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Dipeptidyl peptidase-4 activity is associated with urine albumin excretion in type 1 diabetes

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ABSTRACT

Aims: The inability of kidneys to prevent urinary protein leakage represents the earliest sign of renal damage in diabetic kidney disease (DKD). Recent data suggest the possible nephroprotective role of the dipeptidyl peptidase-4 (DPP-4) inhibitors. We aimed to investigate whether serum DPP-4 activity is associated with urine albumin excretion (UAE) in patients with type 1 diabetes (type 1 DM).
Methods: DPP-4 activity and UAE measurement were performed in 113 patients with type 1 DM and glomerular filtration rate (GFR) within normal range. They were divided into three groups according to UAE tertiles.
Results: Worse lipid profile and higher waist circumference were observed in the group with highest DPP-4 activity. Patients within lowest UAE tertile group had lowest DPP-4 activity value ($p < 0.001$) compared to group within second and third tertile of UAE. DPP-4 activity correlated with systolic blood pressure ($\rho = 0.142$; $p = 0.001$), HbA1c ($\rho = 0.133$; $p = 0.013$) and UAE ($\rho = 0.349$; $p < 0.001$). In the linear regression analysis when DPP-4 activity was adjusted for age, gender, disease duration, HbA1c, waist circumference, the use of ACEI and hypolipemic agents the association remained significant; UAE increased for 8.136 mg/24 h by each increase of DPP-4 activity of 1 U/L ($p < 0.008$).
Conclusion: Our results indicate that serum DPP-4 activity is associated with albuminuria in type 1 diabetes. This arises the question whether the use of DPP-4 inhibitors might serve as an additional therapeutic strategy to prevent proteinuria in patients with DKD.

1. Introduction

Diabetic kidney disease (DKD) represents one of the most frequent microvascular complications of diabetes mellitus (DM) with an increasing prevalence worldwide (Reutens, 2013). It is defined by the presence of albuminuria followed by decreased glomerular filtration rate (GFR) (Kramer et al., 2007). The inability of kidneys to prevent urinary protein leakage represents an important early sign of renal damage in patients with diabetes (Mogensen & Poulsen, 1994) which is accompanied by several histological abnormalities. Characteristic glomerular changes in DKD include podocyte loss, glomerular basement membrane (GBM) thickening and mesangial expansion due to increased mesangial matrix and hypertrophy of mesangial cells (Caramori, Kim, Huang, et al., 2002). Over time, due to mesangiolysis there is a disruption and disintegration of the normal glomerular architecture which leads to microaneurysm formation, decrease in filtration surface area and gradual GFR reduction. Numerous studies have shown that the risk of DKD is tightly linked to poor glucose control accompanied with dyslipidemia and hypertension in both type 1 and type 2 diabetes mellitus (DM) (Adler et al., 2003; Dronavalli, Duka, & Bakris, 2008; Foley et al., 2005; Mogensen, 1999).

Dipeptidyl peptidase-4 (DPP-4) is a serine exopeptidase distributed throughout the body that cleaves numerous substrates such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) which play an important role in glucoregulation by stimulating insulin release in a glucose-dependent manner (Cernea & Raz, 2011). Thus, DPP-4 inhibitors represent a relatively novel class of oral glucose-lowering agents with good efficacy in treatment of type 2 DM. The kidney is where DPP-4 is expressed at the highest level per organ weight (Kanasaki, Shi, Kanasaki, et al., 2014; Mentlein, 1999) and the clinical observations suggest that they might decrease albuminuria in patients with type 2 DM (Cooper, Perkovic, McGill, et al., 2015).

Recent data, however, suggest that DPP-4 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) even when compared to type 2 DM (Firneisz, Varga, Lengyel, et al., 2010; Iwabuchi et al., 2013). So far, there are interesting findings emerged from experimental studies

Conflict of Interest: None.

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which demonstrated the protective effect of DPP-4 inhibitors on the DKD in insulin deficient diabetic mice (Kanasaki et al., 2014; Tanaka, Kume, Chin-Kanasaki, et al., 2016).

Since patients with DKD have a markedly increased risk of cardiovascular complications, (Foley et al., 2005; Mogensen, 1999) the identification of novel DKD biomarkers that might contribute to better understanding of the disease or even provide a pathway to potential novel therapeutic approaches is of a special clinical interest. Thus, we aimed to investigate whether serum DPP-4 activity is associated with albuminuria in patients with type 1 DM.

2. Methods

The study was performed at the In-Patient Department of Diabetology of the Vuk Vrhovac University Hospital, Medical School University of Zagreb, Croatia and included 113 type 1 DM patients. Histories, complete physical examination and laboratory tests were performed in all subjects in order to exclude diseases other than T1DM or medications that might affect cause transient increases in urine albumin excretion (UAE) rate such as fever, marked exercise or exacerbations of congestive heart failure, or resistant hypertension (Jefferson & Shankland, 2008).

Type 1 DM was defined by undetectable meal stimulated C-peptide concentrations (C-peptide <0.2 ng/mL) and positive islet cell and glutamic acid autoantibodies (at least from the previous medical record if the measurement was performed in our Clinic laboratory, respectively). All of the patients were non-smokers and were not using any glucose lowering agent except insulin which was administered by a basal-bolus regimen. Furthermore, patients with glomerular filtration rate <90 mL/min per 1.73 m^2 were not included. In total, 125 patients were approached; two refused to participate, 6 had concomitant respiratory infection and 4 had urinary tract infection. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by local Ethics committee. Written informed consent was obtained from and signed by all patients.

