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Original Research Article

Association of Matrix Metalloproteinase-2 Gene Promoter Polymorphism and the Associated Phenotype Variation with Myocardial Infarction

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Abstract: MatrixMetalloProteinases-2, are zinc dependent endopeptidases which degrade major components of the basal lamina around blood vessels. Myocardial Infarction is mainly due to atherosclerosis of the coronary arteries. MMP-2 destabilizes the arterial wall and breaks atheromatous plaque leading to MI. The gene for human MMP-2 contains 27,862 base pair genomic DNA and is composed of 13 exons. Several common restriction fragment length polymorphisms (RFLPs) have been reported in the MMP-2 gene locus. 100 diagnosed myocardial infarction patients were taken as cases. 100 Age, sex matched individuals taken as controls. Autoimmune, thyroid, neoplastic, hepatic and chronic infections were excluded. Plasma MMP-2 levels were determined by ELISA and Genotyping was done by PCR-RFLP. Plasma MMP-2 level was increased in patients with myocardial infarction. Genotype frequency distribution between cases and controls were compared with a χ^2 test. MMP-2 activity was compared between controls and cases by Student t test, p value < 0.05 was considered significant.
Keywords: Matrixmetalloproteinases, Myocardial Infarction, MMP-2gene, Restriction fragment length Polymorphism, Atheromatous plaque

INTRODUCTION

In Many developing countries Myocardial infarction has become a major problem in public health [1, 2]. MI is a multifactorial disease caused by genetic and environmental factors. The major cause of death in the world is Myocardial infarction [3]. The high plasma lipid levels, high plasma glucose levels, high blood pressure, obesity, smoking, and family history of cardiac disease are considered the most important risk factors for MI. MI is mainly due to atherosclerosis of the coronary arteries. The structural changes which permit the accumulation of cells, extracellular matrix and lipids in the intimate layer of the diseased artery allows the growth of atherosclerotic plaque. Plaque gets ruptured, gives rise to thrombosis, and its complications [4]. Pathophysiology of MI involves a wide variety of proteins including the matrix metalloproteinases (MMPs). Atheromatous plaque formation is facilitated by the action of MMPs. Major extra cellular components of the basal lamina around blood vessels such as type 1V collagen, laminin, and fibronectin are degraded by MMP-2. MMPs also weakens the arterial wall, resulting in destabilization of atheromatous plaque and dissolution of fibrous cap leading to MI. Matrix metalloproteinases are zinc dependent endopeptidases that degrade components of the extracellular matrix (ECM). MMP-2, a 72KDa type IV collagenase is an ubiquitous metalloproteinase involved in various functions such as vascular remodeling, atheromatous plaque rupture and degradation of matrix proteins. In human it is encoded by the MMP-2 gene [6]. MMP-2 is produced as zymogen, pro MMP-2 which binds to its specific inhibitor, called as tissue inhibitor of matrix metalloproteinases-2. There are several pathways for activation of the proenzyme, but the most important pathway is activation by membrane type metalloproteinases-1 (MT1-MMP) [7]. MTI-MMP binds to TIMP-2 and this complex structure moves close to the active site of the MT-MMP enzyme. This results in the removal of two specific pro peptides from pro MMP-2 and the production of an active 72 KDa MMP-2 enzyme [8]. The gene for human MMP-2 contains 27,862 base pair genomic DNA and is composed of 13 exons. It has been localized on chromosome 16q21 [9]. Several common restriction fragment length polymorphisms (RFLPs) have been reported in the MMP-2 gene locus. MMP-2 gene –

1306 C > T promoter region is linked with development of MI [10]. This base transition is situated in CCACC box of the sp1 binding site.

Increased MMP-2 levels have been found in the plasma of patients with MI [11]. Elevated MMP-2 has also been found in atherosclerotic plaques of coronary arteries [12]. In view of this we have evaluated the distribution of MMP-2 promoter gene polymorphism by PCR- RFLP and the concerned phenotype (MMP-2) were analyzed by ELISA.

