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ORIGINAL ARTICLE

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Comparison between metalloproteinases-2 and -9 in healthy subjects, diabetics, and subjects with acute coronary syndrome

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Abstract We hypothesized that matrix metalloproteinase (MMP)-2, -9, and tissue inhibitor metalloproteinase-1, -2 (TIMP-1, -2) would be abnormal in diabetes and in acute coronary syndromes (ACS). We measured MMP-2, -9, and TIMP-1, -2 plasma levels in healthy subjects (controls), in type 2 diabetic patients, in nondiabetic patients with ACS (ACS) and in diabetic patients with ACS (DACS). We enrolled 165 controls, 181 diabetic patients, 78 ACS, and 46 DACS. We measured also BMI (body mass index), HbA_{1c} (glycated hemoglobin) FPG (fasting plasma glucosa), 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), and hs-CRP (high-sensitivity C-reactive protein). A significant increase of BMI was observed in the diabetic group, in ACS and DACS patients compared to controls. A significant increase of SBP and DBP resulted in the diabetic and DACS groups, while only SBP improvement was present in ACS patients with respect to controls. A decrease in SBP and DBP was observed in the ACS group, while SBP variation was present in DACS patients compared to diabetics, and DBP increase was obtained in the DACS group with respect to ACS patients. TC, LDL-C, Tg, and Lp(a) increase was present in diabetics, while TC, Tg, and Lp(a) improvement was present in ACS and DACS patients with a significant decrease of HDL-C levels in diabetic, ACS, and DACS groups compared to controls. A decrease in LDL-C was obtained in ACS and DACS groups, while HDL-C increase was observed in these patients with respect to diabetics. Tg levels were higher in the DACS group compared to diabetics and ACS patients, respectively. Increases in PAI-1, Hct, Fg, and hs-CRP were present in diabetic and DACS groups, while PAI-1, Hct, and hs-CRP improvement was obtained in ACS patients with respect to controls. Higher PAI-1 levels came about in ACS and DACS groups, while HCT and Fg levels were lower in ACS patients compared to diabetics. An increase in Fg was present in the DACS group with respect to ACS patients. A decrease in Hs-CRP was observed in DACS patients compared to diabetics and the ACS group, respectively. Higher MMP-2, MMP-9, TIMP-1, and TIMP-2 levels were present in diabetic, ACS, and DACS patients compared to controls. Significant MMP-2, TIMP-1, and TIMP-2 increases were observed in ACS and DACS groups, while MMP-9 decreased in these patients compared to diabetics. In conclusion, MMP-2, MMP-9, TIMP-1, and TIMP-2 plasma levels were higher in diabetic, ACS, and DACS patients, which may reflect abnormal extracellular matrix metabolism in diabetes and in acute coronary syndrome.

Key words Extracellular matrix · Matrix metalloproteinase · Tissue inhibitor of metalloproteinase · Acute coronary syndrome · Diabetes mellitus

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Introduction

It is well known that the main cause of acute coronary syndromes (ACS) consists in plaque disruption with sub-

sequent superimposed intracoronary thrombus leading to prolonged coronary obstruction.[1] Either matrix metalloproteinases (MMPs)-2 or -9 are synthesized and secreted locally in atherosclerotic lesions, predominantly by monocyte-derived macrophages and endothelial cells.[2] In addition, through their proteolytic activity, these MMPs are capable of degrading fibrous cap of atherosclerotic plaques, thus contributing to plaque destabilization.[3,4]

Earlier studies provide evidence that high MMP plasma values are associated with the presence of ACS.[5,6] In particular, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) have been reported to be markedly increased in patients with ACS both within the atherosclerotic plaque and into the peripheral circulation.[7]

Type 2 diabetic patients are at higher risk for acute coronary events due to an increased propensity of their atherosclerotic plaques to ulceration and overlying thrombosis,[8] with respect to the healthy population.[9] The most common extracellular pathology in diabetes is the thickening of the basement membrane as a result of the deposition of extracellular matrix proteins.[10] Extracellular matrix is a dynamic structure that requires constant synthesis and degradation by MMPs.[11] This is tightly controlled by tissue inhibitors of metalloproteinase (TIMPs).[12]

The expression and activity of MMPs in diabetes thus far have been reported predominantly in relation to microvascular complications.[13,14] On the other hand, there is very little data available on the role of MMPs and TIMPs in macrovascular events in diabetic patients. To date, the only study which 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.[15]

Moreover, as far as is known, there are no data on the association of MMP-2 and MMP-9 with acute coronary events amongst 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 ACS, and whether these values are different compared to those in type 2 diabetic patients with no CAD or in healthy subjects.

Materials and methods

Study design

This multicenter case–control trial 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.

