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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/24h 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.

Key words: albuminuria, dipepidyl peptidase-4, type 1 diabetes mellitus, diabetic kidney disease

1. Introduction

Diabetic kidney disease (DKD) represents one of the most frequent microvascular complications of diabetes mellitus (DM) with an increasing prevalence worldwide [1]. It is defined by the presence of albuminuria followed by decreased glomerular filtration rate (GFR) [2]. The inability of kidneys to prevent urinary protein leakage represents an important early sign of renal damage in patients with diabetes [3] 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 [4]. 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) [5-8].

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 [9]. 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 [10, 11] and the clinical observations suggest that they might decrease albuminuria in patients with type 2 DM [12].

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 [13, 14]. So far, there are interesting findings emerged from experimental studies which demonstrated the protective effect of DPP-4 inhibitors on the DKD in insulin deficient diabetic mice [15, 16].

Since patients with DKD have a markedly increased risk of cardiovascular complications [5, 6] 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 [17].

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/1.73m2 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 haemoglobin (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 [18, 19] .

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 minutes 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, 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, 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-Methyl Coumarin (AMC) which can be measured spectrophotometrically as previously described [20]. 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 minute 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 $\chi 2$ 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 (ρ=0.142; p=0.001), HbA1c (ρ=0.133; p=0.013) and UAE (ρ=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/24h 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. Additionally, beyond already well-established factors associated with albumiuria (HbA1c, obesity and hypertension), we showed that serum DPP-4 activity is significantly higher in type 1 DM patients with albuminuria and that the association remained significant in the linear regression analysis after adjustments for the well established confounders. This finding is partially in support to recent meta-analysis derived from clinical observations which suggest that the use of DPP-4 inhibitors for type 2 DM treatment has pleiotropic effects besides glycaemic control, i.e. that it might be effective in albuminuria reduction [12]. However, none of the studies was primarily designed to test the effect of DPP-4 activity on microalbuminuria and renal function nor the correlation between DPP-4 activity and albumin excretion.

The presence of albuminuria implies either glomerular filtration barrier and/or in tubular reabsorption dysfunction [21]. Podocyte loss is one of the first changes contributing to increased glomerular permeability for albumin [22]. Nevertheless, albuminuria may also

occur in the complete absence of structural changes of podocytes [23]. Tubular dysfunction is another important player promoting albuminuria by lysosomal dysfunction which is promoted by renin-angiotensin system (RAS) activation [24]. It results in increase of circulating levels of renin, angiotensinogen, angiotensin-converting enzyme [ACE), aldosterone and angiotensin II (AngII) increase is associated with DKD. AngII is widely known to adversely affect the progression of renal disease by a sum of mechanismsItleads to induction of intrarenal inflammation and cell apoptosis which is why ACE and AngII inhibitors found their place in DKD prevention and treatment [25]. We did not find the difference in the use of ACE inhibitors (ACEI) between two study groups, neither the difference in the serum DPP-4 activity reached statistical significance betweev ACEI users and nonusers. Moreover, in the regression analysis after adjustment for the ACEI use, DPP-4 activity remained positively associated to UAE.

Although there are several studies linking DPP-4 activity to albumiuria and DKD [11, 15, 16] the exact mechanisms linking DPP-4 activity and renal injury is still largely speculated. DPP-4 is normally bound on the surface of many cell types including kidney proximal tubular and endothelial cells [10]. Recently, Kanasaki et al. (2014) [11] found that DPP-4 protein expression and activity levels of DPP-4 was increased in the whole kidney lysate, endothelial cells, tubules, and glomeruli of streptozotocin (STZ)-induced diabetic kidney while microvesicle-bound DPP-4 secreted from tubular epithelial cells that can be detected in urine may be an early marker of renal damage before the onset of albuminuria [26]. Sun et al. (2012) [26] described higher urinary microvesicle DPP-4 levels in patients with diabetes compared to nondiabetic controls that positively correlated with extent of albuminuria in patients. In addition, Liu et al. (2012) [27] reported that the administration of the DPP-4 inhibitor vildagliptin for 24 weeks prevents kidney damage in STZ-induced diabetic rats. Furthermore, DPP-4 inhibitor linagliptin worked very well in an animal model of type 1

diabetic nephropathy on top of guideline based background medication [28] and it is worth of mention that it works even in non-diabetic CKD models [29].

