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Urinary transferrin as an early biomarker of diabetic nephropathy

Urinarni transferin kao rani marker dijabetesne nefropatije

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Abstract

Background/Aim. Diabetic nephropathy is one of the leading cause of chronic kidney disease and end-stage renal disease. It occurs in 20%–40% of patients with diabetes mellitus and microalbuminuria is still considered as the first sign of diabetic nephropathy. Low sensitivity and specificity of microalbuminuria lead to more sensitive biomarkers that may be used to detect diabetic nephropathy at an earlier stage with a higher accuracy. This study was carried out to determine whether urinary transferrin can serve as an indicator of diabetic nephropathy. **Methods.** Our study included 80 type 2 diabetic patients who were classified into two groups: group 1 – normoalbuminuric patients (albumin excretion up to 30 mg/d); group 2 – microalbuminuric patients (albumin excretion from 30–300 mg/d), and 10 healthy controls. All patients were older than 18, having the diabetic disease more than one year, glomerular filtration rate more than 60 mL/min/1.73 m^2. Serum creatinine, glycosylated hemoglobin (HbA1c), and concentration of transferrin in the 24 h urine samples as well as in spot urine were measured using a highly sensitive one-step sandwich enzyme immunoassay kit. **Results.** Urinary transferrin was significantly higher in the microalbuminuric patients than in the normoalbuminuric ones and healthy control subjects. When comparing these goups according to the urinary transferrin concentration, we found a statistically significant positive correlation r = 0.584 ($p < 0.001$). There was no correlation between level of urinary transferrin and glycoregulation, and no correlation was found between transferrin and duration of diabetes. **Conclusions.** The results from this study provide the evidence that the urinary transferrin levels could be used as an early marker of diabetic nephropathy.

Key words:
diabetes mellitus; diabetic nephropathies; albuminuria; biomarkers; transferrin.

Apstrakt

Uvod/Cilj. Dijabetesna nefropatija predstavlja jedan od vodećih uzroka hronične bubrežne bolesti i terminalne bubrežne insuficijencije. Zastupljena je kod 20%–40% bolesnika sa dijabetes melitusom, a kao prvi znak dijabetesne nefropatije još uvek se smatra mikroalbuminurija. Niska senzitivnost i specifičnost mikroalbuminurije su doveli do ispitivanja novih urinarnih biomarkera koji bi mogli biti rani pokazatelji postojanja dijabetesne nefropatije. Ova studija spovedena je da bi se utvrdilo da li urinarni transferin može biti rani marker dijabetesne nefropatije. **Metode.** U našu studiju bilo je uključeno 80 bolesnika sa tipom 2 dijabetesa, podeljenih u dve grupe: grupa 1 – normoalbuminurični bolesnici (ekskrecija albumina do 30 mg/dan); grupa 2 – mikroalbuminurični (ekskrecija albumina od 30–300 mg/dan) i 10 zdravih osoba. Svi bolesnici bili su stariji od 18 godina, imali su dijabetes melitus duže od jedne godine i jačinu glomerulske filtracije veću od 60 mL/min/1,73 m^2. Svim bolesnicima određivan je nivo serumskog kreatinina, glikozilovanog hemoglobina i transferina u urinu. Koncentracija transferina određivana je u 24 časovnom uzorku urina i u prvom jutarnjem urinu primenom visoko senzitivnog ELISA kita. **Rezultati.** Koncentracija urinarnog transferina bila je značajno veća kod bolesnika koji su imali mikroalbuminuriju u poređenju sa bolesnicima koji su bili normoalbuminurični i zdravim osobama, a Pearson-ov koeficijent korelacije bio je r = 0,584 ($p < 0,001$). Nismo dobili povezanost između nivoa urinarnog transferina i glikoregulacije, kao ni nivoa transferina i dužine trajanja dijabetesa. **Zaključak.** Rezultati ove studije pokazuju da bi urinarni transferin mogao biti rani marker dijabetesne nefropatije.

Ključne reči:
dijabetes melitus; dijabetesne nefropatije; albuminurija; biološki pokazatelji; transferin.

