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Association of vascular indices with novel circulating biomarkers as prognostic factors for cardiovascular complications in patients with type 2 diabetes mellitus

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ABSTRACT

Background: The pathophysiology of atherosclerosis in type 2 diabetes mellitus (T2DM) is multifactorial. The association of vascular indices with circulating biomarkers of inflammation and insulin resistance and their role in the long-term cardiovascular prognosis in T2DM patients were currently investigated.

Patients and methods: Patients with T2DM and poor glycemic control without known cardiovascular diseases (n = 119) at baseline were enrolled and followed for about 9 years. The end-point was the occurrence of any cardiovascular event (coronary heart disease, stroke, peripheral artery disease or cardiovascular death). Aortic pulse wave velocity (PWV), augmentation index (AIx), brachial flow-mediated dilation (FMD), hsCRP, Chitinase-3-like protein 1 (YKL-40), Neutrophil Gelatinase-Associated Lipocalin (NGAL), Fatty Acid Binding Protein (FABP-4) were assessed.

Results: Higher YKL-40 and NGAL were associated with higher PWV, while higher YKL-40 and FABP-4 were related to higher AIx ($p < 0.05$ for all). In univariate Cox regression analysis, PWV > 10 m/s, YKL-40 > 78 ng/ml and NGAL > 42 ng/ml were associated with cardiovascular events ($p < 0.05$ for all). In multivariate analysis, after adjusting for classical risk factors and glycemic control, increased NGAL, YKL-40 and PWV and decreased FMD (i.e. ≤ 2.2%) ($p < 0.05$ for all) were independently associated with cardiovascular events.

Conclusion: In T2DM patients without established cardiovascular disease, novel indices of vascular inflammation (NGAL and YKL-40) were associated with subclinical atherosclerosis (arterial stiffness) but also with adverse clinical prognosis. Arterial stiffness and endothelial dysfunction were also independently related to adverse prognosis.

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the most important risk factors for the development of atherosclerotic cardiovascular diseases [1]. The pathophysiological mechanism underlying the progression of atherosclerosis and the occurrence of cardiovascular events in T2DM is considered multifactorial. Apart from the increased incidence of classical risk factors (i.e. hypertension, dyslipidemia, obesity) in T2DM, other factors such as hyperglycemia, insulin resistance and inflammation may play an important role. Although T2DM patients are considered high risk individuals for the development of cardiovascular events [1], there is a great variability among patients with T2DM regarding their individual cardiovascular risk. Currently, research focuses on the identification of T2DM patient characteristics that could further improve risk stratification in these patients.

Vascular biomarkers that assess the structure and/or function of the

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peripheral vasculature have been proposed as surrogate markers of cardiovascular risk [2,3]. Increased arterial stiffness and arterial wave reflections as well as impaired endothelial function are documented early in the process of atherosclerosis (long before the occurrence of clinically overt cardiovascular diseases) and have been reported to have a prognostic role for cardiovascular events in various populations [3]. Several circulating markers have also been proposed to play a role in cardiovascular prognosis. High sensitivity C-reactive protein (hsCRP), an index of low grade, systemic inflammation, has been widely studied as a predictor of cardiovascular risk in various populations [4–6]. More recently, Chitinase-3-like protein 1 (YKL-40) and Neutrophil Gelatinase-Associated Lipocalin (NGAL) have been proposed as novel inflammatory markers [7–9] with a potential prognostic role for cardiovascular events [10–14], although they are little studied with regard to prognosis in T2DM [15,16]. Fatty Acid Binding Protein-4 (FABP-4) is an adipokine considered to mediate the atherogenic dyslipidemia in T2DM but has have also been related to vascular inflammation and the atherosclerotic process [17,18]. A significant role of FABP-4 in cardiovascular prognosis has been previously shown in various high risk populations [19,20].

The aims of the present study were to investigate the association of indices of subclinical atherosclerosis [increased arterial stiffness and wave reflections i.e. increased carotid-femoral pulse wave velocity (PWV) and aortic augmentation index (AIx) and endothelial dysfunction i.e. reduced brachial artery flow-mediated dilation (FMD)] with classical and novel circulating markers of inflammation (hsCRP, YKL-40, NGAL and FABP-4) and assess the role of these vascular and biochemical biomarkers in the long term cardiovascular prognosis in patients T2DM without known cardiovascular diseases.

