Original Article

Early Change of Extracellular Matrix and Diastolic Parameters in Metabolic Syndrome

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Abstract

Background: Metabolic syndrome (MS) is associated with increased cardiovascular risk. It is not clear whether myocardial changes showed in this syndrome, such as diastolic dysfunction, are due to the systemic effects of the syndrome, or to specific myocardial effects.

Objectives: Compare diastolic function, biomarkers representing extracellular matrix activity (ECM), inflammation and cardiac hemodynamic stress in patients with the MS and healthy controls.

Methods: MS patients (n = 76) and healthy controls (n=30) were submitted to a clinical assessment, echocardiographic study, and measurement of plasma levels of metalloproteinase-9 (MMP9), tissue inhibitor of metalloproteinase-1 (TIMP1), ultrasensitive-reactive-C-Protein (us-CRP), insulin resistance (HOMA-IR) and natriuretic peptide (NT-proBNP).

Results: MS group showed lower E' wave (10.1 ± 3.0 cm/s vs 11.9 ± 2.6 cm/s, p = 0.005), increased A wave (63.4 ± 14.1 cm/s vs. 53.1 ± 8.9 cm/s; p < 0.001), E/E' ratio (8.0 ± 2.2 vs. 6.3 ± 1.2; p < 0.001), MMP9 (502.9 ± 237.1 ng / mL vs. 330.4±162.7 ng/mL; p < 0.001), us-CRP (p = 0.001) and HOMA-IR (p < 0.001), but no difference for TIMP1 or NT-proBNP levels. In a multivariable analysis, only MMP9 was independently associated with MS.

Conclusion: MS patients showed differences for echocardiographic measures of diastolic function, ECM activity, us-CRP and HOMA-IR when compared to controls. However, only MMP9 was independently associated with the MS. These findings suggest that there are early effects on ECM activity, which cannot be tracked by routine echocardiographic measures of diastolic function. (Arq Bras Cardiol. 2013;101(4):311-316)

Keywords: Metabolic Syndrome; Risk Factors; Extracellular Matrix; Diastole / physiopathology.

Introduction

The metabolic syndrome (MS) is defined as a combination of several risk factors associated with cardiovascular disease and type 2 diabetes; estimates suggest that this disease affects approximately 35% of the adult population[1,2]. It is unclear whether myocardial changes associated with the metabolic syndrome are consequences of the systemic effects of the syndrome or due to direct myocardial effects.

The diastolic function evaluation has been used to identify cardiac preclinical changes. Diastolic dysfunction is prevalent in patients with MS, even in the absence of hypertension and diabetes[3], and regardless the left ventricular mass[4,5]. Diastolic dysfunction predicts a worse outcome independently of any other co-morbidity[6]. In MS, diastolic dysfunction is usually attributed to the increased hemodynamic stress[7,8]. Alternatively, diastolic dysfunction may also be secondary to changes in the cardiac extracellular matrix resulting from the altered metabolic-inflammatory milieu and glucose metabolism[9]. Extracellular matrix collagen turnover is tightly regulated by the interaction between metalloproteinases and its plasma tissue inhibitors. Changes in this balance may therefore influence ventricular relaxation and compliance[10].

Aiming to a better understand on the underlying processes involved in the cardiovascular abnormalities seen in MS, we studied echocardiographic parameters of diastolic function and quantified plasma levels of metalloproteinase-9 (MMP9), tissue inhibitor of metalloproteinase-1 (TIMP1), ultrasensitive-reactive-C-Protein (us-CRP), insulin resistance (HOMA-IR) and natriuretic peptide (NT-proBNP) in patients with MS compared to healthy controls.