Fasting venous blood samples were collected for the determination of a complete blood count (CBC), glycated hemoglobin (HbA1c, %, reference interval 3.5–5.7), C-reactive protein (CRP), serum creatinine and liver function tests in order to exclude a wide range of disorders that might affect the study results.

UAE was measured from at least two 24-h urine samples and determined as the mean of 24-h urine from two consecutive days to minimize variability. Serum creatinine was measured in fasting blood sample. Data on serum creatinine levels, age, sex and race were used to calculate the estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, which was shown to be accurate in determining renal function in diabetic patients with normal renal function (Levey et al., 2009; Vučić Lovrenčić et al., 2012).

Blood pressure was measured in the sitting position with a mercury sphygmomanometer with a cuff appropriate to the length and circumference of the arm after a resting period of 10 min and expressed in mmHg. Patients taking blood pressure medications or with blood pressure >140/90 mmHg were considered to have hypertension. Fasting venous blood samples were collected for the determination of biochemistry panel, lipid profile status, and HbA1c. Cholesterol and triglycerides in serum were measured by an enzymatic colorimetric method. Beside the lipid profile status, those patients with history of lipid-lowering agents consumption were considered to have dyslipidaemia. HbA1c was measured spectrophotometrically by turbidimetric immuno-inhibition (Olympus AU600; Beckman-Coulter, CA, USA).

DPP-4 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, DPP-4 cleaves H-Gly-Pro-AMC to release a fluorescent product, 7-amino-4-methylcoumarin (AMC) which can be measured spectrophotometrically as previously described (Blaslov, Bulum, & Duvnjak, 2015a). One unit of activity was defined as the amount of enzyme which will hydrolise the DPP-4 substrate to yield 1.0 μmol of AMC per min at 37 °C.

The data distribution was assessed by Shapiro–Wilk test. All the continuous variables are reported as median and range, whereas categorical variables were reported as numbers and percentages. The differences between study groups were tested by χ^2 test for the attributive variables. Correlations between parameters were determined using parametric Spearmans correlation coefficient. All the tests were two-sided. The association between DPP-4 activity and UAE was further evaluated in multivariate linear regression. Adjustments were performed for gender, age, disease duration, HbA1c value, hypertension, ACEI use, dyslipidaemia (*i.e.* the use of statins). Statistical interference is based on 95% confidence intervals (CIs) and 5% *P* values. All statistical analysis was conducted using the statistical package Statistical Package for the Social Sciences (SPSS) ver.17.0 for Windows.

3. Results

The median age of our study population was 51 (21–74) years with a duration of diabetes 19 (10.5–28) years. Seventy five (66.37%) were male. Patients were divided into three groups according to the tertiles of albuminuria. Eighty-one (71.68%) participants were using angiotensin-converting enzyme (ACE) inhibitors while 50 (44.24%) had diagnosed hypertension. There was no significant difference in the ACEI use (N = 30 *vs* 25 *vs* 26, $p = 0.064$), however, nor there was difference in hypertension presence inbetween groups of UAE (N = 19 *vs* 17 *vs* 22, $p = 0.803$). Furthermore, when patients were divided into two groups according to the ACE use in order to evaluate the potential effect of ACEI on DPP-4 activity, no significant difference was observed (28.82 (21.53–39.53) *vs* 31.92 (21.48–39.92), $p = 0.779$). Fourty nine (43.44%) were using statins with a higher rate in the group with higher UAE (N = 13 *vs* 16 *vs* 20, $p = 0.006$). The detailed clinical and laboratory findings and the difference between them are given in Table 1. The group of patients with higher UAE showed higher DPP-4 activity (<0.001) compared to the group with lower UAE without difference in the GFR, however, they also did have a higher frequency of both non-proliferative and proliferative retinopathy ($p = 0.002$). DPP-4 activity showed positive correlation with systolic blood pressure ($\rho = 0.142$; $p = 0.001$), HbA1c ($\rho = 0.133$; $p = 0.013$) and UAE ($\rho = 0.349$; $p < 0.001$). The simple linear regression with UAE as dependent variable has shown that it increases by 9.764 mg/24 h by each increase of DPP-4 activity of 1 U/L ($p < 0.006$). Furthermore, when DPP-4 activity was additionally adjusted for the possible confounders in five different models (Table 2.), the association remained significant, showing that UAE increases for 8.583 mg/24 h by each increase of DPP-4 activity of 1 U/L ($p < 0.008$) controlled for age, gender, disease duration, HbA1c, BMI, hypertension presence, the use of ACEI and hypolipemic agents (Table 2.). In addition, using a separate linear regression model, we found that by each increase of DPP-4 activity of 1 U/L systolic blood pressure increases for 1.054 (1.006–1.611, $p = 0.004$), while the association with diastolic blood pressure (0.119 (−0.045–0.283), $p = 0.153$) and HbA1c (0.012 (−0.012–0.026), $p = 0.325$) was not significant. Finally, in the binary linear regression, DPP-4 wassignificantly associated with diabetic retinopathy presence (1.377 (1.145–1.655), $p < 0.001$).

4. Discussion

This cross-sectional study was designed in order to examine the association of serum DPP-4 activity with UAE in a population of patients with type 1 DM with GFR within the normal range. It revealed that UAE is higher in those individuals with higher serum DPP-4.