MATERIALS AND METHODS

STUDY POPULATION

CASES

The study sample comprised of 100 newly diagnosed Myocardial Infarction patients (85 male , 15 female) of Mean age 50.34 $\pm$ 9.84 years. Patients with echo findings of more than 50% narrowing of at least one of the major coronary arteries were included. Hospitalized cases with acute attack of Myocardial Infarction were included.

CONTROL SUBJECTS

Controls were recruited from patients attending outpatient clinic for non-cardiac causes. Age, Sex and other confounding factors like diabetes, hypertension, smoking, alcoholism were matched.

Total cholesterol (TC), high density lipoprotein cholesterol (HDL-c). Low density lipoprotein cholesterol (LDL-c) and triglyceride concentration (TGL) were determined by using enzymatic assays .

MMP-2 Gene Polymorphism Screening

DNA was extracted from buffy coat by high salt method [13] 188bp fragment of MMP-2 gene

Was polymerized by using by PCR using forward 5'- CTT CCTTAGGCTGGTCCTTACTGA– 3' and reverse 5'- CTGAGACCTGAAGAG CTA AAG AGCT– 3' primers. Genomic DNA (200ng) was amplified in 25µl (PCR master mix 12.5 µL, Forward primer 0.8 µL,Reverse primer 0.8 µL,DNA 2 .0µL, Distilled water 8.9 µL). After the DNA was denatured for 5 minutes at 94^{o}C, the reaction mixture was subjected to 37 cycles of denaturation for one minute at 94^{o}C, annealing at 60.5^{o}C for 1 minute and extension for 1minute at 72^{o}C. Final extension was carried at 72^{o}C for 10 minutes. MMP-2 gene polymorphism was detected by digestion of the PCR amplified product with 7.5 units of Bfa1 restriction enzyme (New England Biolabs) for 4 hours followed by size fractionation in 3% Ethidium bromide stained Agarose Gel Electrophoresis. C allele does not have the restriction, hence will yield a 188 bp fragment. T allele has the restriction site, hence gets cleaved to give 162bp and 26bp fragment. Heterozygous individuals (CT) allele gets cleaved to give 188bp, 162bp, 26bp fragments. Analysis was done using a low molecular weight DNA ladder (100 bp).

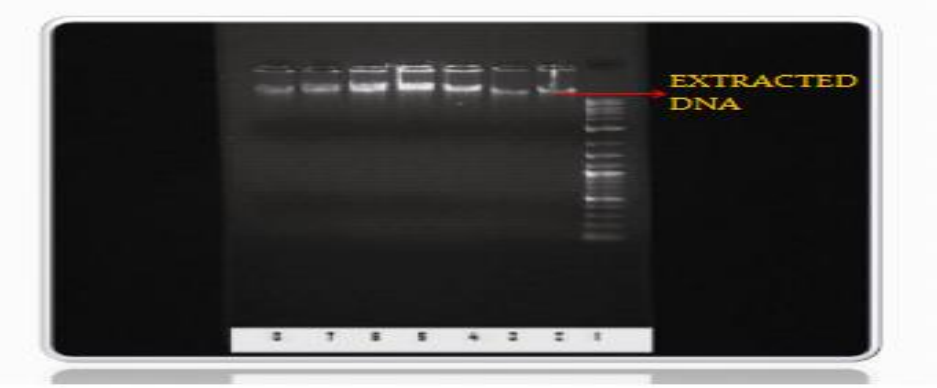

Fig-1:DNA extraction by modified high salt method

Figure 1.N shows extracted DNA(lane 2 to 8) tested on 0.8% agarose gel using 1kbp ladder(lane 1)Ladder shows 10000, 8000, 7000, 6000, 5000, 4000, 3000, 2000 and 1000 bp fragments.