Table 1. Concomitant therapy in patients during the study

	Diabetics	ACS	DACS
n (M/F)	181 (107/74)	78 (58/20)	46 (32/14)
OHA	–	–	40 (30/10)
Insulin	–	–	6 (2/4)
Anti-aggregants	25 (13/12)	28 (12/16)	35 (23/12)
Statins	51 (30/21)	39 (22/17)	36 (25/11)
Fibrates	22 (10/12)	12 (6/6)	5 (4/1)
Antihypertensives	79 (37/42)	67 (49/18)	46 (32/14)

OHA, oral hypoglycemic agents; ACS, nondiabetics with acute coronary syndrome; DACS, diabetics with acute coronary syndrome

Study population

Diabetic and healthy patients

One hundred and eighty-one Caucasian patients aged ≥18 years of either sex were eligible for inclusion in the study if they had type 2 diabetes mellitus according to the American Diabetes Association (ADA) criteria[16] (duration, ≤6 months), diet and exercise-treated and who had not previously taken oral hypoglycemic agents (Table 1). They were of normal weight or overweight (body mass index [BMI], 25.2–28.0 kg/m^2).[17] Suitable patients, identified from review of case notes and/or computerized clinic registers, were contacted by the investigators in person or by telephone.

Patients were excluded if they had a history of ketoacidosis or had unstable or rapidly progressive diabetic retinopathy, nephropathy, or neuropathy; impaired hepatic function (defined as plasma aminotransferase and/or gamma-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 serious cardiovascular disease (CVD) (e.g., New York Heart Association class I–IV congestive heart failure or a history of myocardial infarction or stroke) or cerebrovascular conditions within 6 months before study enrollment also were excluded. As control population we enrolled 165 healthy subjects. They were of normal weight or overweight (body mass index [BMI], 24.9–26.3 kg/m^2). Subjects with infective or inflammatory disorders were excluded, as were those taking anti-inflammatory medications.

Diabetic patients comprised 107 men (59.1%) and 74 women (40.9%) aged 54.1 ± 8.3 years, while healthy control subjects comprised 93 men (56.3%) and 72 women (43.7%) aged 49.5 ± 6.4 years. There were no significant differences between centers in sex distribution, age, and diabetes duration.

CAD 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 of normal weight or overweight (body mass index [BMI], 23.5–29.7 kg/m^2).[17] Exclusion criteria regarding these subjects have been described previously.

Diagnosis of ST-segment elevation AMI (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 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 90 men (72.6%) and 34 women (27.4%) aged 62.8–75.3 years. There were no significant differences between centers in sex distribution, age, CAD, and diabetes duration, and in CAD and diabetes treatment (Table 1).

Cardiologic procedures

All patients underwent coronary angiography by Judkins' technique.[18] 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 one 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.[19]

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 ADA recommendations,[20] 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. Non diabetic patients (healthy and ACS) underwent a diet regimen between 1400 and 1600 kcal. 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, 3 to 5 times per week. The recommended changes in physical activity throughout the study were not assessed.

Laboratory methods

Assessments

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 × *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. Cardiac troponin T 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.

Body mass index 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.[21]

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. Blood pressure was measured by the same investigator at each visit, in the morning 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). Plasma insulin was assayed with Phadiaseph Insulin RIA (Pharmacia, Uppsala, Sweden). Total cholesterol (TC) and triglyceride (Tg) levels were determined using fully enzymatic techniques on a clinical chemistry analyzer (Hitachi 737; Hitachi, Tokyo, Japan). High-density lipoprotein cholesterol (HDL-C) level was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid. Low-density lipoprotein-cholesterol (LDL-C) level was calculated by the Friedewald formula.

Plasminogen activator inhibitor-1 (PAI-1) was assayed with a commercial two-stage indirect enzymatic assay (Spectrolyse, Biopool, Umea, Sweden). Homocysteine (Hct) was measured by a modified procedure of Araki and Sako.[22]

High-sensitivity C-reactive protein (Hs-CRP) was measured with use of latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, DE, USA). Lipoprotein(a) [Lp(a)] was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) method, that is insensitive to the presence of plasminogen, using the commercial kit Macra-Lp(a) (SDI, Newark, Delaware, USA).[23]

