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Subclinical diastolic dysfunction in type 2 diabetic patients with and without carotid atherosclerosis: Relationship with glyco-oxidation, lipid-oxidation and antioxidant status

Giovanni Sartore [a], Francesco Piarulli [a], Eugenio Ragazzi [b], Silvia Burlina [a,*], Nino Cristiano Chilelli [a], Cristiano Sarais [c], Raffaella Marin [a], Enzo Manzato [a], Domenico Fedele [a], Annunziata Lapolla [a]

[a] Department of Medical and Surgical Sciences, University of Padova, Italy
[b] Department of Pharmacology and Anesthesiology, University of Padova, Italy
[c] Department of Cardiologic, Thoracic and Vascular Sciences, University of Padova, Italy

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ABSTRACT

Objectives: The aim of this study was to evaluate subclinical diastolic dysfunction in type 2 diabetic patients and its relationship with glyco-oxidation, lipo-oxidation and antioxidant capacity in the presence or absence of carotid plaques.
Background: Subclinical diastolic dysfunction is the early stage of diabetic cardiomyopathy, the pathogenic mechanisms of which are still little known. In particular, few data are available on the role of glyco-oxidation, lipo-oxidation and antioxidant status, factors known to be involved in the atherosclerotic process.
Methods: We assessed myocardial systolic and diastolic functions in 57 consecutive asymptomatic type 2 diabetic patients (24 patients with no carotid plaques; 33 with plaques) and 27 healthy volunteers using transthoracic echocardiography. Glyco-oxidation and lipo-oxidation parameters and antioxidant status were also evaluated in fasting venous blood samples.
Results: Systolic function was similar between diabetic patients and controls, while most of the diastolic parameters (A, e′, E/A, E/e′) differed significantly between diabetics and controls, being worse in the former. Among the diastolic parameters, only the peak late diastolic velocity A differed significantly between the two groups of diabetic patients with no carotid plaques and with plaques (0.72 ± 0.16 m/s vs 0.84 ± 0.25 m/s, $p<0.05$). The diastolic parameters A and E/e′ related to glycemic control, glyco-oxidation and antioxidant capacity, and to LDL size and density.
Conclusions: Glyco-oxidation and antioxidant status, combined with the presence of small, dense LDL correlate with subclinical diastolic dysfunction in type 2 diabetic patients. Atherosclerotic lesions are associated with an altered atrial function.

1. Introduction

Diabetic patients have a higher incidence of heart failure (HF). The Framingham Study [1], the United Kingdom Prospective Diabetes Study [2] and the Cardiovascular Health Study [3] have all suggested that diabetes may independently raise the risk of incident HF. This is attributable to diabetic cardiomyopathy, defined as a ventricular dysfunction occurring irrespective of any coronary artery disease and hypertension [4]. The natural history of diabetic cardiomyopathy involves the normal heart developing a subclinical diastolic dysfunction (an underlying etiology of HF), which progresses to systolic dysfunction and finally to symptomatic HF. Subclinical diastolic dysfunction is characterized by impaired relaxation and passive filling during the diastole. The main characteristics of diabetic cardiomyopathy are an increased myocyte apoptosis, myocardial fibrosis and collagen deposition [4,5]. Chronic hyperglycemia seems to be central to the pathogenesis of this disease, through the promotion of collagen formation in the myocardium, which results in interstitial fibrosis, and the increase in advanced glycation end-products (AGEs) [6], which may alter the structure of proteins and contribute to myocardial stiffness. In particular, collagen interacts with glucose, forming a stable form of cross-linked collagen [4,5]. Glyco-oxidation products sustain a condition of oxidative stress that has an important role in the pathogenesis of diabetic cardiomyopathy, with an increased production of reactive oxygen species (ROS) that outweighs their degradation by antioxidant defenses [4,5]. On the other hand, antioxidants – and vitamin E, in particular – have been shown to have a protective role in the early stages of type 1 diabetic cardiomyopathy in animal models [7]. As regards human diabetic cardiomyopathy, few studies have shown a correlation between glycemic control (particularly

* Corresponding author at: Department of Medical and Surgical Sciences, University of Padova, Via dei Colli 4, 35143 Padova, Italy. Tel.: +39 0498216848.
E-mail address: silvia.burlina@studenti.unipd.it (S. Burlina).

