income countries, including 85% of population, worldwide (Kazemi and Sharifzadeh, 2006). Coronary artery disease (CAD) including myocardial infarction (MI) affects nearly 1–1.5 million people in United States, out of which approximately 33% people die annually due to disease severity (Mehralian, 2007). The prevalence of MI is approximately 64.3 per 1000 people, and common cause of death in Indian population (Lim *et al*., 2010). The patho-physiology of MI is multifactorial and various risk factors were contributed towards the progression of atherosclerotic lesions and plaque rupture. Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteases basically involved in degradation of extra cellular matrix (ECM) having key role in the left ventricular dysfunction, MI and heart failure (Yabluchanskiy *et al*., 2013; Ma *et al*., 2012; DeLeon-Pennell *et al*., 2017). MMPs are identified in myocardium and well expressed in tissue macrophages, endothelial cells and smooth muscle cells involved in weakening and degradation of the fibrous cap of atherosclerotic lesions (Pasterkamp *et al*., 2000), destabilizing the plaque (Galis *et al*., 1994). Up-regulated levels of MMPs are strongly correlated with progression of left ventricular dimension, MI and cardiac remodelling. The role of matrix metalloproteinase-9 (MMP-9) in weakening and rupture of atherosclerotic plaques has been well-established and reported (Loftus *et al*., 2000). MMP-9 (also called as gelatinase B, 92 kDa gelatinase, or 92 Da type IV collagenase) is mainly associated with degradation of ECM and vascular remodelling which lead to the progression of atherosclerosis or restenosis (Galis and Khatri 2002). Previous studies have already been reported that higher levels of plasma MMP-9 may be

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associated with the prognosis of a cardiovascular disease risk (Blankenberg *et al*., 2003), and also found to be elevated in patients with MI (Squire *et al*., 2004; Guzel *et al*., 2013). Several recent findings regarding the genetic background of patients have indicated that DNA polymorphisms in MMP-9 gene may be associated with a high risk of MI (Yabluchanskiy *et al*., 2013; Sheikhvatan *et al*., 2018; El-Aziz *et al*., 2017; Wang *et al*., 2011). Two of major MMP-9 gene variants are R279Q A/G (rs17576) and C-1562T (rs3918242), located in exon-6 and -1562 in the promoter region respectively, were studied in patients with MI and acute coronary syndrome (ACS) in different populations (Sheikhvatan *et al*., 2018; El-Aziz *et al*., 2017; Wang *et al*., 2011). A very recent study in Iranian population suggested that C-1562T and R279Q polymorphisms were positively associated in patients with MI, whereas few studies have reported with conflicting results (Wang *et al*., 2011, 2012). A previous study in North Indian population was reported in patients with CAD (Mishra *et al*., 2012). However, no significant differences were observed in the frequency distribution for R279Q A/G polymorphism in angiographically confirmed CAD.

MMP-9 gene is related to inflammation and is characterized by disintegration of the fibrous cap, leading to plaque rupture and progression of MI; we attempted to explore the association between MMP-9 single nucleotide polymorphisms (SNPs) and MI. Two candidate SNPs of MMP-9 gene involved in the patho-physiology cardiovascular diseases, including MI and ACS i.e, R279Q (rs17576) and C-1562T (rs3918242) –were selected based on genetic analysis and literature survey, reported earlier (Sheikhvatan *et al*., 2018; El-Aziz *et al*., 2017; Wang *et al*., 2011, 2012). The MMP-9 gene polymorphisms (R279Q & C-1562T) in patients with myocardial infarction have not been reported so far in Indian study subjects. In our previous study, we observed that advanced glycation end products (AGEs) was significantly associated with up-regulated levels of MMP-9 in AMI patients with diabetes mellitus as compared to healthy controls and diabetic patients (Rajdan *et al*., 2017). These reports suggested that MMP-9 gene was closely associated with the etiopathogenesis of vulnerable plaque. Therefore, the present study is a step towards investigating the role of MMP-9 gene polymorphism and serum MMP-9 levels, if any, in patients with or without family history of myocardial infarction.

MATERIALS AND METHODS

Study Subjects

A total of 125 patients (men, women) of myocardial infarction (MI) within 24 hrs of the event were recruited from the Department of Cardiology, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, New Delhi, India for the present study. Exclusion criteria applied for the patients of diabetes mellitus, cancers, patients of chronic liver disease, chronic kidney disease and diseases of inflammatory pathology like arthritis, were excluded from the study. Additionally, patients were sub-divided further into two groups: i) patients with family history (F/H) of MI (N=52) and ii) patients who did not had any family history (without F/H) of MI (N= 73). All the participants were interviewed using a questionnaire with regard to their lifestyles, smoking habit, alcohol consumption, salt intake and family history of MI. At the time of recruitment, written informed consent was obtained from each participant. An approval of ethics committee of Lady Hardinge Medical College & Associated Hospitals and Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, New Delhi, India was obtained prior to the study. The study group was diagnosed according to the evidence of significant stenosis of the epicardial coronary artery more than 50% stenosis in at least one of the arteries confirmed by coronary artery angiography. The present study was conducted according to the Declaration of Helsinki principles (1964) and its later amendments (2008). The results of the study were not used to change or alter the treatment given to the patient.