Renal effects of DPP-4 inhibitors appear to be mediated by targeting diabetes related abnormalities: hyperglycaemia, dyslipidaemia along with oxidative stress and low grade inflammation in a direct or indirect pathway. Hyperglycaemia is recognized as the most important factor for DKD development and progression in type 1 DM [7]. Increased systemic DPP-4 activity degradates incretine hormones GLP-1 and GIP and thus might contribute to glycaemic control deterioration and the increase in advanced end glycosilation production and thus causes renal injury [30]. Dyslipidaemia, defined as high total cholesterol, high low-density lipoprotein cholesterol and low high-density cholesterol with race cut-off's may cause renal mesangial and epithelial cell injury and promote renal disease progression [31, 32]. Recently, Tanaka et al. (2016) [15] provided the evidence on the possible renoprotective effect of DPP-4 inhibitors in cultured mouse proximal tubular cells by inhibiting tubulointerstitial injury induced by inflammation, fibrosis, and apoptosis in mice without altering systemic characteristics including body weight, fasting blood glucose, and food intake. The increased serum DPP-4 activity might increase the degradation process of endogenous GLP-1 resulting in worsening of the mentioned parameters. According to current literature, DPP‑4 inhibition and the GLP‑1 receptor each play an important role in this process that appears to be independent of any associated impact on blood glucose which is also in support with our study results since we did not found a significant association between DPP-4 activity and HbA1c in the linear regression model. The relevance of the GLP‑1 receptor is supported due to its expression on podocytes. In line with this, there are data showing that kidney protection in diabetic animals can be achieved with the GLP‑1 analogues, as well. The pathway comprising DPP‑4, GLP‑1 and the GLP‑1 receptor presents several an interesting features in the setting of DKD [33]. Furtermore, an observation

that DPP‑4 activity inhibition decreases NHE3 exchanger function, a transporter that mediates sodium reabsorption in renal proximal tubule could provide an additional connection between glucose homeostasis, renal function and blood pressure that links DPP-4 activity with DKD.

In our study, we found that DPP-4 activity is associated with systolic blood pressure that as already mentioned might cause podocyte loss [23-25]. It is proposed that DPP-4 inhibition reduces microvascular tone through direct nitric oxide (NO) system preventing acute podocyte ischemia events. Therefore, we can generate the conclusion that increased serum DPP-4 activity might increase vascular tonus resulting in podocyte and/or tubular lesions that gradually lead to albumin leakage, endothelial proliferation, nodule formation, decrease in filtration surface area and gradual GFR reduction that is similar patophysiological process to diabetic retinopathy developmet, and we did found a significant association between retinopathy presence and DPP-4 activity as previously described [34] .Our results indicate only that serum DPP-4 activity is strongly associated with albuminuria in type 1 DM patients and thus that it might be of a great importance in DKD development in addition to already known risk factors. However, the lack of control group and the cross-sectional study design clearly diminishes its power for deriving any general conclusions. In addition, we did not measure urinary DPP4 activity which would be much closer to the local kidney DPP4 activity as compared to serum DPP4 activity and provided us with more relevant results. However, we do believe that even this results raise the question whether the use of DPP-4 inhibitors might serve as an additional therapeutic strategy to protect proximal tubular cells against proteinuria in patients with DKD. Thus, the relationship between DPP-4-activity and DKD progression in the prospective analysis would be of great interest.