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Introduction

Diabetes mellitus (DM) is a chronic disease whose incidence and prevalence show a steady increase. According to the International Diabetes Federation about 415 million people suffer from diabetes around the world, and it is estimated that by 2040 the number of people with diabetes will be around 642 million, with prevalence of 10% [1]. An increasing number of diabetic patients, mostly with the type 2 diabetes (90%) is associated with enhanced rate of diabetic complications, including diabetic kidney disease [2]. Diabetes is considered a leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD). Costs of care for patients with diabetic kidney disease (DKD) are extremely high, especially after they enter ESRD, and it is necessary to establish the diagnosis of diabetic nephropathy as soon as possible [3, 4]. Microalbuminuria (MA) is generally considered to be the earliest non-invasive marker of kidney damage and it was described for the first time in 1960s [5]. Microalbuminuria is defined as persistent elevation of albumin in the urine, of 30–300 mg/day, and it is generally considered the earliest non-invasive marker for the development of diabetic nephropathy (DN), even though the specificity and sensitivity of MA are limited [6, 7]. Some patients with diabetes mellitus progress to DN even if urinary albumin levels are in the normal range, indicating that albuminuria is not the perfect marker for the early detection of DN [8, 9]. Recent studies have shown that some relevant biomarkers associated with DN were found and they potentially could be used to predict DN or progression of the disease [10]. Several different markers of tubular and glomerular damage were investigated to discover DN in its early phase and to start therapy as soon as possible [11].

Urinary transferrin is considered to be an early marker of glomerular injury in diabetic patients. It is a protein, slightly higher molecular weight than albumin (76.5 kDa). Due to its low molecular weight and its ionic load, it filters easily through the glomerular membrane [12]. Some previous studies have shown that increased urinary transferrin excretion can be reported before MA in the normoalbuminuric patients with DM type 2. Because of that, urinary transferrin is considered to be a more sensitive marker of glomerular damage in the diabetic patients [13]. Excretion of transferrin was not associated with glycemic control (hemoglobin A1c), but some studies showed that urinary transferrin concentration was higher in the patients with diabetic retinopathy [14]. The aim of this study was to determine if urinary transferrin can be classified into a group of early biomarkers of DN.

Methods

This cross-sectional study was carried out between September 2015 and December 2016, with the aim to investigate the correlation between MA and urinary transferrin in DN. The study was approved by the Ethics Committee of the Military Medical Academy, Belgrade, Serbia and written informed consent was taken from all patients involved. Eighty patients with type 2 diabetes mellitus (DM2) with duration of the disease for one year or more, estimated glomerular filtration rates more than 60 mL/min/1.73 m^2, and without albuminuria were included in the study. The patients with overt albuminuria (> 300 mg/day), previous renal diseases, urinary tract infection in the last 4 weeks, the use of nephrotoxic drugs, systemic disease, malignant diseases except for basocellular skin carcinoma, were excluded.

The selected patients were studied in detail for the history and physical examination, including ultrasonography of the kidney. Age, gender, duration of diabetes mellitus, weight, height, blood pressure and smoking habits were noted too. The body mass index (BMI) was calculated according to the formula based on the height and weight measurements of the patients. The blood samples were taken after overnight fasting for at least 8 hours and the following parameters were analyzed: serum level of glycaemia, urea, creatinine, glycated hemoglobin (HbA1C). Glomerular filtration rate (GFR) was calculated based on the CKD-EPI formula. [GFR = 141 × min (Scr /κ, 1) α × max (Scr /κ, 1)-1.209 × 0.993 years × 1.018 (for women) = mL/min/1.73 m^2] [15]. Transferrin concentration (ng/mL) and transferrin to creatinine ratio (mg/g of creatinine) were determined in a spot morning urine sample, and albuminuria (30 mg/day or greater) measured in a 24h urine collected on the subsequent day. All samples of urine were immediately processed within 4 hours of collection to ensure optimal protein stability. Urine was centrifuged (1000 × g, 20 min), then divided into 1.5 mL aliquots and frozen at -80°C until the analysis. The levels of urinary transferrin were determined by the commercially available ELISA kits from Elabscience Biotechnology Co., Ltd. Minimum and detectable concentration for urinary transferrin was 1.56 ng/mL.

Statistics

Statistical analyses were performed using the Statistical Package for the Social Science (SPSS) version 19.0. Basic descriptive statistical parameters were presented by the measures of central tendency (mean and median), a measure of variability (standard deviation and variation interval) and were expressed in percentages. To compare continuous variables, the Student's *t*-test was used for independent samples or the Mann-Whitney test, depending on the normality of distribution, which was checked by the Kolmogorov-Smirnov test. For comparison of frequencies for categorical variables, the χ^2-test was used. A statistical hypothesis was tested at 0.05 level of significance, and probability (*p*) value less than 0.05 was regarded statistically significant.

Results

Our study included 80 type 2 diabetic patients, 44 (55%) males and 36 (45%) females, mean age 59.85 ± 8.87 years (range 38–73 years). Prevalence of MA was 41.25% (33 patients) and 58.75% (47 patients) were normoalbuminuric. Among the patients with MA 17 (51.52%) were males and 16 (48.48%) were females. The average duration of diabetes was 13.29 ± 7.69 years, and the average estimated GFR was 86.86 ± 14.18. There were no significant differences in baseline clinical characteristics among examined groups (Table 1).

