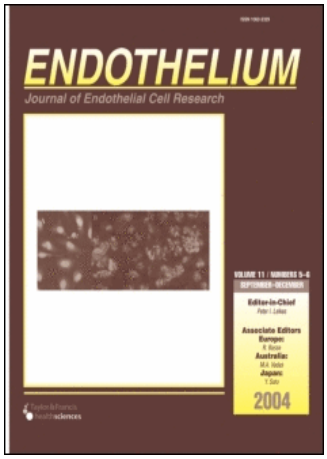

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Metalloproteinase-2 and -9 in Diabetic and Nondiabetic Subjects during Acute Coronary Syndromes

Giuseppe Derosa
Department of Internal Medicine and Therapeutics, University of Pavia, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy

Arrigo F. G. Cicero
"G Descovich" Atherosclerosis Study Center, "D Campanacci" Department of Clinical Medicine and Applied Biotechnology, University of Bologna, Bologna, Italy

Filippo Scalise
Catheterization Laboratory, Cardiovascular Department at Policlinico of Monza, Monza, Italy

Maria A. Avanzini
Research Laboratories for Pediatric Oncohematology and Immunology, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy

Carmine Tinelli
Biometric Unit, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy

Mario N. Piccinni, Emmanouil Peros, Diego Geroldi, Elena Fogari, and Angela D'Angelo
Department of Internal Medicine and Therapeutics, University of Pavia, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy

The authors hypothesized that matrix metalloproteinase (MMP)-2, -9, and tissue inhibitor metalloproteinase (TIMP)-1, -2 would be abnormal in acute coronary syndromes (ACSs). MMP-2, -9, and TIMP-1, -2 plasma levels were measured in diabetic patients with ACSs compared to nondiabetic patients with ACSs. A total of 46 diabetic and 78 nondiabetic patients with ACSs were enrolled. The following parameters were measured: body mass index (BMI), glycosylated hemoglobin (HbA_{1c}), fasting plasma glucose (FPG), fasting plasma insulin (FPI), homeostasis model assessment index (HOMA index), systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (Tg), lipoprotein(a) [Lp(a)], plasminogen activator inhibitor-1 (PAI-1), homocysteine (Hct), fibrinogen (Fg), high-sensitivity C-reactive protein (hs-CRP), and plasma levels of MMP-2, MMP-9, TIMP-1, and TIMP-2. Significant HbA_{1c}, FPG, FPI, HOMA index, DBP, Tg, Hct, and Fg increases were present in the diabetic group with ACSs, whereas hs-CRP was lower in these patients compared to nondiabetic patients with ACSs. MMP-9, TIMP-1, and TIMP-2 plasma levels were higher in diabetic patients with ACSs compared to nondiabetic patients with ACSs. MMP-9, TIMP-1, and TIMP-2 plasma levels were increased in diabetic patients with ACSs, which may reflect abnormal extracellular matrix metabolism in diabetes during acute event.

Keywords Acute Coronary Syndromes, Extracellular Matrix, Matrix Metalloproteinases, Tissue Inhibitors of Metalloproteinases

It is well-known that the main cause of acute coronary syndromes (ACSs) is in plaque disruption, with subsequent superimposed intracoronary thrombus leading to prolonged coronary obstruction (Falk et al. 1995). Either matrix metalloproteinase (MMP)-2 or -9 is synthesized and secreted locally in atherosclerotic lesions, predominantly by monocyte-derived macrophages and endothelial cells (Death et al. 2003). In addition, through their proteolytic activity, these MMPs are capable of degrading the fibrous cap of atherosclerotic plaques, thus contributing to plaque destabilization (Kai et al. 1998). Earlier studies provide evidence that high MMP plasma values are associated with the presence of ACSs (Jones et al. 2003). In particular, MMP-2

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Address correspondence to Giuseppe Derosa, MD, PhD, Department of Internal Medicine and Therapeutics, University of Pavia, Fondazione IRCCS Policlinico S. Matteo, Pavia, P. le C. Golgi, 2-27100 Pavia, Italy. E-mail: giuseppe.derosa@unipv.it

(gelatinase A) and MMP-9 (gelatinase B) have been reported to be markedly increased in patients with ACSs both within the atherosclerotic plaque and into the peripheral circulation (Brown et al. 1995).

