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EVALUATION OF THE ROLE OF MATRIX METALLOPROTEINASES IN THE PATHOGENESIS AND COURSE OF ACUTE MYOCARDIAL INFARCTION

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ABSTRACT

Matrix Metalloproteinases (MMPs), a class of Zn containing enzymes are involved in the erosion of the fibrous cap and rupture of the plaque which leads to Acute Myocardial Infarction (AMI). Though the exact mechanism of the involvement of MMPs in AMI is not clear, evidence suggest that they play a major role in different phases of AMI. Our aim was to evaluate the role of MMP2 and MMP9 in AMI patients, based on certain risk factors and also to study their variation in these risk groups and to identify which MMP among these two shows a significant variation when compared to the other so that it can be used as a specific marker for the diagnosis of AMI. For that the blood samples from three hundred AMI patients and hundred sex and age matched control subjects were analysed for MMP2 and MMP9 by sandwich enzyme immuno assay and the values were noted. It is found that both MMP2 and MMP9 were found to be significantly elevated in all the AMI patients irrespective of the risk factors taken into account. However MMP 9 shows a considerable elevation when compared to MMP2, which indicates its potential role in AMI, and hence can be used as a marker for the disease.

KEYWORDS: ***Atherosclerosis, Acute Myocardial Infarction, Matrix Metalloproteinases, Extra Cellular Matrix***

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INTRODUCTION

Acute myocardial infarction (AMI), an important manifestation of coronary artery disease (CAD) is the most prevalent cause of premature death in advanced societies[1]. It is an outcome of the remodeling of the vascular wall due to erosion or uneven thinning of the fibrous cap due to the formation of unstable plaque through the degradation of the collagenous fibers and conjunctive tissue[2]. Recent studies have suggested that the different phases of atherosclerosis and its sequlae acute myocardial infarction may be mediated by the family of metalloproteinases (MMPs)[3], Zn dependent physiological regulators of the extra cellular matrix. Though it is not completely certain how MMP production is induced by the extra cellular matrix, these gelatinases are necessary for infiltration of monocytes and T-lymphocytes to occur in the sub endothelial spaces[4]. Activated endothelial cells express adhesion molecules, such as vascular cell adhesion molecule – 1, which promotes infiltration of circulating monocytes and T-lymphocytes. Adhesion of these cells to the endothelial cells could induce production of MMP2 (72 KD, Gelatinase A), which facilitates breakdown of the extra cellular matrix[5]. More over contact with type I collagen and laminin in the matrix increases expression of MMP9 (92 KD, Gelatinase B). Inter leukin-1 (IL-1) secretion can activate pro-MMP2 and pro-MMP9 produced by smooth muscle cells, which could lead to endothelial disruption or intra plaque hemorrhage[4]

Though the molecular mechanism of the regulation of MMPs in AMI is still unclear, it is generally considered that ECM degradation is a major risk factor for unstable plaque in which ECM degradation is remarkably enhanced[6]. Hence the present study was undertaken to estimate the amount of MMP2 and MMP9 in AMI patients based on certain risk factors and to identify whether they are elevated in all the AMI patients, and if so, in all the risk groups or particularly in certain risk groups. Further the study compares the variation between these two MMPs and which MMP is elevated more when compared to the other, so that it can be used as a specific marker for the early diagnosis of AMI.

MATERIALS AND METHODS

(i) Patients

All patients admitted to the Intensive Coronary Care Unit (ICCU) of Amala Cancer Hospital with a diagnosis of acute myocardial infarction presenting within 24 hours of onset of chest pain were included in the study. Present study included 300 AMI patients and 100 sex and age matched control subjects. Myocardial infarction was diagnosed by at least 0.1-mv ST segment elevation in two or more contiguous limb leads or 0.2-mv ST segment elevation in two or more chest leads associated with typical chest pain. Patients with cardiogenic shock, cerebrovascular accident and significant hepatic or renal diseases were excluded. Patients with clear evidence of infection anywhere in the body were also excluded. Written informed consent was obtained from each subject and the study was approved by the institutional ethics committee.