Sample collection and processing

Five milliliter (5 ml) of peripheral blood was collected using all aseptic procedures, from the participants within 24 hours of MI after confirmation of the diagnosis by clinical evaluation, ECG. The samples were taken in two vacutainers, One ml of whole blood was used for the total DNA isolation and remaining volume of blood sample was used for the serum separation at 2000 rpm for 10 minutes. Serum was stored at -20^0C (in suitable aliquots) before it was batch analysed for MMP-9 ELISA test. The pack cell volume (PCV) was used for the purpose of genomic DNA isolation.

DNA extraction and Genotyping

Extraction of genomic DNA from peripheral whole blood was carried out by using QIAamp® DNA Blood Mini Kit (QIAGEN®), a silica-based membrane with spin column method according to the manufacturer's instructions. Genomic DNA was quantified by Biospectrometer (eppendorf®), and stored at 4°C until further processing.

Genotyping of MMP-9 polymorphisms were analysed by Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method, as described earlier (Wang *et al*., 2011) with slight modifications.

MMP-9 C-1562T (rs3918242)

The C-1562T variant of MMP-9 gene was amplified by PCR with the use of specific primers (Wang *et al*., 2011), Forward: 5'-GCCTGGCACATAGTAGGCCC-3'; Reverse: 5'-CTTCCTAGCCAGCCGGCATC-3'. Amplification was carried out in the Thermal cycler (eppendorf®) under the following conditions: denaturation at 94°C for 5 mins followed by 35 cycles (denaturation at 94°C for 30 sec, annealing at 63.7°C for 30 sec and extension at 72 °C for 30 sec) and final extension at 72 °C for 10 min. The PCR products were separated on 2.0% agarose gel electrophoresis. The PCR amplified 436-base pairs (bp) products were digested by the restriction enzyme Sph1, separated by 2.0% agarose gel electrophoresis and visualized by Gel documentation system (Syngene® G:Box). Individuals homozygous for allele, i.e homozygous CC (only a single 436 bp band appeared on the gel) for homozygous TT two bands i.e. 242, 194 bp appeared on gel and heterozygous CT (all 3 bands, i.e 436 bp, 242-bp and 194-bp appeared on the gel).

MMP-9 R279Q (rs17576)

The R279Q variant of MMP-9 gene was amplified by PCR with the use of specific primers (Wang *et al*., 2011), Forward: 5'-ATGGGTCAAAGAACAGGA-3'; Reverse: 5'-GGTAGACAGGGTGGAGG-3'. Amplification was carried out in the Thermal cycler (Eppendorf) under the following conditions: denaturation at 94°C for 5 mins followed by 35

cycles (denaturation at 94°C for 30 sec, annealing at 57.5°C for 30 sec and extension at 72°C for 30 sec) and final extension at 72°C for 10 min. The PCR products were separated on 2.0% agarose gel electrophoresis. The PCR product of 277-bp was digested by the restriction enzyme Sma1, separated in 2.0% agarose gel and visualized by Gel documentation system (Syngene G:Box). Individuals homozygous for allele, ie GG, a 96-bp band and a 181-bp band appeared on the gel, homozygous AA (only a single 277-bp band appeared on the gel), or heterozygous GA (all 3 bands i.e. 96-bp, 181-bp & 277-bp, appeared on the gel)

Serum matrix metalloproteinase-9 (MMP-9) levels

The levels of serum MMP-9 were estimated by commercially available Human-MMP-9 ELISA kits (Cat No. QY-E02978, QAYEE-BIO, Shanghai, China), and used according to the manufacturer's instructions. Serum samples were diluted 5-fold in assay buffer. All assays were performed in duplicates. The absorbance was recorded by ELISA reader (Infinite® 200 PRO, TECAN, Switzerland) at a wavelength of 450 nanometer (nm).

Statistical analysis

All statistical analysis was performed using GraphPad Prism version 5 (GraphPad Software Inc., San Diego, CA, USA) and SPSS version 16 (SPSS, IBM Corporation, Hong Kong). Chi-square goodness of fit was used to verify the agreement of observed genotype frequencies with those expected (Hardy–Weinberg equilibrium). The analysis of variance (ANOVA) was used to calculate the difference between genotype groups using Bonferroni's method for multiple comparisons between genotype classes. An odds ratio at [95% confidence intervals (CI)] was calculated as an index of the association of the gene with the disease. Other quantitative variables were tested with Student's t test (unpaired) between the two groups. p value < 0.05 was considered statistically significant.

