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Fasting serum dipeptidyl peptidase-4 activity is independently associated with alanine aminotransferase in type 1 diabetic patients

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ABSTRACT

Objectives: Dipeptidyl peptidase-4 (DPP4) was recently proposed as a novel adipokine linked to insulin resistance (IR). As IR represents a cluster of disorders in hepatic and muscle cell insulin signalisation, we aimed to assess the possible correlation between fasting serum DPP4 activity, IR and liver enzymes in order to elucidate the question of hepatic contribution to serum DPP4 activity.

Design and methods: This cross-sectional study comprised 44 T1DM patients aged 18 to 65 years. IR was estimated using the equation derived from euglycemic–hyperinsulinemic clamp studies-estimated glucose disposal rate (eGDR). DPP4 serum activity was determined spectrophotometrically as a rate of cleavage of 7-amino-4-methyl coumarin (AMC) from H-Gly-Pro-AMC. The patients were divided into two groups according to the mean value of fasting serum DPP4 activity (31.42 U/L).

Results: The group with lower fasting serum DPP4 activity had lower mean rate of liver biomarkers alanine aminotransferase (ALT) ($p = 0.001$) and aspartate aminotransferase (AST) ($p = 0.002$) while higher eGDR ($p = 0.003$) compared to group with higher DPP4 activity. DPP4 activity showed positive correlation with AST ($r = 0.358$, $p = 0.017$) and ALT ($r = 0.364$, $p = 0.015$) while negative correlation with eGDR ($r = -0.612$, $p < 0.001$). ALT remained positively associated with fasting serum DPP4 activity after controlling for age, gender, diabetes duration, the use of statins and antihypertensives ($p = 0.025$).

Conclusions: Fasting serum DPP4 activity might be associated with hepatic IR in T1DM patients and a part of soluble DPP4 activity might be of a hepatic origin. Further study investigation is warranted to elucidate this topic.

Introduction

Dipeptidyl peptidase-4 (DPP4) is a serine exopeptidase that cleaves X-proline dipeptides from the polypeptide N-terminus. It is expressed on the various cell types surface and in soluble form in the circulation [1]. A fraction of soluble DPP4 originates from the immune system cells which explains its altered abundance and the circulating activity in various immune mediated conditions [2] including type 1 diabetes mellitus (T1DM) [3]. Recent data suggest that DPP4 activity is higher in patients with T1DM compared to healthy controls independently of islet-cell antibody status, C-peptide concentration, disease duration or glycated hemoglobin (HbA1c) level [3,4] and an inverse correlation with body mass index (BMI) and insulin sensitivity. However, the major source of soluble DPP4 fraction in T1DM remains unknown [4]. Recently Lamers et al. (2011) [6] documented that a portion of soluble DPP4 might originate from differentiated adipocytes performing a comprehensive proteomic media derived from primary human adipocytes. They proposed it to be a novel adipokine linking adipose tissue to insulin resistance (IR). IR is inability of insulin to produce its peripheral tissue actions; predominantly in the liver and muscle. It refers either to interference of insulin binding to its s receptor and/or impairment of insulin signalization distal from the cell surface [7–9]. Although IR typically characterizes type 2 diabetes mellitus (T2DM), while the insulin deficiency is a primary defect in T1DM patients, there are suggestions that a certain degree of IR exists in T1DM [10,11]. The mechanisms of IR development in T1DM are likely due to a combination of supraphysiologic level of exogenous insulin and obesity, although it was thought to be primarily related to hyperglycemia for a long time [12]. It is now known that adults with T1DM have both impaired glucose utilization and impaired insulin-induced non-esterified fatty acid suppression, independent of glycemic control [13]. Skeletal muscle IR is a known feature of T1DM and is due to decreased glucose transport into myocytes [14]. Hepatic IR has been recognized in T1DM as well as in T2DM [15] but whether it is related to hepatic steatosis like in T2DM remains unclear [16,17]. The Insulin Resistance Atherosclerosis Study (IRAS) results indicate that elevated alanine aminotransferase (ALT) is independently associated with IR which is in accordance with the hypothesis that liver fat might contribute to its development [9].