MMP-2, MMP-9, TIMP-1, and TIMP-2 levels were determined by a two-site ELISA methods using commercial reagents (Amersham Biosciences, Uppsala, Sweden). The intra- and interassay CsV for measuring MMP-2 levels were 5.4%, and 8.3%, respectively, while those for measuring MMP-2 activities were 5.4%, and 17.9%, respectively.[24] The intra- and interassay CsV to evaluate MMP-9 levels were 4.9%, and 8.6%, respectively while those for measuring MMP-9 activities were 3.4%, and 20.7%, respectively.[25] The intra- and interassay CsV for measuring TIMP-1 levels were 9.3%, and 13.1%, respectively,[26] while those for measuring TIMP-2 levels were 5.4%, and 5.9%, respectively.[27] Quantitative cTnT was determined with the second generation troponin T ELISA (Enzymun-Test Troponin-T) on ES 300 system (Boehringer Mannheim, Mannheim, Germany). The detection limit was 0.04 µg/l. Quantitative cTnI was measured with the Opus Troponin I assay (Behring Diagnostics, Westwood, MA, USA) performed on the Opus Plus Analyser, with a detection limit of 0.5 µg/l. Concentrations of CK were measured with dry chemistry using Ektachem 950ICR System (Johnson & Johnson Clinical Diagnostics, Rochester, NY, USA). CK-MB mass was analyzed by Microparticle Enzyme Immunoassay (MEIA) technology with AxSYM system (Abbott Diagnostics, Abbott Park, IL, USA).

Statistical analysis

Nonparametric tests were employed in the statistical analysis because data were not normally distributed (Kolmogorov–Smirnov test). Mann–Whitney *U*-test was used to compare two independent groups. Correlations between parameters were analyzed with Pearson's coefficient; only correlations with coefficient (r) of 0.40 or more were considered. A P value of less than 0.05 was considered statistically significant. All tests were two-sided. Statistica 6.0 (Statsoft. 2003, Tulsa, OK, USA) was used for statistical computations.

Results

Study sample

A total of 470 patients were enrolled in this trial. The characteristics of the patient population at study entry are shown in Table 2.

Body mass index

Significant BMI change ($P < 0.0001$) was observed in diabetic patients with respect to controls. A BMI increase ($P < 0.0001$) was obtained in ACS and DACS group compared to the control baseline value. No BMI variation was present in ACS and DACS subjects with respect to diabetic patients. No BMI change resulted in DACS with respect to the ACS group, as reported in detail in Table 3.

Glycemic control

HbA_{1c}, FPG, FPI, and HOMA index increases ($P < 0.0001$) were present in the diabetic group compared to the control baseline value. No HbA_{1c}, FPG, or FPI variation was obtained in the ACS group compared to controls, while a HOMA index increase ($P < 0.05$) was observed in ACS patients with respect to controls. HbA_{1c}, FPG, FPI, and HOMA index improvement ($P < 0.0001$) was present in the DACS group compared to the control baseline value. A significant HbA_{1c}, FPG, FPI, and HOMA index decrease ($P < 0.0001$) was observed in ACS subjects with respect to the diabetic group. No significant HbA_{1c}, and HOMA index change was obtained in the DACS group compared to the diabetic baseline value, while an FPG and FPI increase ($P < 0.0001$) was present in DACS patients with respect to diabetics. A significant HbA_{1c}, FPG, FPI, and HOMA index increase ($P < 0.0001$) was observed in the DACS group compared to the ACS group baseline value (Table 3).

Blood pressure control

Values of SBP and DBP ($P < 0.0001$) were higher in patients with diabetes with respect to controls. An SBP increase ($P < 0.0001$) was obtained in the ACS group, while no DBP variation was present in ACS patients compared to controls. Significant SBP and DBP change ($P < 0.0001$) was obtained in DACS compared to the control baseline value.

A significant SBP and DBP decrease ($P < 0.01$, and $P < 0.0001$ respectively) was observed in ACS subjects with respect to the diabetic group. Variation in SBP ($P < 0.05$) was obtained in DACS compared to the diabetic baseline

Table 2. General characteristics of the patients in the study

	Controls	Diabetics	ACS	DACS
n	165	181	78	46
Sex (M/F)	93/72	107/74	58/20	32/14
M/F (%)	56.3/43.7	59.1/40.9	74.4/25.6	69.6/30.4
Age, mean (years)	49.5 ± 6.4	54.1 ± 8.3	68.3 ± 5.5	70.7 ± 4.6
Age, median (years) [IQR]	51.0 [44.0–53.0]	53.0 [49.0–61.0]	66.0 [58.0–72.0]	72.0 [67.0–74.0]
Diab. Dur., mean (years)	–	4.6 ± 1.5	–	8.0 ± 1.2
median (years) [IQR]		5.0 [3.0–6.0]		8.0 [7.0–9.0]

Data are mean ± SD, median, and interquartile range [IQR]; ACS: nondiabetics with acute coronary syndrome; DACS: diabetics with acute coronary syndrome