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HbA1c) and subclinical diastolic dysfunction [8,9], and no data are available on the direct correlation between the latter and glyco-oxidation or anti-oxidant status.

Diabetic patients also have a higher risk of atherosclerosis than the general population. Some mechanisms behind the development of diabetic cardiomyopathy are involved in the atherosclerotic process, particularly increased glyco-oxidation and decreased anti-oxidant status. In fact, AGEs may cause vascular damage through the formation of abnormal cross-links in collagen, contributing to arterial stiffening [10,11]. Immunohistochemical studies have shown that AGEs accumulate in coronary atherosclerotic plaques and are associated with an increased carotid intima-media wall thickness [12,13]. Reduced antioxidant defenses are also described in diabetic patients and this also contributes to the onset of chronic complications [14]. In addition, the lipid oxidation and the quantitative and qualitative alterations of the lipid profile have an important role. As regards lipid oxidation, malondialdehyde (MDA), an important indicator of lipid peroxidation in vivo, is elevated in diabetic patients with macroangiopathy [15]. As for qualitative lipid changes, the presence of small and dense LDL particles has an important role because they remain in the bloodstream for longer, preferentially penetrating through the endothelial barrier, and they are more susceptible to oxidation than larger LDL particles [16].

Considering the hypothesis of a common pathogenesis for diabetic cardiomyopathy and atherosclerotic complications, we evaluated subclinical diastolic dysfunction in type 2 diabetic patients and its relationship with glyco-oxidation, lipid oxidation and antioxidant capacity in the presence or absence of carotid plaques.

2. Methods

The study involved 57 consecutive asymptomatic type 2 diabetic patients (32 men and 25 women) aged <75, who were attending our outpatient clinic between November and December 2009. They had no history of coronary artery or valve disease, as confirmed by normal resting 12-lead electrocardiograms and the maximal treadmill exercise stress test. Twenty-seven healthy volunteers free of cardiovascular disease, none of whom had a personal or family history of any illness, were also recruited in the same period as controls. The study complied with the Declaration of Helsinki and the approval of local institutional review boards, and informed consent was obtained from each patient. Any presence of carotid plaque (focal plaques>1.5 mm thick) was investigated in all patients and controls by echo color Doppler B-mode imaging on a high-resolution imaging system (ATL HDI 5000), always by the same operator.

All patients were assessed in terms of body mass index (BMI), diastolic and systolic pressure, any hypertension and/or use of antihypertensive drugs and lipid-lowering drugs.

Blood was obtained from fasting venous samples for biochemical analysis.

Fasting plasma glucose (FPG) was determined using a glucose-oxidase method [17]. HbA1c was measured by liquid chromatography [18] (Bio-Rad Laboratories, Milan, Italy). Total cholesterol and HDL-cholesterol were measured using enzymatic analytical chemistry [19,20] (CHOP-PAP method; Roche, Milan, Italy), as were triglycerides [21] (GPO-PAP colorimetric enzyme test; Roche Diagnostic System). LDL relative flotation (LDL-rf), a measure of LDL particle size and density, was ascertained by Single Vertical Spin Ultracentrifugation, as described elsewhere [22]. OxLDL were measured with an ELISA method [23] (Mercodia Oxidized LDL ELISA kit, Uppsala, Sweden), as were oxLDL Ab [24,25] (Anti-oxLDL ELISA, IMMCO Diagnostics, Buffalo, NY).

AGEs were measured using an ELISA method [26], while liquid chromatography was used to measure pentosidine [27], and MDA [28]. Vitamin E levels were measured by high-precision chromatography in a reverse-phase column using a diode array spectrophotometric detector, according to Laidman and Hall [29]. Nitrotyrosine (NT) was measured with a chemiluminescent method [30] (HBT ELISA test kit for nitrotyrosine).

2.1. Resting echocardiography

Subclinical diastolic dysfunction can be studied by echocardiography (using mitral flow velocities) and by tissue Doppler imaging. The latter is preload-independent and more sensitive than conventional echocardiography, and it enables a non-invasive assessment of regional myocardial diastolic and systolic functions. Color M-mode flow propagation velocity measurements can also be used to study subclinical diastolic dysfunction and are relatively independent of volume load [31].