5. Conflict of Interest: None.

6. References

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Table 1. Patients anthropometric and laboratory characteristics according to the DPP-4 activity median value

Variable	Urine albumin excretion <23.55 mg/24h N=37	Urine albumin excretion >23.55-41.08 mg/24h N=40	Urine albumin excretion >41.08-365.6 mg/24h N=36	***p***
Age (years)	50 (21-74)	51 (23-71)	53 (27-65)	0.812
Gender (M/F)	23/14	25/15	22/14	0.056
BMI (kg/m2)	25 (20-29)	23 (19-30)	26.5 (23-33)	0.067
Waist circumference (cm)	87.5 (81-97.5)	91 (68-102)	91 (72-111)	0.019
Diabetes duration (years)	24 (21-46)	26 (25-34)	24 (22-36)	0.563
HbA1c (%)	7.1 (5.2-9.3)	7.2 (5.5-11.7)	7.0 (6.2-8.1)	0.886
Heart rate (beats per minute)	72 (46-104)	75 (53-100)	71 (50-106)	0.067
Systolic blood pressure (mmHg)	125 (100-145)	120 (110-160)	120 (100-155)	0.975
Diastolic blood pressure (mmHg)	80 (65-100)	80 (70-100)	80 (75-90)	0.553
Total serum cholesterol (mmol/L)	4.98 (3.22-6.70)	5.31 (4.49-6.48)	4.73 (3.77-5.61)	0.573
HDL cholesterol (mmol/L)	1.56 (1.03-2.89)	1.74 (1.22-2.25)	1.51 (1.03-1.72)	0.347
LDL cholesterol (mmol/L)	2.81 (0.99-3.95)	2.70 (1.60-3.75)	2.87 (1.92-3.39)	0.452
Triglycerides (mmol/L)	1.11 (0.52-2.72)	1.02 (0.87-2.58)	1.10 (0.71-1.53)	0.214
Serum creatinine (μmol/L)	72 (49-84)	57 (41-90)	78 (64-90)	0.058
CKD-EPI (mL/min/1.73 m^2)	98 (90-130)	108 (94-110)	98 (28-90)	0.558
Urine albumin excretion (mg/24h)	5.4 (1.55-23.55)	31.80 (23.55-41.08)	174.80 (41.10-365.6)	<0.001
Non-proliferative retinopathy presence (N, %)	6 (16.21)	11 (27.5)	18 (50.0)	0.002
Proliferative retinopathy presence (N, %)	0 (0)	1 (2.5)	8 (22.2)	<0.001
C reactive protein (mg/L)	1.1 (0.1-4.9)	2.6 (0.1-3.6)	4.8 (0.1-4.9)	0.347
AST (U/L)	22 (10-44)	24 (17-34)	24 (17-34)	0.602
ALT (U/L)	21 (13-58)	26 (10-31)	20 (17-35)	0.860
GGT (U/L)	19 (9-63)	23 (18-56)	24 (11-42)	0.278
AP (U/L)	77 (61-115)	87 (75-99)	78 (66-109)	0.479
DPP-4 activity (U/L)	24.55 (19.21-36.20)	30.86 (22.41-38.31)	37.79 (27.86-39.53)	<0.001

Legend: DPP-4: dipeptidyl peptidase; AST: aspartat aminotransferase; ALT: alanin aminotransfrerase; GGT: gamma glutamil transferase; AP:alkaline phosphatase; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration formula

Table 2. Linear regression analysis for the serum DPP4 activity (U/L) and the UAE (mg/24h) derived from twoseparate models

	B	β	p	95% confidence interval	
				Lower bound	Upper bound
MODEL 1					
Serum DPP activity (U/L)	9.764	0.270	0.006	2.821	16.707
MODEL 2					
Serum DPP activity (U/L)	8.136	0.309	0.009	2.112	14.160
Age (years)	1.606	0.067	0.549	-3.696	6.908
Gender (male)	3.617	0.142	0.241	-5.251	7.485
Disease duration (years)	2.743	0.110	0.305	-2.546	8.031
HbA1c (%)	14.254	0.066	0.518	-57.933	29.424
ACEI use (%)	-1.161	-0.002	0.985	-127.499	125.177
Systolic blood pressure (mmHg)	1.182	0.071	0.514	-4.769	2.404
Waist circumference (cm)	2.172	0.090	0.483	-3.962	8.306