Original article

Levels of cathepsins in acute myocardial infarction

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KEYWORDS

Acute myocardial infarction
Cathepsins
Cystatin C
Stable angina

ABSTRACT

Aims/objective: Over expression of matrix degrading enzymes have been implicated in plaque destabilisation and rupture. Cathepsins associated with extracellular matrix breakdown make them intriguing suspects. The aim of the study was to analyse peripheral levels of cathepsin B and cathepsin K and their inhibitor cystatin C during acute myocardial infarction (AMI).

Materials and methods: Study population included AMI patients at acute event (AMI group, $n=48$), stable angina patients (stable angina group $n=17$), and healthy individuals (Control group, $n=31$). Cathepsin B, cathepsin K, cystatin C, and matrix metalloproteinases (MMP)-9 were analysed by enzyme-linked immunosorbent assay (ELISA) method.

Results: Cathepsin B (45.9%) and cathepsin K (92.31%) at acute event of myocardial infarction (AMI group) increased ($P=0.001$) while cystatin C decreased marginally (12.5%) as compared to controls. Stable angina group, demonstrated only marginal reduction in all the parameters studied as compared to controls.

Conclusion: Cathepsin B and cathepsin K can be further evaluated as biomarkers in identifying high-risk individuals for AMI.

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Introduction

In spite of the current modern diagnostic and treatment approaches to combat acute myocardial infarction (AMI), the morbidity and mortality associated with this disease remains very high. More than a decade of advances in our understanding of the complex mechanisms underlying the initiation and progression of atherothrombosis and its complications has stimulated efforts to identify and characterise new markers associated with this disease. Recent investigations have been directed towards analysing components, involved in the pathogenesis of AMI, upstream from biomarkers of necrosis, one of them being components of plaque destabilisation and rupture.

Pathoanatomic studies have described major determinants of a plaque's vulnerability and rupture.¹ They are lack of smooth muscle cells, impaired healing, size of the lipid-rich core, inflammation with plaque degradation, thin fibrous cap,

inflammatory cell infiltration of the fibrous cap and the adventitia, positive arterial remodelling around the plaque, and increased plaque neovascularity. Protease contribution to these processes is supported by many experimental studies. Over expression of serine proteases, matrix metalloproteinases (MMP), and their respective inhibitors have been implicated in atherogenesis.² Potential significance of imbalance between matrix degrading enzymes, cathepsins, and their most abundant inhibitor, cystatin C have been highlighted in human atherosclerosis and aortic aneurysms.^{3,4} Specifically in the atherosclerotic lesions, increased expression of cathepsin S, B, K, D, and L has been reported.^{5–10} In Extracts of human atheroma as well as cytokine stimulated smooth muscle cells (SMCs), increased elastolytic activity has been observed which could be inhibited up to 40% by cysteine protease inhibitors.^{5,6} The above findings imply that these cathepsins may play a role in plaque destabilisation and rupture.

Beside protease function and vascular effects, protease detection and quantisation in peripheral blood may help detect atheromatous disease stages and aid in clinical decision-making. Among lysosomal cysteine proteases, patients with

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coronary artery stenosis have been demonstrated to have increased serum cathepsin L levels than those without lesions detectable by quantitative angiography¹¹ and serum cathepsin S levels are reported to be elevated in patients with atherosclerosis and diabetes.¹² Increased lysosomal aspartyl protease, cathepsin D, has been demonstrated both in plasma and monocytes of acute coronary syndrome (ACS) patients.¹³

Other important enzymes of lysosomal cysteine proteases are cathepsin B and cathepsin K. Jormsjö et al.⁶ have demonstrated expression of cathepsin B along with D and L in macrophage-derived foam cells localised in the necrotic cores of plaques and in the fibrous cap. In another experiment by Chen et al.,⁸ cathepsin B mRNA and protein levels were found to be increased in atherosclerotic lesions of apo-E deficient mice and cathepsin B immuno-reactivity was shown to be highest in the areas next to the lumen and macrophages. Cathepsin K expression was demonstrated to be high in macrophages and SMCs of the fibrous cap of advanced atherosclerotic plaques as compared to the intima and medial SMCs of normal arteries and in early atherosclerotic lesions.^{5,9}

There are no reports as yet on circulating levels of cysteine proteases, cathepsin B that exhibits elastolytic activity and cathepsin K that harbours collagenolytic activity in AMI. In the present study, aim was to analyse circulating levels of these enzymes and their inhibitor cystatin C from serum of AMI patients and correlate with degree of severity of the disease.

Materials and methods

Study population

Subjects in the age group of 25–75 years were selected for this study. Patients were categorised into two groups as reported previously.¹⁴ The AMI Group group ($n=48$) at presentation and stable angina group ($n=17$). Control group included healthy individuals ($n=31$).

