Zagazig University Medical Journal
www.zumj.journals.ekb.eg

ORIGINAL ARTICLE

Assessment of Urinary Podocin Level as an Early Indicator in Diabetic Nephropathy

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Submit Date	2019-03-09
Revise Date	2019-05-29
Accept Date	2019-06-01

ABSTRACT

Background: Diabetic nephropathy one of the leading causes of end stage renal disease in which podocytes damage play a vital role. It will be interesting to recognize podocyturia as a first noninvasive marker of subclinical early renal damage. The current study was aimed to assess the Urinary podocin level (podocyte-specific protein detected in urine using antipodocin antibody ELISA kit) as an early indicator for the glomerular lesion in patients with type 2 diabetes mellitus. **Methods:** This study included 45 clinically stable type 2 diabetic patients with glomerular filtration rate (GFR) (>60 ml/min/1.73m^2) and excluded patients with fever, urinary tract infection, uncontrolled hypertension, congestive heart failure or malignancy collected from Zagazig University Hospitals and Air force Hospital. In addition to 15 apparently healthy volunteers matched in age and sex as a control group. Patients were classified according to albumin/creatinine (A/C) ratio into three groups 15 patients for each group.
Results: There was statistically significant difference between all studied groups as regards urinary podocin levels (P <0.001). urinary podocin levels appeared even in normoalbuminuria group with median 9.2 (7.1 – 12 ng/ml) which was significantly higher compared to control group with median 3.9 (2.2 – 4.8) ng/ml and the higher level was found in macroalbuminuria group with median 41.5 (38.5 – 48.5)ng/ml.
Conclusion: Urinary podocin levels were highly sensitive early marker to predict diabetic nephropathy in patients with type 2 diabetes mellitus for further studies.
Keywords: Urinary Podocin Level, Diabetic Nephropathy

INTRODUCTION

Diabetes mellitus is one of the most chronic diseases affecting 451 million people worldwide and the incidence will rise to 693 million by 2045 **[1].**

Diabetic kidney disease (DKD) is defined by characteristic structural and functional changes. The predominant structural changes include mesangial expansion, glomerular basement membrane(GBM) thickening, podocyte injury and ultimately glomerular sclerosis **[2].**

Diabetic nephropathy (DN) can be prevented to a great degree by early detection using novel biomarkers in body fluids **[3]**.

Podocytes are key structural elements of the glomerular filtration barrier (GFB). It is accepted that podocytes' injuries play an essential role in the progression of diabetic kidney disease. Monitoring urine podocytes and podocyte-specific proteins can reveal potentially important urinary markers for early diagnosis of DKD **[4].**

Podocytes are normally absent or seen in small numbers in urine of normal persons or those with inactive renal disease. Podocyturia increases with active renal disease even before proteinuria appears and also seen to be improved with treatment **[5]**. Podocytes is detected in urine by indirect immune florescence using antibodies against podocin. Number of patients with podocyturia and micro-albuminuria increased as duration of diabetes increased, proving podocyturia can

also act a marker of diabetic nephropathy in type 2 DM patients **[6].**

The current study was aimed to assess the urinary podocin level as an early marker in diabetic nephropathy.

METHODS

This study was conducted in outpatient clinic of Endocrinology Unit of Zagazig University Hospitals and Air Force Hospital. The study including 60 subjects, 45 of them were patients with type 2 diabetes mellitus, in addition to 15 apparently healthy volunteers matched in age and sex as a control group.

Study design & Grouping:

A cross-sectional study was carried out including 45 Patients with type 2 diabetes mellitus.

They were divided into 3 groups according to A/C ratio:

Group 1: 15 patients with normoalbuminuria (<30mg/gm)

Group 2: 15 patients with microalbuminuria (30-300mg/gm)

Group 3: 15 patients with macroalbuminuria (>300 mg/gm)

In addition to 15 apparently healthy volunteers matched in age and sex as a control group.

