Matrix metalloproteinase-9 (MMP9) and high sensitivity C – Reactive protein (hs-CRP) in coronary artery ectasia

Waleed Ammar a,*, Mahmoud Kappary a, Yasser Baghdady a, Mohamed Shehata b

a Departments of Cardiology, Cairo University, Egypt
b Departments of Clinical Pathology, Cairo University, Egypt

Received 31 December 2012; accepted 26 April 2013
Available online 2 June 2013

Abstract  Objective: The specific causative mechanisms of abnormal luminal dilatation in coronary artery ectasia (CAE) are essentially unknown. Destruction of the extracellular matrix may be responsible for ectasia formation. Thus, we investigated the role of matrix metalloproteinases (MMP9), and inflammatory marker (high-sensitive C-reactive protein) in CAE patients.

Methods: This study consisted of 30 consecutive CAE patients, 30 obstructive coronary artery disease (CAD) patients, and 20 controls with normal coronary arteries undergoing cardiac catheterization. Plasma levels of MMP-9, and hs-CRP were measured.

Results: Hs-CRP level was significantly higher in the CAE group than both in the CAD and control groups (2.3 ± 0.5, 1.19 ± 0.54, 0.8 ± 0.3 mg/l, respectively, both p < 0.001), while, MMP-9 level was significantly higher in both CAE group and CAD than control groups (27.71 ± 4.7, 25.2 ± 4.1, 18.6 ± 3.3 ng/ml, respectively, both p < 0.001). In subgroup analyses, MMP-9 level was significantly higher in CAE patients with multivessel involvement compared to those with single-vessel ectasia (29.4 ± 3.1 vs. 25.2 ± 5.5 ng/ml, P = 0.01), while hs CRP level was comparable in both groups (2.3 ± 0.52 vs. 2.4 ± 0.45 ng/ml, P = 0.82).

Conclusion: Our results suggest that the increased levels of MMP-9, hs-CRP may be responsible for ectasia formation in patients with CAE and plasma level of MMP-9 is correlated with the severity of CAE.

© 2013 Production and hosting by Elsevier B.V. on behalf of Egyptian Society of Cardiology.

1. Introduction

Coronary artery ectasia (CAE) was defined as a dilation exceeding 1.5-fold normal diameter in major coronary arteries. It causes adverse coronary events like vasospasm, dissection, and thrombosis and was found in the range of 1.2–4.9% in different series. Extensive structural damage was observed in different layers of the vessel especially in the tunica media.
and intima in histological examinations.\textsuperscript{3,5} Therefore, CAE is considered to be a different form of vascular remodeling in response to atherosclerosis; however, the underlying mechanisms responsible for ectasia formation are clearly unknown.

Earlier studies have reported that CAE is associated with concomitant aneurysms in other vascular beds such as ascending thoracic aorta, abdominal aorta and its major branches possibly because of a common underlying mechanism. These studies have proved that development of aneurysm is associated with the destruction of extracellular matrix.\textsuperscript{6,7} A few studies investigating the proteolytic role of MMPs in CAE patients are found.\textsuperscript{8–11} These studies have shown divergent results.

Similarly, studies evaluating the role of inflammatory markers including C-reactive protein (CRP) have also demonstrated different results.\textsuperscript{9–13} Therefore, we aimed to investigate the role of MMP-9 and inflammatory markers such as high-sensitive CRP (hs-CRP) in CAE patients and the relationship between these markers and the severity of CAE.

2. Patients and methods

Eighty patients with angina pectoris and objective evidence of ischemic heart disease indicated by echocardiographic evidence of regional wall motion abnormality, positive stress tests (stress ECG, Exercise or pharmacological stress myocardial perfusion imaging or positive dobutamine stress echocardiography) and those with equivocal stress ECG and high pretest likelihood for CAD who underwent coronary angiography were selected and prospectively enrolled in the study. On the basis of their angiographic findings, they were sorted into three groups:

- Group1: 30 patients with isolated CAE (CEA group),
- Group2: 30 patients with obstructive CAD (CAD group),
- Group3: 20 patients with angiographically normal coronary arteries (Control group).