Type 2 diabetic patients are at high risk for acute coronary events due to an increased propensity of their atherosclerotic plaques to ulceration and overlying thrombosis (Cooper et al. 2001). The most common extracellular pathology in diabetes is the thickening of the basement membrane as a result of the deposition of extracellular matrix proteins (Vranes et al. 1999). Extracellular matrix is a dynamic structure that requires constant synthesis and degradation by MMPs (Nagase and Woessner 1999). This is tightly controlled by tissue inhibitors of metalloproteinase (TIMPs) (Vincenti 2001).

The expression and activity of MMPs in diabetes thus far have been reported predominantly in relation to microvascular complications (Noda 2003; Maxwell et al. 2001). Instead, there are very little data available about the role of MMPs and TIMPs in macrovascular events in diabetic patients. To date, the only study that undertook the latter investigation has reported significantly higher levels of MMP-2 and MMP-9 in diabetic patients with coronary artery disease (CAD) compared to nondiabetics with CAD (Marx et al. 2003). Moreover, as far as it is known, there are no data on the association of MMP-2 and MMP-9 with CAD among individuals with type 2 diabetes. Therefore, in the present study we investigated whether plasma MMP-2, MMP-9, and their inhibitors TIMP-1 and TIMP-2 concentration increases are linked with acute coronary events in type 2 diabetic and nondiabetic patients, when dosed during ACSs.

MATERIALS AND METHODS

Study Design

This multicenter case-control trial was conducted in the Department of Internal Medicine and Therapeutics at University of Pavia, in the "G. Descovich" Atherosclerosis Study Center, "D. Campanacci" Clinical Medicine and Applied Biotechnology Department at the University of Bologna, and in the Catheterization Laboratory, Cardiovascular Department at Policlinico of Monza, Italy. The study protocol was approved at each site by institutional review boards and was conducted in accordance with the Declaration of Helsinki and its amendments. All patients provided written informed consent to participate.

Study Population

We recruited 124 patients of either sex admitted to the coronary care unit (CCU) with a diagnosis of ACS associated with at least one of the following findings: (1) ischemic electrocardiographic changes consisting of new (or presumably new) ST-segment depression, persistent (>20 min) ST-segment elevation, T-wave inversion; (2) elevated cardiac markers, including cardiac troponin T (cTnT) and cardiac troponin I (cTnI). They were normoweight or overweight (body mass index [BMI], 23.5 to 29.7 kg/m^2) (World Health Organization 1997).

Exclusion criteria regarding subjects with a history of impaired hepatic function (defined as plasma aminotransferase and/or γ-glutamyltransferase level higher than the upper limit of normal [ULN] for age and sex), impaired renal function (defined as serum creatinine level higher than the ULN for age and sex), severe anemia, and neoplastic, infectious, or autoimmune disease. Patients with cerebrovascular conditions within 6 months before study enrollment also were excluded.

Diagnosis of ST-segment elevation acute myocardial infarction (STEMI) was based on chest pain for 30 min and ST-segment elevation >1 mm in 2 or more contiguous leads on the 12-lead electrocardiogram (ECG). Patients with unstable angina (UA-NSTEMI) were included if they presented with recurrent chest pain at rest associated with ischemic ST-segment or T-wave changes. Most of these patients also had elevated values of creatine kinase (CK)-MB and cTnT.

Participants comprised of 90 men (72.6%) and 34 women (27.4%) aged 62.8 to 75.3 years. There were no significant differences between centers in sex distribution, age, CAD and diabetes duration, and CAD and diabetes treatment.

Cardiologic Procedures

All patients underwent coronary angiography in Judkins' technique (Judkins 1967). Two experienced blinded observers visually assessed the coronary angiographies. When needed, quantitative assessment was performed by a third blinded observer. Hemodynamically relevant CAD was defined as ≥75% area reduction with respect to prestenotic segment area in at least 1 major epicardial coronary artery or major branch (>2.5-mm diameter). Patients were classified as having 1-, 2-, or 3-vessel disease. Coronary artery territories were defined from the angiogram in patients with 1-vessel disease by using the American Heart Association/American College of Cardiology guidelines (Fox 2004).

Diet and Exercise

All diabetic patients had received dietary advice prior to enrolling in the study and were taking a controlled-energy diet (~600 kcal daily deficit), based on American Diabetes Association (ADA) recommendations (American Diabetes Association 2001), that contained 50% of calories from carbohydrates, 30% from fat (6% saturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/day and 35 g/day of fiber. Each center's standard diet advice was given by a dietitian and/or specialist physician. Individuals with no CAD were also encouraged to increase their physical activity by walking briskly or riding a stationary bicycle for 20 to 30 min, three to five times per week. The recommended changes in physical activity throughout the study were not assessed.