In patients included in the study, detailed history was taken and complete physical examination was carried out. A 12 lead ECG with V_3R and V_4R was recorded immediately on admission and repeated after 2hrs, 6hrs, 12hrs, 24hrs, 48hrs and pre-discharge. 10 ml of blood was drawn from the peripheral vein on admission for biochemical analysis. Serial creatine kinase assays were done to confirm the myocardial infarction and it was done at 2hrs, 6hrs, 12hrs, 24hrs, and 48hrs after admission. Routine hematological parameters and lipid profile were also done. For the MMP assays, the blood was collected in heparinised tubes. After centrifugation the plasma samples were frozen and stored at -80^{o}C until the MMP assay was carried out. Chest X- ray was done at the time of discharge from the ICCU. An echocardiographic examination was performed at the time of

discharge or at the end of the first week or early second week after admission. All patients were seen in the cardiology out-patient department 4 to 6 weeks after discharge, when a detailed history was taken and complete physical examination was again carried out. A symptom limited treadmill test was done as per the Bruce protocol and maximum heart rate (HR), blood pressure (BP), double product, time to 1 mm ST depression, metabolic equivalences (METs) achieved, duration of exercise, angina, dyspnoea and arrhythmias were recorded along with ST segment changes.

The normal volunteers had no past history or evidence of cardiovascular disease, hypertension or diabetes mellitus. The present study does not include control subjects with a history of neoplastic, hepatic, infectious or autoimmune disease or any surgical procedure in the preceding 6 months.

The patients were allowed to relax and on the second day they were subjected to an oral questionnaire as described in our Proforma, in order to collect the history of these patients. They were asked about the type of chest pain, the time of onset of the pain, radiation to other parts of the body and any previous history of chest pain. They were also asked about the symptoms associated with the chest pain, history of diabetes, history of hypertension, habits of smoking or alcohol or pan chewing, food habits (vegetarians or non vegetarians) and any positive family history of AMI. Then these patients were categorized according to the following risk factors and based on these risk factors the study has been designed.

1) Age and sex
2) Time of onset of chest pain
3) History of diabetes and hypertension
4) A cholesterol value of $\leq$200 mg/dl and >200 mg/dl
5) Habits of smoking and alcohol intake
6) Food habits and a positive and a negative family history of AMI

(ii) Methods

The assay of MMPs are based on the principle that the antibodies immobilized on a bead matrix, in combination with enzyme-labeled antibodies, directed against different antigenic sites on the same MMP molecule[7,8]. Upon addition of an MMP containing specimen, an MMP molecule gets sandwiched between the solid phase and enzyme labeled antibodies. After removing unbound enzyme-labeled antibody, the bead containing the sandwich is incubated with enzyme substrate and O-phenylenediamine, resulting in the development of color. The activity of the peroxidase enzyme is proportional to the amount of antigen, MMP, so that MMP concentration in specimens can be determined from a standard curve.

The assay mixture contained 50µl of standard or specimen with 300µl enzyme labeled antibody solution and one anti-MMP coated bead. The mixture was incubated at 17-27^{0}C for 1 hour. The reaction was then stopped by the addition of 3ml of washing solution and it was aspirated and this was repeated at least 3 times. Each washed bead was then transferred in to a clean fresh tube and 300µl of coloring solution was added and incubated at 17-27^{0}C for 1 hour. The reaction was then stopped by the addition of 1.5ml of stop solution. Using deionized water as blank, the absorbance for the standard curve solutions and specimens were taken at 492 nm (A_{492}).

(iii) Statistical analysis

The statistical analysis was done by using Sigmastat version 2 and the values obtained were expressed as mean $\pm$ SD . Statistical significance was found out by using the 'z- test' and by the analysis of variance (ANOVA). The 'z' value was determined and the results obtained by 'z- test' were expressed in terms of probability (p). 'z' value $\geq$ 2.88 ($p<0.01$) and $\geq$1.96 ($p<0.05$) were considered to be statistically significant. The inter group comparison has been done by using the one-way analysis of variance (ANOVA) using the same programme.The results of ANOVA were expressed in terms of alphabets. The same alphabets denote the homogeneity between the values and different alphabets denote the heterogeneity between the values. Always the

highly significant value is expressed by the alphabet 'a' followed by b, c and d. The'd' denotes the least significant value among the four.