RESULTS

Distribution of genotype and allele frequencies of MMP-9 C-1562T

The distribution of genotypes in the patients with F/H (χ^2=0.18, p=0.66) and patients without F/H (χ^2=1.43, p=0.2) groups was in accordance with the Hardy-Weinberg equilibrium. The CC genotype was most frequent among patients with F/H group as well as in patients without F/H group as compared to CT and TT genotypes, respectively. The observed allele frequencies in patients with F/H group were 0.83 and 0.17 for C and T alleles respectively, whereas 0.79 and 0.21 was observed in patients without F/H groups. We have not observed any significant association in the MMP-9 genotypes CC Vs (CT+TT) between both the study groups [χ^2 = 0.69, p= 0.40, Odds ratio = 0.71 (0.32-1.59) at 95% CI]. The adjusted odds ratio for C Vs T allele frequencies was found to be 0.70 (0.36-1.36) [χ^2 =1.13, p= 0.28 at 95% CI] (Table 1).

Table 1 Genotype and Allele Frequencies of C-1562T variant of the Matrix metalloproteinase (MMP-9) gene in the study subjects

Subjects	Genotypes*			Allele frequency**	
	CC (%)	CT (%)	TT (%)	C	T
Patients with F/H (N=52) (χ^2=0.18, p=0.66; NS)	36(69)	14(27)	2(4)	0.83	0.17
Patients without F/H (N=73) (χ^2=1.43, p=0.2; NS)	47(63)	21(29)	5(7)	0.79	0.21

*Adjusted odds ratio at 95% confidence interval (CI) for genotypes and alleles:
CC Vs CT = 0.77 (0.33-1.80) [χ^2 = 0.36, p= 0.55]
CC Vs TT = 0.42(0.07-2.46) [χ^2 = 0.91,p= 0.339]
CC Vs CT+TT = 0.71 (0.32-1.59) [χ^2 = 0.69, p= 0.40]
C Vs T = 0.70 (0.36-1.36) [χ^2 =1.13, p= 0.28]
Patients groups were compared with controls with chi-square (χ^2) test at one degree of freedom with Odds ratio and relative risk in both genotypes and alleles. P< 0.05 is considered to be significant. F/H = Family history; NS= Non significant.

There was no significant difference found in the genotypes and alleles for MMP-9 C-1562T polymorphism between patients with F/H group and in patients without F/H group.

Intergenotypic (C-1562T variant of the MMP-9 gene) variations in serum MMP-9 levels in study subjects

A statistically non-significant (p=0.62) intergenotypic variation in the serum MMP-9 levels in patients with F/H group i.e. CC, CT and TT genotypes was found to be 56.13±13.37 ng/ml, 50.14±16.52 ng/ml and 55.11±11.31 ng/ml respectively. Similarly, there was no statistically significant results were found between the genotypes and MMP-9 levels in patients with F/H group, when compared with CC Vs CT (p=1.11), CC Vs TT (p=0.08) and CC Vs CT+TT (p=1.04), respectively. The intergenotypic variation in the serum MMP-9 levels in patients without F/H group i.e. CC, CT and TT genotypes was found as statistically non significant (p=0.10) and values are 49.34±19.54 ng/ml, 58.44±13.13 ng/ml and 54.61±16.18 ng/ml respectively. Also, there was no statistically significant results were observed for genotypes and MMP-9 levels in patients without F/H group, when compared with CC Vs CT (p=2.07), CC Vs TT (p=0.67) and CC Vs CT+TT (p=2.05), respectively (Table 2).

Table 2 Intergenotypic (C-1562T variant of the MMP-9 gene) variations in Serum MMP-9 levels in study subjects

Subjects	Genotypes Vs MMP-9 levels (ng/ml)			p*
	CC	CT	TT	
Patients with F/H (N=52)	56.13±13.37	50.14±16.52	55.11±11.31	0.62
Patients without F/H (N=73)	49.34±19.54	58.44±13.13	54.61±16.18	0.10
Comparison of Genotypes*	p-value			
	Patients with F/H		Patients without F/H	
CC Vs CT	1.11		2.07	
CC Vs TT	0.08		0.67	
CC Vs CT+TT	1.04		2.05	

F/H, Family history; MMP-9, Paraoxonase-2 gene; ng/ml, nanogram per milli liter.
MMP-9 levels were compared with respect to genotypes with t-test of significance test at one degree of freedom. p < 0.05 is considered to be significant.
*analysis of variance (ANOVA) using Bonferroni's method for multiple comparisons between genotype classes.