Methods

Population

In this cross-sectional analysis, we selected subjects ranging from 30-55 years of age with MS and healthy controls (CTR). The MS group consisted of all subjects

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recruited for a randomized clinical trial whose protocol and results have already been published[11]. From the initial sample of 471 evaluated volunteers, 76 matched the clinical trial eligibility criteria, which were: Body Mass Index (BMI) ≥ 30 kg/m^2 and ≤ 40 kg/m^2, waist circumference ≥ 95 cm and at least two other Metabolic Syndrome criteria according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP/ATP III)[12]. Moreover, all patients had an oral glucose tolerance test that was negative for diabetes. The exclusion criteria were pregnancy, lactation, creatinine ≥ 1.5 mg/dL, musculoskeletal dysfunction, inflammatory or chronic liver disease, thyroid dysfunction and/or use of corticosteroids or anorectic drugs. The baseline data in this group were compared with locally recruited healthy subjects of same gender and age range as that of MS group. The study was approved by the Ethics Committee of our Institution and by the Research Committee, and all participants signed a written informed consent prior to enrollment.

Clinical Assessment

Blood pressure and heart rate were measured in triplicate after five minutes at rest using an aneroid sphygmomanometer (Tycos, Welch Allyn, USA), with reported average. Height was measured by a wall-mounted stadiometer, and patients had their weight measured by an electronic scale, wearing light clothes and no shoes. Waist circumference was measured midway between the costal border and the iliac crest.

Biochemical Analysis

Blood samples were collected in a fasting state. Insulin was measured via electrochemiluminescence (Roche, Switzerland), and ultra-sensitive plasma C-reactive protein (us-CRP) was measured via immunonephelometry (Roche, Switzerland). The lipid profile was enzymatically measured (Roche, Switzerland), and the LDL-cholesterol level was calculated according to the Friedewald formula whenever the triglycerides were below 400 mg/dL. The homeostasis model assessment for insulin resistance (HOMA-IR) was performed to determine insulin resistance[13]. The coefficients of variation for these parameters were all below 6%.

Echocardiographic Study

Images were obtained with an EnVisor C HD ultrasound system (Philips Medical, Andover, MA, USA) equipped with a 4 to 2 MHz sectorial transducer. The cine loops and static images were digitally recorded, including the M-mode, 2-dimensional, and Doppler modalities. Images were read off-line in a dedicated workstation (ComPACS, Medimatic Srl, Italy) by a single investigator.

The left ventricle (LV) internal dimension, septum and posterior wall thicknesses were measured from the parasternal longitudinal two-dimensional image. The left atrial volume index (LAVi) was measured at the end-ventricular systole from the apical 4-chamber view, using the simple Simpson's rule and indexed to the body surface area.

The diastolic function was evaluated from the mitral inflow Doppler[14] and tissue Doppler[15] measurements, including: mitral inflow early (E wave) and late (A wave) diastolic velocities, deceleration time (DT) of early diastolic velocity, and early (E' wave) and late (A' wave) diastolic annular velocities assessed at the septal mitral anulus. The E/A ratio and the E/E' ratio were calculated from previous parameters.

All measurements and definitions of relevant cut-offs followed the American Society of Echocardiography recommendations; all of the data were an average of up to 3 consecutive cardiac cycles[16,17]. Left ventricle volume and ejection fraction were calculated using the Teichholz formula. Left ventricle mass index (LVMI) was calculated according to the American Society Echocardiography formula (16) and indexed to the body height to the power of 2.7[18]. Relative wall thickness (RWT) was defined as (septum + posterior wall thickness)/LV diastolic diameter. The intra-reader reproducibility was assessed in 16 participants as a coefficient of variation (CV) and intra-class correlation (ICC), which were, respectively, E' wave (CV: 5.2% and ICC: 0,99;95%CI: 0.97-0.99), E wave (CV 4.5% and ICC: 0.98; IC95% 0.96-0.99) and A wave (CV 3.5% and ICC: 0.98;IC95% 0.97-0.99). Additionally, for the two-dimensional measurements, CVs ranged between 8% and 13% and ICCs were above 0.75, whose values are similar to those described in previous studies[19,20].