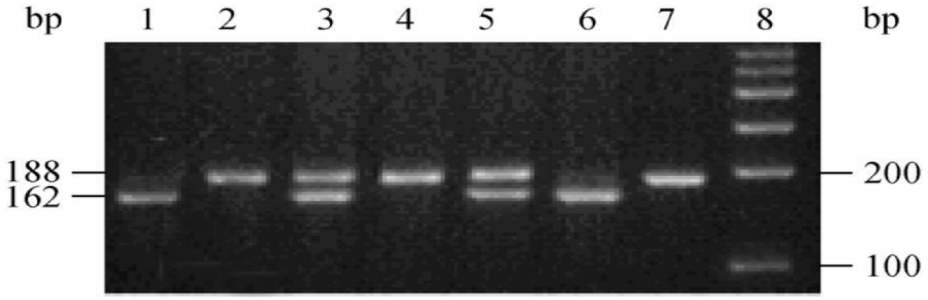

Fig-2: Restriction digestion products

Fig 2. shows genotype analysis done on 2%agarose gel electrophoresis using 100bp DNA ladder (lane1) Lane8-Ladder Lane (1,6)-TT Lane (2,4,7)-CC, Lane (3,5)-CT

STATISTICAL ANALYSIS

Allele frequencies were calculated by allele counting .Age , BMI, serum lipid levels were compared between control subjects and cases by students t test. Genotype frequency distribution between cases and controls were compared with a $\chi^{2\ test}$. MMP-2 activity was compared between control and cases by Student t test $p<0.05$ was considered significant.MMP-2 activity for both cases and controls was entered into a Microsoft Excel Spread Sheet. MMP-2 activity was compared between MMP-2 genotypes by using one-way ANOVA. Independent variables included in the analysis were age, sex, smoking, Alcoholism, Hypertension, Diabetes, Serum Levels of Cholesterol and Triglycerides.

Table 1: Characteristics of patients with mi and of controls

Variables	Case	Control	P value
Age	55.0±8.8	55.0±7.6	0.273 –NS
Sex: Male	85(85%)	85 (85%)	0.495 –NS
Female	15 (15%)	15 (15%)	0.495-NS
DM	34 (34%)	44(44%)	0.094–NS
HT	45(45%)	35 (35%)	0.149 –NS
SMK	54(54%)	48(48%)	0.991 -NS
ALC	46(46%)	42(42%)	0.565-NS
BMI	25.69±0.57	24.91±5.80	0.638-NS
CHOLESTEROL	184.73±5.05	158.47±4.25	0.001-S
TRIGLYCERIDES	162.01±8.40	128.45±5.04	0.001-S
HIGH DENSITY LIPOPROTEIN	39.21±1.90	45.98±1.84	0.004-S
LOW DENSITY LIPOPROTEIN	108.25±4.77	83.29±4.97	0.001-S

Table 2: Genotype distribution of human mmp-2 gene

			Type		Total	P Value
			Cases	Control		
MMP2_Genotype	CC	Count	76	59	135	
		% within MMP2_Genotype	56.3%	43.7%	100.0%	Chi – sq. = 6.87 P = 0.032
	CT	Count	16	30	46	
		% within MMP2_Genotype	34.8%	65.2%	100.0%	
	TT	Count	8	11	19	
		% within MMP2_Genotype	42.1%	57.9%	100.0%	
Total		Count	100	100	200	
		% within MMP2_Genotype	50.0%	50.0%	100.0%	