Table 3. Data at baseline in control, diabetic ACS and DACS group

	Controls	Diabetics	ACS	DACS
HbA_{1c}, mean (%)	5.1 ± 0.4	7.0 ± 0.4	5.3 ± 0.4	7.4 ± 0.5
HbA_{1c}, median (%) [IQR]	5.2 [4.8–5.4]	6.9 [6.7–7.3]•••	5.3 [5.1–5.6]^^^	7.5 [6.9–7.8]•••**
FPG, mean (mg/dl)	88.0 ± 9.5	130.6 ± 13.3	92.4 ± 7.6	146.2 ± 11.4
FPG, median (mg/dl) [IQR]	89.0 [79.0–94.0]	128.0 [121.0–142.0]•••	93.0 [88.0–96.0]^^^	147.5 [137.0–154.0]•••^^^**
FPI, mean (μU/ml)	7.1 ± 1.7	12.7 ± 4.3	8.3 ± 2.8	14.6 ± 3.8
FPI, median (μU/ml) [IQR]	7.3 [6.2–8.5]	13.2 [7.9–16.8]•••	7.75 [6.2–10.3]^^^	15.8 [10.4–17.6]•••^^^**
HOMA index, mean	1.6 ± 0.5	4.2 ± 1.7	1.9 ± 0.6	5.2 ± 1.5
HOMA index, median [IQR]	1.6 [1.3–2.0]	4.2 [2.4–5.4]•••	1.9 [1.5–2.4]•^^^	5.3 [3.6–6.3]•••**
SBP, mean (mmHg)	129.4 ± 5.7	144.2 ± 6.8	139.8 ± 12.3	140.0 ± 10.5
SBP, median (mmHg) [IQR]	130.0 [126.0–134.0]	144.0 [140.0–150.0]•••	140.0 [130.0–150.0]•••^^	140.0 [135.0–150.0]•••^
DBP, mean (mmHg)	79.3 ± 5.7	86.4 ± 7.2	81.7 ± 9.2	88.0 ± 8.1
DBP, median (mmHg) [IQR]	80.0 [75.0–85.0]	85.0 [80.0–90.0]•••	80.0 [75.0–86.0]^^^	90.0 [85.0–95.0]•••**
TC, mean (mg/dl)	195.2 ± 8.9	210.2 ± 19.6	208.5 ± 25.8	215.8 ± 25.2
TC, median (mg/dl) [IQR]	195.0 [189.0–203.0]	210.0 [198.0–224.0]•••	205.5 [197.0–220.0]•••	209.0 [197.0–235.0]•••
LDL-C, mean (mg/dl)	122.6 ± 13.6	137.1 ± 19.4	129.0 ± 24.1	127.5 ± 27.7
LDL-C, median (mg/dl) [IQR]	124.0 [106.0–135.0]	149.0 [121.0–152.0]•••	132.0 [110.0–141.0]^	116.5 [107.0–159.0]^^
HDL-C, mean (mg/dl)	48.8 ± 4.9	38.9 ± 4.4	42.6 ± 6.5	42.5 ± 6.6
HDL-C, median (mg/dl) [IQR]	48.0 [46.0–51.0]	39.0 [35.0–41.0]•••	41.0 [38.0–48.0]•••^^	43.0 [36.0–49.0]•••^^
Tg, mean (mg/dl)	121.7 ± 31.7	169.8 ± 43.8	183.2 ± 56.5	221.3 ± 28.0
Tg, median (mg/dl) [IQR]	123.0 [93.0–147.0]	171.0 [152.0–195.0]•••	198.0 [140.0–228.0]•••	225.0 [212.0–240.0]•••^^^*
Lp(a), mean (mg/dl)	9.2 ± 12.4	14.8 ± 7.2	15.2 ± 15.0	18.8 ± 16.1
Lp(a), median (mg/dl) [IQR]	4.7 [2.8–11.0]	16.8 [9.3–19.1]•••	10.1 [6.3–18.7]•••	10.3 [6.0–28.3]•••
PAI-1, mean (ng/ml)	18.4 ± 2.6	33.7 ± 5.2	73.9 ± 27.3	77.0 ± 30.3
PAI-1, median (ng/ml) [IQR]	18.2 [16.3–21.1]	34.5 [28.5–37.3]•••	75.0 [57.5–97.5]•••^^^	76.3 [58.7–105.0]•••^^^
Hct, mean (μmol/l)	6.9 ± 1.7	11.0 ± 2.7	10.2 ± 7.2	18.5 ± 23.0
Hct, median (μmol/l) [IQR]	6.5 [5.4–8.6]	10.3 [9.3–12.4]•••	7.9 [6.1–10.6]••^^^	10.4 [6.5–15.9]•••
Fg, mean (mg/dl)	337.4 ± 48.9	368.0 ± 43.8	329.0 ± 92.2	383.5 ± 98.3
Fg, median (mg/dl) [IQR]	348.0 [294.0–385.0]	374.0 [336.0–393.0]•••	312.0 [253.0–423.0]^^	378.0 [321.0–447.0]••*
Hs-CRP, mean (mg/dl)	0.4 ± 0.2	1.2 ± 0.3	1.5 ± 1.2	1.2 ± 1.2
Hs-CRP, median (mg/dl) [IQR]	0.4 [0.3–0.6]	1.2 [0.9–1.4]•••	1.2 [0.9–1.6]•••	0.8 [0.2–1.6]•••^^*