For more comparative analysis, CEA and CAD groups were further divided into those with single vessel and multivessel involvement (Fig. 1). CAD and control groups were selected on the basis of maximum available matching to the CEA group regarding age, sex, risk factors and current medications to minimize confounding factors. Medical history, atherosclerotic risk factors, and medications were interrogated in case report forms. Blood samples were obtained at least 7 days after coronary angiography to avoid the possible effect of angiography on inflammatory situations.

Patients with acute coronary syndromes, known aortic aneurysms, hematological disorders, acute/chronic infectious diseases, hepatitis or previously known inflammatory/autoimmune disorders, acute/chronic renal failure, documented cancer, use of steroids, previous PCI or CABG, Combined ectasia and obstructive CAD were excluded from the study.

All patients were provided informed consent for participation in this study.

3. Assay of proteolytic and inflammatory markers

Blood samples were drawn into tubes including EDTA after an 8-h fasting period in the morning with minimal trauma from antecubital vein. Plasma was separated by centrifugation at 3000 g for 10 min and then stored at \(-20\)°C.

According to the manufacturer’s instructions, plasma levels of MMP-9 were assayed by a specific two-site enzyme linked immunosorbent assay (ELISA) sandwich method using their specific trade kits (Biotrak MMP-9 human ELISA systems, Amersham Pharmacia Biotech, Buckinghamshire, UK). Plasma hs-CRP level was measured according to the manufacturer’s instructions (Dade Behring Inc., Marburg, Germany).

4. Angiographic evaluation

Coronary angiography was performed using standard technique. Images were recorded in digital format and stored for later analyses using Toshiba infinity model DFP-8000D catheterization machine.

Evaluations were visually performed by two experienced angiographers blinded to the patient’s biochemical data. The vessel diameter was calculated quantitatively in case of the presence of conflict about CAE.

Coronary ectasia was defined as a dilation exceeding the 1.5-fold normal diameter in major coronary arteries.\textsuperscript{1,2} A luminal narrowing greater than 50% in the coronary artery was
considered obstructive CAD. Absence of any atherosclerotic plaques was regarded as normal coronary artery.

4.1. Statistical analysis

Numerical data were presented as mean ± SD. They were compared using Student’s t-test, Mann–Whitney U test and/or ANOVA/post hoc tests when appropriate. Categorical data were presented as frequencies (%). Association between different categorical variables were done using chi square test (X²).

Receiver operating characteristic (ROC) curves were instructed with Area under the curves (AUC) for MMP9 and hs-CRP alone and for them combined were compared. Sensitivity, specificity, PVP, NPV and accuracy together with Odds ratio (OR) were calculated at chosen cut-off values. Receiver operating characteristic (ROC) curve analysis (Fig. 2), showed hs-CRP levels greater than or equal to 1.3 mg/l identified CAE patients with 96.7% sensitivity and 80% specificity(area under curve = 0.96, P < 0.001), while MMP-9 was comparable in both groups (29.4 ± 3.1 vs. 27.57 ± 2.8 ng/ml, P = 0.06).

Receiver operating characteristic (ROC) curve analysis (Fig. 2), showed hs-CRP levels greater than or equal to 1.3 mg/l identified CAE patients with 96.7% sensitivity and 80% specificity(area under curve = 0.96, P < 0.001), while MMP-9 was comparable in both groups (29.4 ± 3.1 vs. 27.57 ± 2.8 ng/ml, P = 0.06).

Receiver operating characteristic (ROC) curve analysis (Fig. 2), showed hs-CRP levels greater than or equal to 1.3 mg/l identified CAE patients with 96.7% sensitivity and 80% specificity(area under curve = 0.96, P < 0.001), while MMP-9 was comparable in both groups (29.4 ± 3.1 vs. 27.57 ± 2.8 ng/ml, P = 0.06).

5. Results

5.1. Patient characteristics, risk factors and medications

All the three groups were age, sex, risk factors and medications matched as shown in Table 1.

5.2. Hs-CRP and MMP-9 in study population

Hs-CRP level was significantly higher in the CAE group (2.3 ± 0.5 mg/l) than both in the CAD (1.19 ± 0.54 mg/l, p < 0.001), and the control groups (0.8 ± 0.3 mg/l, p < 0.001), while MMP-9 level was significantly higher in both the CAE group (27.71 ± 4.7 ng/ml) and CAD (25.2 ± 4.1 ng/ml) than the control groups (18.6 ± 3.3 ng/ml, p < 0.001).