Table 1

Baseline clinical characteristics of type 2 diabetic patients according to the levels of urinary albumin

Characteristics of the patients	All patients (n = 80)	Normoalbuminuric (n = 47)	Microalbuminuric (n = 33)	Healthy persons (n = 10)	*p*
Gender (M/F), n/n	44/36	27/20	17/16	5/5	0.276
Age (years), mean ± SD	59.85 ± 8.871	60.49 ± 8.73	58.94 ± 9.13	54 ± 10.59	0.014
Duration of DM (Years), mean ± SD	13.29 ± 7.69	13.34 ± 7.74	13.21 ± 7.73	n/a	0.942
BMI (kg/m^2), mean ± SD	27.36 ± 4.42	26.64 ± 3.56	28.38 ± 5.31	25.73 ± 4.77	0.325
Current smoker (%), mean ± SD	39 (48.8)	26 (55.3)	13 (39.4)	3 (30)	
Systolic BP (mmHg), mean ± SD	134.60 ± 14.08	133.47 ± 12.87	136.21 ± 15.71	122 ± 17.02	0.411
Diastolic BP (mmHg), mean ± SD	81.56 ± 7.53	80.96 ± 7.42	82.42 ± 7.72	75.5 ± 10.39	0.389
Serum creatinine (μmol/L), mean ± SD	75.38 ± 15.04	76.64 ± 15.97	73.58 ± 13.64	73.6 ± 7.6	0.360
GFR (mL/min /1.73 m^2), mean ± SD	86.86 ± 14.18	85.78 ± 13.55	88.39 ± 15.12	92.84 ± 9.06	0.430
HbA1c (%), mean ± SD	7.59 ± 1.34	7.25 ± 1.15	8.07 ± 1.45	4.93 ± 0.3	0.074

DM – diabetes mellitus; BMI – body mass index; BP – blood pressure; HbA1c – hemoglobin A1c; GFR – glomerular filtration rate; SD – standard deviation.

Table 2

Correlation of transferrinuria and microalbuminuria

Transferrin concentration (μg/gC$_r$)	Microalbuminuric patients (n = 33) (≥ 30 mg/24h)	Normoalbuminuric patients (n = 47) (< 30 mg/24h)	Perason's *r*	*p*
	mean ± SD	mean ± SD		
Transferrin concentration 24h urine	91.76 ± 68.45	22.56 ± 31.46	0.489	< 0.001
Transferrin concentration spot urine	85.07 ± 56.54	25.63 ± 29.85	0.354	< 0.001

C$_r$ – creatinine; Pearson's test, *r* – correlation coefficient; SD – standard deviation.

Urinary transferrin concentration in spot samples and urinary transferrin concentrations in 24h urine samples showed significant linear correlation, therefore, we used results from spot urine samples for further analyses.

The mean concentration of urinary transferrin in the MA patients was 85.07 ± 56.54 μg/gC$_r$, and for the normoalbuminuric patients it was 25.63 ± 29.85 μg/gC$_r$. We found a statisticaly significant correlation in the transferrin concentration between these two groups (Table 2).

Table 3

Correlation of transferinuria with independent variables

Parameters	All patients (n = 80)	Pearson's *r*
	mean ± SD	
Age (years)	59.85 ± 8.87	0.003
BMI (kg/m^2)	27.3 ± 4.42	0.053
HbA1c (%)	7.59 ± 1.34	0.132
Duration of DM (years)	13.29 ± 7.69	0.127
Microalbuminuria	40.42 ± 40.89	0.584

Pearson's test, *r* – correlation coefficient; SD – standard deviation; BMI – body mass index; HbA1c – hemoglobin A1c; DM – diabetes mellitus.

The correlation analysis for the concentration of urinary transferrin with independent variables is shown in Table 3. Among all variables, we found significant correlation only with MA (Table 3).

Diabetic retinopathy was found in 24 (30%) patients. Those patients had significantly higher urinary transferrin levels, albumin excretion and duration of diabetes.

Sensitivity and specificity of urinary transferrin concentrations expressed as an area under the reciever operating characteristics (ROC) curve (AUC), and it was 87.1%, with the sensitivity 81.8%, and specificity 80.9% [95% confidence interval (CI) – from 0.796 up to 0.945; $p < 0.001$] (Figure 1).