RESULTS

Table 1 represents the values of MMP2 and MMP9 in both normal as well as in AMI patients. When compared to the normal individuals the values of both MMP2 and MMP9 were found to be increased significantly ($p<0.01$) in AMI patients.

Table: 1
Values of MMP2 and MMP9 in normal and in AMI patients

Parameters	Groups	
	Normal (n = 100)	AMI (n = 300)
MMP 2 (ngm/ml)	468.34 ± 46.74	899.84 ± 140.02 **
MMP 9 (ngm/ml)	30.17 ± 6.59	96.32 ± 23.89 **

Values are mean ±SD, **p<0.01

Table: 2
Values of MMP2 and MMP9 in normal and in AMI patients according to the age and sex.

Parameters	Age (years)						Sex			
	< 40		40 - 60		> 60		Male		Female	
	Normal (n=22)	AMI (n=27)	Normal (n=54)	AMI (n=160)	Normal (n=24)	AMI (n=113)	Normal (n=85)	AMI (n=261)	Normal (n=15)	AMI (n=39)
MMP2 (ng/ml)	497.68± 48.22	908.07± 136.38**	460.41± 44.57	893.35± 151.85**	459.29± 38.58	907.06± 121.78	471.26± 47.70	895.08± 143.99**	451.80± 36.75	931.69± 104.40
MMP9 (ng/ml)	31.18± 6.59	120.21± 20.21^{a**}	30.54± 7.18	95.05± 25.34^{b**}	28.38± 6.07	92.41± 18.83^{bc**}	30.57± 6.74	98.29± 24.50^{a**}	27.85± 5.11	83.14± 13.19^{b**}

Values are mean ±SD, **p<0.01
Values are mean ± SD, **p<0.01, Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogeneous values.

The values of MMP2 and MMP9 in normal as well as in AMI patients according to the categorization of age and sex are given in table 2. In all the three age groups (<40 years, 40-60 years and >60 years) with AMI, both MMP2 and MMP9 were found to be increased ($p<0.01$) when compared to their normal controls. Among the three age groups (intra groups with AMI) MMP9 showed a statistically significant elevation in <40 years age group when compared to the other two groups. However, MMP2 did not show any significant alteration within these groups. MMP2 and MMP9 were found to be elevated both in males and females with AMI when compared to their normal controls ($p<0.01$). When AMI males were compared with AMI females, MMP9 was found to be more in AMI males than in AMI females.

Table 3 represents the values of MMP2 and MMP9 in normal as well as in AMI patients based on the time of onset of chest pain. When compared to normal all the four groups showed statistically significant elevation in MMP2 and MMP9 ($p<0.01$). Within the group the patients

with an onset of chest pain at 12 midnight to 6 am showed an elevation in the value of MMP9 when compared to the other three groups. MMP2 showed a statistically significant elevation in patients with the onset of chest pain at 6pm to 12 midnight.

Table: 3

Values of MMP2 and MMP9 in normal and in AMI patients according to the time of onset of chest pain.

Parameters	Time of Onset of Chest Pain				
		AMI			
	Normal (n=100)	12midnight to 6am (n=50)	6am to 12 noon (n=94)	12noon to 6pm (n=90)	6pm to 12midnight (n=66)
MMP2 (ng/ml)	468.34 ± 46.74	922.92 ± 174.71^{ab**}	868.04 ± 148.58^{cd**}	898.38 ± 111.32^{abc**}	929.65 ± 122.14^{a**}
MMP9 (ng/ml)	30.17 ± 6.59	113.19^{a**} ± 25.11	91.57 ± 25.15^{cd**}	91.91 ± 18.39^{c**}	96.31 ± 21.91^{b**}

Values are mean ± SD, **p<0.01, Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabet denote heterogeneous values.

Table 4 represents the values of MMPs in normal as well as in AMI patients with diabetes and hypertension. Both MMPs were increased in diabetic as well as in nondiabetic AMI patients, and hypertensive and nonhypertensive AMI patients when compared to their normal controls respectively. MMP9 values are significantly elevated in hypertensive AMI patients when compared to nonhypertensive AMI patients, where as MMP2 did not show any significant difference between the groups. Both MMP2 and MMP9 did not show any significant change in diabetic AMI patients when compared to nondiabetic AMI patients

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Table : 4

Values of MMP2 and MMP in normal and in AMI patients according to the history of Diabetes and Hypertension.