Data are mean ± SD, median, and interquartile range [IQR]
ACS, nondiabetics with acute coronary syndrome; DACS, diabetics with acute coronary syndrome; 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, high sensitivity C-reactive protein
• $P < 0.05$ vs control; •• $P < 0.01$ vs control; ••• $P < 0.001$ vs control; ^ $P < 0.05$ vs diabetics; ^^ $P < 0.01$ vs diabetics; ^^^ $P < 0.0001$ vs diabetics; * $P < 0.01$ vs ACS; ** $P < 0.0001$ vs ACS

value, while no DBP change was present in DACS with respect to diabetics. No SBP variation was observed in the DACS group, while a significant DBP increase ($P < 0.0001$) was obtained in the DACS group with respect to the ACS group, as reported in Table 3.

Lipid profile and lipoprotein variables

A Significant TC, LDL-C, Tg, and Lp(a) increase ($P < 0.0001$) was observed in the diabetic group while a significant decrease of HDL-C levels ($P < 0.0001$) was present in diabetic patients with respect to controls. TC, Tg, and Lp(a) levels ($P < 0.0001$) were higher in DACS and ACS groups while HDL-C variation ($P < 0.0001$) was present in both groups compared to controls. No LDL-C change was obtained in ACS and DACS patients with respect to controls.

A significant LDL-C decrease ($P < 0.05$) was observed in ACS subjects while an HDL-C increase ($P < 0.01$) was present in the ACS group with respect to diabetics. No TC, Tg, and Lp(a) variation was obtained in ACS patients compared to the diabetics baseline value.

A significant LDL-C decrease ($P < 0.01$) was observed in the DACS group, while an HDL-C and Tg increase ($P < 0.01$ and $P < 0.0001$, respectively) was present in the DACS group with respect to diabetics. No TC, and Lp(a) change was obtained in either group. Tg levels ($P < 0.0001$) were higher in DACS, while no TC, LDL-C, HDL-C, and Lp(a) variation was present in either group (Table 3).

Coagulation, fibrinolytic, and inflammation parameters

A significant PAI-1, Hct, Fg, and hs-CRP increase ($P < 0.0001$) was present in patients with diabetes compared to the control baseline value. Significant PAI-1, Hct, and hs-CRP increases ($P < 0.01$, and $P < 0.0001$ respectively) were observed in ACS subjects, while no Fg change was present in this group with respect to controls. Increases in PAI-1, Hct, Fg, and hs-CRP ($P < 0.01$, and $P < 0.0001$ respectively) were obtained in the DACS group compared to the control baseline value.

PAI-1 was higher ($P < 0.0001$), while Hct and Fg were lower ($P < 0.01$, and $P < 0.0001$, respectively) in patients

with ACS compared to the diabetic group. No hs-CRP change was obtained in either group.

A significant PAI-1 increase ($P < 0.0001$) was observed in DACS while a hs-CRP decrease ($P < 0.01$) was obtained in this group with respect to the diabetic baseline value. No Hct and Fg variation was present in either group (Table 3). Fg was higher ($P < 0.01$), while hs-CRP was lower ($P < 0.01$) in the DACS group compared to the ACS group. No significant PAI-1 and Hct change was present in either group (Table 3).

Enzymatic characterization

MMPs, TIMP-1, and TIMP-2 levels quantified in control, diabetic, and in ACS and DACS groups are reported in Table 2. MMP-2 and MMP-9 levels were significantly higher ($P < 0.0001$) in diabetic patients than in controls (Table 4).

A significant increase was observed for TIMP-1 and TIMP-2 levels ($P < 0.0001$) in patients with diabetes compared to controls (Table 4). MMP-2, MMP-9, TIMP-1, and TIMP-2 levels were higher ($P < 0.0001$) in ACS and DACS patients compared to the controls baseline value (Table 4).

A significant MMP-2, TIMP-1, and TIMP-2 increase ($P < 0.05$, and $P < 0.0001$ respectively) was observed in ACS and DACS groups, while a decrease in MMP-9 ($P < 0.0001$) was obtained in these patients compared to diabetics (Table 4). No significant changes in MMP-2, MMP-9, TIMP-1, and TIMP-2 levels were observed in DACS with respect to the ACS group, as reported in Table 4.