This study conducted over 45 diabetic patients 51.1% were males and 48.9% were females with mean of age 49.62 ± 7.01, ranging from 35 – 65years, with mean of BMI(kg/m^2) 26.96±2.27 ranging from 22.1-32, median of duration of DM (in months) 48 ranging from7 – 156, mean of GFR 87.34 ± 17.86 (ml/min/1.73m^2) and about 53.3% of patients on insulin treatment while 46.7% of patients on oral hypoglycemic drugs and Hypertension was present in 42.2% of all cases (table 1).

However, in control group 53.3% were male and 46.7% were female with mean of age 46.27±7.58, ranging from 36-63 years, with mean of BMI (kg/m^2) 25.69±1.32 ranging from 23.8-28.4. Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University and Air Force Hospital. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Inclusion criteria:

Clinically stable type 2 diabetic patients with GFR (>60 ml/min/1.73 m^2) was included.

Exclusion criteria:

All patients had history of any of the following were excluded:

Patients with type I DM, history of diabetic ketoacidosis or hypoglycemic coma in the past 3 months preceding the study was excluded.

Any general medical disease other than diabetes that may affect the general condition of the patients e.g. cardio vascular diseases (CVD), chronic liver diseases, autoimmune diseases, inflammatory diseases, pregnancy, infections, hematological diseases, neoplastic diseases and other endocrine diseases such as (thyroid and adrenal disorders).

Any urinary system disorders (macroscopic hematuria, urinary tract infection, tumors and past history of glomerulonephritis or renal diseases other than DN).

Previous treatment with immunosuppressive drugs, chemotherapy or radiotherapy was excluded.

Methods:

After obtaining an informed written consent all participants were subjected to the following data:

Full History & clinical Examination

All enrolled subjects underwent full history taking & clinical examination with special concern to age and gender, history of co-morbidities including hypertension (HTN), hepatitis C virus (HCV), Cerebrovascular stroke (CVS) and Ischemic heart disease (IHD), Current medications, Duration of diabetes and other diabetic complications.

BMI was computed using the equation:

Body Mass Index: weight (in kg) / height (in meter^2).

Laboratory Investigations

Blood sample:

Seven milliliters of venous blood were collected under complete aseptic precautions after 10 hours fating. Five milliliters were put in plain test tubes without anticoagulant, and the remaining two milliliters were put in a test

tube with ethylene diamine tetra-acetate (EDTA) (1.2mg/ml) as an anticoagulant, to be used for performing hemoglobin level and HbA1c test. After clotting, samples were centrifuged at 1500 x g for 15 minutes. Part of the separated serum was used to perform fasting blood glucose (FBG), serum urea, creatinine, albumin and serum lipids (cholesterol, HDL, LDL and triglycerides) tests.

GFR was calculated by The CKD-EPI equation (7)

GFR (mL/min/1.73 m^2) = 141 × min (serum creatinine/*k*, 1)$^{\alpha}$ × max (serum creatinine/*k*, 1)$^{-1.209}$ × 0.993^{Age} × 1.018 (if female) × 1.159

Where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is –0.329 for females and –0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1**.**

Urine sample

Routine: 15 ml of morning urine samples were collected aseptically from all subjects included in the study, 1 ml was used for Urinary albumin/creatinine ratio (ACR), 10 ml were used for urine analysis. Special: 4 ml of sample was centrifuged for 20 minutes at 3000 rpm then supernatant was collected into aliquots and stored at -20ºC for podocin levels estimation by enzyme-linked immuno-sorbent assay (ELISA). Repeated freezing and thawing was avoided.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

The used tests were:

Chi-square test: For categorical variables, to compare between different groups. Fisher's Exact or Monte Carlo correction: Correction for chi-square when more than 20% of the cells have expected count less than 5.

Kruskal Wallis test: For abnormally quantitative variables, to compare between more than two studied groups.

F-test (ANOVA): For normally quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons.

RESULTS

Our study shows that urinary podocin levels were significantly higher in patients group with median 20.5 compared to control group with median 3.9 (table 2) (P<0.01).

Also, GFR was significantly lower in patients with macroalbuminuria then patients with microalbuminuria then patients with normoalbuminuria when compared to control group with mean 71.8±15.93, 87.89±8.64, 102.31±13.25 and 110.37±9.56 respectively. (P<0.01) (table 3).