Distribution of genotype and allele frequencies of MMP-9 R279Q

The distribution of genotypes in the patients with F/H (χ^2=0.089, p=0.76) and patients without F/H (χ^2=0.13, p=0.71) groups was in accordance with the Hardy-Weinberg equilibrium. The AG genotype was most frequent among patients with F/H group as well as in patients without F/H group as compared to AA and GG genotypes, respectively. The observed allele frequencies in patients with F/H group were 0.60 and 0.40 for A and G alleles respectively, whereas 0.52 and 0.48 was observed in patients without F/H groups.

We have not observed any significant association in the MMP-9 genotypes AA Vs (AG+GG) between both the study groups [$\chi 2$= 1.93, p= 0.16, Odds ratio = 0.57(0.26-1.26) at 95% CI]. The adjusted odds ratio for A Vs G allele frequencies was found to be 0.71(0.42-1.18) [$\chi 2$= 1.75, p= 0.18 at 95% CI] (Table 3). There was no significant difference found in the genotypes and alleles for MMP-9 R279Q polymorphism between patients with F/H group and in patients without F/H group.

Table 3 Genotype and Allele Frequencies of R279Q variant of the Matrix metalloproteinase (MMP-9) gene in the study subjects

Subjects	Genotypes*			Allele frequency**	
	AA (%)	AG (%)	GG (%)	A	G
Patients with F/H (N=52) (χ^2=0.089, p=0.76; NS)	19(37)	24(46)	9(17)	0.60	0.40
Patients without F/H (N=73) (χ^2=0.13, p=0.71; NS)	19(26)	38(52)	16(22)	0.52	0.48

*Adjusted odds ratio at 95% confidence interval (CI) for genotypes and alleles:
AA Vs AG = 0.59(0.26-1.36) [$\chi 2$= 1.52, p= 0.21]
AA Vs GG = 0.48(0.17-1.42) [$\chi 2$= 1.75, p= 0.18]
AA Vs AG+GG = 0.57(0.26-1.26) [$\chi 2$= 1.93, p= 0.16]
A Vs G = 0.71(0.42-1.18) [$\chi 2$= 1.75, p= 0.18]
Patients groups were compared with controls with chi-square (χ^2) test at one degree of freedom with Odds ratio and relative risk in both genotypes and alleles. P< 0.05 is considered to be significant.
F/H = Family history; NS= Non significant.

Intergenotypic (R279Q variant of the MMP-9 gene) variations in serum MMP-9 levels in study subjects

A statistically non-significant (p=0.72) intergenotypic variation in the serum MMP-9 levels in patients with F/H group i.e. AA, AG and GG genotypes was found to be 57.96±8.68 ng/ml, 51.85±23.43 ng/ml and 54.18±11.57 ng/ml respectively. Similarly, there was no statistically significant results were found between the genotypes and MMP-9 levels in patients with F/H group, when compared with AA Vs AG (p =1.06), AA Vs GG (p=0.49) and AA Vs AG+GG (p=1.01), respectively. The intergenotypic variation in the serum MMP-9 levels in patients without F/H group i.e. AA, AG and GG genotypes was found as statistically non significant (p=0.91) and values are 54.76±15.77 ng/ml, 51.32±19.84 ng/ml and 51.68±16.43 ng/ml respectively. Also, there was no statistically significant results were observed for genotypes and MMP-9 levels in patients without F/H group, when compared with AA Vs AG (p=0.66), AA Vs GG (p=0.49) and AA Vs AG+GG (p=0.67), respectively (Table 4).

Table 4 Intergenotypic (R279Q variant of the MMP-9 gene) variations in Serum MMP-9 levels in study subjects

Subjects	Genotypes Vs MMP-9 levels (ng/ml)			p*
	AA	AG	GG	
Patients with F/H (N=52)	57.96±8.68	51.85±23.43	54.18±11.57	0.72
Patients without F/H (N=73)	54.76±15.77	51.32±19.84	51.68±16.43	0.91
Comparison of Genotypes*	**p-value**			
	Patients with F/H		Patients without F/H	
AA Vs AG	1.06		0.66	
AA Vs GG	0.49		0.49	
AA Vs AG+GG	1.01		0.67	

F/H, Family history; MMP-9, Matrix metalloproteinase gene; ng/ml, nanogram per milli liter.
MMP-9 levels were compared with respect to genotypes with t-test of significance test at one degree of freedom. p < 0.05 is considered to be significant.
*analysis of variance (ANOVA) using Bonferroni's method for multiple comparisons between genotype classes.

Serum matrix metalloproteinase-9 (MMP-9) levels

The levels of serum MMP-9 was not significantly differed (p=0.053) between the patients with F/H group (54.48±17.41) and the patients without F/H group (52.29±17.97) (Table 5).