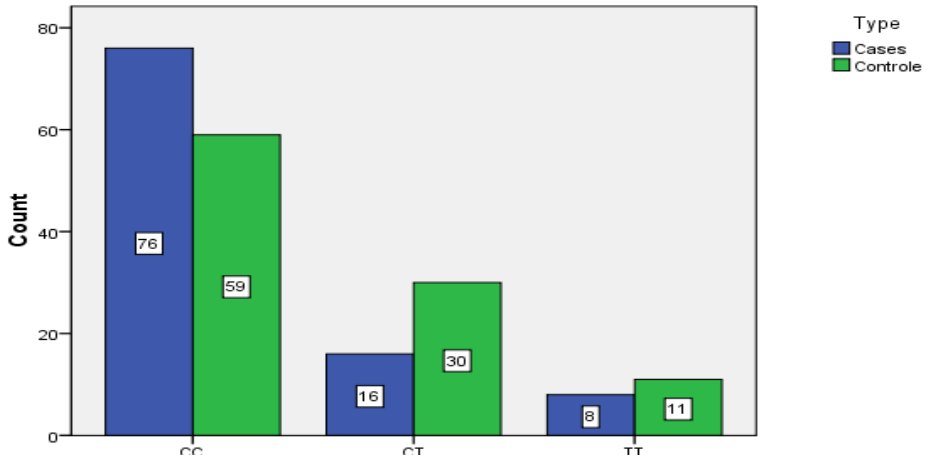

Fig-3: Genotype distribution of mmp-2 gene

Table 3: Comparison of mmp-2 activity among cases and controls

Variable	Case	Control	P value
ACTIVITY (ng/mL)	698.02±178.05	481.35±90.45	0.001 –S

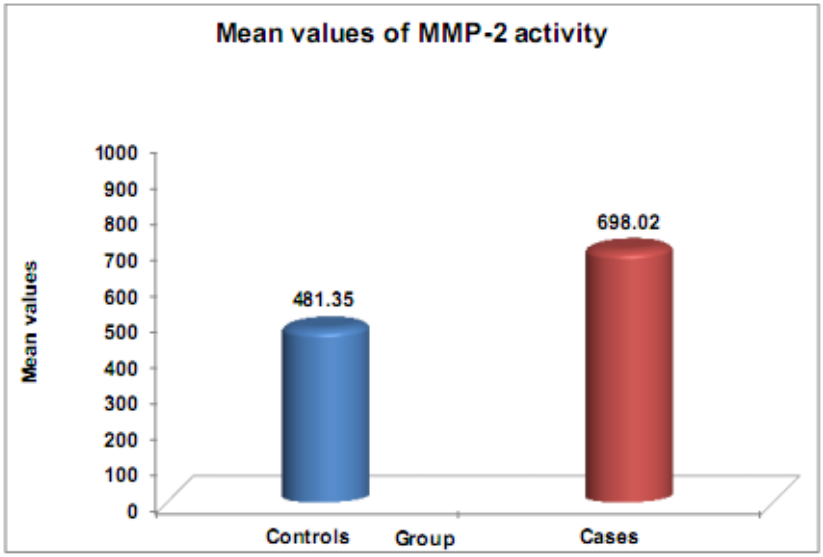

Fig-4: Comparison of mmp-2 activity among cases and controls

Table 4: Relationship between mmp-2 activity and genotype

Genotype	MMP-2 Activity(ng/ml)	P Value
CC	655±174	0.0001-S
CT	472±78	0.0001-S
TT	402±42	0.0001-S

