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Circulating dipeptidyl peptidase-4 activity is associated with insulin resistance in type 1 diabetic patients

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ABSTRACT

Aim: The pathophysiology of insulin resistance (IR) comprises a complex adipokine mediated cross-talk between white adipose tissue and other organs. Dipeptidyl peptidase-4 (DPP4) is protease recently proposed as a novel adipokine linked to IR. We aimed to assess the relationship between fasting serum DPP4 activityand IR in type 1 diabetic (T1DM) patients.
Methods: A cross-sectional study comprised 44 T1DM patients aged >18 and <65 years. IR was esimated using the equation for insulin sensitivity 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.
Results: Patients were divided according to DPP4 activity tertiles (<25.40; ≥36.54 U/L). Fasting serum DPP4 activity was related to disease duration ($p = 0.012$), systolic ($p = 0.009$) and diastolic ($p = 0.047$) blood pressure, waist circumference ($p = 0.037$), urine albumin excretion ($p = 0.022$) and conversely related to eGDR ($p = 0.004$). The linear regression has shown that eGDR decreases for 0.203 mg kg^{-1} min^{-1} by each increase of serum DPP4 activity of 1 U/L ($p < 0.001$) after adjustment for adjusted for age, gender, disease duration, albuminuria and the use of antihypertensives and statins.
Conclusion: Serum DPP4 activity is associated with IR in T1DM patients and it might play an important role in its pathophysiology.

1. Introduction

Insulin resistance (IR) is an inability of insulin to produce its actions in peripheral tissue derived either from interference of insulin binding to its surface receptor or by impairment of insulin signalisation distal from the cell surface (Fraser et al., 2009; Hanley, Wagenknecht, Festa, D'Agostino, & Haffner, 2007; Reaven, 1995). Although IR typically characterises type 2 diabetes mellitus (T2DM), while the insulin deficiency is considered as a primary defect in type 1 diabetes mellitus (T1DM), a consistent body of literature suggests that there is a certain degree of IR in patients with T1DM (DeFronzo, Hendler, & Simonson, 1982; Ghosh, Collier, Hair, Malik, & Elhaad, 2010). The mechanisms of IR in T1DM are likely due to a combination of supraphysiologic levels of exogenous insulin and obesity. In the past, it was thought that IR in T1D was primarily related to hyperglycemia (Yki-Järvinen, Helve, & Koivisto, 1987). It was recently proposed that adults with T1DM have both impaired glucose utilisation and impaired insulin-induced non-esterified fatty acid suppression, independent of glycemic control (Schauer et al., 2011). White adipose tissue has been recognized as major endocrine organ producing a huge diversity of adipokines which build a complex inter- and intra-cellular feedback loops that could link obesity to IR (Arner, 2003; Breitling, 2003). Lamers, Famulla, Wronkowitz, et al. (2011) (Lamers et al., 2011) have performed a comprehensive proteomic profiling of the media derived from primary human adipocytes and proposed dipeptidyl peptidase-4 (DPP4) as a novel adipokine linking adipose tissue to IR.

DPP4 is a serine exopeptidase also known as adenosine desaminase complexing protein 2 (ADCP 2) or T-cell activation antigen CD26 which cleaves X-proline dipeptides from the N-terminus polypeptides such as chemokines, neuropeptides and peptide hormones (Ansorge, Nordhoff, Bank, et al., 2011). It exists in two forms: as an integral membrane glycoprotein expressed ubiquitously on the cell surface and in a soluble form in the circulation. 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 (Yazbeck, Howarth, & Abbott, 2009) although the major