Parameters	Normal (n=100)	AMI			
		Diabetes		Hypertension	
		Diabetic (n=101)	Non diabetic (n=199)	Hypertensive (n=62)	Non hypertensive (n=238)
MMP2 (ng/ml)	468.34 ± 46.74	876.80 ± 175.17**	911.54 ± 116.51**	875.88 ± 154.62**	906.08 ± 135.26**
MMP9 (ng/ml)	30.17 ± 6.59	97.89 ± 25.41**	95.52 ± 23.04**	104.65 ± 28.27^{a**}	94.15 ± 22.10^{b**}

Values are mean ± SD, **p<0.01

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogeneous values.

Table 5 shows the values of both MMP2 and MMP9 in AMI patients with the cholesterol value >200 mg/dl and $\leq$ 200 mg/dl. The values of both MMP2 and MMP9 were found to be statistically

(p<0.01) elevated in both these groups when compared to the normal controls. The comparison of AMI patients with cholesterol value >200 mg/dl with cholesterol value ≤200 mg/dl have shown the elevated value of MMP9 in AMI patients with cholesterol value >200 mg/dl. However, the value of MMP2 did not show any significant difference between these two groups.

Table 6 represents the values of MMPs in normal as well as in AMI patients with the habit of smoking and intake of alcohol. Here both the values of MMP2 and MMP9 were found to be increased significantly (p<0.01) in AMI patients both with and without the habit of smoking as well as intake of alcohol when compared to their normal controls. A comparison of the AMI patients with the habit of smoking with AMI patients without the habit of smoking has shown the elevated value of MMP9 in AMI patients with the habit of smoking. MMP2 is not significantly different in either of these groups. The values of MMP2 and MMP9 did not show any significant difference in alcoholic AMI patients when compared to nonalcoholic patients.

Table: 5

Values of MMP2 and MMP9 in normal and in AMI patients according to the value of Cholesterol

Parameters	Cholesterol (mg/dl)		
	Normal (n=100)	AMI	
		> 200 (n=105)	≤ 200 (n=195)
MMP2 (ng/ml)	468.34 ± 46.74	879.36 ± 172.70**	910.87 ± 117.28**
MMP9 (ng/ml)	30.17 ± 6.59	118.14 ± 21.91[a**]	84.57 ± 15.01[b**]

Values are mean ± SD, **p<0.01,
Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogeneous values.

Table : 6

Values of MMP2 and MMP9 in normal and in AMI patients with the habits of Smoking and Alcohol intake.

Parameters	Smoking				Alcohol intake			
	Yes		No		Yes		No	
	Normal (n=62)	AMI (n=222)	Normal (n= 38)	AMI (n=78)	Normal (n= 20)	AMI (n=160)	Normal (n= 80)	AMI (n=140)
MMP2 (ng/ml)	473.64 ± 51.76	891.28 ± 144.63**	459.68 ± 35.45	924.21 ± 122.76**	505.15 ± 44.01	894.04± 155.89**	459.13 ± 42.71	906.47 ± 118.97**
MMP9 (ng/ml)	30.96 ± 6.48	99.60 ± 25.76[a**]	28.86 ± 6.57	86.98 ± 13.75[b**]	31.88 ± 6.57	96.70 ± 13.74**	29.74 ± 6.91	95.88 ± 22.06**

Values are mean ±SD, **p<0.01,
Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogeneous values.

Table 7 represents the values of MMP2 and MMP9 in normal as well as in AMI patients with different food habits as well as with and without the history of AMI. When compared to the normal the vegetarian AMI patients and the nonvegetarian AMI patients showed elevated values of both MMP2 and MMP9 ($p<0.01$). However the comparison of nonvegetarian AMI patients with the vegetarian AMI patients have shown a significant difference for MMP9 only. AMI patients with and without the family history of AMI showed elevated values of both MMP2 and MMP9 when compared with normal controls ($p<0.01$). A comparison between AMI patients with and without the history of AMI have shown significant elevation of MMP9 in AMI patients without the history of AMI. MMP2 did not show any significant difference.