Laboratory Methods

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical

examination, vital signs, a 12-lead electrocardiogram, measurements of fasting plasma glucose (FPG), fasting plasma insulin (FPI), homeostasis model assessment (HOMA index), blood pressure, lipid profile, coagulation, fibrinolytic, and inflammation parameters, MMP-2, MMP-9, TIMP-1, and TIMP-2.

All plasmatic parameters were determined after a 12-h overnight fast, determined 2 h after lunch. Venous blood samples were taken for all patients between 08.00 and 09.00 and were drawn from an antecubital vein with a 19-gauge needle without venous stasis.

We used plasma obtained by addition of Na_2-EDTA, 1 mg/mL, and centrifuged at $3000 \times g$ for 15 min at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at −80°C for no more than 3 months. All measurements were performed in a central laboratory except biochemical markers for the diagnosis of myocardial injury, which were determined within 24 h of admission. cTnT and cTnI levels exceeding the upper normal limit of each local laboratory were considered as increased. Elevated CK and/or CK-MB levels within 24 h of admission were considered those values exceeding twice the upper normal limit of each local laboratory.

BMI was calculated by the investigators as weight in kilograms divided by the square of height in meters. The estimate of insulin resistance was calculated by HOMA index with the formula: [FPI (μU/mL) × FPG (mmol/L)]/22.5, as described by Matthews and coworkers (1985). Blood pressure (BP) measurements were obtained from each patient (using the right arm) in the seated position, using a standard mercury sphygmomanometer (Erkameter 3000, ERKA, Bad Tolz, Germany) (Korotkoff I and V) with a cuff of appropriate size. BP was measured by the same investigator at each visit, in the morning before daily drug intake and after the patient had rested for ≥10 min in a quiet room. Three successive BP readings were obtained at 1-min intervals, and the mean of the three readings was calculated.

Plasma glucose was assayed by glucose-oxidase method (GOD/PAP; Roche Diagnostics, Mannheim, Germany) with intra- and interassay coefficients of variation (CsV) of <2% (European Diabetes Policy Group 1999). Plasma insulin was assayed with Phadiaseph Insulin RIA (Pharmacia, Uppsala, Sweden) by using a second antibody to separate the free and antibody-bound ^{125}I-insulin (intra- and interassay CsV: 4.6% and 7.3%, respectively) (Heding 1972).

Total cholesterol (TC) and triglyceride (Tg) levels were determined using fully enzymatic techniques (Klose et al. 1978; Wahlefeld 1974) on a clinical chemistry analyzer (HITACHI 737; Hitachi, Tokyo, Japan); intra- and interassay CsV were 1.0% and 2.1% for TC measurement, and 0.9% and 2.4% for Tg measurement, respectively. High-density lipoprotein cholesterol (HDL-C) level was measured after precipitation of plasma apo B–containing lipoproteins with phosphotungstic acid (Havel et al. 1955); intra- and interassay CsV were 1.0% and 1.9%, respectively; low-density lipoprotein cholesterol (LDL-C) level was calculated by the Friedewald formula (Friedewald et al. 1972).

Plasminogen activator inhibitor-1 (PAI-1) was assayed with a commercial two-stage indirect enzymatic assay (Spectrolyse; Biopool AB, Umea, Sweden) intra- and interassay CsV were 5.9% (Juhan-Vague and Collen 1992). Fibrinogen (Fg) was determined according to Clauss. The intra-assay CV for the Fg method was less than 5% (Clauss 1959).

Homocysteine (HCT) was measured by a modified procedure of Araki and Sako (Araki and Sako 1987) with high-pressure liquid chromatography and fluorescence detection. The intra-assay CV of the method was 2.5%.

High-sensitivity C-reactive protein (hs-CRP) was measured with use of latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, Delaware). The intra- and interassay CsV were 5.7% and 1.3%, respectively (Rifai et al. 1999).

Lipoprotein (a) [Lp(a)] was measured by a sandwich enzyme-linked immunosorbent assay (ELISA), which is insensitive to the presence of plasminogen, using the commercial kit Macra-Lp(a) (SDI, Newark, Delaware) (Scanu and Scandian 1991; Uterman and Weber 1987); the intra- and inter-assay CsV of this method were 5% and 9%, respectively.