In all patients, the transthoracic echocardiographic assessment was performed by the same operator in the left lateral decubitus position, using an Acuson Sequoia 512 ultrasound machine. Images were obtained in the standard views of the left ventricular (parasternal long and short axis, and apical four-chamber, two-chamber and long axis views) and the different cardiac chambers were measured according to the recommendations of the American Society of Echocardiography [32]. The ejection fraction (EF) was calculated as the percentage change in left ventricular chamber volume between the diastole and the systole, based on apical four- and two-chamber views using Simpson's rule, and considering three measurements.

Mitral inflow velocities, the peak early diastolic velocity (E) and the peak late diastolic velocity (A) were recorded using conventional pulsed-wave Doppler echocardiography, positioning a sample volume at the level of the mitral leaflet tips in the apical four-chamber view. The E/A ratio (an index of diastolic function) was also calculated.

Pulsed-wave TDI was performed with the same machine, after enabling the TDI function: the sample volume was located on the septal side of the mitral annulus. Early diastolic mitral annulus velocity (e′) and the E/e′ ratio (a marker of left ventricular filling pressure) were recorded.

2.2. Statistical analysis

Values are expressed as mean ± standard deviation. Student's *t* test was used for the multiple comparison of continuous variables. Data occurring as categorical variables were analyzed with Pearson chi-square test. Differences were deemed statistically significant when $p<0.05$.

A useful multivariate technique for reducing the dimensions of the data set is the Principal Component Analysis (PCA), which can help identify new, meaningful underlying variables. The reduced set contains what are called 'principal factors', which are linear combinations of the original variables. The first principal component accounts for as much of the variability in the data possible, with each successive component accounting for the remaining variability. *Biplot* software was used as an Excel add-in [33]. The first three components were considered for data classification. A biplot graphic display was used to present the variables' behavior in order to examine their correlation. The most useful variable is the cosine of the eigenvectors suggesting correlations between different variables [34]. When the angle between eigenvectors nears 0°, the variables are positively correlated, while the angle for negative correlations approaches 180° and angles of 90° indicate no correlation.

3. Results

Patients were divided into two groups on the basis of the supra-aortic vessel echo color Doppler findings: Group 1 included 24 diabetic patients with no carotid plaques; Group 2 contained 33 diabetic patients with plaques. None of the 27 healthy controls had carotid plaques.

3.1. Clinical characteristics

As shown in Table 1, the two groups of diabetic patients differed in terms of age only (65.33 ± 9.11 years vs 57.58 ± 8.53 years; $p<0.01$). No statistically significant difference was found for medication use, including statins, between the two groups. Diabetic patients differed significantly from controls in terms of FPG, HbA1c and LDL-rf (Table 1).

3.2. Glyco-oxidation, lipid oxidation and antioxidant status

Table 2 shows the oxidation parameters and antioxidant status. AGEs were significantly higher in Group 2 than in Group 1 or controls (9.63 ± 3.52 μg/mg proteins vs 7.71 ± 2.62 μg/mg proteins and vs 6.69 ± 2.54 μg/mg proteins, respectively; $p<0.05$). Pentosidine (a parameter of glyco-oxidation) was significantly higher in diabetic patients than in controls; among the diabetic patients, it was higher in Group 2 than in Group 1 (97.63 ± 34.46pmol/ml vs 74.13 ± 36.64pmol/ml; $p<0.05$) (Table 2).

Among the lipid oxidation parameters, only MDA differed significantly between diabetic patients and controls; it was higher in Group 1 and in Group 2 than in controls (0.26 ± 0.16 μmol/l and 0.22 ± 0.17 μmol/l, respectively, vs 0.10 ± 0.08 μmol/l; $p<0.001$). No differences emerged between the two groups of diabetic patients.

Diabetic patients and controls had similar NT levels. Vitamin E levels were significantly lower in Group 2 than in Group 1 diabetic patients (15.82 ± 8.03 μmol/l vs 25.79 ± 4.06 μmol/l; $p<0.001$), while no differences were observed between Group 1 and controls.