Acute myocardial infarction group: The AMI patients were those admitted in the emergency department of cardiac unit with clinical diagnosis of myocardial infarction (MI) with prolonged chest pain more than 30 minutes and ST-segment elevation >0.1 mV on at least two adjacent electrocardiogram (ECG) leads. All these patients had their first episode of chest pain and they were not on any medications for the same. Their blood samples were collected in emergency department at the time of admission before administration of any treatment for routine analysis such as electrolytes, cardiac enzymes, complete blood count, and prothrombin time. The remaining serum was stored at -80°C and ethylene diamine tetraacetic acid (EDTA) blood sample at 4°C for further analysis. Thus, patients in this group were not on any medications before or at the time of collection of the blood sample. **Stable angina group:** These patients were with exertional angina, positive stress test and angiographically verified coronary artery disease (CAD) having at least 50% stenosis in at least one major coronary artery. They were on statins and antihypertensives

and antidiabetics if needed. *Exclusion criteria were:* Valvular heart disease, known cardiomyopathy, malignancy, renal, or liver diseases as well as subjects with systemic inflammatory disease, current use of anti-inflammatory (except aspirin or statin) or immunosuppressive drugs or with systemic infection. For stable angina patients, blood sample was collected prior to coronary intervention on the day of the coronary angiography. **The control group:** The controls were healthy individuals with blood pressure 135/85 mmHg or less, with no risk factors of CAD or clinical symptoms of any other organic disease. For healthy individuals, 12-hour fasting blood samples were collected. As per the selection criteria in each group, subjects were recruited with their informed consent. Information regarding their demographic status, clinical history, family history, and medication were noted down in detail. The ethical committee of Sir HN Hospital and Research Centre and Rajawadi Municipal Hospital approved the study protocol.

Methodology

The peripheral blood samples were collected in plain, sodium EDTA, sodium citrate, and sodium fluoride bulbs. One aliquot of serum was sent to the Pathology Department of the hospital to test the biochemical parameters and anticoagulated blood with sodium EDTA for haematological parameters. Those subjects with fasting glucose levels >110 mg/dL, serum transaminases, blood urea nitrogen (BUN), and creatinine levels beyond normal range or abnormal ECGs were excluded from the control group.

The other aliquot of serum stored at -80°C was used for the estimation of levels of MMP-9, cathepsin B, K, and cystatin C by the commercially available enzyme-linked immunosorbent assay (ELISA) kits with monoclonal antibodies against each, according to the manufacturer's instructions. For determination of cathepsin B and cystatin C, ELISA kits from R and D systems were used while cathepsin K was estimated by ELISA kit from Alpco Diagnostics. The minimum detectable level of cathepsin B, K, and cystatin C, were 0.016 ng/mL, 1.1 pmol/L, and 0.102 ng/mL, respectively. The intra-assay coefficient of variation of cathepsin B, K, and cystatin C were 5.5%, 5%, and 5%, respectively and inter-assay coefficient of variation were 7.5%, 7%, 7% respectively. MMP-9 estimation and analysis of all data was as described previously.¹⁴

Statistical analysis

Results are expressed as frequency and percentages, mean $\pm$ standard deviation for parametric variables and median with inter quartile ranges for non-parametric variables. For parametric variables, analysis of significance of difference between two groups was performed by student's unpaired *t*-test and by non-parametric test such as Mann Whitney U-test was applied for comparing significance between two medians. Correlations were evaluated by Spearman's rank correlation test. A *P* value <0.05 was considered statistically significant.

Table 1
Demographic and lipid profile.

	Controls	AMI	Stable angina
No. (male/female)	31 (14/17)	48 (35/13)	17 (10/7)
Age (yr)	47.6±7.57	50.7 [‡] ±10.9	57.8**±10.2
Systolic BP (mmHg)	115.5±9.7	130.2 [‡] ±27	131.5 [‡] ±3.25
Diastolic BP (mmHg)	78.7±5.7	81.2 [‡] ±25.4	87.0 [‡] ±1.77
Total cholesterol mg/dL (mmol/L)	199.63±33 (5.1±0.84)	181.5**±43.5 (4.65±1.1)	183.8 [‡] ±44.8 (4.24±1.15)
HDL cholesterol mg/dL (mmol/L)	48.1±9.97 (1.23±0.26)	39.6 [‡] ±8.2 (1.03±0.2)	41.3*±7.5 (1.01±0.19)
Total cholesterol/HDL cholesterol	4.2±0.67	4.45 [‡] ±0.66	4.51*±0.05
Triglyceride mg/dL (mmol/L)	117.3±47.2 (1.34±0.54)	136.6 [‡] ±66.9 (1.56±0.76)	141.6 [‡] ±50.3 (1.45±0.5)
LDL cholesterol mg/dL (mmol/L)	127.9±26.1 (3.30±0.67)	112*±31.3 (2.87±0.81)	111.6**±9.84 (2.88±1.07)
Smokers (%)	5 (16.1)	15 (30.6)	2 (11.1)
Alcohol (%)	5 (16.1)	9 (18.4)	1 (5.6)
Hypertension (%)	0	10 (20.4)	6 (33.3)
Diabetes (%)	0	11 (22.4)	2 (11.1)