Chronic kidney disease stage was significantly higher in macroalbuminuric patients compared to other studied groups (P<0.01) (table 3).

Urinary podocin levels were significantly higher in patients with macroalbuminuria, microalbuminuria and normoalbuminuria compared to control group with median (41.5,19,9.2,3.9) respectively. (P<0.01)(table 4). Also, urinary podocin levels were significantly higher in patients with macroalbuminuria and microalbuminuria compared to normoalbuminuric patients (P<0.01) (table 4).

Urinary podocin levels were significantly higher in macroalbuminuric patients compared to microalbuminuric patients (P<0.01) (table 4).

Table 1. Comparison between different studied groups according to demographic data.

		Control group	Normoalbuminuria group	Microalbuminuria group	Macroalbuminuria group	Test value	P-value	Sig.
		No. = 15	No. = 15	No. = 15	No. = 15			
Age	Mean ± SD	46.27 ± 7.58	46.33 ± 5.64	50.27 ± 6.92	52.27 ± 7.44	2.770 •	0.050	S
	Range	36 – 63	35 – 55	39 – 61	38 – 65			
Sex	Female	7 (46.7%)	5 (33.3%)	7 (46.7%)	10 (66.7%)	3.404 *	0.333	NS
	Male	8 (53.3%)	10 (66.7%)	8 (53.3%)	5 (33.3%)			
BMI (kg/m 2)	Mean ± SD	25.69 ± 1.32	25.65 ± 2.18	27.02 ± 1.42	28.19 ± 2.43	6.155 •	0.001	HS
	Range	23.8 – 28.4	22.1 – 29	24.7 – 30.1	23.7 – 32			

BMI: Body Mass Index

Table 2. Comparison between patients and control groups according to urinary podocin Ab (ng/ml).

Urinary podocin(ng/ml)	Control group	Patients group	Test value≠	P-value	Sig.
	No. = 15	No. = 45			
Median (IQR)	3.9 (2.2 – 4.8)	20.5 (11.3 – 38.5)	-5.745	0.000	HS
Range	1.49 – 6	5.8 – 65.7			

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
≠: Mann-Whitney test

Table 3. Comparison between different studied groups accordiong to GFR and CKD stage.

		Control group	Normoalbuminuria group	Microalbuminuria group	Macroalbuminuria group	Test value	P-value	Sig.
		No. = 15	No. = 15	No. = 15	No. = 15			
GFR	Mean ± SD	110.37 ± 9.56	102.31 ± 13.25	87.89 ± 8.64	71.81 ± 15.93	29.017•	0.000	HS
	Range	97.8 – 129	76.7 – 130.2	65.6 – 100	60.1 – 116.1			
CKD Stage	0	15 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	86.638*	0.000	HS
	1	0 (0.0%)	14 (93.3%)	12 (80.0%)	3 (20.0%)			
	2	0 (0.0%)	1 (6.7%)	3 (20.0%)	12 (80.0%)			

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
*: Chi-square test; •: One Way ANOVA test; ≠: Kruskal-Wallis test
GFR: Glomerular Filteration Rate
CKD stage : Chronic Kidney Disease stage

Table 4. Urinary podocin levels (ng/ml) in different study groups

Urinary podocin levels (ng/ml)	Control group	Normoalbuminuria group	Microalbuminuria group	Macroalbuminuria group	Test value ≠	P-value	Sig.
	No. = 15	No. = 15	No. = 15	No. = 15			
Median (IQR)	3.9 (2.2 – 4.8)	9.2 (7.1 – 12)	19 (14.5 – 22.3)	41.5 (38.5 – 48.5)	52.667	0.000	HS
Range	1.49 – 6	5.8 – 22.5	10 – 35.5	30.9 – 65.7			

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
≠: Kruskal-Wallis test

DISCUSSION

Diabetic nephropathy is one of the leading causes of end-stage renal disease and creates heavy healthcare burdens globally [8].

Podocytes are highly specialized epithelial cells lining the urinary surface of the glomerular capillary tuft. They are part of the filtration barrier together with capillary endothelial cells and the glomerular basement membrane (GBM), ensuring the selective permeability of the glomerular capillary wall [9].