Table : 7

Values of MMP2 and MMP9 in normal and in AMI patients according to the food habits and family history of AMI.

Parameters	Food habits				Family history		
	Vegetarians		Non Vegetarians		Normal (n=100)	AMI	
	Normal (n=16)	AMI (n=27)	Normal (n=84)	AMI (n=273)		+ve (n=82)	-ve (n=218)
MMP2 (ng/ml)	454.81 ± 37.45	922.25 ± 97.58**	470.91 ± 47.88	897.63 ± 143.34**	468.34 ± 46.74	904.62 ± 130.86**	898.05 ± 143.27**
MMP9 (ng/ml)	27.46 ± 5.17	84.01 ± 14.17^{b**}	30.68 ± 6.71	97.54 ± 24.28^{a**}	30.17 ± 6.59	85.87 ± 21.23^{b**}	100.25 ± 23.65^{a**}

Values are mean ±SD, **p<0.01,

Inter group comparisons were done by one way ANOVA. Same alphabets denote homogenous values and different alphabets denote heterogeneous values.

DISCUSSION

Irrespective of the risk factors taken into account, both MMP2 and MMP9 are found to be significantly elevated in all the AMI patients when compared to normal healthy individuals, indicating increased proteolysis in the plaques due to an enhanced expression of these MMPs. MMP system components are expressed in atherosclerotic tissues and in their active form they may contribute to vascular remodeling and plaque disruption[9] However there are certain important findings obtained from this study. Out of these two MMPs, it is MMP9 which has been predominantly elevated in most of the risk groups taken for the study when compared to MMP2, indicating the specific role of MMP9 in AMI. This may be due to the availability of the substrates of MMP 9, which include type IV collagen and elastin. Type collagen is an essential component of the plaque basement membrane and fibrous cap of the plaque. Thus MMP9 plays a significant role in the progress of extra cellular matrix degradation in the plaques[10]. MMP9 showed a statistically significant elevation in AMI patients with age <40 years when compared to other two age groups. This is an indication of the involvement of MMP 9 in the early onset of AMI in younger generation due to the focal accumulation of cells that over express activated forms of MMPs, especially MMP9, which may promote local destruction of ECM in atheroma, leading to plaque destabilization and rupture[11] leading to severe AMI. Further its value is significantly increased in AMI males when compared to AMI females. A variation in the elevation of MMP2 and MMP9 with respect to the onset of chest pain shows the early release of MMP9 from the tissues into the blood stream than the MMP2 as indicated by the time of onset of chest pain, which occur as an outcome of infarction followed by angina due to plaque rupture by the MMPs.

MMP9 has considerably elevated in hypertensive AMI patients when compared to MMP2 and is increased in patients with cholesterol value >200mg/dl. These results again support the potential role of MMP9 in AMI. MMP9 was significantly elevated in patients with the habit of smoking, while MMP2 did not show significant alteration between smoker and non smoker AMI patients. More over MMP9 was elevated in patients consuming non vegetarian food and with no family history of AMI. The significant alteration of MMP9 in these subgroups when compared to that of MMP2 may be due to the increased expression of MMP9 in response to the risk factors at the genetic level. Further, the substrate of MMP9 (type IV collagen) is more in the atherosclerotic plaque and that can also contribute to the increased activity of MMP9. The monocyte interaction with collagen and platelets is required for the leukocytes to synthesize MMP9 and that the interactions are produced particularly in areas of the vessel where inflammatory phenomena develop in response to injury[12]. Hence MMP9 can be considered as a potential indicator of AMI. So in comparison with MMP2, MMP9 can be used as a marker for the diagnosis of AMI along with other cardiac markers.

CONCLUSION

Thus the present study indicates elevated levels of MMP2 and MMP9 in all the acute myocardial infarction patients. MMP9 has shown a considerable elevation in its activity than MMP2 in different risk groups studied. Hence MMP9 can be used as a diagnostic marker for Acute Myocardial Infarction.

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