Table 5 Levels of serum Matrix metalloproteinase (MMP-9) in study subjects

Subjects	MMP-9 levels (ng/ml) *	p-value#
Patients with F/H (N=52)	54.48±17.41	0.053
Patients without F/H (N=73)	52.29±17.97	

MMP-9, Matrix metalloproteinase gene; ng/ml, nanogram per milli liter.
*Data are means ± SD. #Patients and controls were compared with respect to serum MMP-9 levels with t-test of significance test, p = 0.053. #p < 0.05 is considered to be significant.

DISCUSSION

The development of thrombus formation mainly involves two important events: rupture of the atheromatous fibrous cap and superficial damage and exposure of the endothelium. Any damage to the endothelium triggers monocyte and T-lymphocyte adhesion with the help of cell-surface adhesion molecules known as a vascular cell adhesion molecule-1 (VCAM-1), which further promotes their differentiation into macrophages. The increased permeability of endothelium facilitates the entry of low density lipoproteins (LDL) into intima of artery, where oxidation of LDL takes place due to production of reactive oxygen species (ROS) and nitrogen species (NOS) by endothelial cells. Now oxidized LDL (Ox-LDL) taken up by macrophages through phagocytosis and subsequently become lipid rich known as 'foam cells', collection of such foam cells constitutes fatty streaks. These fatty streaks further evolve into two forms of atherosclerotic plaques, namely stable and unstable plaque. Stable plaques are developed due to gradual deposition of lipid in foam cells and proliferation of smooth muscle cells, having thick fibrin caps that are not procumbent to rupture. On the other hand, unstable plaques are fast growing that give rise to more lipid deposition, and having thin fibrin caps that are much prone to rupture, ultimately leads to thrombosis.

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes, which found in the myocardium and acts towards the myocardial remodelling including changes in cardiac myocytes and in the extracellular matrix (ECM). MMPs involve more than 25 members, having common basic domain structures and salient features towards their functions [21] such as, degradation of ECM, activity at neutral pH, inhibition by means of certain tissue inhibitors of metalloproteinases (TIMPs), presence of zinc for catalytic domain and requirement of calcium for their stability. Based on their structure and substrate specificity, MMPs were divided into four groups known as collagenases, gelatinases, stromelysins and membrane-type (MT) MMPs respectively. MMP-9 comes under second group gelatinases, as they are able to degrade the gelatins and collagen type IV in basement membranes. It has been reported that the synthesis of MMPs are stimulated by various pro-inflammatory cytokines, epidermal and platelets derived growth factors (Creemers *et al*., 2001), and a cell-surface protein named ECM metalloproteinase inducer

(Schmidt *et al*., 2006). The activity of MMPs in tissue is mainly inhibited by TIMPs. All four types of TIMPs (1 to 4) have ability to inhibit all available types of MMPs with different peculiarity. TIMP combine with the zinc binding site of the active MMPs and hinders the substrate availability. Animal studies suggested that the disproportion among MMPs and TIMPs levels may lead to the further changes in cardiomyocyte and infarct expansion in myocardial infarction (Mukherjee *et al*., 2003; Zhang *et al*., 2004). Human MMP-9 gene is composed of 13 exons and 12 introns, located on chromosome 20q13.12. Two most common polymorphisms of MMP-9 gene are R279Q (rs17576) and C-1562T (rs3918242), studied in different population with conflicting results (Wang *et al*., 2011, 2012; Mishra *et al*., 2012). A polymorphism (rs17576) in the exon-6 regions of MMP-9 gene lead to the substitution of adenine (A) to guanine (C) base at the 46011586 position as the missense variant. Another SNP (rs3918242) located at -1562 in the promoter region of MMP-9 gene leads to substitution of cytosine (C) for thymine (T) as an upstream variant on human chromosome 20. A recent meta-analysis comprised of seven related studies suggested that MMP-9 C-1562T polymorphism was associated with susceptibility of MI in white population; however it was not significantly associated with the disease progression in Asian population (Juan *et al*., 2015). Similarly, another meta-analysis comprised of sixteen case-control studies reported that MMP-9 C-1562T polymorphism was significantly associated with the CAD and MI in East Asians, although no significant association was observed in West Asians or Western population (Wang and Shi 2014). Wang *et al*. (2011) reported that MMP-9 C-1562T polymorphism was significantly associated with pathogenesis of acute coronary syndrome (ACS), however no association for MMP-9 R279Q polymorphism with ACS was observed in Uygur population of China. Most-recent case control and cohort study in Iranian population revealed that R279Q and C-1562T polymorphisms of MMP-9 gene are associated with the susceptibility of premature MI (Sheikhvatan *et al*., 2018).