ELISA Assays

Fasting venous blood samples (15 mL) were collected in ethylenediamine tetraacetic acid-containing tubes. Samples were immediately centrifuged at 4°C at 3,000 x g for 20 min, and the plasma removed and stored at –70°C. The plasma samples were all blinded analyzed simultaneously by a laboratory technician. MMP9 and TIMP1 levels were measured in duplicate using commercially available ELISA kits (R & D Systems, Minneapolis, MN, USA). The MMP9 assay sensitivity was <0.156 ng/mL, and the TIMP1 assay sensitivity was <0.08 ng/mL; the intra- and inter-assay coefficients of variation were 6% and 10%, respectively. The NT-proBNP level was also measured with a commercial ELISA kit (Roche Diagnostic, France). The NT-proBNP assay sensitivity was <0.6 pmol/L with intra- and inter-assay coefficients of variation of 1.9% and 3.1%, respectively.

Statistical Analysis

Results are expressed as mean and SD, or as percentage. Groups were compared with a Chi-square or independent Student's *t* test analysis. The associations between continuous variables were tested with Pearson correlation coefficient. Multivariable linear regression analyses models were performed to identify which variables were independently associated with the presence of the MS.

We calculated a sample size of 66 MS and 33 CTR, considering an alpha value of 0.05, a power of 0.8 and 0.6 standard deviation of difference in MMP9 levels between groups. This value was estimated based on

MMP9 differences described by Tayebjee et al[21] - between hypertensive patients – which frequently showed diastolic dysfunction - and normal controls. P values <0.05 were considered to be statistically significant. All of the statistical analyses were performed with the SPSS software package (SPSS 15.0 Inc., USA).

Results

We studied 76 patients in the MS group (43.3 ± 7.9 years, 65% male), and 30 healthy controls (CTR; 40.9 ± 6.6 years, 63% male). Further clinical characteristics and laboratory parameters of the groups are shown in Table 1. The MS group, as expected, had increased weight, waist circumference, heart rate, blood pressure, and cholesterol levels when compared with the CTR group.

Left ventricular mass index was higher in the MS group (Table 2). Left atrial volume index and ejection fraction did not differ between groups.

The diastolic function parameters showed that MS had higher A wave (63.4 ± 14.1 cm/s vs. 53.1 ± 8.9 cm/s; $p < 0.001$), and lower E' wave (10.1 ± 3.0 cm/s vs. 11.9 ± 2.6 cm/s; $p = 0.005$) compared with controls, but with mean values within the normality range[17]. These differences resulted in a reduced E/A ratio ($p = 0.05$) and an increased E/E' ratio ($p < 0.001$) in the MS group. E wave ($p = 0.45$) and deceleration time ($p = 0.98$) did not differ between the groups (Table 2).

Extracellular matrix activity biomarkers showed that MMP9 levels were higher in the MS group (502.9 ± 237.1 ng / mL vs. 330.4 ± 162.7 ng/mL; $p < 0.001$), but with no differences in TIMP1 (210.2 ± 55.6 ng/mL vs 220.2 ± 57.2 ng/mL; $p = 0.41$) levels (Figure 1). Insulin resistance measured by HOMA-IR ($p < 0.001$) and us-CRP ($p = 0.001$) levels were higher in the MS group, while NT-proBNP levels ($p = 0.19$) did not show statistically significant difference between the groups (Table 3).

In a multiple linear regression, we investigated the independent associations of blood pressure, BMI, waist circumference, HDL-cholesterol, triglyceride, HOMA-IR, us-CRP, MMP9, TIMP1, NT-proBNP, E wave, A wave, E' wave, and A' wave with MS. Only MMP9 ($\beta = 0.13$, $p = 0.03$) was independently associated to MS.

Discussion

In this study, the MS group showed difference in diastolic function parameters and higher levels of HOMA-IR, us-CRP and MMP9 when compared to healthy controls, with no difference in TIMP1 and NT-proBNP levels. However, when adjusted for covariates, only MMP9 was independently associated with the MS.

De las Fuentes et al[4], investigating echocardiographic parameters of diastolic function in MS patients, showed increased A wave, decreased E' wave, and no difference in E wave. Although we have found similar results for these parameters, they were not independently associated with MS after adjustment for covariates, whereas MMP9 was still significant. We could infer that, in the early phases of metabolic syndrome, modulations in ECM activity measured by the increase in MMP9 levels, anticipate measurable changes in cardiac pressures measured by diastolic Doppler parameters, NT- proBNP levels or left atrial dimensions, which are frequently used as surrogate markers of loading conditions[22,23].