* $P<0.05$, ** $P<0.01$, [‡] $P<0.001$, [‡]non-significant. AMI: acute myocardial infarction, BP: blood pressure, HDL: high-density lipoprotein, LDL: low-density lipoprotein.

Analyses were performed using statistical software SPSS (version 15.0, Chicago, IL).

Results

Table 1 depicts the demographic and lipid profile of all the groups. Levels of cathepsins, MMP-9, and cystatins C expressed in medians with 25th and 75th percentiles for AMI group and stable angina group are depicted in Figure 1 and Figure 2, respectively. Analysis demonstrated significant ($P=0.001$) elevation of cathepsin B (45.9%), cathepsin K (92.31%), MMP-9 (46.3%), and marginal decrease in cystatin C (12.5%, $P=0.033$) at acute phase of MI as compared to controls. In stable angina group cathepsin B (13.1%, NS), MMP-9 (25.5%, NS), cathepsin K (23.3%, $P=0.003$), and cystatin C (16.4%, $P=0.016$) decreased marginally as compared to controls. Age matched analysis demonstrated similar trend as seen overall. Within the group coronary risk factors such as presence or absence of hypertension or diabetes or habits of smoking or alcohol consumption demonstrated no variation in any parameters studied.

Within control group positive correlations were observed of cathepsin B (r_s [spearman correlation]=0.378, $P=0.040$) and cystatin C ($r_s=0.496$, $P=0.008$) with age. Within AMI group cathepsin B demonstrated strong positive correlation

Figure 1 Circulating levels of cathepsins, cystatin C, and MMP-9 in AMI group. AMI: acute myocardial infarction, MMP: matrix metalloproteinases.

Figure 2 Circulating levels of cathepsins, cystatin C, and MMP-9 in stable angina group. MMP: matrix metalloproteinases.

($r_s=0.481$, $P=0.001$) while cystatin C demonstrated negative correlation ($r_s=-0.308$, $P=0.047$) with MMP-9.

Discussion

Several characteristics of cathepsins make them intriguing suspects for causing matrix destruction in vascular disease. Initially thought to be strictly lysosomal enzymes functioning to degrade intracellular proteins optimally within acidic environment and non-functional in the extracellular spaces, they are now shown to be secreted from cells such as macrophages, SMCs, and endothelial cells.⁵ Also, macrophages are demonstrated to form cathepsin-containing acidic microenvironment outside the cell that would enable these enzymes to function. Some of these proteases are even demonstrated to function at neutral pH as well.^{5,7}

In the present study, the circulating levels of cathepsin B, K, and cystatin C in AMI patients at acute phase and in angiographically verified stable angina patients have been analysed. These 48 AMI patients were with first episode of chest pain and had neither past history of such clinical symptoms nor were on any known medications for the same. We observed elevated levels of cathepsin B and K at acute phase and marginal

decrease in their inhibitor cystatin C in AMI group. As compared to this observation, the peripheral blood levels of these enzymes and cystatin C in stable angina patients were marginally reduced as compared to controls. Moreover, plasma concentration of MMP-9, identified as a novel predictor of cardiovascular mortality in patients with CAD and also marker for plaque destabilisation and rupture,¹⁵ demonstrated strong positive correlation with cathepsin B and negative correlation with cystatin C in AMI group.

Several studies have found that increased levels of cystatin C are associated with healthy aging.¹⁶ Similar to this finding, in the present study, cystatin C demonstrated positive correlation with age in the control group. Cystatin C is also known as marker of renal function. Patients with mild renal impairment have demonstrated cystatin C to be associated with both the occurrence and the severity of CAD independent of other risk factors.¹⁷ However, in the present study, as mentioned above, patients with renal disease were excluded and cystatin C levels were in fact marginally reduced in AMI and stable angina group. This finding of the present study, however, is contradictory to the reports wherein cystatin c is also demonstrated to be associated with the risk of death and several types of cardiovascular disease (stroke, heart failure, peripheral arterial disease, metabolic syndrome, and MI).¹⁸

Thus in the present study significant elevation in the levels of cathepsin B and K was observed in patients with AMI. This preliminary pilot data provided insights into the pathophysiological contribution of extracellular proteolysis that plays a role in the development of AMI.

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