In our study, we observed a co-dominance pattern of inheritance among study subjects, as the genotype frequencies in both groups were all in accordance with the Hardy-Weinberg equilibrium. We found that CC genotype was most frequent as compared to CT and TT genotypes in the patients with family history group as well as in patients without family history group for MMP-9 C-1562T polymorphism. In context to MMP-9 R279Q genetic polymorphism, AG genotype was most frequent as compared to AA and GG genotypes in the patients with family history group as well as in patients without family history group. To the best of our knowledge, no such study on MMP-9 R279Q and C-1562T gene polymorphism in the patients with family history group as well as in patients without family history group has been reported in Asian Indian context. In our study, we did not observed any significant association between both the groups studied for MMP-9 R279Q (p = 0.18, odds ratio = 0.71 [0.42-1.18]) and C-1562T (p= 0.28, odds ratio = 0.70 [0.36-1.36]) gene polymorphism with the MI. Our results for MMP-9 R279Q polymorphism also are in agreement with the previous study carried out in Chinese populations in patients with ACS (Wang *et al*., 2011). Blankenberg *et al*. (2003) has been reported that 279Q allele was significantly associated with elevated levels of MMP-9 and prognosis of cardiovascular death and MI (Squire *et al*., 2004). However, several studies suggested that R279Q variant of MMP-9 was not significantly associated with the risk of CAD and stable angina (Morgan *et al*., 2003; Kim *et al*., 2002; Lamblin *et al*., 2002). Similarly, our findings for MMP-9 C-1562T gene polymorphism are in consistent with the studies reported in Asians (Juan *et al*., 2015) and West Asians populations (Wang *et al*., 2014), as there was no association found in patients with CAD and premature MI, respectively. In a recent meta-analysis Zhang *et al*. (2017) reported that MMP-9 -1562T allele was a risk factor for CAD and MI, however, no statistically significant association was observed between rs3918242 polymorphism and CAD either in allelic or recessive models in Caucasians.

On further analysis, we studied the serum MMP-9 levels in both the subject groups for present study. Serum MMP-9 levels was not significantly differs (p=0.053) between patients with family history group as compared to patients without family history group. Similarly, we did not find any statistically significant difference between intergenotypic variations and serum MMP-9 levels in both the study groups for MMP-9 R279Q (p= 0.72 & 0.91) and C-1562T (p= 0.62 & 0.10) gene polymorphism, respectively. The association between Genetic polymorphism and MMP-9 levels is also varied among different ethnic populations and races. Some studies are reported that no positive association found between MMP-9 polymorphism and plasma MMP-9 levels in white control subjects (Demacq *et al*., 2008; Lacchini *et al*., 2010), However, Metzger *et al*. (2012) suggested their positive association in black study subjects. The variations in MMP-9 levels might be due to the effects of different drugs (e.g. statins) that usually prescribed in CAD, as reported earlier (Souza-Costa *et al*., 2007; Izidoro-Toledo *et al*., 2011). Orn *et al*. (2007) reported that MMP-9 levels were not associated with the scar size in post MI condition, suggested that different mechanisms were might be associated in the regulation of plasma MMP-9 levels in patients with long term complicated MI. Atherosclerotic Disease, Vascular Function and Genetic Epidemiology (ADVANCE) study was conducted for different SNPs & serum profiling for various MMPs in patients with acute MI, stable angina and healthy controls suggested that circulating levels of MMP-2 and MMP-9 are independently associated with progression of an acute MI contrary to stable angina (Hlatky *et al*., 2007).

CONCLUSIONS

The results of the present study suggested that the MMP-9 (C-1562T and R279Q) gene polymorphisms and serum MMP-9 levels may not contribute to the development of myocardial infarction in patients having no family history as compared to patients with family history. Further studies in extended sample size with patients follow-up are warranted to confirm the association of MMP-9 gene polymorphisms and MMP-9 levels with the risk of developing MI.

Conflict of Interest

The authors have no potential conflict of interests to declare.

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References

Blankenberg, S., Rupprecht, H.J., Poirier, O., Bickel, C., Smieja, M., Hafner, G., Meyer, J., Cambien, F., Tiret, L.; AtheroGene Investigators. 2003. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation, 2003. 107: 1579-85.

Creemers, E.E., Cleutjens, J.P., Smits, J.F., Daemen, M.J. 2001. Matrix metalloproteinase inhibition after myocardial infarction: A new approach to prevent heart failure? Circ Res., 89: 201-10.

DeLeon-Pennell, K.Y., Meschiari, C.A., Jung, M., Lindsey, M.L. 2017. Matrix Metalloproteinases in Myocardial Infarction and Heart Failure. Prog Mol Biol Transl Sci., 147: 75-100.

Demacq, C., Vasconcellos, V.B., Marcaccini, A.M., Gerlach, R.F., Silva, W.A. Jr., Tanus-Santos, J.E. 2008. Functional polymorphisms in the promoter of the matrix metalloproteinase-9 (MMP-9) gene are not linked with significant plasma MMP-9 variations in healthy subjects. Clin Chem Lab Med., 46: 57-63.