Extracellular matrix activity has been associated with relaxation and LV stiffness[9]. The increased MMP9 in the metabolic syndrome may represent a collagen turnover state in the extracellular matrix, which may contribute to adverse ventricular remodeling and left ventricular stiffness. Gonçalves et al[24], studying 25 patients with MS and 25 healthy controls, found increased levels of both MMP9 and TIMP1 in the MS group; reflecting the advanced dysmetabolic state in those patients compared to our sample.

Table 1 – Clinical characteristics and laboratory parameters of the metabolic syndrome (MS) and healthy control (CTR) groups

	MS (n = 76)	CTR (n = 30)	p
Male (%)	65.3	63.3	0.85
Age (y)	43.3(7.9)	40.9(6.6)	0.14
BMI (kg/m²)	34.7(2.8)	24.9(2.6)	< 0.001
Waist (cm)	106.7(7.3)	86.1(8.9)	< 0.001
SBP (mmHg)	128.0(12.7)	115.8(10.2)	< 0.001
DBP (mmHg)	81.2(9.7)	76.3(9.1)	0.02
Heart rate (bpm)	86.6(10.4)	69.9(11.0)	< 0.001
Total cholesterol (mg/dL)	215.8(38.9)	194.1(31.8)	0.01
HDL-cholesterol (mg/dL)	45.6(10.9)	49.2(14.4)	0.17
Triglyceride (mg/dL)	189.6(224)	124.7(80.2)	0.13
Glucose (mg/dL)	93.8(8.45)	86.7(7.4)	< 0.001

Values showed as mean (SD) or percentage. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2 - Echocardiographic parameters and diastolic function of the metabolic syndrome (MS) and healthy control (CTR) groups

	MS (n = 76)	CTR (n = 30)	p
LVMI (g/m$^{2.7}$)	37.8(7.5)	32.4(7.2)	0.001
Relative Wall Thickness	0.39(0.07)	0.36(0.05)	0.17
LV Ejection Fraction (%)	68.5(5.4)	66.9(7.1)	0.22
LAVI (mL/m²)	24.3(6.0)	25.6(5.9)	0.35
E wave (cm/s)	76.9(15.7)	74.4(15.6)	0.45
A wave (cm/s)	63.4(14.1)	53.1(8.9)	< 0.001
Deceleration time (ms)	205.2(28.6)	205.3(35.3)	0.98
E' wave(cm/s)	10.1(3.0)	11.9(2.6)	0.005
A' wave (cm/s)	11.0(2.3)	10.1(1.9)	0.04
E/A ratio	1.26(0.38)	1.42(0.3)	0.05
E/E' ratio	8.0(2.2)	6.3(1.2)	< 0.001

Values showed as mean (SD). LVMI: left ventricular mass index; LAVI: left atrial volume index; E wave: mitral inflow early diastolic velocity; A wave: mitral inflow late diastolic velocity; E' wave: early diastolic annular velocity; A' wave: late diastolic annular velocity.