Correlation analyses

Correlation analyses did not indicate various patterns of associations in MMP-2 and MMP-9, TIMP-1, and TIMP-2 with any other parameters in control, diabetic, and in ACS and DACS groups (Table 5).

Discussion

The interest of the scientific community toward the MMPs has been rapidly increasing during the last years. MMPs represent a marker of vascular disease,[14] as also demonstrated by our group in children and adolescents with type 1 diabetes,[28] and in patients with hypertension,[29] pathologi-

Table 4. MMPs, TIMP-1, and TIMP-2 levels in controls, diabetic, ACS and DACS patients

	Controls	Diabetics	ACS	DACS
MMP-2 levels, mean (ng/ml)	628.1 ± 267.4	1351.3 ± 153.9	1674.3 ± 344.7	1578.9 ± 333.5
MMP-2 levels, median (ng/ml) [IQR]	639.3 [415.0–804.0]	1363.4 [1250.3–1461.3]•	1572.2 [1428.0–1843.1]•^^	1534.2 [1282.0–1753.1]•^
MMP-9 levels, mean (ng/ml)	53.7 ± 16.0	521.7 ± 63.9	196.2 ± 83.0	309.0 ± 112.1
MMP-9 levels, median (ng/ml) [IQR]	55.3 [39.2–68.2]	523.4 [476.5–566.6]•	170.0 [129.0–272.0]•^^	320.1 [231.1–391.0]•^^
TIMP-1 levels, mean (ng/ml)	166.9 ± 56.4	510.7 ± 49.9	924.0 ± 61.2	1000.6 ± 60.5
TIMP-1 levels, median (ng/ml) [IQR]	156.7 [125.7–195.9]	512.7 [472.9–550.2]•	927.9 [882.5–963.2]•^^	984.0 [965.5–996.8]•^^
TIMP-2 levels, mean (ng/ml)	80.1 ± 5.9	99.9 ± 3.5	141.2 ± 7.7	190.2 ± 9.8
TIMP-2 levels, median (ng/ml) [IQR]	80.1 [74.9–85.1]	99.5 [97.2–102.7]•	139.9 [136.9–144.6]•^^	189.3 [182.9–196.1]•^^

Data are mean ± SD, median, and interquartile range [IQR]
ACS, nondiabetics with acute coronary syndrome; DACS, diabetics with acute coronary syndrome; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitors of metalloproteinase-1; TIMP-2, tissue inhibitors of metalloproteinase-2
• $P < 0.0001$ vs control; ^ $P < 0.05$ vs diabetics; ^^ $P < 0.0001$ vs diabetics

Table 5a. Correlation data between MMPs/TIMPs and various parameters considered in the study in control group

	MMP-2		MMP-9		TIMP-1		TIMP-2	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
HbA_{1c} (%)	0.025	0.751	–0.059	0.452	0.002	0.984	–0.014	0.856
FPG (mg/dl)	–0.128	0.103	–0.112	0.152	–0.239	0.002	0.073	0.354
FPI (μU/ml)	–0.019	0.804	0.003	0.967	0.328	0.00002	0.175	0.025
HOMA index	–0.126	0.107	0.005	0.950	0.115	0.140	0.020	0.801
SBP (mmHg)	–0.083	0.292	–0.024	0.760	–0.048	0.543	0.054	0.490
DBP (mmHg)	0.050	0.522	0.021	0.793	0.002	0.984	–0.040	0.607
TC (mg/dl)	–0.044	0.578	0.183	0.019	0.118	0.131	–0.062	0.432
LDL-C (mg/dl)	–0.058	0.463	0.169	0.030	0.129	0.100	–0.047	0.546
HDL-C (mg/dl)	0.099	0.208	–0.142	0.070	–0.003	0.970	–0.036	0.648
Tg (mg/dl)	–0.080	0.305	0.028	0.726	–0.121	0.121	0.009	0.907
Lp(a) (mg/dl)	–0.064	0.418	–0.041	0.597	0.095	0.224	0.033	0.675
PAI-1 (ng/ml)	–0.022	0.779	0.008	0.920	0.049	0.529	–0.026	0.741
Hct (μmol/l)	0.189	0.015	0.047	0.550	–0.051	0.515	–0.077	0.326
Fg (mg/dl)	–0.084	0.283	0.150	0.055	0.120	0.124	0.001	0.993
Hs-CRP (mg/dl)	–0.039	0.618	–0.099	0.205	–0.076	0.334	–0.070	0.373

Table 5b. Correlation data between MMPs/TIMPs and various parameters considered in the study in the diabetic group