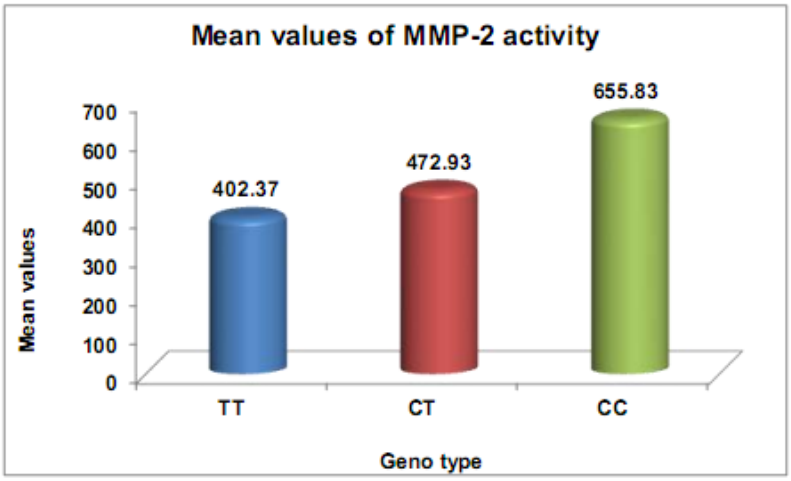

Fig-5: Relationship between mmp-2 activity and genotype

RESULTS

Table 1 shows Age , Sex , BMI , High Density Lipoprotein levels and conventional risk factor distribution among cases and control subjects. We obtained a non-significant p value with respect to all the confounding variables like age, sex, BMI, history of diabetes, hypertension, smoking and alcoholism. There was a significant difference in Total cholesterol levels (high in cases 184.73±5.05), low in controls 158.47±4.25), Triglycerides level (high in cases 162.01±8.40),low in controls 128.45±5.04), High Density Lipoprotein level (low in cases 39.21 +1.90),high in controls 45.98±1.84), Low Density Lipoprotein level (high in cases 108.25±4.77), low in controls (83.29±4.97).Table 2 shows Genotype distribution of human matrix metallo proteinase-2 gene in patients with MI and control subjects.

Table 2 shows that CC genotype was more frequent among cases (76%) when compared to controls (59%). In contrast TT genotype was more common among controls (11%) when compared to cases (8%). Distribution of CT genotype is visualized in both cases (16%) and controls (30%). P value was 0.032. The Allele frequencies were CC=135, CT=46, TT=19. This was found to be in Hardy Weinberg equilibrium. Table 3 shows the comparison of Matrix Metalloproteinase-2 activity among cases and controls. Significantly high MMP-2 activity was observed among cases (698± 178) when compared to controls. (481 ± 90). P value was < 0.001. Table 4 shows the difference in MMP-2 activity between CC, CTgenotype and TT genotype. The activity was significantly higher among CC genotype individuals (655±174) when compared to CT (472± 78) and TT (402 ± 42) genotype individuals p value was < 0.001.

DISCUSSION

Genetic factors and various environmental factors in combination lead to the development of MI. The susceptibility to MI is a complex trait [14]. This study was conducted to determine the association of MMP-2 polymorphism and its related MMP-2 activity with Myocardial Infarction. The three human MMP-2 genotypes and phenotypes were determined in 100 patients with MI confirmed by angiography and 100 control subjects.

The insignificant p value with respect to all the confounding variables like age ,sex, BMI, history of diabetes, hypertension, smoking and alcoholism, showed that the cases and controls groups were perfectly matched. The plasma MMP-2 levels were increased in cases when compared to controls. The significantly high MMP-2 levels in cases(698±178) and low levels in controls (481±90) p (<0.001) re-emphasizes the fact that it causes atherogenesis and plaque rupture.

When genotype analysis was performed , distribution of CC genotype was significantly higher among cases (76%) when compared to controls (59%). P value (0.032) showing its significance. This indicates that CC genotype is an independent risk factor for atherosclerotic plaque rupture. The evidence available showed that there is a significantly high MMP-2 activity among cases (698±178) when compared to controls (481±90). P value was less than 0.001. This shows that high MMP-2 activity is an independent risk factor for atherosclerosis.

MMP-2 activity when compared between MMP-2 genotypes there was a significantly high MMP-2 activity among CC genotypic individuals (655±174) and CT genotypic individuals (472±78), when compared to TT genotypic individuals (402±42). p value was (<0.001), suggesting the fact that CC genotype is associated with high activity and this high activity makes a person more susceptible to atherosclerosis. Hence CC genotype and the resultant high MMP-2 activity can be considered as an independent risk factor for atherosclerotic plaque rupture.

CONCLUSION

The high plasma MMP-2 activity and the CC genotype may be considered as an independent risk factor for Myocardial Infarction. MMP-2 activity can be used as a parameter for assessing Myocardial Infarction risk. MMP-2 activity can also be used to assess the outcome of atherosclerotic plaque rupture.

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