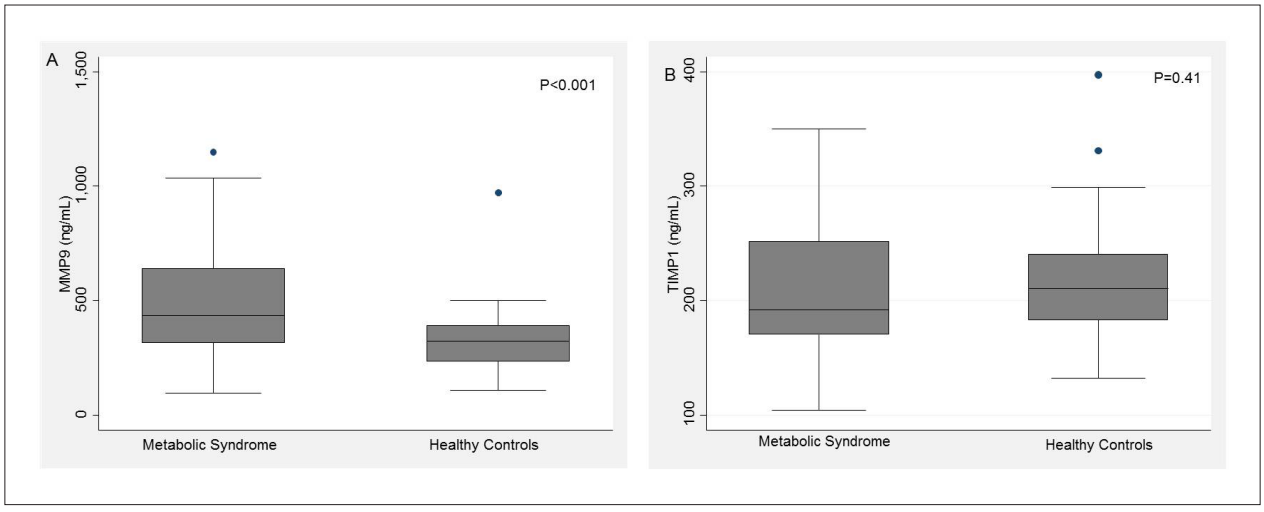

Figure 1 - *Circulating biological markers of cardiac remodeling in the metabolic syndrome (MS) and healthy control (CTR) groups. A. Metalloproteinase-9 levels (MMP9). B. Plasma Tissue Inhibitor of Metalloproteinase-1 levels (TIMP1).*

Table 3 – Circulating biomarkers in the metabolic syndrome (MS) and healthy control (CTR) groups

	MS (n = 76)	CTR (n = 30)	p
MMP9 (ng/mL)	502.9(237.1)	330.4(162.7)	< 0.001
TIMP1 (ng/mL)	210.2(55.6)	220.2(57.2)	0.41
NT-proBNP (ng/mL)	29.9(21.9)	23.6(21.7)	0.19
HOMA-IR units	3.4(1.6)	1.6(0.8)	< 0.001
us-CRP (mg/dL)	3.9(3.6)	1.5(1.5)	0.001

Values showed as mean (SD). MMP9: metalloproteinase 9; TIMP1: Plasma Tissue Inhibitor of Metalloproteinase-1; NT-proBNP: natriuretic peptide; HOMA-IR: homeostasis model assessment for insulin resistance; us-CRP: ultrasensitive C-reactive protein.

Oversimplification of multifactorial mechanisms based upon a limited subset of markers is inherent to this study design and precludes causal inferences. A potential bias of this analysis was the non-blinded echocardiographic acquisition for the groups, minimized by the off-line reading by a single investigator. It must also be brought to attention the potential role of newer technologies, such as the speckle tracking, which could more accurately find these early adaptive changes related to the metabolic syndrome.

Conclusions

We have found that patients with MS showed differences in echocardiographic measures of diastolic function, in ECM activity measured by MMP9, us-CRP and HOMA-IR when compared to healthy controls. However, only MMP9 was independently associated with the MS. These findings suggest that there are early effects on extracellular matrix activity in metabolic syndrome, which cannot be tracked by routine echocardiographic measures of diastolic function.

Author contributions

Conception and design of the research: Santos ABS, Junges M, Silvello D, Macari A, Araújo BS, Seligman BG, Duncan BB, Clausell N, Foppa M; Acquisition of data: Santos ABS, Junges M, Silvello D, Macari A, Araújo BS, Seligman BG, Foppa M; Analysis and interpretation of the data: Santos ABS, Junges M, Silvello D, Seligman BG, Rohde LEP, Clausell N, Foppa M; Statistical analysis: Santos ABS, Foppa M; Obtaining funding: Santos ABS, Duncan BB, Foppa M; Writing of the manuscript: Santos ABS, Seligman BG, Duncan BB, Clausell N, Foppa M; Critical revision of the manuscript for intellectual content: Santos ABS, Seligman BG, Duncan BB, Rohde LEP, Clausell N, Foppa M.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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