5.3. Hs-CRP and MMP-9 in study subgroups

Subgroup analysis showed that 12 patients in the CAE group had single vessel ectasia and 18 patients had multivessel ectasia while in the obstructive CAD group 11 patients had single vessel disease and 19 patients had multivessel disease. In subgroup analyses within the CAE group, MMP-9 level was significantly higher in CAE patients with multivessel involvement compared with those with single-vessel ectasia (29.4 ± 3.1 vs. 25.2 ± 5.5 ng/ml, P = 0.01) signifying the relation of MMP-9 to the severity of CAE, while, hs-CRP level was comparable in both groups (2.3 ± 0.52 vs. 2.4 ± 0.45 ng/ml, P = 0.82).

In addition, MMP-9 and hs-CRP levels were significantly higher in CAE with single vessel involvement compared to CAD with single vessel involvement (25.1 ± 5.5 vs. 21 ± 2.1 ng/ml, P = 0.03) and (2.36 ± 0.45 vs. 1.1 ± 0.47 mg/l, P < 0.001), respectively.

Also, hs-CRP level was significantly higher in CAE with multivessel involvement compared with CAD with multivessel involvement (2.3 ± 0.52 vs. 1.2 ± 0.59 mg/l, P < 0.001), while MMP-9 was comparable in both groups (29.4 ± 3.1 vs. 27.57 ± 2.8 ng/ml, P = 0.06).

Chi-square test showed a strong association between CAE and high MMP9 level 22/30 (73.3%) vs. b35/50 (70%) with an estimated risk of (6.4 times) more than patients with normal MMP9 (OR = 6.4, 95% CI = 2.33–17.6).

Similarly, there is a strong association between CAE and high hs CRP level 29/30(96.7%) vs. 40/50(80%) with an estimated risk of 116 times more than patients with normal hs-CRP (OR = 116, 95% CI = 14.1–957.3).

6. Discussion

Histopathological characteristics of CAE are similar to those of coronary atherosclerosis, which most likely points out a common shared mechanism between CAE and atherosclerosis. The accumulation of inflammatory cells and damage of the extracellular matrix, elastin and collagen fibrils have been demonstrated in ectatic arteries. The severity of these changes may be related to the diameter of ectatic vessels. In contrast, it is unknown why the atherosclerotic process rarely leads to abnormal vessel dilatation while it most frequently results in obstructive CAD during the progression of atherosclerosis. A limited number of studies have evaluated the role of inflammatory (CRP and IL-6) and proteolytic markers (MMPs, TIMPs) in the CAE patients. They provided divergent results.

In our study, we found significantly higher levels of hs-CRP in CAE when compared to CAD and controls. Also in subgroup analysis, hs-CRP was significantly higher in the single vessel ectasia group than in the single vessel obstructive

<table>
<thead>
<tr>
<th>Variables</th>
<th>CAE group (n = 30)</th>
<th>CAD group (n = 30)</th>
<th>Control group (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50.7 ± 6.4</td>
<td>53.5 ± 6.7</td>
<td>48.3 ± 8.2</td>
<td>NS</td>
</tr>
<tr>
<td>Male/female</td>
<td>27/3</td>
<td>26/4</td>
<td>17/3</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8 (26.7%)</td>
<td>11 (36.7%)</td>
<td>6 (30%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>9 (30%)</td>
<td>12 (40%)</td>
<td>5 (25%)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking</td>
<td>24 (80%)</td>
<td>25 (83.3%)</td>
<td>12 (60%)</td>
<td>NS</td>
</tr>
<tr>
<td>B-Blockers</td>
<td>26(96%)</td>
<td>26(96%)</td>
<td>18 (82%)</td>
<td>NS</td>
</tr>
<tr>
<td>Aspirin</td>
<td>29(96.7%)</td>
<td>30(100%)</td>
<td>19 (95%)</td>
<td>NS</td>
</tr>
<tr>
<td>Nitrates</td>
<td>9 (32%)</td>
<td>12 (44%)</td>
<td>8 (36%)</td>
<td>NS</td>
</tr>
<tr>
<td>Statins</td>
<td>8 (28%)</td>
<td>11 (41%)</td>
<td>8 (36%)</td>
<td>NS</td>
</tr>
</tbody>
</table>
CAD group and in CAE with multivessel involvement compared with those CAD with multivessel involvement. These findings highlight the importance of the inflammatory process in the pathogenesis of CAE.