MMP-2 and MMP-9 levels and activities and TIMP-1 and TIMP-2 levels were determined by two-site ELISAs using commercial reagents (Amersham Biosciences, Uppsala, Sweden). The intra- and interassay CsV for measuring MMP-2 levels were 5.4% and 8.3%, respectively, whereas those for measuring MMP-2 activities were 5.4% and 17.9%, respectively (Fujimoto et al. 1993a). The intra- and interassay CsV to evaluate MMP-9 levels were 4.9% and 8.6%, respectively, whereas those for measuring MMP-9 activities were 3.4% and 20.7%, respectively (Fujimoto et al. 1994). The intra- and interassay CsV for measuring TIMP-1 levels were 9.3% and 13.1%, respectively, (Clark et al. 1991), whereas those for measuring TIMP-2 levels were 5.4% and 5.9%, respectively (Fujimoto et al. 1993b).

Quantitative cTnT was determined with the second-generation troponin T ELISA (Enzymun-Test Troponin-T) on ES 300 system (Boehringer Mannheim GmbH, Mannheim, Germany). This assay uses the two cardiac-specific monoclonal antibodies M11.7 and M7 (Muller-Bardoff et al. 1997). The detection limit was 0.04 μg/L. The intra- and interassay CsV for measuring cTnT levels were 3.2% and 6.2%, respectively. Quantitative cTnI was measured with the Opus Troponin I assay (Behring Diagnostics, Westwood, MA) performed on the Opus Plus Analyser, with a detection limit of 0.5 μg/L (Larue et al. 1993). The intra- and interassay CsV for measuring cTnI levels were 3.9% and 7.6%, respectively.

Concentrations of CK were measured with dry chemistry using Ektachem 950ICR System (Johnson & Johnson Clinical Diagnostics, Rochester, NY). The CV for measuring CK levels was 8.6% at 192 U/L (Toffaletti et al. 1983).

CK-MB mass was analyzed by Microparticle Enzyme Immunoassay (MEIA) technology with AxSYM system (Abbott Diagnostics, Abbott Park, IL). The CV for measuring

CK-MB mass levels was 8.3% at 3.8 μg/L (Jockers-Wretou and Pfleiderer 1975).

Statistical Analysis

Nonparametric tests were employed in the statistical analysis of the data because data were not normally distributed (Kolmogorov-Smirnov test). Mann-Whitney U test was used to compare two independent groups. A p value of less than .05 was considered statistically significant. All tests were two-sided. Statistica 6.0 (Statsoft, 2003, Tulsa, OK) was used for statistical computations.

RESULTS

Study Sample

A total of 124 patients were enrolled in the trial. The characteristics of the patient population at study entry are shown in Table 1.

TABLE 1
Data at baseline in ACS and DACS group

	ACS	DACS
n	78	46
Sex (M/F)	58/20	32/14
Age (years)	68.3 ± 5.5	70.7 ± 4.6
Duration of diabetes (years)	—	8.0 ± 1.2
BMI (kg/m^2)	26.5 ± 3.0	27.0 ± 2.7
HbA_{1c} (%)	5.3 ± 0.4	7.4 ± 0.5***
FPG (mg/dL)	92.4 ± 7.6	146.2 ± 11.4***
FPI (μU/mL)	8.3 ± 2.8	14.6 ± 3.8***
HOMA index	1.9 ± 0.6	5.2 ± 1.5***
SBP (mm Hg)	139.8 ± 12.3	140.0 ± 10.5
DBP (mm Hg)	81.7 ± 9.2	88.0 ± 8.1***
TC (mg/dL)	208.5 ± 25.8	215.8 ± 25.2
LDL-C (mg/dL)	129.0 ± 24.1	127.5 ± 27.7
HDL-C (mg/dL)	42.6 ± 6.5	42.5 ± 6.6
Tg (mg/dL)	183.2 ± 56.5	221.3 ± 28.0***
Lp(a) (mg/dL)	15.2 ± 15.0	18.8 ± 16.1
PAI-1 (ng/mL)	73.9 ± 27.3	77.0 ± 30.3
Hct (μmol/L)	10.2 ± 7.2	18.5 ± 23.0*
Fg (mg/dL)	329.0 ± 92.2	383.5 ± 98.3**
hs-CRP (mg/dL)	1.5 ± 1.2	1.2 ± 1.2*

Note. Data are means ± *SD*. ACS: nondiabetics with acute coronary syndrome; DACS: diabetics with acute coronary syndrome. $^*p < .05$ versus ACS; $^{**}p < .01$ versus ACS; $^{***}p < .0001$ versus ACS. BMI: body mass index; HbA_{1c}: glycated hemoglobin; FPG: fasting plasma glucose; FPI: fasting plasma insulin; HOMA index: homeostasis model assessment index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; Tg: triglycerides; Lp(a): lipoprotein(a); PAI-1: plasminogen activator inhibitor-1; Hct: homocysteine; Fg: fibrinogen; hs-CRP: C-reactive protein.