Progressive chronic nephropathies in diabetes nephropathy are typically characterized with podocyte depletion and associated glomerulosclerosis due to oxidative stress that leads to podocytes depletion resulting in abnormal proteinuria, progressive renal damage and eventual loss in renal function [10].

Our results go in agreement with most of other studies as Roberto et al [11] study in which demonstrated that urinary podocin levels were increased in glomerular lesion in patients compared to healthy controls. Another study, show that urinary podocin levels were markedly elevated in podocytes effacement which occur in diabetic nephropathy [12]. Another study reported that urinary podocin levels could be useful in monitoring disease activity in LN (Lupus Nephritis) patients [12].

We found that there was statistically significant difference between all studied groups as regards urinary podocin levels (P <0.001). Urinary podocin appeared even in normoalbuminuria group (9.2 (7.1 – 12) ng/ml) which was significantly higher compared to control (median 3.9 (2.2 – 4.8 ng/ml) and the significantly higher level was found in macroalbuminuria group (median 41.5 (38.5 – 48.5) ng/ml) in comparison to other groups.

This can be explained as diabetes is associated with glomerular filtration barrier GFB damage and submits podocytes to injurious factors by several mechanisms such as neurohormonal changes, oxidative stress and diminished expression of adhesion molecules, resulting in podocyte injury and loss leading to increased glomerular permeability manifested clinically as albuminuria [13]. Our results above go in agreement with the study of Trimarchi [14] in the identification of urinary podocin could be an additional tool to be considered by nephrologists to assess the activity of glomerulopathies. Furthermore, our study showed highly significant positive correlation between duration of diabetes and urinary podocin levels as the more the duration of diabetes the more the level of urinary podocin with P value of <0.001.

In line with our study, it was reported in Petrica et al [15] study that podocin were detected in the urine of 10% of the healthy controls, 24% of the normoalbuminuric, 40% of the microalbuminuric, and 82% of the macroalbuminuric patients. In multivariate logistic regression analysis, urinary podocin levels correlated with urinary albumin:creatinine ratio. In agreement with Lioudaki et al [16] who states that Diabetic and control groups differed in the presence of hypercholesterolemia, hypertriglyceridemia, central obesity and HTN, namely, factors pathogenetically associated with DM.

Regarding our study, we found that Sensitivity of urinary podocin as an early marker to predict diabetic nephropathy in normoalbuminuria group (versus control) is 94.7% while specificity is 92%, with a positive predictive value of 94.7 and negative predictive value of 92% at cut off value >6.0 ng/ml ($p<0.001$) which comes in agreement with El Shaarawy et al [17] as urinary podocin is highly sensitive and specific marker to predict diabetic nephropathy. Our study also comes in agreement with Hayman et al [18] as urinary podocin levels may function as a more specific marker of ongoing glomerular damage as it was significantly increased in normoalbuminuric group compared to control group.

Finally, our study show multiple strength points as it was started by a full bibliographic citation, full biographical information about the author was given, the general problem area and intentions toward it was discussed building on past researches and the objective of the research was clearly stated. Also, further large cohort study for urinary podocin level as prognostic marker of diabetic nephropathy as a larger number may improve the validity.

Conclusion

Urinary podocin was a highly sensitive early marker to predict diabetic nephropathy in patients with type 2 diabetes mellitus with normoalbuminuria and may associated with increase albuminuria and progression of diabetic nephropathy for further studies.

Recommendations

Further large cohort study for urinary podocin level as prognostic marker of diabetic nephropathy as a larger number may improve the validity. Further interventional studies about effect of glycemic control drugs and anti-proteinuric drugs on urinary podocin levels in diabetic patients.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding information None declared

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To Cite This Article: Abdel Rahman HS, Hadhoud K, Bakr HG, Youssef KM. Assessment of Urinary Podocin Level as an Early Indicator in Diabetic Nephropathy. ZUMJ 2019; 25 (5): 683-688. Doi 10.21608/zumj.2019.10380.10960.