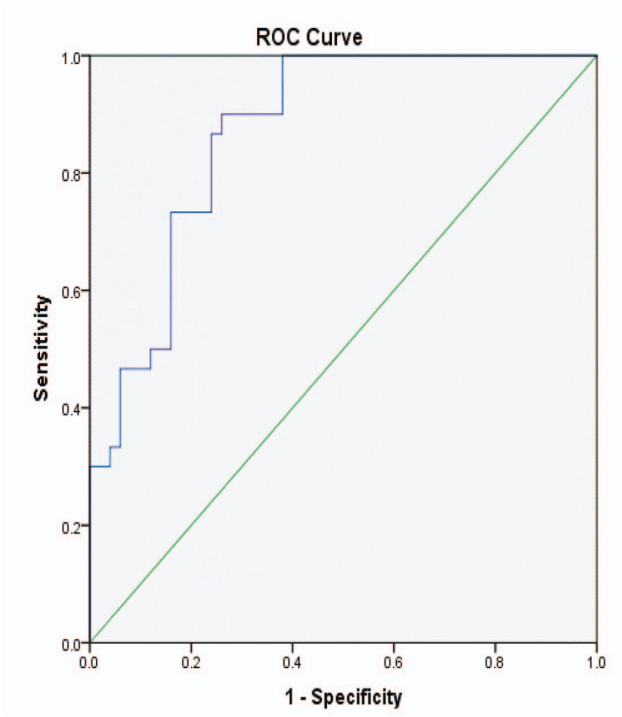

Fig. 1 – Sensitivity and specificity of urinary transferrin concentration.
ROC – receiver operating characteristic.

Discussion

Albuminuria is considered as a marker of kidney (glomerular) damage, and the first clinical indicator of DN presence [16]. Even today it is a clinically useful tool for predicting the outcome and for monitoring a response to the therapy [11]. Discordance between the presence of albuminuria and the decline in renal function is crucial point of clinical significance of albuminuria. The presence of albuminuria is not mandatory in all patients with reduced GFR. Perkins et al. [9] reported the development of advanced CKD (GFR < 60 mL/min 1.73 m^2) without a concomitant progression of albuminuria in the type 1 diabetic patients. Chen et al. [17] compared several studies from 1977 to the present and showed that a portion of diabetic patients with normoalbuminuria had progressive decline in renal function, referred to nonalbuminuric DN. In different studies, the number of nonalbuminuric diabetic kidney disease was from 21.8% reported by Boronat et al. [18] to 56.6% reported in 2014 by Penno et al. [19]. Nonalbuminuric renal impairment was not associated with HbA1c and retinopathy, but some studies found that gender is correlated with nonalbuminuric renal impairment. That is why we need a new biomarker with higher sensitivity and specificity for an earlier detection of DN and more accurate prediction of the progression to ESRD. Therefore, we analyzed urinary transferrin as a biomarker of glomerular injury implicated in the early DN, in nonalbuminuric diabetic patients.

The results of our study showed that increased urinary excretions of transferrin was higher in the patients with MA than in the normoalbuminuric type 2 diabetic patients. We found statistically significant correlation between concentration of urinary transferrin and MA. This is in accordance with the results obtained by Narita et al. [13] who reported that increased urinary transferrin found in the diabetic patients independently of microalbuminuria could also predict the development of MA in the normoalbuminuric DM2 patients. In the 24-month follow-up study with the DM2 patients, Kazumi et al. [20] found that 31% of patients who had transferrinuria at baseline subsequently developed MA, compared with 7% of patients without transferrin excretion. They concluded that in the patients with type 2 diabetes without MA, increased urinary transferrin excretion may predict the development of MA. The same results were found by Kanauchi et al. [21] in the group of 60 DM2 patients. They presented a significant correlation between the urinary excretion of transferrin and albumin. Their findings indicate that urinary transferrin may be useful in detecting DN at an early stage. Al-Rubeaan et al. [22] described similar results in a cross-sectional study in the group of 467 DM2 patients.

Similarly to our results, O'Donnell et al. [23] found no correlation between urinary transferrin levels and glycemic control in the group of 40 DM2 patients at first day of the disease diagnosis and after 6 and 12 weeks of treatment. Urinary excretion rates of transferrin were measured, and they showed that urinary transferrin was not correlated with glycemic control. We found no correlation between the urinary transferrin levels and the duration of diabetes, as well.

Several studies found a relationship between excretion of urinary albumin and diabetic retinopathy, and the famous one was the Japanese study of Moriya et al. [24], which included 2,205 DM2 patients aged 40–70 years. We found similar results. The patients with retinopathy had significantly higher values of urinary transferrin excretion as well as higher levels of MA.

Our sensitivity and specificity analysis of urinary transferrin excretion showed that it could be more sensitive indicator of an early glomerular damage in diabetes mellitus than MA.

Conclusion

Urinary transferrin was significantly increased in DM2 patients with MA. It was independent of diabetes duration and glycemic control. According to our results, the level of urinary transferrin excretion could be used as an early biomarker of diabetic nephropathy.

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