El-Aziz, T.A.A. and Mohamed, R.H. 2017. Matrix metalloproteinase -9 polymorphism and outcome after acute myocardial infarction. Int J Cardiol., 227: 524-528.

Galis, Z.S. and Khatri, J.J. 2002. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res., 90: 251–262.

Galis, Z.S., Sukhova, G.K., Lark, M.W., Libby, P. 1994. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest., 94: 2493–2503.

Guzel, S., Serin, O., Guzel, E.C., Buyuk, B., Yılmaz, G., Güvenen, G. 2013. Interleukin-33, matrix metalloproteinase-9, and tissue ınhibitor of matrix metalloproteinase-1 in myocardial infarction. The Korean Journal of Internal Medicine, 28: 165-173.

Hlatky, M.A., Ashley, E., Quertermous, T., Boothroyd, D.B., Ridker, P., Southwick, A., Myers, R.M., Iribarren, C., Fortmann, S.P., Go, A.S. 2007. Atherosclerotic Disease, Vascular Function and Genetic Epidemiology (ADVANCE) Study. Matrix metalloproteinase circulating levels, genetic polymorphisms, and susceptibility to acute myocardial infarction among patients with coronary artery disease. Am Heart J., 154: 1043-51.

Izidoro-Toledo, T.C., Guimaraes, D.A., Belo, V.A., Gerlach, R.F., Tanus-Santos, J.E. 2011. Effects of statins on matrix metalloproteinases and their endogenous inhibitors in human endothelial cells. Naunyn Schmiedebergs Arch Pharmacol., 383: 547-54.

Juan, Z., Wei-Guo, Z., Heng-Liang, S., Da-Guo, W. 2015. Association of Matrix Metalloproteinase 9 C-1562T Polymorphism with Genetic Susceptibility to Myocardial Infarction: A Meta-Analysis. Curr Ther Res Clin Exp., 77: 40-5.

Kazemi, T. and Sharifzadeh, G.R. 2006. Changes in risk factors, medical care and rate of acute myocardial infarctions in Birjand (1994-2003). ARYA J., 1: 271–4.

Kim, J.S., Park, H.Y., Kwon, J.H., Im, E.K., Choi, D.H., Jang, Y.S., Cho, S.Y. 2002. The roles of stromelysin-1 and the gelatinase B gene polymorphism in stable angina. Yonsei Med J., 43: 473-481.

Lacchini, R., Metzger, I.F., Luizon, M., Ishizawa, M., Tanus-Santos, J.E. 2010. Interethnic differences in the distribution of matrix metalloproteinases genetic polymorphisms are consistent with interethnic differences in disease prevalence. DNA Cell Biol., 29: 649-55.

Lamblin, N., Bauters, C., Hermant, X., Lablanche, J.M., Helbecque, N., Amouyel, P. 2002. Polymorphisms in the promoter regions of MMP- 2, MMP-3, MMP-9 and MMP-12 genes as determinants of aneurysmal coronary artery disease. J Am Coll Cardiol., 40: 43-48.

Lim, S.S., Vos, T., Flaxman, A.D., Danaei, G., Shibuya, K., Adair-Rohani, H., *et al*. 2012. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet, 380: 2224-60.

Loftus, I.M., Naylor, A.R., Goodall, S., Crowther, M., Jones, L., Bell, P.R., Thompson, M.M. 2000. Increased matrix metalloproteinase-9 activity in unstable carotid plaques. A potential role in acute plaque disruption. Stroke, 31: 40-7.

Ma, Y., Halade, G.V., Lindsey, M.L. 2012. Extracellular matrix and fibroblast communication following myocardial infarction. J Cardiovasc Transl Res., 5: 848–857.

Mehralian, H.A. and Salehi, S. 2007. Myocardial infarction risk factors in the patients referred to Chaharmahal and Bakhtiary province hospitals, 2005. J Shahrekord Univ Med Sci., 9: 77–84.

Metzger, I.F., Luizon, M.R., Lacchini, R., Tanus-Santos, J.E. 2012. Genetic variants in matrix metalloproteinase-9 gene modify metalloproteinase-9 levels in black subjects. DNA Cell Biol., 31: 504-10.

Mishra, A., Srivastava, A., Mittal, T., Garg, N., Mittal, B. 2013. Association of matrix metalloproteinases (MMP2, MMP7 and MMP9) genetic variants with left ventricular dysfunction in coronary artery disease patients. Clin Chim Acta., 413: 1668-74.

Morgan, A.R., Zhang, B., Tapper, W., Collins A., Ye, S. 2003. Haplotypic analysis of the MMP-9 gene in relation to coronary artery disease. J Mol Med., 81: 321-326.