	MMP-2		MMP-9		TIMP-1		TIMP-2	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
HbA_{1c} (%)	0.094	0.211	–0.062	0.406	–0.107	0.150	–0.069	0.356
FPG (mg/dl)	–0.023	0.759	0.098	0.190	–0.003	0.973	–0.067	0.369
FPI (μU/ml)	–0.058	0.438	0.051	0.497	–0.053	0.483	–0.096	0.198
HOMA index	–0.050	0.507	0.059	0.432	–0.025	0.742	–0.077	0.302
SBP (mmHg)	0.111	0.137	–0.005	0.950	0.158	0.034	–0.066	0.375
DBP (mmHg)	–0.031	0.674	0.017	0.824	0.009	0.900	–0.049	0.513
TC (mg/dl)	0.121	0.104	–0.055	0.459	0.067	0.369	–0.092	0.216
LDL-C (mg/dl)	0.103	0.166	0.027	0.718	0.073	0.331	–0.036	0.629
HDL-C (mg/dl)	–0.147	0.049	–0.102	0.173	–0.106	0.156	–0.033	0.659
Tg (mg/dl)	0.130	0.082	–0.128	0.087	0.068	0.362	–0.118	0.115
Lp(a) (mg/dl)	0.024	0.753	0.083	0.268	–0.048	0.523	0.095	0.202
PAI-1 (ng/ml)	–0.091	0.221	–0.050	0.507	–0.152	0.040	0.071	0.339
Hct (μmol/l)	0.217	0.003	0.112	0.132	–0.079	0.289	–0.106	0.154
Fg (mg/dl)	0.027	0.714	–0.088	0.239	–0.004	0.960	–0.156	0.036
Hs-CRP (mg/dl)	–0.191	0.010	–0.054	0.471	–0.089	0.231	0.070	0.349

Table 5c. Correlation data between MMPs/TIMPs and various parameters considered in the study in the ACS group

	MMP-2		MMP-9		TIMP-1		TIMP-2	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
HbA_{1c} (%)	–0.173	0.130	–0.211	0.064	0.095	0.408	0.028	0.806
FPG (mg/dl)	–0.027	0.816	–0.318	0.005	0.131	0.254	0.137	0.233
FPI (μU/ml)	–0.118	0.304	0.106	0.357	0.126	0.272	0.032	0.779
HOMA index	–0.167	0.144	–0.021	0.852	0.043	0.707	0.030	0.797
SBP (mmHg)	0.133	0.247	0.107	0.350	–0.096	0.405	0.116	0.311
DBP (mmHg)	–0.165	0.150	–0.070	0.541	–0.031	0.786	0.006	0.962
TC (mg/dl)	–0.084	0.464	–0.165	0.150	–0.086	0.453	–0.151	0.188
LDL-C (mg/dl)	–0.122	0.288	–0.242	0.033	–0.059	0.608	–0.111	0.333
HDL-C (mg/dl)	–0.227	0.045	–0.053	0.647	–0.133	0.247	–0.015	0.896
Tg (mg/dl)	0.013	0.910	0.231	0.042	0.021	0.855	0.149	0.194
Lp(a) (mg/dl)	–0.182	0.111	–0.067	0.562	–0.191	0.093	0.023	0.844
PAI-1 (ng/ml)	–0.086	0.453	0.013	0.910	0.039	0.737	–0.243	0.032
Hct (μmol/l)	0.225	0.047	0.044	0.704	0.002	0.984	0.187	0.100
Fg (mg/dl)	0.091	0.430	–0.094	0.414	0.027	0.816	0.115	0.316
Hs-CRP (mg/dl)	0.052	0.652	0.024	0.836	0.054	0.641	0.081	0.483

Table 5d. Correlation data between MMPs/TIMPs and various parameters considered in the study in the DACS group

	MMP-2		MMP-9		TIMP-1		TIMP-2	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
HbA_{1c} (%)	–0.262	0.079	0.168	0.266	0.027	0.861	0.022	0.886
FPG (mg/dl)	0.513	0.0003	–0.002	0.991	0.152	0.314	–0.118	0.434
FPI (μU/ml)	–0.194	0.196	0.059	0.698	–0.218	0.146	–0.265	0.075
HOMA index	0.013	0.930	0.096	0.526	–0.193	0.198	–0.230	0.124
SBP (mmHg)	–0.093	0.537	0.106	0.482	–0.168	0.266	0.127	0.399
DBP (mmHg)	–0.067	0.657	0.122	0.420	0.117	0.439	0.176	0.242
TC (mg/dl)	0.135	0.372	0.039	0.796	0.022	0.882	–0.034	0.822
LDL-C (mg/dl)	0.044	0.769	0.135	0.371	–0.016	0.917	0.044	0.772
HDL-C (mg/dl)	0.139	0.358	–0.379	0.009	0.068	0.653	–0.059	0.698
Tg (mg/dl)	0.168	0.265	0.085	0.573	0.148	0.326	–0.133	0.377
Lp(a) (mg/dl)	–0.097	0.519	–0.178	0.238	0.268	0.072	0.015	0.922
PAI-1 (ng/ml)	0.028	0.852	0.159	0.292	0.288	0.052	–0.137	0.364
Hct (μmol/l)	–0.100	0.509	–0.160	0.288	0.108	0.474	0.072	0.636
Fg (mg/dl)	–0.158	0.296	0.148	0.327	0.119	0.431	–0.031	0.836
Hs-CRP (mg/dl)	–0.165	0.273	0.187	0.212	–0.144	0.340	–0.038	0.802