2. Methods

2.1. Study subjects

This was a prospective open-label observational study. A total of 119 patients with previously diagnosed T2DM were consecutively recruited from the Endocrinology outpatient clinics of the University Hospital and Hatzikosta General Hospital of Ioannina, Ioannina, Greece from January 2003 to December 2005. Eligible patients were between 40 and 80 years old, under treatment with oral antidiabetic medications (metformin or/and sulfonylureas) or/and insulin therapy with poor glycemic control. The duration of diabetes was confirmed by the patients' clinical records. Patients with a history of macrovascular disease (coronary artery, cerebrovascular, or peripheral vascular disease), diabetic retinopathy or symptomatic neuropathy, macroalbuminuria, stage 4 or 5 chronic renal disease [i.e. estimated glomerular filtration rate (GFR) $<$ 30 ml/min/1.73 m^2], chronic heart failure, atrial fibrillation, liver disease (or abnormal liver enzymes at study entry), anemia, thyroid dysfunction, other endocrine diseases, alcoholism or severe chronic condition were excluded from the study. From the enrolled patients 26% had GFR $\geq$ 90 ml/min/1.73 m^2 (stage 1 chronic renal disease), 61% had GFR 60–89 ml/min/1.73 m^2 (stage 2 chronic renal disease) and 13% had GFR 30–59 ml/min/1.73 m^2 (stage 3 chronic renal disease).

The study was approved by the Ethics Committee of the “Michaelidion” Cardiac Center, University of Ioannina, Greece and informed consent was obtained from all patients. The study complies with the Declaration of Helsinki.

2.2. Data collection

All participants underwent a medical interview concerning disease and risk factor history and general use of medications. A physical examination was performed including measurement of blood pressure (BP), height and weight while body mass index was calculated as weight/height^2 (kg/m^2). The minimum waist circumference between the pelvic brim and the costal margin was measured. Hypertension was defined by systolic BP $>$ 140 mm Hg or diastolic BP $>$ 85 mm Hg or both or treatment with antihypertensive medications. Hypercholesterolemia was defined by low density lipoprotein cholesterol (LDL-C) $>$ 2.6 mmol/l (100 mg/dl) or treatment with lipid-lowering agents (i.e. statins). Patients who were smoking at the time of study entry or stopped smoking during the last 12 months prior to study entry were considered as current smokers. The estimated GFR was calculated using the chronic kidney disease epidemiology collaboration (CKD-EPI) formula (ml/min/1.73 m^2). Participants were followed-up with periodic visits at the outpatient clinics. A direct telephone interview of the patients or their relatives was performed for all participants from June 2012 through June 2013, with a median duration of 106 months (minimum 91 and maximum 120 months) follow-up (ca. 9 years). Events were confirmed by the patients' medical records or interview with their physicians.

2.3. Definition of endpoints

The outcome measured at follow-up was the combined cardiovascular endpoint, defined as the first incidence of non-fatal coronary heart disease (CHD) or stroke, peripheral artery disease (PAD) or cardiovascular death. CHD was defined as an acute coronary syndrome (acute myocardial infarction or unstable angina) or chronic stable angina confirmed by electrocardiography, stress test, myocardial enzymes, coronary angiography or coronary revascularization (percutaneous coronary intervention or coronary artery bypass graft surgery) based on patient's medical history. The diagnosis of stroke was confirmed by the presence of a relevant hospitalization and brain imaging indicative of cerebral ischemia in patient's medical history. PAD was defined by the presence of symptoms suggestive of critical limb ischemia or ulcers with a positive diagnostic angiography or the history of amputation or revascularization of lower extremities' arteries. During the follow-up period there were 21 patients (18%) who suffered the combined cardiovascular endpoint (first event recorded): 4 deaths due to cardiovascular causes, 17 cases with CHD, 5 cases with stroke and 2 cases with PAD. In total, 11 patients died (9%) including cardiovascular and non-cardiovascular causes.

2.4. Blood Chemistry measures

Blood samples were obtained during the morning hours after an overnight fasting, 30–60 min prior to the assessment of vascular biomarkers. Blood chemistry including measurements of serum fasting glucose, creatinine, lipids and hsCRP along with whole blood HbA1c levels were performed as described previously [21].

Serum levels of YKL-40 were determined using a sandwich ELISA assay (Quidel corporation, San Diego, CA, US). According to manufacturer, the intra-assay and inter-assay coefficients of variance (CVs) for YKL-40- range between 5.6 and 6.6% and 6–7% respectively. Serum levels of NGAL were determined in duplicate by solid phase ELISA techniques (R&D Systems, Minneapolis, MN, US). According to manufacturers, the intra-assay and inter-assay CVs for NGAL range between 3.1 and 4.1% and 5.6 and 7.9% respectively. Serum levels of FABP-4 were determined using a commercial human enzyme immunoassay kit (BioVendor Laboratory Medicine, Inc., Modrice, Czech Republic). The assay was conducted according to the manufacturer's instructions. The antibodies in human a-FABP ELISA are highly specific for human FABP-4, with no detectable cross-reactivity to other members of human FABP family and also to leptin and adiponectin. The intra- and inter-assay CVs were $<$ 5.5%. The measurements were performed in the Department of Clinical Biochemistry, “Aghia Sophia” Children's Hospital and the Clinical Biochemists were blind in clinical data.