Mukherjee, R., Brinsa, T.A., Dowdy, K.B., Scott, A.A., Baskin, J.M., Deschamps, A.M., Lowry, A.S., Escobar, G.P., Lucas, D.G., Yarbrough, W.M., Zile, M.R., Spinale, F.G. 2003. Myocardial infarct expansion and matrix metalloproteinase inhibition. Circulation, 107: 618–25.

Orn, S., Manhenke, C., Squire, I.B., Ng, L., Anand, I., Dickstein, K. 2007. Plasma MMP-2, MMP-9 and N-BNP in long-term survivors following complicated myocardial infarction: relation to cardiac magnetic resonance imaging measures of left ventricular structure and function. J Card Fail., 13(10):843-9.

Pasterkamp, G., Schoneveld, A.H., Hijnen, D.J., de Kleijn, D.P., Teepen, H., van der Wal, A.C., Borst, C. 2000. Atherosclerotic arterial remodeling and the localization of macrophages and matrix metalloproteases 1, 2 and 9 in the human coronary artery. Atherosclerosis, 150: 245-53.

Rajdan, V., Singh, R., Tyagi, S. 2017. Poorly controlled diabetes mellitus type 2 is associated with increased mmp-9 levels and occurance of myocardial infarction-a pilot study. IJCAR, 6: 3584-88.

Schmidt, R., Bultmann, A., Ungerer, M., Joghetaei, N., Bülbül, O., Thieme, S., Chavakis, T., Toole, B.P., Gawaz, M., Schömig, A., May, A.E. 2006. Extracellular matrix metalloproteinase inducer regulates matrix metalloproteinase activity in cardiovascular cells: Implications in acute myocardial infarction. Circulation, 113: 834-41.

Sheikhvatan, M., Boroumand, M.A., Behmanesh, M., Ziaee, S. 2018. Association of R279Q and C1562T polymorphisms of matrix metalloproteinase 9 gene and increased risk for myocardial infarction in patients with premature coronary artery disease. J Clin Lab Anal., 32: e22218.

Souza-Costa, D.C., Sandrim, V.C., Lopes, L.F., Gerlach, R.F., Rego, E.M., Tanus-Santos, J.E. 2007. Anti-inflammatory effects of atorvastatin: modulation by the T-786C polymorphism in the endothelial nitric oxide synthase gene. Atherosclerosis, 193: 438-44.

Squire, I.B., Evans, J., Ng, L.L., Loftus, I.M., Thompson, M.M. 2004. Plasma MMP-9 and MMP-2 following acute myocardial infarction in man: correlation with echocardiographic and neurohumoral parameters of left ventricular dysfunction. J Card Fail., 10: 328-33.

Wang, L., Ma, Y.T., Xie, X., Yang, Y.N., Fu, Z.Y., Li, X.M., Liu, F., Huang, Y., Ma, X., Chen, B.D., Yuan, S., Sun, M.H., Peng, X., Wang, B.Z. 2012. Interaction between MMP-9 gene polymorphisms and smoking in relation to myocardial infarction in a Uighur population. Clin Appl Thromb Hemost., 18: 72–78.

Wang, L., Ma, Y.T., Xie, X., Yang, Y.N., Fu, Z.Y., Liu, F., Li, X.M., Chen, B.D. 2011. Association of MMP-9 gene polymorphisms with acute coronary syndrome in the Uygur population of China. World J Emerg Med., 2: 104-110.

Wang, X. and Shi, L.Z. 2014. Association of matrix metalloproteinase-9 C1562T polymorphism and coronary artery disease: a meta-analysis. J Zhejiang Univ Sci B., 15: 256-63.

Yabluchanskiy, A., Chilton, R.J., Lindsey, M.L. 2013. Left ventricular remodeling: One small step for the extracellular matrix will translate to a giant leap for the myocardium. Congestive heart failure, 19: E5–8.

Yabluchanskiy, A., Ma, Y., Iyer, R.P., Hall, M.E., Lindsey, M.L. 2013. Matrix metalloproteinase-9: Many shades of function in cardiovascular disease. Physiology (Bethesda), 28: 391-403.

Zhang, M.M., Chang, X.W., Hao, X.Q., Wang, H., Xie, X., Zhang, S.Y. 2017. Association between matrix metalloproteinase 9 C-1562T polymorphism and the risk of coronary artery disease: an update systematic review and meta-analysis. Oncotarget, 9: 9468-9479.

Zhang, P., Yang, Y.J., Chen, X., Ruan, Y.M., Zhou, Y.W., Tian, Y., Chen, Z.J. 2004. Effects of doxycycline on the expression of matrix metalloproteinase and tissue inhibitor of MMP in myocardium after acute myocardial infarction: An animal experiment with rats. Zhonghua Yi Xue Za Zhi., 84: 1288-93.

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