DPP4 is expressed on bile canaliculi, hepatocytes and hepatic stellate cells [18]. Current data suggest the association of serum DPP4 activity

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with different chronic liver diseases. Furthermore, DPP4 was recently proposed as a novel non-alcoholic fatty liver disease (NAFLD) biomarker, a state considered to be a hepatic manifestation of IR [19]. High serum DPP4 activity in NAFLD correlates with liver tests but not with the fasting plasma glucose or HbA1C in T2DM patients and in normal glucose tolerance controls.

Thus, the speculation is that hepatic IR contributes to DPP4 serum concentration and/or activity. In this cross-sectional study we aimed to assess the possible correlation between fasting serum DPP4 activity, IR and liver enzymes in order to elucidate the question of hepatic contribution to serum DPP4 activity in T1DM patients.

Material and methods

Patients and study design

This cross-sectional study was undertaken at the University Clinic for diabetes, endocrinology and metabolic diseases Vuk Vrhovac (Zagreb, Croatia). Forty four T1DM patients aged over 18 and below 65 years comming for their comprehensive annual review were recruited. The sample size was in accordance with G power 3.1.7 calculation for correlations (two tailed t test, total sample size $= 44$, $\alpha = 0.05$, $1 - \beta = 0.8$, $\rho = 0.4$). The inclusion criteria were: age at onset of diabetes younger than 40 years, positive autoantibodies and time to definite insulin therapy less than a year. Non inclusion criteria were: medical history of cardiovascular diseases or electrocardiogram (ECG) evidence of ischemic heart disease, any systemic disease and any infection in the previous month, thyroid hormone therapy, medications that might affect glucose metabolism and insulin sensitivity such as glucocorticoids or oral contraceptives. The study subjects could be using antihypertensive or lipid lowering drugs (i.e., statins: atorvastatin and simvastatin).

Insulin sensitivity was calculated using the equation derived from euglycemic–hyperinsulinemic clamp studies-estimated glucose disposal rate (eGDR): 24.31–12.2X(WHR)–3.29X(AHT)–0.57X(HbA1c), where the units are $mgkg^{-1}$ min^{-1}, WHR indicates the waist to hip ratio, AHT indicates blood pressure, and is expressed as: 0—no, 1—yes. Those on blood pressure medications or with blood pressure >140/90 mm Hg were considered to have hypertension [20], the equation was derived from a substudy of 24 EDC (Epidemiology of Diabetes Complications) participants who underwent euglycemic–hyperinsulinemic clamp studies [21]. Lower eGDR levels indicate greater insulin resistance. The study was conducted according to the guidelines laid down in the Declaration of Helsinki. This study has been cleared by Merkur University Hospital Ethics Review Board for human studies (No 6565) and written informed consent was obtained from and signed by all patients.