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source of soluble DPP4 fraction remains unknown. Since T1DM is an immune mediated condition, the reports on elevated DPP4 activity in serum are not surprising (Varga et al., 2011). The data from two independent studies suggest that DPP4 activity is higher in patients with T1DM compared to healthy controls but independently of islet-cell antibody status, C-peptide level, disease duration or glycated haemoglobin (HbA1c) level (Iwabuchi et al., 2013; Varga et al., 2011) However, they do report an inverse correlation with body mass index and insulin sensitivity (Iwabuchi et al., 2013). Serum DPP4 activity is also higher in T2DM individuals with IR compared to those without (Firneisz, Varga, Lengyel, et al., 2010). DPP4 is highly expressed on kidney cell surface and data are available to suggest that DPP4 levels may associated with of renal function (Sun, Deng, Guan, et al., 2012). Moreover, insulin sensitivity, assessed by hyperinsulinemic–euglycemic clamp, is continuously associated with a greater risk of increasing albuminuria (Pilz, Rutters, Nijpels, et al., 2014). Accordingly, the aim of this study was to investigate relationship between fasting serum DPP4 activity, insulin resistance and renal function in T1DM patients.

2. Subjects

This cross-sectional was study undertaken at the University Clinic for diabetes, endocrinology and metabolic diseases Vuk Vrhovac (Zagreb, Croatia). We recruited 44 T1DM C-peptide negative (C-peptide <0.3 ng/mL) patients aged >18 and <65 years coming for their comprehensive annual review. 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 diagnosis of T1DM was defined as suggested by American Diabetes Association, 2010. The inclusion criteria were: age at onset of diabetes younger than 40 years, positive autoantibodies and time to definite insulin therapy less then 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). The study was conducted according to the guidelines laid down in the Declaration of Helsinki. Written informed consent was obtained from and signed by all patients.

2.1. Subjects and methods

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 mg kg^{-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 mmHg were considered to have hypertension; the equation was derived from a substudy of 24 EDC (Epidemiology of Diabetes Complications) participants who underwent euglycemic–hyperinsulinemic clamp studies (Williams, Erbey, Becker, Arslanian, & Orchard, 2000). Lower eGDR levels indicate greater insulin resistance.

2.2. Laboratory analysis

The detailed description of the methods concerning anthropometric measurement and standard laboratory procedures was as previously described (Blaslov, Bulum, Zibar, & Duvnjak, 2010). Fasting venous blood samples were collected for the determination of biochemistry panel, lipid profile status, glycated haemoglobin A1c (HbA1c) and serum DPP4 activity. After clotting, the sera were separated and kept at −70 °C until the determination of enzymatic activity. Urine albumin excretion (UAE) was measured from at least two 24-h urine samples and determined as the mean of 24-h urine collections and expressed as mg/24 h. Patients performed collections on two consecutive days to minimise variability. Data on serum creatinine levels, age, sex and race were used to calculate the estimated GFR (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, which has been shown to be accurate in determining renal function in diabetic patients with normal renal function (Levey et al., 2009; Vučić Lovrenčić et al., 2012). 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 fluorescent 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 °C, 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. DPP4 was expressed as pmol/min/mL (U/L). One unit of activity was defined as the amount of enzyme which will hydrolise the DPP4 substrate to yield 1.0 μmol of AMC per minute at 37 °C.

2.3. 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 three study groups were tested by one-way ANOVA followed by Bonferroni's correction for multiple comparisons while the categorical variables were analysed 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 eGDR value was further evaluated in multivariate linear regression. Adjustments were performed for age, gender, disease duration, eGFR, UAE, the use of statins and antihypertensive agents 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.

3. 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, i.e. angiotensin-converting enzyme inhibitors (ACEI). Patients were divided into three groups according to the tertiles (25th, 50th and 75th) of fasting serum DPP4 activity. The detailed clinical and laboratory findings and the difference between them are given in Table 2. The group of patients in the 1st tertile of fasting serum DPP4 activity had a significantly shorter disease duration ($p = 0.012$) and the lowest systolic ($p = 0.009$) and diastolic ($p = 0.047$) blood pressure, waist circumference ($p = 0.037$) and UAE ($p = 0.022$) compared to the second and third tertiles. The eGDR was significantly higher in the group with lowest