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Association of plasma fetuin-a levels with peripheral arterial disease and lower extremity arterial calcification in subjects with type 2 diabetes mellitus

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ABSTRACT

Aims: Fetuin-A is a hepatic glycoprotein that is involved in insulin resistance and atherosclerosis. Herein we examined the association of plasma fetuin-A levels with peripheral arterial disease (PAD) in patients with type 2 diabetes mellitus (T2DM).
Material and Methods: A total of 71 patients with T2DM and 57 non-diabetic individuals were recruited. Diagnosis of PAD was based on the absence of triphasic waveform at pedal arteries, while ankle–brachial index (ABI) was calculated. Radiographs of both feet and ankles were taken for the assessment of lower extremity arterial calcification (LEAC). Plasma fetuin-A levels were measured using ELISA.
Results: Patients with T2DM had higher fetuin-A levels than non-diabetic participants. Participants with diabetes and PAD had lower fetuin-A levels than non-PAD diabetic patients. In subjects with T2DM fetuin-A levels were associated with ABI. Multivariate analysis demonstrated that in patients with T2DM the odds of PAD increased with long diabetes duration, smoking, presence of arterial hypertension and dyslipidemia, as well as with lower fetuin-A levels. A trend towards higher fetuin-A levels in subjects with less severe LEAC was found.
Conclusion: Plasma fetuin-A levels are lower in patients with T2DM and PAD and are associated with PAD, irrespective of traditional cardiovascular risk factors. Moreover, fetuin-A may be involved in arterial calcification.

1. Introduction

Fetuin-A is a multifunctional hepatic glycoprotein that exerts its effects on the cardiovascular system by two different mechanisms. On the one hand it inhibits insulin signaling and induces insulin resistance contributing to the onset of atherosclerosis (Stefan et al., 2006) and on the other hand it inhibits calcium deposition and protects from vascular calcification (Schafer et al., 2003).

Insulin resistance is a main pathophysiological mechanism involved in the development of type 2 diabetes mellitus (T2DM) and previous data showed that persons with T2DM have increased levels of fetuin-A in comparison with individuals without T2DM (Ou et al., 2011).

Contradictory results have been reported regarding the role of fetuin-A in peripheral arterial disease (PAD) in persons with diabetes (Eraso et al., 2010; Lorant et al., 2011; Roos et al., 2010). Two studies have reported that fetuin-A levels are lower in individuals with T2DM and PAD (Eraso et al., 2010; Roos et al., 2010), while another study reported opposite findings (Lorant et al., 2011). Nevertheless, none of the studies tried to elucidate the association of fetuin-A levels with lower extremity arterial calcification. It is known that PAD is a state of advanced and systemic atherosclerosis and that in patients with diabetes is often accompanied by vascular calcification of the tunica media of the arterial wall (Mönckeberg sclerosis) (Jude, Eleftheriadou, & Tentolouris, 2010). The aim of the present study was to investigate the association of plasma fetuin-A levels with PAD in patients with T2DM and to further elucidate the association of plasma fetuin-A levels with lower extremity arterial calcification (LEAC).

2. Subjects, materials and methods

2.1. Study population

The study population consisted of 71 patients with T2DM attending the outpatient Diabetes and Foot Clinics of our hospital and 57 individuals without diabetes who were hospital staff or who attended the outpatient clinics of our hospital for minor health problems, such as

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management of arterial hypertension, dyslipidemia or osteoarthritis. Diabetes status was confirmed from the medical records and the treatment with antidiabetic medications. Individuals without diabetes had fasting serum glucose levels <126 mg/dl and HbA_{1c} levels <6.5%.

Exclusion criteria were prior bypass surgery or percutaneous angioplasty to the lower limb arteries, acute limb ischemia, moderate or severe renal disease (creatinine clearance (CrCl) <45 ml/min), significant hepatic or cardiorespiratory disease, malignancy, connective tissue disease, hormone replacement therapy and acute illness. Patients with all four Fontaine Classification stages were included in the study.

The study was conducted according to the principles of the declaration of Helsinki and was approved by the ethics committee of our hospital (ethics committee approval number ES 77/27-1-06). The purpose of the study was clearly explained to all participants and written informed consent was obtained.

2.2. Methods

This is a cross-sectional study and participants attended once the Diabetic Laboratory of our Department.

All measurements were performed in the morning after **10–12 h fast** and in a room temperature of 23 ± 1 °C. The antidiabetic or the other medications were withheld and were administered to the participants at the end of the examination and after blood sampling. Blood samples were collected from the antecubital vein, immediately centrifuged and deep frozen at −80 °C.

A complete physical examination was performed and a detailed history for current and previous diseases, use of current medications and smoking habits was obtained. Body weight and height were measured for each participant in light clothing and body mass index (BMI) was calculated.

Blood pressure at the brachial artery was measured in the sitting position using an appropriate cuff three consecutive times, one minute apart, and the mean value of the last two measurements was used in the analysis. Arterial hypertension was defined according to the current guidelines if systolic blood pressure was ≥140 mmHg and/or diastolic blood pressure was ≥90 mmHg and/or if patients were on antihypertensive treatment (Mancia et al., 2007). Dyslipidemia was defined as use of lipid lowering drugs, and/or diabetes status and LDL cholesterol levels ≥70 mg/dl, and/or primary elevation of LDL cholesterol ≥190 mg/dl, and/or clinical atherosclerotic cardiovascular disease and LDL cholesterol ≥70 mg/dl (Stone et al., 2014). Diagnosis of peripheral neuropathy was based on neuropathy symptom score, neuropathy disability score and vibration perception threshold (Boulton, Malik, Arezzo, & Sosenko, 2004).