Anthropometric measurements and laboratory analysis

Basic anthropometric measurements were performed as follows: waist circumference was measured on bare skin as the narrowest circumference between the 10th rib and the iliac crest while hip circumference was measured at the widest point of the gluteal muscles using a tailor measure and expressed in centimeters in order to calculate WHR. Weight was measured using a balanced beam scale with light clothing without shoes and expressed in kilograms (kg) and height was measured using a wallmounted stadiometer and expressed in centimeters (cm) in order to calculate BMI. Blood pressure was measured twice in the sitting position with a mercury sphygmomanometer after a resting period of 10 min (mm Hg, reference interval 130/80). Fasting venous blood samples were collected for the determination of lipid profile status [total cholesterol (mmol/L, reference interval 5.0), HDL cholesterol (mmol/L, reference interval [1.0 for men, [1.3 for women), LDL cholesterol (mmol/L, reference interval\3.0), VLDL cholesterol (mmol/L), and triglycerides (mmol/L, reference interval\1.7)], HbA1c (%, reference interval 3.5–5.7), liver biochemistry [AST (units/L, reference range 11–38), ALT (units/L, reference range 12–48), GGT (units/L, reference range 11–55), ALP (units/L, reference range 60–142)] and serum DPP4 activity. HbA1c was measured spectrophotometrically by turbidimetric immunoinhibition (Olympus AU600;p Beckman-Coulter, USA). Total serum cholesterol, HDL cholesterol and triglycerides in serum were measured by an HDL-C were estimated by homogenous enzymatic colorimetric method [20–22]. LDL cholesterol was estimated using Friedewald formula: LDL-C = TC 2 HDL-C 2 TG/2.2 (mmol/L). In this formula, TG stands for triglycerides, and TG/2.2 (or TG/5) serves as a proxy for VLDL cholesterol. The ratio of the mass of triglyceride to that of cholesterol in VLDL is assumed to be relatively constant [23]. AST, ALT, GGT and ALP were measured using standard laboratory methods coupling of the respective reactions with dehydrogenase reactions: oxaloacetate → malate and for ALT pyruvate → lactate. Both reactions oxidize NADH to NAD^+ with the disappearance of NADH, subsequently measured at 340 nanometers. GGT is assayed by measurement of a chromogenic end product when GGT catalyzes gamma-glutamyl-p-nitroanilide to liberate p-nitroaniline. AP is measured by an international accepted standard method for serum enzyme activity assessment is the International Federation of Clinical Chemistry/American Association for Clinical Chemistry (AACC) reference method [24–26]. After clotting, the sera were separated and kept at −70 °C until the determination of DPP4 enzymatic activity. DPP4 activity was measured by a colorimetric assay procured from Sigma, St. Louis, MO, USA in a microplate reader (Cary Eclipse Varian, Agilent Technologies) at 460 nm, 37 °C in a continuous monitoring for 35 min. In this assay, DPP4 cleaves H-Gly-Pro-AMC to release a florecent product, 7-amino-4-methyl coumarin (AMC) which can be measured spectrophotometrically. All the DPP4 assays were run in duplicates. Briefly, 50 μL of serum sample was added to 96-well plates, followed by the addition of 10 μL assay buffer. After 10 min of pre-incubation at 37°, the enzymatic reaction was started with the addition of 40 μL of Master Reaction Mix containing 2 μL substrate and 38 μL of the assay buffer. Liberation of AMC was monitored continuously at excitation 360 nm and emission 460 nm every 5 min for up to 35 min in a 96-well black flat bottom plate. Fluorometric catalysis rates were determined from the linear portion of the curve of the increase in fluorescence and were calculated as the slope of the regression line determined from the line. DPP-4 was expressed as pmol/min/mL (U/L). One unit of activity was defined as the amount of enzyme which will hydrolyze the DPP4 substrate to yield 1.0 μmole of AMC per minute at 37 °C.

Data analysis and statistics

The data distribution was assessed by Shapiro–Wilk test. All the continuous variables were log-transformed and reported as mean values and 95%CI of means, whereas categorical variables were reported as numbers and percentages. Because we found normal distribution of the data, the differences between two study groups were tested by Student's t-test while the categorical variables were analyzed by the χ^2 test. Correlations between fasting serum DPP4 activity with anthropometric and metabolic variables were determined using Pearson's correlation coefficient. All the tests were two-sided. The association between fasting serum DPP4 activity and AST value was further evaluated in multivariate linear regression. Adjustments were performed for age, gender, disease duration, HbA1c and the use of statins since it is yet to be clarified whether they affect serum DPP4 activity. Level of statistical significance was chosen to be 0.05. Statistical analysis was performed by Statistical Package for the Social Sciences (SPSS) ver. 17.0 and MedCalc 11.0 for Windows.

Results

The clinical and biochemical characteristics of all 44 T1DM patients are given in Table 1. Out of 44 study participants, 28 (63.6%) were male, mean age approximately 45 years and 21 years of diabetes duration. Thirty patients (68.2%) were using statins and 22 (50%) antihypertensive agents. Patients were divided into two groups according to the