These findings were concordant with Turhan et al., findings in which 32 patients with isolated CAE compared to 32 patients with obstructive CAD showed significantly higher hs-CRP levels in patients with CAE.

Whereas, Finkelstein et al. found no significant difference in hs-CRP levels in CAE compared to CAD. This conflicting result may be due to confounding factors and patient’s heterogeneity including the use of statins or ACE inhibitors at the beginning of the study and these agents might suppress the inflammatory process. We avoided these confounding factors by having age, sex and risk factors matched groups.

The matrix metalloproteinases (MMPs) are a large family of zinc dependent, extracellularly acting endopeptidases, the substrates of which are proteins of the extracellular matrix and adhesion proteins. Matrix metalloproteinase-9 (MMP9), also known as gelatinase B, 92 kDa gelatinase, or 92 Da type IV collagenase. The matrix metalloproteinase family of enzymes is involved in arterial wall extracellular matrix degradation and remodeling. The latter activities have been implicated in a number of normal and pathologic processes, such as atherosclerotic lesion formation and progression, plaque destabilization and rupture, and also in plaque stabilization and healing. Konstantino et al. pointed out the prominent role of MMP9 in plaque formation, destabilization, and rupture, and postulated that MMP9 levels may serve as a biomarker for acute coronary syndrome. An association of MMP9 levels with atherosclerotic changes has been previously found in patients with atherosclerosis of the femoral artery. In a large prospective study of middle-aged men (465 cases, 1076 controls), Welsh et al. showed an association of serum MMP9 with the incidence of coronary heart disease in the general population.

The human MMP9 gene was mapped to the chromosome region 20q11.2–q13.1 and several polymorphisms of this gene were identified. Plasma levels of MMP-9 were significantly higher in CAE and CAD patients than in controls. However it was statistically insignificant in CAE compared to CAD (P = 0.06). It is worth noting that within the CAE group, MMP-9 level was significantly higher in CAE patients with multivessel ectasia compared to those with single-vessel ectasia. This finding suggests a correlation of MMP-9 level with extent and severity of CAE. Moreover, MMP-9 level was significantly higher in CAE with single vessel involvement compared with those CAD with single vessel involvement.

 Destruction of extracellular matrix by MMPs may weaken the connective tissue in the vessel wall, thereby leading to thinning and dilatation of the vascular wall. MMP-9 digests gelatin and several types of collagens. The activity of MMPs is regulated predominantly by TIMP-1. TIMPs are inactivated by both MMPs and different ILs.

Our findings agree and confirmed the results of Dogan et al.’s study in which they examined MMP-3, MMP-9, and TIMP-1 in 28 consecutive CAE patients, 27 obstructive CAD patients, and 22 controls with normal coronary arteries. MMP-9 levels were significantly higher in CAE compared with CAD and control groups suggesting that more severe inflammation may be involved in the pathogenesis of CAE.

Finally, while most experimental and clinical trials of MMP inhibitors have not demonstrated significant benefits, some trials still showed promising results. With the advent of new genetic and pharmacological tools, disease-specific MMP inhibitors with fewer undesirable effects are being developed and could be useful in the management of vascular disease.

7. Limitations

The population of our study was rather small to provide a definite conclusion, in particular subgroups’ size. Destruction of the extracellular matrix in the cardiovascular system involves enzymes other than the MMP family, such as cathepsins and cystatins. These catabolic enzymes were not assessed in our study.

8. Conclusions

Our results suggest that the increased level of MMP-9 and hs-CRP may be responsible for ectasia formation in patients with CAE, and plasma level of MMP-9 is correlated with the severity of CAE. These findings provide further evidence for the role of proteolytic and inflammatory activities in ectasia formation.

References


14. SPSS Statistics 17.0 is a comprehensive system for analyzing data. (<http://www.spss.com>).