2.2.1. ABI measurement

The American Diabetes Association defines PAD as ankle–brachial index (ABI) ≤0.90, normal ABI values 0.91–1.30 and Mönckeberg sclerosis as ABI values >1.30 (Diabetes Care, 2003). All individuals in the study underwent ABI measurements of the lower limbs and all measurements were performed by the same experienced examiner. Bilateral brachial and ankle pressures (dorsalis pedis and posterior tibial artery) were measured using a hand-held Doppler device with a 5–10 mHz probe (dopplex II, Huntleigh Healthcare Ltd., Cardiff, UK, intra-observer cv <3%) and ABI was calculated as a ratio of systolic blood pressures in the lower and upper extremities. The higher brachial systolic pressure was used as the denominator of the ABI. The higher of the ankle pressures (dorsalis pedis or posterior tibial artery) was used as the numerator of the ABI for each leg. ABI was calculated for both feet.

2.2.2. Arterial ultrasound examination for PAD diagnosis

All individuals underwent qualitative waveform analysis of the lower limbs and all measurements were performed by the same experienced examiner. Arterial ultrasound examination and Doppler qualitative analysis of the waveform have been demonstrated to accurately estimate the severity of PAD. Qualitative analysis of spectral waveforms of the posterior tibial arteries was performed by visual interpretation of continuously displayed waveforms. Presence of biphasic, monophasic or blunted waveforms is highly suggestive of hemodynamically significant vessel disease (Makrilakis, 2010; Williams, Harding, & Price, 2005). Arterial ultrasound and qualitative waveform analysis were performed in the supine position on the posterior tibial artery and dorsalis pedis artery of both feet using a high resolution Kretz Technik (Kretz Technik, Austria) ultrasound system with a 7.5 mHz linear probe. Arterial flow velocity was measured using a pulsed Doppler signal at a 60° angle at the center of the artery. Diagnosis of PAD was based on the absence of triphasic waveform with loss of reverse flow on the pedal arteries (Makrilakis, 2010). Participants were categorized as having or not PAD according to arterial ultrasound examination and Doppler qualitative analysis of the waveform.

2.2.3. Assessment of lower extremity arterial calcification

In all participants plain radiographs were taken of both tibia, ankles and feet. Posterior tibial artery calcification was assessed at the tibia and ankle level and dorsalis pedis artery calcification in the foot x-rays. Lower extremity arterial calcification (LEAC) was independently assessed by two observers and graded as absent (score = 0), barely visible calcification (score = 1), slightly visible calcification (score = 2), outline of the artery wall (score = 3), very dense calcification of length ≤ 2 cm (score = 4), very dense of length > 2 cm (score = 5) at two locations (posterior tibial artery and dorsalis pedis artery) for each limb. A similar LEAC scoring system has been used in the literature (Costacou, Huskey, Edmundowicz, Stolk, & Orchard, 2006). Total LEAC score for each patient (0–20) was calculated by adding the scores from all four locations on both lower limbs. In case of disagreements the two observers reviewed the radiographs together and agreed on a score (Costacou et al., 2006).

2.2.4. Fetuin-A, insulin and other measurements

Fetuin-A levels were measured in EDTA plasma using a commercially available ELISA kit (R&D Systems, Inc., Minneapolis, USA, intra-assay cv = 4.4%). Plasma glucose, total serum cholesterol, HDL cholesterol, triglycerides, serum urea and creatinine were measured on an automatic analyzer. LDL cholesterol was estimated using the Friedewald formula, while CrCl was estimated with the Cockroft–Gault formula. HbA_{1c} levels were determined using a DCA analyzer (DCA 2000+, Bayer HealthCare LLC, Elkhart, IN 46514 USA). Albumin to creatinine ratio (ACR) was measured in a first morning void urine sample using the same DCA analyzer. Plasma insulin levels were measured using Luminex Multiplex immunoassay (Millipore's MILLIPLEX MAP Human Bone Metabolism Panel 1A, Millipore Corp., Missouri, USA, intra-assay cv = 3.8%). Insulin resistance was assessed by the homeostasis model assessment of insulin resistance (HOMA-IR). Measurements of fetuin-A and plasma insulin levels were performed by the same experienced researcher.

2.3. Statistical analysis

Except for the study population description, we performed a limb-specific analysis. Statistical analysis was performed using the SPSS 15.0 statistical package (SPSS, Inc., Chicago, IL, USA).

The power was calculated at the beginning of the study using the Power and Precision V4 statistical power analysis software package (Biostat Inc., Englewood, NJ, USA); **it was estimated, using ANOVA, that a number of 30 participants per group would provide adequate power (>0.80) at a = 0.05 to detect differences in plasma fetuin-A concentrations among the study groups.**

All data were assessed for normal distribution of their values. Parameters are presented as means ± standard deviation (SD) or as median value and interquartile range. Categorical variables were compared with a Chi-squared test and/or Fisher's exact test. Comparisons between groups of normally distributed data were performed by the independent samples Student's t test or one-way analysis of variance

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