Body Mass Index

No BMI change was observed in diabetic patients with ACSs compared to nondiabetic patients with ACSs, as reported in detail in Table 1.

Glycemic Control

Significant HbA_{1c}, FPG, FPI, and HOMA index increases ($p < .0001$) were present in the diabetic group with ACSs compared to the nondiabetic with ACSs baseline values (Table 1).

Blood Pressure Control

No systolic BP (SBP) change was observed in either group, whereas a significant diasystolic BP (DBP) increase ($p < .0001$) was obtained in diabetic patients with ACSs compared to nondiabetic patients with ACSs, as reported in Table 1.

Lipid Profile and Lipoprotein Variables

A significant Tg increase ($p < .0001$) was observed in diabetic group with ACSs, whereas no TC, LDL-C, HDL-C, and Lp(a) variation were present in both groups (Table 1).

Coagulation, Fibrinolytic, and Inflammation Parameters

Significant Hct and Fg increases ($p < .05$, and $p < .01$ respectively) were present in diabetic patients with ACSs, whereas hs-CRP was lower ($p < .05$) in these patients compared to nondiabetic patients with ACSs. No significant PAI-1 change was present in both groups (Table 1).

Enzymatic Characterization

MMPs, TIMP-1, and TIMP-2 levels quantified in diabetic group with ACSs and nondiabetic group with ACSs are reported in Table 2. No significant MMP-2 variation was present in either group, whereas MMP-9 levels were significantly higher ($p < .0001$) in diabetic patients with ACSs respect to nondiabetic patients with ACSs, as reported in Table 2. Significant increase was observed for TIMP-1 and TIMP-2 levels ($p < .0001$) in the diabetic group with ACSs compared to the nondiabetic with ACSs baseline values.

Correlation Analyses

Correlation analyses did not indicate any patterns of associations in MMP-2, MMP-9, TIMP-1, and TIMP-2 with any other parameters in diabetic and nondiabetic patients with ACSs.

DISCUSSION

The interest of the scientific community toward the MMPs has rapidly increased during the recent years. MMPs represent a marker of vascular disease (Maxwell et al. 2001), as also demonstrated by our group in children and adolescents with type 1 diabetes (Derosa et al. 2004) and in patients with hypertension (Derosa et al. 2006) or pathological conditions associated with

TABLE 2
MMPs, TIMP-1, and TIMP-2 levels in ACS and DACS patients

Enzyme	ACS	DACS
MMP-2	1674.3 ± 344.7^a	1578.9 ± 333.5
	1572.2 [1428.0–1843.1][b]	1534.2 [1282.0–1753.1]
MMP-9	196.2 ± 83.0	309.0 ± 112.1
	170.0 [129.0–272.0]	320.1 [231.1–391.0]***
TIMP-1	924.0 ± 61.2	1000.6 ± 60.5
	927.9 [882.5–963.2]	984.0 [965.5–996.8]***
TIMP-2	141.2 ± 7.7	190.2 ± 9.8
	139.9 [136.9–144.6]	189.3 [182.9–196.1]***

Note. Data are [a]means ± *SD* (ng/mL), [b]median (ng/mL) [interquartile range, IQR]. ACS: nondiabetics with acute coronary syndrome; DACS: diabetics with acute coronary syndrome. *** $p < .0001$ versus ACS. MMP-2: matrix metalloproteinase-2; MMP-9: matrix metalloproteinase-9; TIMP-1: tissue inhibitors of metalloproteinase-1; TIMP-2: tissue inhibitors of metalloproteinase-2.

chronic endothelial dysfunction and unstable plaque formation (Nakazawa et al. 2005).

Furthermore, it has been postulated that they could be relevant targets for atherothrombotic cardiovascular diseases treatment (Sierevogel et al. 2003). In fact, circulating MMPs levels are elevated in patients with acute myocardial infarction, unstable angina, and also after coronary angioplasty, which is related to late loss index after the procedure: these observations suggest that MMP expression may be not only related to instability of the plaque but also to the formation of restenotic lesions (Ikeda and Shimada 2003).