cal conditions associated with chronic endothelial dysfunction, and unstable plaque formation.[30]

Furthermore, it has been postulated that MMPs could be a relevant target for atherothrombotic cardiovascular disease treatment.[31] 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 related not only to instability of the plaque, but also to the formation of restenotic lesions.[32] Diabetes is also associated with plaque vulnerability and a high risk for acute coronary events due to overlying thrombosis; therefore the MMP/TIMP system may provide a novel mechanism to account for the increased incidence of acute vascular events in diabetic patients.[33]

In our study we observed that diabetics and ACS and DACS patients have MMP-2, MMP-9, TIMP-1, and TIMP-2 levels significantly higher than controls. It is possible that peripheral macrophages and leukocytes might be a source of elevated MMPs because monocytes can be activated in patients with ACS.[34] In this sense, our findings are in agreement with the most part of the available literature, except for those results very recently reported by Eckart et al., who observed an increased level of MMP-2 in myocardial infarction patients while, paradoxically, MMP-9 decreased.[35] On the other hand, Ferroni and colleagues reported that MMP-9 is markedly elevated in myocardial infarction patients, as compared to healthy controls, while MMP-2 does not change.[36]

Several lines of evidence have implicated MMPs in the rupture of atherosclerotic plaques and subsequent ACS. Much of the existing data implicating MMPs in plaque rupture has 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.[7] MMP-2 is highly activated in coronary plaques, and its activation is correlated with plaque calcification.[37–39]

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 ACS, suggesting a pathogenic role of MMPs in the development of ACS.[3] Previous epidemiological data also showed that both MMP-9 and TIMP-1 plasma level are markedly increased in coronary artery with unstable atherosclerotic plaque of patients affected by ACS.[40] 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.[41]

Another clinical study demonstrated elevated plasma MMP-2 levels and activity in patients with acute myocardial infarction. These data may implicate this MMP in post-myocardial infarction complications, but not plaque rupture.[42] 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.[43]

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.[44]

In our study we measured MMP-2, MMP-9, TIMP-1, and TIMP-2 levels also in diabetic patients without ACS. 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.[45] These findings are in agreement with the results from Death et al.[2] In fact in their study on cultured endothelial cells and monocyte-derived macrophages, they found out that high glucose exposure could induce not only the expression but also the gelatinolytic activity of MMP-9, and in our previous study we demonstrated that type 2 diabetic patients have significant MMP-2, MMP-9, TIMP-1, and TIMP-2 levels higher than healthy subjects.[46] Enhanced MMP-9 activity was also observed by Uemura et al., in their study on type 1 and type 2 diabetic rats.[33] In contrast with our results are the findings from the study of Baugh et al., where no significant difference in MMP-9 production was observed between controls and type 2 diabetes groups.[47]

Furthermore, diabetes and/or hyperglycemia per se are associated with significant change to the structure and function of cardiac and vascular tissue.[48] These changes indicate alterations of MMPs that are central factors in the control of ECM turnover. There is increasing evidence of the role of MMP-9 in atherogenesis.[33,49]

Recently Galis et al. used an MMP-9 knockout mouse carotid artery model to demonstrate that an MMP-9 deficiency leads to a decrease in intimal hyperplasia and lumen loss, but an accumulation of interstitial collagen.[50] 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 with respect to nondiabetics.

Although there was a significant positive correlation with FPG and MMP-2 ($r = 0.513$, $P < 0.0003$) in the DACS group, but not in the diabetic group and the ACS group, we think that this result was irrelevant in the context of the study. However, we could explain this positive correlation by considering the instability of the plaque (through the increase of MMP-2 levels) given by fasting hyperglycemia in patients with ACS. In conclusion, in our study MMP-2, MMP-9, TIMP-1, and TIMP-2 levels are higher in diabetics, ACS, and DACS patients than in controls, and these findings might contribute to clarify the potential role of MMPs in the progression of atherosclerosis and plaque vulnerability.

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