In our study, we observed that diabetic patients affected by ACSs have MMP-9, TIMP-1, and TIMP-2 levels significantly higher that nondiabetics ACS patients, whereas MMP-2 levels are not significantly higher.

It is possible that peripheral macrophages and leukocytes might be a source of elevated MMPs because monocytes can be activated in patients with ACSs (Jude et al. 1994). In this sense, our findings are in agreement with the most part of the available one, except for those very recently reported by Eckart et al., who observed an increased level of MMP-2 in myocardial infarction patients, while, paradoxically, MMP-9 decreased (Eckart et al. 2004). On the other hand, Ferroni and colleagues reported that MMP-9 is markedly elevated in myocardial infarction patients, as compared to healthy control, whereas MMP-2 does not change (Ferroni et al. 2003).

Several lines of evidence have implicated MMPs in the rupture of atherosclerotic plaques and subsequent ACSs.

Much of the existing data implicating MMPs in plaque rupture have been obtained from patients undergoing coronary atherectomy and carotid endarterectomy. In one such report, specimens from patients with unstable angina showed a 70% increase in intracellular MMP-9, indicating active synthesis, compared to specimens from patients with stable angina (Brown et al. 1995). MMP-2 is highly activated in coronary plaques, and its activation is correlated with plaque calcification (Noji et al. 2001; Galis et al. 1944; Zempo et al. 1994). Although convincing data exist demonstrating the association of MMPs with atheromatous plaques and colocalization of MMPs in the shoulder region of vulnerable lesions, a direct association with actual plaque rupture is less established.

Kai et al. obtained serial changes in MMP-2 and MMP-9 levels in patients with ACSs, suggesting a pathogenic role of MMPs in the development of ACSs (Kai et al. 1998).

Previous epidemiological data also showed that both MMP-9 and TIMP-1 plasma levels are markedly increased in coronary artery with unstable atherosclerotic plaque of patients affected by ACSs (Inokubo et al. 2001); because TIMP-1 is a potent inhibitor of MMP-9, its increase during the acute phase of acute myocardial infarction may indicate the induced production of MMP-9 in the infarcted myocardium (Dollery et al. 1995).

Another clinical study demonstrated elevated plasma MMP-2 level and activity in patients with acute myocardial infarction. This data may implicate this MMP in post–myocardial infarction complications, but not plaque rupture (Hojo et al. 2001).

Moreover, other investigators showed that MMP-2, MMP-9, and total gelatinolysis activities were increased in patients with CAD who underwent coronary artery bypass graft surgery (Kameda et al. 2003).

There remain several limitations of the currently available clinical studies of MMPs and acute coronary events. For instance, the extent to which plasma levels or activity of MMPs reflect levels or activity within atherosclerotic lesions remains unclear. Furthermore, it is known that thrombin generation can also activate MMP expression (Duhamel-Clerin et al. 1997).

In our study we measured MMP-2, MMP-9, TIMP-1, and TIMP-2 levels in ACS patients with or without diabetes. Diabetes mellitus is a key player in cardiovascular morbidity and mortality, where it is closely linked to the genesis and progression of coronary atheroma, generalized vascular atherosclerosis, hypertension, and dyslipidemia (Lim et al. 2004).

Furthermore, diabetes and/or hyperglycemia per se are associated with significant change to the structure and function of cardiac and vascular tissue. These changes indicate alterations of MMPs that are central factor in the control of extracellular matrix (ECM) turnover. There is increasing evidence of the role of MMP-9 in atherogenesis (Uemura et al. 2001; Shah and Galis 2001).

Recently, Galis and Khatri used an MMP-9 knockout mouse carotid artery model to demonstrate that MMP-9 deficiency leads to a decrease in intimal hyperplasia and lumen loss, but an accumulation of interstitial collagen (Galis and Khatri 2002). This finding showed that the MMP-9 inhibition could increase the mechanical stability of arteries by increasing their collagen content and decreasing lumen loss, indicating the involvement of MMP-9 in the pathogenesis of atherosclerosis. This observation could explain the increase of MMP-9 levels in our diabetic patients with ACS respect to nondiabetics.

In conclusion, in our study, MMP-9, TIMP-1, and TIMP-2 levels are higher in ACS-affected diabetics than in nondiabetics, and these findings might contribute to clarify the potential role of MMPs in the progression of atherosclerosis and plaques vulnerability.

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